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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

**Abha Malik, Navjeevan Singh, Arora, V. K. and Arati Bhatia.** Association of microfilarae with leprosy and other diseases. Acta Cytol. **46** (2002) 69–70.

This paper addresses the association of microfilariae with other granulomatous diseases, particularly leprosy. The histopathological findings in a patient with borderline tuberculoid leprosy in a type 1 reaction and associated microfilaria are presented. It is emphasized that overall evaluation of the clinical setting assumes importance in the interpretation of the cytological findings, since such disorders share a common cytomorphological picture.—Trop. Dis. Bull.

**Aoki, Y.** Leprosy prevention law and healthcare professionals in Japan. Int. Hist. Nurs. J. **7** (2002) 68–74.

This article considers the issues surrounding leprosy, and focuses on the social stigma and mistreatment associated with the disease. In particular, the history of leprosy patients and leprosy-prevention law in Japan is examined in both a historical and social context. Matters related to infringement of human rights are also considered along with issues for nurses caring for patients with leprosy. Eugenics and its legal approval are then discussed, as is the abolition of the leprosy laws in Japan. Finally, the author argues that problems surrounding this infectious disease are not country-specific; nurses may be ignorant of the number of patients with this debilitating disease worldwide, and of the policies and laws surrounding it.—Author's Abstract

**Bundit, C., Sampoonachot, P. and Mahotarn, K.** Present status and future vision of the International Medical Co-operation for Leprosy. Nihon Hansenbyo Gakki Zasshi **71** (2002) 211–213.

In the year 2002, leprosy situation in Thailand has been steadily progress. However, the prevalence rate and percentage of leprosy patients are still quite high in the North-Eastern part of Thailand. Therefore, we have focused our plan of action 2001–2 on "The strengthening of Leprosy Elimination and Prevention of Disability in the North-Eastern Region." The objective of which is to improve and sustain the ability of leprosy related staff to conduct activities such as case finding, complication diagnosis, treatment of disabilities, rehabilitation, supervision and evaluation. The International Medical Co-operation for Leprosy in 2001, we received funds from Netherland Leprosy Relief Association (NLR) for 9 programs concerning training of leprosy for health officers and assessment of the quality of life for leprosy affected persons living in northeastern colonies. There are 3 training courses of leprosy for new medical doctors, lab technicians from community and provincial hospitals and 2 workshops on Rehabilitation and Development of Leprosy Affected Persons' "Quality of Life" under the Germany Leprosy Relief Association (GLRA) support. From Japan we received funding from Sasakawa Memorial Health Foundation (SMHF) for 4 projects in immunological studies since 1997 and 2 projects concerning dental services for leprosy patients in the north and northeast regions from Umemoto Memorial Dental Service Group (UMDSG). The medical co-operation between Japan and Thailand should increase in many aspects especially, for new chemotherapy, immunotherapy and vaccine study in leprosy. The future vision of leprosy, we plan

to set up the International Center of Leprosy for medical officers, technicians, etc. for the South-East Asian Countries. You are welcome to join and work together with us.—Authors' Abstract

**Convit, J.** Fighting leprosy and other endemic diseases. Rev. Panam. Salud Publica. **12** (2002) 1–4.

As part of its 100th-anniversary celebration, the Pan American Health Organization has named 12 persons as "Public Health Heroes of the Americas" in recognition of their noteworthy contributions to public health in the Region of the Americas. Over the course of this year, the Revista Panamericana de Salud Publica/Pan American Journal of Public Health will be carrying pieces written by or about these heroes. Como parte de la celebracion de su Centenario, la Organizacion Panamericana de la Salud (OPS) ha distinguido con el titulo de Heroes de la Salud Publica a 12 personalidades que se han destacado por su valiosa contribucion a la salud en el continente americano. A lo largo de este ano, la Revista Panamericana de Salud Publica/Pan American Journal of Public Health publicara una serie de escritos de los mismos galardonados o acerca de ellos.—Author's Abstract

**Hatano, K.** What is possible for us to corroborate developing countries in the leprosy field? Nihon Hansenbyo Gakki Zasshi **71** (2002) 215–221. (In Japanese)

Developing countries have their own unique characteristics, histories, and situation. There are great differences from country to country. From the experiences worked in both Bangladesh and Myanmar which share their border, some similarities and dissimilarities among these two greatly different countries are discussed. Considering this, common problems on leprosy in the developing countries are analyzed. The needs of developing countries in the field of leprosy are studied, and the possible way of corroboration for us, Japanese leprosy workers, are suggested.—Author's Abstract

**Kumaresan, J. A. and Maganu, E. T.** Socio-cultural dimensions of leprosy in northwestern Botswana. Soc. Sci. Med. **39** (1994) 537–541.

A study to determine some sociocultural factors influencing knowledge and attitudes of the community toward leprosy was carried out in northwestern Botswana, where cases of leprosy have been known to exist over the years. The study was largely qualitative, using ethnographic approaches. The research was tailored in a way to capture the ethnic diversity of the region, in particular 2 ethnic groups, namely Bayei and Bambukushu. The name or symptom complex associated with leprosy was 'ngara' or 'lepero' and this was associated with bad blood. Knowledge on disease causation was lacking which in turn influenced health seeking behavior of patients. Patients were well integrated and accepted into the social structure of communities. Women caring for these patients did experience some additional burden and identified time as their major constraint in caretaking. It was apparent that the degree of rejection correlated with seriousness of the disease and extent of disabilities and dysfunction. The present pattern of health seeking behavior needs to be altered, so that an early diagnosis can be made at the health facility. This will aid appropriate management and prevent occurrence of deformities and disabilities, which in turn will reduce rejection and isolation of patients. Education of community, patients, traditional and religious healers on various aspects of the disease, especially causation, is essential to achieve a change in the health seeking behavior.—Authors' Abstract

**Li Huan-Ying, Shun-Peng, R. and Rong-De, Y.** Leprosy control in a prefecture of Yunnan Province in the Peoples' Republic of China, using intensive health education of the public and primary health care workers for the detection of cases, 1998–1999. Lepr. Rev. **73** (2002) 83–87.

Between January and December 1998, the bacterial indices (BI) of slit skin smears (SSS) were compared with the bacterial indices of granuloma (BIG) of skin biopsies

of 108 leprosy patients (80 men and 28 women) from the Department of Dermatology, Osmania General Hospital in Hyderabad, India. SSS were positive for acid fast bacilli (AFB) in 23 of 108 patients (21%). The highest BI value was 4+ in six patients. When BIG were studied, AFB were observed in 42 of 108 (38.8%) specimens. 22 of 23 skin smear positive cases were also positive for BIG. In contrast, skin smears were negative in 20 of 42 patients who were BIG positive. The difference between values of BIG and BI of SSS was highly significant ($p < 0.005$). BIG of skin biopsies have shown uniformly higher values compared with skin smears in all types of leprosy except in one patient. Moreover, in patients with BIG values of 4+ or more, SSS were positive for AFB in 72% of cases compared with only 23% of SSS positivity in patients with BIG values less than 4+ ($p < 0.001$).—Trop. Dis. Bull.

**Opala, J. and Boillot, F.** Leprosy among the Limba: illness and healing in the context of world view. Soc. Sci. Med. **42** (1996) 3–19.

The study analyzes the traditional beliefs and practices concerning leprosy of the Limba people of Sierra Leone. It shows that this dialectically diverse ethnic group has two views of leprosy and its cause, and two varieties of stigma associated with the disease. The Limba have abandoned their traditional treatments for leprosy in response to an effective leprosy control program, but retained their traditional world view, including its definition of illness, which holds a person seriously ill only when he/she has severe pain or disability. Thus, they seek treatment from the program, but often at a relatively advanced stage of the disease. The study shows that the Limba have reinterpreted the notion of 'germs' as introduced by medical workers, and that leprosy control workers have their own misunderstandings of Limba beliefs and practices. The study points the way to improved communication between leprosy workers and Limba patients by focusing on the points at which their views differ and by identifying concepts within Limba world view that can be adapted by leprosy workers to help convey their message. The study emphasizes the importance of world view as a key to understanding patient attitudes and behavior in developing countries and to making valid cross-cultural comparisons, but notes that it can take years for an investigator to understand the world view of a particular culture. It argues that in short-term research projects there is an advantage to working with an anthropologist who has in-depth knowledge of the culture, but who may not be a specialist in medical anthropology.—Authors' Abstract

**Teo, S., Resztak, K., Scheffler, M., Kook, K., Zeldis, J., Stirling, D. and Thomas, S.** Thalidomide in the treatment of leprosy. Microbes Infect. **4** (2002) 1193.

Leprosy is a chronic infection of the skin and nerves caused by *Mycobacterium leprae*. Erythema nodosum leprosum (ENL) is a reactive state in lepromatous leprosy. Thalidomide has been used to treat ENL since the 1960s. One of its mechanisms of action is anti-inflammatory through selective inhibition of the pro-inflammatory cytokine TNF-alpha produced by monocytes.—Authors' Abstract

**Toweir, A. A. and Chaudhary, R. C.** Review of leprosy cases in Genghazi, Libyan Arab Jamahiriya, 1994–98. East Mediterr. Health J. **6** (2000) 1098–1102.

A descriptive study was conducted using case records from the Leprosy Clinic, Benghazi for the period 1994–98. A constant decline in the number of leprosy cases registered for multidrug treatment (MDT) was observed, from 18 in 1994 to 4 in 1998. The ratio of multibacillary to paucibacillary cases was 1.3:1. Most of the patients were young male adults who were socially and economically productive. An hypopigmented patch was the most common lesion present on easily accessible sites. Early registration, compliance with MDT and follow-up will enhance the cure rate and lead to a reduction in disability rates. Despite elimination surveillance for new leprosy cases is essential.—Authors' Abstract

**Yuasa, Y.** Present and future of leprosy works. Nihon Hansenbyo Gakkai Zasshi **71** (2002) 187–193. (In Japanese)

Convinced with an effectiveness of MDT for curing leprosy as an infectious disease since 1982, WHO has presented to the 44th World Health Assembly (WHA) in May 1991, a resolution on "The Elimination of Leprosy, as a public health problem, by year 2000," with a numerical target of achieving a prevalence of leprosy of one case per 10,000 population, and it was unanimously adopted. Since then all the leprosy endemic countries of the world have expanded their MDT programs to cover the whole country, aided by free availability of MDT drugs through WHO since 1995, and succeeded in reaching the target on global basis at the end of 2000, with reduction of leprosy endemic countries down to 12 from nearly 100. At the WHA of 2000, WHO has put a new resolution to achieve the same target, at a national level, by 2005, and the program is progressing reasonably well in terms of reducing the number of cases registered. However this single minded endeavor of WHO is causing some difficulties, in terms of more comprehensive care of patients, especially in POD and rehabilitation activities. In addition, WHO's public announcements give a strong impression that by the end of 2005 all leprosy problems will be solved with nothing more to do beyond that time. In this presentation, what has been achieved so far, and what needs to be done will be presented briefly. Then various issues facing us currently will be discussed in relation to a realistically perceived final goal, which the speaker defines as "A World without Leprosy related Problems, both medical and social," rather than more commonly accepted "Eradication of Leprosy" or "A World without Leprosy," and explains the reasons. Finally leprosy within the context of human history is discussed rather briefly, pointing out that leprosy patients, throughout history and almost everywhere in the world, suffered a worst case of human rights violation to any minority groups, because they have been conceived as a group of people totally alien to the society. The speakers believe that true understanding of the basic nature of leprosy problems and efforts to solve them will contribute to improved human relationship in general in the world, where any minorities need not to suffer any more, and able to coexist with the surrounding majorities.—Author's Abstract

## Chemotherapy

**Costa Queiroz, R., de Souza, A., Sampaio, S. and Melchior, E.** Biochemical and hematological side effects of clofazimine in leprosy patients. Pharmacol. Res. **46** (2002) 191.

Gastrointestinal toxicity and red skin discoloration were the major side effects observed in leprosy patients undergoing long-term treatment with clofazimine (CFZ). Hematological and biochemical alterations have been cited among other side effects; however, their real magnitude and clinical significance at the doses currently employed in therapy have not been sufficiently documented. We therefore investigated the correlation between CFZ plasma concentration and biochemical (transaminases, bilirubins, alkaline phosphatase, gamma-glutamyltransferase, amylase, urea, creatinine, and potassium plasma levels) as well as hematological changes, blood and reticulocyte counts, osmotic fragility, detection of Heinz bodies and methemoglobinemia (MHM), following in two regimes of treatment: CFZ as a single drug and CFZ as part of multidrug (MDT) therapy, in combination with dapsone and rifampin. MHM and hemolytic anemia were detected in the MDT group only. Eosinophilia was found in patients of either group. Determination of hepatic, pancreatic and renal biochemical parameters showed rare, occasional changes of apparently no clinical significance. We conclude that CFZ is a generally well tolerated and safe drug when given as a daily dose of 50 mg, which is currently used in leprosy patients.—Authors' Abstract

**Muthukumar, T., Jayakumar, M., Fernando, E. M. and Muthusethupathi, M. A.** Acute renal failure due to rifampicin: a study of 25 patients. Am. J. Kidney Dis. **40** (2002) 690–696.

BACKGROUND: Acute renal failure (ARF) caused by rifampin typically occurs on intermittent administration. There are isolated case reports and only one series reported in the literature. Systematic data, especially from countries endemic for tuberculosis and leprosy, are sparse. METHODS: We studied demographic, clinical, biochemical, and histopathologic features and prognosis of 25 consecutive patients with rifampin-associated ARF admitted from July 1990 to June 2000. RESULTS: rifampin-associated ARF constituted 2.5% of all cases of ARF seen during the study period. The most common pattern of drug intake resulting in ARF (40%) was ingestion of a single dose preceded by a drug-free period (range, 10 days to 6 years) after a course of daily rifampin (range, 8 days to 18 months). Onset was with gastrointestinal and flu-like symptoms 4 hours (median) after drug intake. All patients were oliguric. Anemia and thrombocytopenia each occurred in 60% of patients. Acute hepatitis was present in 32%. Among 12 patients who underwent kidney biopsy, 7 patients (58%) had acute interstitial nephritis (AIN). Crescentic glomerulonephritis was seen in 1 patient, and mesangial proliferation, in 3 patients. No single feature at presentation predicted the severity of renal failure. There were no deaths, and all patents recovered renal function. CONCLUSION: Patients with rifampin-associated ARF were oliguric and presented with gastrointestinal and flu-like symptoms, typically after reintroduction of the drug after a drug-free period. Anemia and thrombocytopenia were common. AIN was the most common biopsy finding. No factor predicted severity, but the renal prognosis was good.—Authors' Abstract

**Narang, A. S. and Srivastava, A. K.** Evaluation of solid dispersions of Clofazimine. Drug Dev. Ind. Pharm. **28** (2002) 1001–1013.

Clofazimine (CLF) was formulated with polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP) as a solid solid dispersion (SSD) to increase the aqueous solubility and dissolution rate of the drug. Different molecular weights of PEG (1500, 4000, 6000, and 9000 Da) and PVP (14,000 and 44,000 Da) were used in different drug : carrier weight ratios (1:1, 1:5, and 1:9) and their effect on the dissolution performance of the drug was evaluated in USP Type 2 apparatus using 0.1 N HCl medium. The dissolution rate was compared with corresponding physical mixtures, a currently marketed soft gelatin capsule product, and free CLF. The effect of different methods of preparation (solvent/melt) on the dissolution rate of CLF was evaluated for PEG solid dispersions. Saturation solubility and phase solubility studies were carried out to indicate drug : carrier interactions in liquid state. Infrared (IR) spectroscopy and X-ray diffraction (XRD) were used to indicate drug : carrier interactions in solid state. Improvement in the drug dissolution rate was observed in solid dispersion formulations as compared to the physical mixtures. The dissolution rate improved with the decreasing weight fraction of the drug in the formulation. Polyvinyl pyrrolidone solid dispersion systems gave a better drug release profile as compared to the corresponding PEG solid dispersions. The effect of molecular weight of the PEG polymers did not follow a definite trend, while PVP 14,000 gave a better dissolution profile as compared to PVP 44,000. Improvement in saturation solubility of the drug in the solid dispersion systems was noted in all cases. Further, IR spectroscopy indicated drug : carrier interactions in solid state in one case and XRD indicated reduction in the crystallinity of CLF in another. It was concluded that solid-dispersion formulations of Clofazimine can be used to design a solid dosage form of the drug, which would have significant advantages over the currently marketed soft gelatin capsule dosage form.—Authors' Abstract

# Clinical Sciences

**Gomes Guerra, J., Oliveira Penna, H., Miranda de Castro, L. C., Turchi Martelli, C. M. and Araújo Stefani, M. M.** Erythema nodosum leprosum: clinical and therapeutic up-date. An. Bras. Dermatol. **77** (2002) 389–407.

Erythema Nodosum Leprosum (ENL) is a syndrome that occurs before, during or after leprosy treatment, often interrupting the chronic course of *M. leprae* infection. ENL is considered an important cause of morbidity among lepromatous patients, which may lead to nerve injury, paralysis and deformity without prompt medical care. Nowadays, ENL episodes are considered one of the main causes of hospitalization among lepromatous patients in many endemic Brazilian regions. This paper reviews the epidemiological data on ENL according to different settings. The histopathological pattern and a concise description of the immunologic mechanism underlying type 2 reactions are presented. A semi-objective clinical criterion for grading ENL severity, applicable under field conditions, is proposed. Thalidomide is considered the drug of choice for ENL treatment according to the official Leprosy Control Program. Systemic steroid is mandatory for the treatment of peripheral neuritis, iritis and orchitis associated with ENL. This paper points out the lack of reliable data on reactive episodes and the widespread and possible abuse of steroid therapy on a routine basis. An effective surveillance system including pharmacovigilance is needed for detecting steroid-associated adverse events in this novel leprosy elimination context.—Authors' Abstract

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

Screening for human immunodeficiency viruses type 1 and 2 (HIV-1 and HIV-2) antibodies was carried out in the serum of 1245 leprous patients and 5731 controls selected in 9 different centers from the Congo, Ivory Coast, Senegal, and Yemen Arab Republic. In Yemen, all sera were negative. In the Congo, the seropositivity among patients and controls was, respectively, 3.8 and 5.2%; in Senegal, it was 1.3 and 0.6%; and in the Ivory Coast 4.8 and 3.9%. Differences were not statistically significant, even considering lepromatous or tuberculoid forms (3.6% and 3.7%, respectively). HIV-2 antibodies were only detected in subjects from the Ivory Coast and Senegal. Using appropriate criteria for seropositivity (confirmation by Western blot, reactivity to HIV envelope glycoproteins), and a large selection of patients (several countries with several centers), it appears that leprosy (especially the lepromatous form) is not a factor for HIV infection.—Authors' Abstract

**Low, W. K., Ngo, R. and Qasim, A.** Leprosy: otolaryngologist's perspective. ORL J. Otorhinolaryngol. Relat. Spec. **64** (2002) 281–283.

A patient with hemi-facial erythematous swelling as a result of borderline leprosy and reversal reaction is reported. This uncommon presentation of the disease poses initial diagnostic difficulties to the otolaryngologist. The otolaryngologist must be familiar with otolaryngologic manifestations of leprosy, since early diagnosis and treatment reduces the risk of transmission of the disease and may avoid permanent nerve damage.—Authors' Abstract

**Namisato, M., Goto, M., Gidoh, M., Hosokawa, A., Sugita, Y., Ishii, N., Nagao, E. and Ozaki, M.** Clinical cure of leprosy—a criteria in Japan (2002). Nihon Hansenbyo Gakkai Zasshi **71** (2002) 235–238.

In Japan, a cautious definition of clinical cure of leprosy has been used since 1988. This report presents a new definition of clinical cure for leprosy patients after multidrug treatment is completed. When the patients complete the standard treatment published in 2000, they are defined as "clinically cured." The doctor in charge should inform the patient of the cure of the disease clearly. On the release from the treatment, it is important to explain necessary cares for protection against injuries and prevention from deformities. The patient should be careful about signs of relapse and reactions.—Authors' Abstract

**Thappa, D. M. and Jayanthi, S.** Leg ulcers in active lepromatous leprosy associated with cryoglobulinaemia. Clin. Exp. Dermatol. **27** (2002) 451–453.

A 40-year-old male agricultural laborer presented with active lepromatous leprosy and painful leg ulcers of 2 months' duration. Biopsy from the ulcer showed nonspecific changes. Raised erythrocyte sedimentation rate and positive rheumatoid factor made us suspect underlying cryoglobulinemia. Presence of cryoprecipitate in the serum, demonstration of cryoglobulins by serum electrophoresis and raised cryocrit were compatible with cryoglobulinemia as the cause of atypical leg ulcers in this case. The ulcers healed with bed rest, aspirin and specific anti-leprosy treatment. Though 95% of lepromatous leprosy patients can have cryoglobulinemia, the presence of atypical ulcers as seen in our patient has not previously been related to the presence of cryoglobulinemia.—Authors' Abstract

**Toledano Fernandez, N., Garcia Saez, S., Arteaga Sanchez, A. and Diaz Valle, D.** Bilateral lagophthalmos in lepromatous leprosy. Case report. Arch. Soc. Esp. Oftalmol. **77** (2002) 511–514. (In Spanish)

CASE REPORT: A case of bilateral facial palsy with paralytic ectropion, lagophthalmos and corneal damage secondary to corneal exposure in a long-standing patient with lepromatous leprosy is presented. Correction of paralytic ectropion was performed by medial cantoplasty, tarsal strip and Medpor (R) lower eyelid spacer implantation. Lagophthalmos was corrected by gold weight implant in the upper tarsus. DISCUSSION: Ocular findings in leprosy appear in 72% of patients. Facial nerve palsy occurs in 3–19.8%, being bilateral in 5%. In long standing cases with corneal complications secondary to exposure, surgical treatment is required.—Authors' Abstract

**Tomimori-Yamashita, J., Maeda, S. M., Sunderkotter, C., Kaminsky, S. K., Michanlany, N. S., Rotta, O. and Castro, R. M.** Leukomelanodermic leprosy. Int. J. Dermatol. **41** (2002) 513–515.

A 39-year-old woman presented with a 6-year history of asymptomatic macules on her back. The patient had no other complaints, and she did not report any case of infectious disease in her family. Examination revealed hyperpigmented macules, presenting irregular edges but with a sharp demarcation, on the midline of the back from the cervical to the lumbar region. Within these hyperpigmented areas, islands of normal appearing skin were observed. There were also some hypopigmented macules on the lateral and posterior aspects of the trunk. The patient presented thickening of the left ulnar nerve and sensory loss to temperature on the lateral aspect of the left arm. Biopsy specimens were obtained from the hyper- and hypopigmented areas. In the hyperpigmented macule, the biopsy revealed focal areas of hypomelanosis in the epidermis and the presence of melanophages in the superficial dermis, but no acid-fast bacilli were found. The Masson-Fontana stain showed an evident pigmentary incontinence. The biopsy obtained from the hypopigmented lesion also revealed focal areas of hypomelanosis, but in the superficial dermis an infiltrate of foamy macrophages was observed, as typically found in lepromatous leprosy; acid-fast bacilli were found by Fite-Faraco stain. The focal hypomelanosis was confirmed by Masson-Fontana stain. Mitsuda's reaction in this patient was negative and slit-skin smears (cutaneous lesion, earlobes, elbows, and knees) were negative for acid-fast bacilli. The patient

was treated with multidrug therapy for multibacillary leprosy: rifampin, 600 mg once monthly (supervised), clofazimine, 300 mg once monthly (supervised), dapsone, 100 mg daily, and clofazimine, 50 mg daily, all given for 2 years. After 7 months of treatment, the hypopigmented lesions diminished and the hyperpigmented lesions improved after 2 years of treatment and the sensory loss to temperature was restored.—Authors' Abstract

**Tosh, K., Meisner, S., Siddiqui, M. R., Balakrishnan, K., Ghei, S., Golding, M., Sengupta, U., Pitchappan, R. M. and Hill, A. V.** A region of chromosome 20 is linked to leprosy susceptibility in a South Indian population. J. Infect. Dis. **186** (2002) 1190–1193.

A major susceptibility locus for leprosy has recently been mapped on chromosome 10 (10p13) by genome-wide linkage analysis. Microsatellite markers from this genome screen that showed suggestive evidence of linkage to leprosy were evaluated in an additional 140 families with affected sib pairs. A second region of linkage has thus been identified on chromosome 20 (20p12). The peak of linkage lies at marker D20S115, which has a significant single-point maximum logarithm of odds score of 3.48 ($p = 0.00003$). Transmission disequilibrium testing of the microsatellite markers in 20p12 showed that the marker D20S835 is associated with protection against leprosy ($p = 0.021$), which suggests that a locus controlling susceptibility lies close to this marker.—Authors' Abstract

## Immuno-Pathology

**Azulay, R. D.** Antileprosy vaccinations. An. Bras. Dermatol. **77** (2002) 489–494.

Antileprosy vaccination can be immunoprophylactic or immunotherapeutic. The first vaccine used was BCG, for therapeutic purposes but with inferior results.

Other bacteria were used without results. Intradermal or oral BCG for achieving a change from negative to positive Mitsuda reaction came to be used in the prophylaxis of leprosy.

Other vaccines appeared with the same purpose:

- *Mycobacterium w,* proposed by Talwar, 1978.
- ICRC (*M. avium intracellulare* proposed by Deo and cols., 1981.
- BCG + heat-killed *M. leprae,* propose by Convit, 1992.
- *Mycobacterium tufu,* proposed by Iushin and Kalianina, 1995.
- *Mycobacterium habana,* proposed by Singh and cols., 1997.

The subject is discussed particularly in relation to its prophylactic and therapeutic effect. The results are contradictory. Nevertheless, the majority report beneficial results, both as a prophylaxis and for the therapeutics in association with multidrug therapy.—Author's Abstract

**Geluk, A., Meijgaarden, K. E., van Franken, K. L. M. C., Subronto, Y. W., Weiles, B., Arend, S. M., Sampaio, E. P., Boer, T., de Faber, W. R., Naafs, B. and Ottenhoff, T. H. M.** Identification and characterization of the ESAT-6 homolog of *Mycobacterium leprae* and T-cell cross-reactivity with *Mycobacterium tuberculosis.* Infect. Immun. **70** (2002) 2544–2548.

In this paper, we describe the identification and characterization of *Mycobacterium leprae* ESAT-6 (L-ESAT-6), the homolog of *M. tuberculosis* ESAT-6 (T-ESAT-6). T-ESAT-6 is expressed by all pathogenic strains belonging to the *M. tuberculosis* complex but is absent from virtually all other mycobacterial species; it is a promising antigen for immunodiagnosis of tuberculosis (TB). Therefore, we analyzed whether L-ESAT-6 is a similarly powerful tool for the study of leprosy by examining T-cell responses against L-ESAT-6 in lep-

rosy patients, TB patients, and exposed or nonexposed healthy controls from areas where leprosy and TB are endemic (Rio de Janeiro, Brazil) and areas where they are not endemic (Netherlands). The subjects included 49 Dutch individuals; of which, 21 were leprosy patients, 10 were TB patients, 7 were healthy individuals who had contact with TB patients and 11 were healthy controls with no known previous exposure to *M. leprae* or *M. tuberculosis.* The Brazilian subjects were composed of 9 leprosy patients, 15 TB patients and 7 healthy individuals. L-ESAT-6 was recognized by T cells from leprosy patients, TB patients, individuals who had contact with TB patients, and healthy individuals from an area where TB and leprosy are endemic, but not by T cells from individuals who were not exposed to *M. tuberculosis* and *M. leprae.* Moreover, leprosy patients who were not responsive to *M. leprae* failed to respond to L-ESAT-6. A very similar pattern was obtained with T-ESAT-6. These results show that L-ESAT-6 is a potent *M. leprae* antigen that stimulates T-cell-dependent gamma interferon production in a large proportion of individuals exposed to *M. leprae.* Moreover, our results suggest that there is significant cross-reactivity between T-ESAT-6 and L-ESAT-6, which has implications for the use of ESAT-6 as tool for diagnosis of leprosy and TB in areas where both diseases are endemic.—Trop. Dis. Bull.

**Lockwood, D. N., Colston, M. J. and Khanolkar-Young, S. R.** The detection of *Mycobacterium leprae* protein and carbohydrate antigens in skin and nerve from leprosy patients with type 1 (reversal) reactions. Am. J. Trop. Med. Hyg. **66** (2002) 409–415.

Type 1 (reversal) reactions are the most common immunological complications of leprosy. These episodes of delayed hypersensitivity produce severe local immunopathology and ultimately nerve damage. To date, the *Mycobacterium leprae* antigens associated with type 1 reactions have not been identified. Using monoclonal antibodies to defined protein and carbohydrate *M. leprae* epitopes (65, 35 and 28 kd and lipoarabinomannan [LAM]) in a two-step immunoperoxidase staining technique, *M. leprae* antigens were demonstrated in skin and nerve biopsies from patients in reversal reaction. Antigen presence and staining patterns were similar in skin and nerve lesions, implying that the pathological processes are similar in the two sites. Antigens were present both in macrophages and Schwann cells but also as a diffuse extracellular infiltrate associated with the inflammatory infiltrate. The 28-kd antigen was present most strongly and may be a potential candidate antigen for initiating type 1 reactions. LAM also stained strongly and persisted after treatment. The possible roles of LAM and 65-kd in the cellular events of type 1 reactions are discussed.—Authors' Abstract

**Martinuzzo, M. E., de Larranafa, G. F., Forastiero, R. R., Pelegri, Y., Farina, M. H., Alonso, B. S., Kordick, L. C. and Carreras, L. O.** Markers of platelet, endothelial cell and blood coagulation activation in leprosy patients with antiphospholipid antibodies. Clin. Exp. Rheumatol. **20** (2002) 477–483.

OBJECTIVE: To evaluate plasma levels of markers of platelet, endothelial cell and blood coagulation activation in leprosy patients with or without antiphospholipid antibodies (aPL) and to compare them to those found in patients with antiphospholipid syndrome (APS). METHODS: 42 patients with leprosy (35 lepromatous and 7 borderline): 29 aPL(+) and 13 aPL(–), as well as 26 healthy subjects as normal controls (NC) and 79 control aPL patients without leprosy (59 with and 20 without APS) were included in the study. Plasma soluble P and E selectin (sPsel and sEsel), and VCAM-1 (sVCAM-1), prothrombin F1 + 2 fragment (F1 + 2), thrombin-antithrombin complexes (TAT) and D dimer (DD) were measured by ELISA. The protein C pathway was assessed by the ProC global test. RESULTS: Leprosy patients with aPL presented increased median levels of sPsel [ng/ml (82.0 vs 36.0, $p < 0.001$)] and sVCAM-1 [ng/ml (495 vs 335, $p < 0.001$)] compared to NC, as observed in control aPL patients without leprosy. Levels of sPsel in aPL(+) patients with leprosy were

significantly higher than in aPL(–) ones (52.5 ng/ml), p = 0.005. However, plasma markers of thrombin generation were increased in control aPL patients without leprosy but not in those with leprosy. ProcC global test was abnormal in 24.1% of leprosy patients with aPL compared to 4.4% of NC (p <0.024), and to 57.2% of control patients with aPL without leprosy (p = 0.005). CONCLUSIONS: We demonstrated that although patients with leprosy present a high prevalence of aPL, and platelet and endothelial cell activation *in vivo* to the same extent than patients with APS, they do not show a procoagulant state.—Authors' Abstract

**Ottenhoff, T. H., Verreck, F. A., Lichtenauer-Kaligis, E. G., Hoeve, M. A., Sanal, O. and Van Dissel, J. T.** Genetics, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae. Nat. Genet. **32** (2002) 97–105.

Host genetic factors are important in determining the outcome of infections caused by intracellular pathogens, including mycobacteria and salmonellae, but until now have been poorly characterized. Recently, some individuals with severe infections due to otherwise weakly pathogenic mycobacteria (non-tuberculous mycobacteria or *Mycobacterium bovis* bacille Calmette-Guerin) or *Salmonella* species have been shown to be unable to produce or respond to interferon-gamma. This inability results from mutations in any of five genes encoding essential proteins of the type 1 cytokine cascade: interleukin-12p40, interleukin-12Rbeta1, interferon-gammaR1, interferon-gammaR2 or STAT1. Ten syndromes have thus far been identified. Recent insights in genetically controlled host defense and susceptibility to mycobacterial disease are discussed.—Authors' Abstract

**Patrus Ananias, M. T., Grossi Araújo, M., Dias Gontijo, E. and Martins Guedes, A. C.** Study of antiPGL-1 in patients with Hansen's disease using ultramicroelisa. An. Bras. Dermatol. **77** (2002) 425–433.

BACKGROUND—Phenolic glycolipid 1 (PGL-1), described by Brenner and Barrow in 1980, is a carbohydrate antigen present in the cellular surface of *Mycobacterium leprae.*

OBJECTIVES—1. Compare the ultramicroelisa technique, to detect anti-PGL-1 antibodies by means of the Elisa technique. 2. Study serological levels in leprosy patients, relating them to clinical condition, bacilloscopy, Mitsuda test, number of cutaneous lesions, immunosuppression, neural involvement, conditions of treatment (whether treatment-free or not), family history of leprosy and use of medication. Methods—A longitudinal study, with follow-up of 52 patients. A clinical and laboratory evaluation was made based on blood sampling and performance of the Mitsuda test at the beginning and end of the study. The patients were followed up on average for 13.8 months.

RESULTS—The negative result of the Mitsuda test (p = 0.00), positive bacilloscopy (p = 0.03) and the greater number of lesions (p = 0.00) were significantly associated with the positivity of the anti-PGL-1. The difference between the initial and final fluorescence readings was significantly associated with the family history of leprosy (p = 0.02), with a treatment-free condition showing a tendency to influence this difference (p = 0.06). The ultramicroelisa technique for detecting IgM anti-PGL-1 provided results similar to those using the disaccharide conjugate with, bovine albumin, as provided by the World Health Organization (WHO).

CONCLUSION—The negative Mitsuda test, positive bacilloscopy and greater number of lesions were significantly associated with the positivity of anti-PGL-1. Patients with a leprosy-positive family history present a greater difference between the initial and final fluorescence values, and the fact of being treatment free indicates a tendency to influence this difference. The Umelisa-Hansen ultramicroelisa technique is a good alternative for the study of anti-PGL-1.—Authors' Abstract

**Sampaio, E. P., Hernandez, M. O., Carvalho, D. S. and Sarno, E. N.** Management of erythema nodosum leprosum by thalidomide: thalidomide analogues in-

hibit *M. leprae*-induced TNF-alpha production *in vitro.* Biomed. Pharmacother. **56** (2002) 13–19.

Thalidomide is being successfully used for the treatment of erythema nodosum leprosum (ENL), among other disorders with inflammatory and immunological bases. Although the active molecules responsible for the diverse therapeutic activities of the drug and the sequence of reactions triggered inside the cells remain unclear, it was demonstrated that thalidomide (THAL) inhibits TNFalpha mRNA expression and protein production by stimulated monocytes and activated T lymphocytes. Patients treated with THAL experienced a reduction in serum TNFalpha levels and it diminished cytokine gene expression at the lesion site, with a concomitant abrogation of clinical symptoms. It has been reported that thalidomide as well as some its analogs decrease *M. leprae*-induced TNFalpha and IL-12 mRNA *in vitro.* THAL also reduced monocyte apoptosis in the cultures. The present data further support thalidomide's effects on TNFa synthesis and the growing need to search for new specific TNFalpha inhibitors (non-teratogenic compounds) that might be potentially used in clinical disorders such as leprosy.—Authors' Abstract

**Shannon, E. J. and Sandoval, F. G.** Thalidomide can costimulate or suppress CD4+ cells' ability to incorporate [H3]-thymidine-dependence on the primary stimulant. Int. Immunopharmacol. **2** (2002) 1143–1153.

Thalidomide is a drug that can enhance mitogen- and antigen-stimulated cells' ability to synthesize IL-2. To assess if thalidomide could concomitantly enhance the synthesis of IFN-gamma and incorporation of [H3]-thymidine, peripheral blood mononuclear cells (PBMC) were incubated in the presence or absence of thalidomide and staphylococcal enterotoxin A (SEA), anti-CD3, Con-A or PHA. After 18 hr, the cultures were sampled for IL-2. At the termination of the 3-day cultures, they were assayed for IFN-gamma and incorporation of [H3]-thymidine. Regardless of the mitogen used to stimulate the PBMC, the thalidomide-treated PBMC produced more IL-2 than controls. Thalidomide enhanced IFN-gamma synthesis in the Con-A and anti-CD3-stimulated PBMC. It suppressed the ability of SEA and PHA-stimulated PBMC to incorporate [H3]-thymidine, whereas it enhanced incorporation of [H3]-thymidine in PBMCs stimulated with anti-CD3. When the PBMC were enriched for CD4+ or CD8+ cells, the SEA- and anti-CD3-stimulated CD4+ cells responded far better than the CD8+ cells in the synthesis of IL-2 and incorporation of [H3]-thymidine. In the thalidomide-treated SEA-stimulated CD4+ and CD8+ cells, thalidomide acted as a costimulant to enhance the synthesis of IL-2. In the anti-CD3-stimulated thalidomide-treated cultures of PBMC enriched for CD4+ cells, thalidomide acted as a costimulant to enhance the incorporation [H3]-thymidine. Thalidomide cooperated with all of the mitogens to enhance T-cell synthesis of IL-2. However, depending on the stimulant, thalidomide could suppress or enhance PBMC incorporation of [H3]-thymidine. The SEA-stimulated cells targeted by thalidomide to suppress incorporation of [H3]-thymidine were CD4+. CD4+ cells stimulated with anti-CD3 were enhanced by thalidomide in their ability to synthesize IL-2 and to incorporate [H3]-thymidine. Increased production of IL-2 by activated T cells may be a mechanism through which thalidomide exerts its immunomodulatory effects.—Authors' Abstract

**Srivinas, D., Rao, P. N., Lakshmi, T. S., and Suneetha, S.** Bacterial index of granuloma and its relevance compared to BI of skin smears. Lepr. Rev. **73** (2002) 79–80.

This report describes a leprosy control program carried out in selected townships of Wenshan Prefecture in Yunnan Province, China in order to accelerate the elimination process, maintain vigilance for newly arising cases and assess the potential of primary health care workers in the context of integration. The aim of integrated leprosy control is early diagnosis, prompt, free delivery of multiple drug therapy and 100% area coverage. The main results of the project are discussed.—Trop. Dis. Bull.

# Microbiology

**Bardarov, S., Bardarov, Jr. S. Jr., Pavelka, Jr. M. S. Jr., Sambandamurthy, V., Larsen, M., Tufariello, J., Chan, J., Hatfull, G. and Jacobs, Jr. W. R. Jr.** Specialized transduction: an efficient method for generating marked and unmarked targeted gene disruptions in *Mycobacterium tuberculosis, M. bovis* BCG and *M. smegmatis.* Microbiology **148** (2002) 3007–3017.

The authors have developed a simple and highly efficient system for generating allelic exchanges in both fast- and slow-growing mycobacteria. In this procedure a gene of interest, disrupted by a selectable marker, is cloned into a conditionally replicating (temperature-sensitive) shuttle plasmid to generate a specialized transducing mycobacteriophage. The temperature-sensitive mutations in the mycobacteriophage genome permit replication at the permissive temperature of 30 degrees C but prevent replication at the non-permissive temperature of 37 degrees C. Transduction at a non-permissive temperature results in highly efficient delivery of the recombination substrate to virtually all cells in the recipient population. The deletion mutations in the targeted genes are marked with antibiotic-resistance genes that are flanked by gammadelta-res (resolvase recognition target) sites. The transductants which have undergone a homologous recombination event can be conveniently selected on antibiotic-containing media. To demonstrate the utility of this genetic system seven different targeted gene disruptions were generated in three substrains of *Mycobacterium bovis* BCG, three strains of *Mycobacterium tuberculosis,* and *Mycobacterium smegmatis.* Mutants in the lysA, nadBC, panC, panCD, leuCD, Rv3291c and Rv0867c genes or operons were isolated as antibiotic-resistant (and in some cases auxotrophic) transductants. Using a plasmid encoding the gammadelta-resolvase (tnpR), the resistance genes could be removed, generating unmarked deletion mutations. It is concluded from the high frequency of allelic exchange events observed in this study that specialized transduction is a very efficient technique for genetic manipulation of mycobacteria and is a method of choice for constructing isogenic strains of *M. tuberculosis,* BCG or *M. smegmatis* which differ by defined mutations.—Authors' Abstract

**Broxmeyer, L., Sosnorska, D., Miltner, E., Chacon, O., Wagner, D., McGarvey, J., Barletta, R. G. and Bermudez, L. E.** Killing of *Mycobacterium avium* and *Mycobacterium tuberculosis* by a mycobacteriophage delivered by a non-virulent mycobacterium: a model for phage therapy of intracellular bacterial pathogens. J. Infect. Dis. **186** (2002) 1155–1160.

*Mycobacterium avium* causes disseminated infection in patients with acquired immune deficiency syndrome. *Mycobacterium tuberculosis* is a pathogen associated with the deaths of millions of people worldwide annually. Effective therapeutic regimens exist that are limited by the emergence of drug resistance and the inability of antibiotics to kill dormant organisms. The present study describes a system using *Mycobacterium smegmatis,* an avirulent mycobacterium, to deliver the lytic phage TM4 where both *M. avium* and *M. tuberculosis* reside within macrophages. These results showed that treatment of *M. avium*-infected, as well as *M. tuberculosis*-infected, RAW 264.7 macrophages, with *M. smegmatis* transiently infected with TM4, resulted in a significant time- and titer-dependent reduction in the number of viable intracellular bacilli. In addition, the *M. smegmatis* vacuole harboring TM4 fuses with the *M. avium* vacuole in macrophages. These results suggest a potentially novel concept to kill intracellular pathogenic bacteria and warrant future development.—Authors' Abstract

**Constant, P., Perez, E., Malaga, W., Laneelle, M. A., Saurel, O., Daffe, M.**

**and Guilhot, C.** Role of the pks15/1 gene in the biosynthesis of phenolglycolipids in the *M. tuberculosis* complex: evidence that all strains synthesize glycosylated p-hydroxybenzoic methyl esters and that strains devoid of phenolglycolipids harbour a frameshift mutation in the pks15/1 gene. J. Biol. Chem. **277** (2002) 38148–38158.

Diesters of phthiocerol and phenolphthiocerol are important virulence factors of *M. tuberculosis* and *M. leprae,* the two main mycobacterial pathogens in humans. They are both long-chain beta-diols and their biosynthetic pathway is beginning to be elucidated. Although the two classes of molecules share a common lipid core, phthiocerol diesters have been found in all the strains of the *M. tuberculosis* complex examined but phenolphthiocerol diesters are produced by only a few groups of strains. To address the question of the origin of this diversity 8 reference strains and 10 clinical isolates of *M. tuberculosis* were analyzed. We report the presence of glycosylated p-hydroxybenzoic acid methyl esters, structurally related to the type-specific phenolphthiocerol glycolipids, in the culture media of all reference strains of *M. tuberculosis,* suggesting that the strains devoid of phenolphthiocerol derivatives are unable to elongate the putative p-hydroxybenzoic acid precursor. We also show that all the strains of *M. tuberculosis* examined and deficient in the production of phenolphthiocerol derivatives are natural mutants with a frameshift mutation in pks15/1 whereas a single open reading frame for pks15/1 is found in *M. bovis* BCG, *M. leprae* and strains of *M. tuberculosis* that produce phenolphthiocerol derivatives. Complementation of the H37Rv strain of *M. tuberculosis,* which is devoid of phenolphthiocerol derivatives, with the fused pks15/1 gene from *M. bovis* BCG restored phenolphthiocerol glycolipids production. Conversely, disruption of the pks15/1 gene in *M. bovis* BCG led to the abolition of the synthesis of type-specific phenolphthiocerol glycolipid. These data indicate that Pks15/1 is involved in the elongation of p-hydroxybenzoic acid to give p-hydroxyphenylalkanoates which in turn are converted, presumably by the PpsA-E synthase, to phenolphthiocerol derivatives.—Authors' Abstract

**Kordulakova, J., Gilleron, M., Mikusova, K., Puzo, G., Brennan, P. J., Gicquel, B. and Jackson, M.** Definition of the first mannosylation step in phosphatidylinositol mannoside synthesis: PimA is essential for growth of mycobacterium. J. Biol. Chem. **30** (2002) 31335–31344.

We examined the function of the pimA (Rv2610c) gene, located in the vicinity of the phosphatidylinositol synthase gene in the genomes of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis,* which encodes a putative mannosyltransferase involved in the early steps of phosphatidylinositol mannoside synthesis. A cell-free assay was developed in which membranes from *M. smegmatis* overexpressing the pimA gene incorporate mannose from GDP-[14C]Man into di- and tri-acylated phosphatidylinositol monomannosides. Moreover, crude extracts from *Escherichia coli* producing a recombinant PimA protein synthesized diacylated phosphatidylinositol mono-mannoside from GDP-[14C]Man and bovine phosphatidylinositol. In order to determine if PimA is an essential enzyme of mycobacteria, we constructed a pimA conditional mutant of *M. smegmatis.* The ability of this mutant to synthesize the PimA mannosyltransferase was dependent on the presence of a functional copy of the pimA gene carried on a temperature-sensitive rescue plasmid. We demonstrate here that the pimA mutant is unable to grow at the higher temperature at which the rescue plasmid is lost. Thus, the synthesis of phosphatidylinositol mono-mannosides and derived higher PIM in *Mycobacterium smegmatis* appears to be dependent on PimA and essential for growth. This work provides the first direct evidence of the essentiality of phosphatidylinositol mannosides for the growth of mycobacteria.—Authors' Abstract

## Epidemiology and Prevention

**Bakker, M. I., Hatta, M., Kwenang, A., Klatser, P. R. and Oskam, L.** Epidemiology of leprosy on five isolated islands in the Flores Sea, Indonesia. Trop. Med. Int. Health **7** (2002) 780–787.

We conducted a population-based survey on five small islands in South Sulawesi Province (Indonesia) to collect baseline data previous to a chemoprophylactic intervention study aiming at interrupting the transmission of *Mycobacterium leprae*. Here we describe the present leprosy epidemiology on these geographically isolated islands. Of the 4774 inhabitants living in the study area 4140 were screened for leprosy (coverage: 87%). We identified 96 leprosy patients (85 new and 11 old patients), representing a new case detection rate (CDR) of 205/10,000 and a prevalence rate of 195/10,000. CDRs were similar for males and females. Male patients were more often classified as multibacillary (MB) than women. Of the new patients, 33 (39%) were classified as MB, 16 (19%) as paucibacillary (PE) 2–5 lesions and 36 (42%) as PE single lesion. In this area of high leprosy endemicity leprosy patients were extensively clustered, i.e., not equally distributed among the islands and within the islands among the houses.—Authors' Abstract

**Ishii, N., Obara, A., Ozaki, M., Kumano, K., Sugita, Y., Namisato, M., Nogami, R., Hosokawa, A., Makino, M. and Sasaki, S.** Survey of newly diagnosed leprosy patients in Japan (1993–2000). Nihon Hansenbyo Gakki Zasshi **71** (2002) 223–233. (In Japanese)

We analyzed the medical and social problems of newly registered leprosy patients in the past 8 years from 1993 to 2000 in a low endemic country, Japan. There were 56 registered Japanese patients (males, 32; females, 24), and 76 registered foreign patients (males, 56; females, 20). The number of Japanese patients in each year was between 5 and 9, and 2/3 of them were from Okinawa Prefecture, located in subtropical zone. But the number of foreign patients in each year was between 5 and 18, and 2/5 of them were from Brazil. The number of foreign patients was greater than that of Japanese patients. Male/female ratio has decreased among the Japanese.—Authors' Abstract

## Other Mycobacterial Diseases and Related Entities

**Abate, G., Koivula, T. and Hoffner, S. E.** *In vitro* activity of thiacetazone on mycobacterial species belonging to the *Mycobacterium tuberculosis* complex. Int. J. Tuberc. Lung Dis. **6** (2002) 1933–1935.

Thiacetazone, despite frequent side-effects, may still be considered for the treatment of new tuberculosis cases when there is a shortage of drugs and for the management of multidrug-resistant tuberculosis. Fifty-four strains of *M. tuberculosis* complex were characterized based on the minimum inhibitory concentration (MIC) of thiacetazone and the growth pattern in the presence of different concentrations of the drug. The results showed that the MIC of thiacetazone to type II *M. africanum* strains was significantly higher than for other strains in the study ($p < 0.01$). Thiacetazone showed a paradoxical effect on 63% of strains where lower concentrations exhibited a better inhibiting activity than higher concentrations.—Authors' Abstract

**Camus, J. C., Pryor, M. J., Medigue, C. and Cole, S. T.** Re-annotation of the genome sequence of *Mycobacterium tu-*

*berculosis* H37Rv. Microbiology **148** (2002) 2967–2973.

Original genome annotations need to be regularly updated if the information they contain is to remain accurate and relevant. Here the complete re-annotation of the genome sequence of *Mycobacterium tuberculosis* strain H37Rv is presented almost 4 years after the first submission. Eighty-two new protein-coding sequences (CDS) have been included and 22 of these have a predicted function. The majority were identified by manual or automated re-analysis of the genome and most of them were shorter than the 100 codon cut-off used in the initial genome analysis. The functional classification of 643 CDS has been changed based principally on recent sequence comparisons and new experimental data from the literature. More than 300 gene names and over 1000 targeted citations have been added and the lengths of 60 genes have been modified. Presently, it is possible to assign a function to 2058 proteins (52% of the 3995 proteins predicted) and only 376 putative proteins share no homology with known proteins and thus could be unique to *M. tuberculosis.*—Authors' Abstract

**Dega, J., Bentoucha, A., Robert, J., Jartier, V. and Grosset, J.** Bactericidal activity of rifampin-amikacin against *Mycobacterium ulcerans* in mice. Antimicrob. Agents Chemother. **46** (2002) 3193–3196.

To identify the most active curative treatment of Buruli ulcer, two regimens incorporating the use of rifampin (RIF) were compared with the use of RIF alone in a mouse footpad model of *Mycobacterium ulcerans* infection. Treatments began after footpad swelling from infection and continued for 12 weeks with five doses weekly of one of the following regimens: (i) 10 mg of RIF/kg alone; (ii) 10 mg of RIF/kg and 100 mg of amikacin (AMK)/kg; and (iii) 10 mg of RIF/kg, 100 mg of clarithromycin (CLR)/kg, and 50 mg of sparfloxacin (SPX)/kg. The activity of each regimen was assessed in terms of the reduction of the average lesion index and acid-fast bacillus (AFB) and CFU counts. All three regimens displayed various degrees of bactericidal activity against *M. ulcerans*. The ranking of bactericidal activity was found to be as follows: RIF-AMK > RIF-CLR-SPX > RIF. RIF-AMK was able to cure *M. ulcerans*-infected mice and prevent relapse 26 weeks after completion of treatment. To determine the impact of different rhythms of administration of RIF-AMK on the suppression of *M. ulcerans* growth, mice were given the RIF-AMK combination for 4 weeks but doses were administered either 5 days a week or twice or once weekly. After completion of treatment, the mice were kept under supervision for 30 additional weeks. *M. ulcerans* was considered to have grown in the footpad if swelling was visually observed and harvests contained more than $5 \times 10^5$ AFB per footpad. The proportion of mice in which growth of *M. ulcerans* occurred, irrespective of drug dosage, was compared with the control mice to determine the proportion of *M. ulcerans* killed. Each dosage of RIF-AMK was bactericidal for *M. ulcerans* ($p < 0.001$), but the effect was significantly stronger in mice treated 5 days per week. The promising results of RIF-AMK treatment in *M. ulcerans*-infected mice support the clinical trial that is currently in progress under World Health Organization auspices in Ghana.—Authors' Abstract

**Engelhardt, H., Heinz, C. and Niederweis, M.** A tetrameric porin limits the cell wall permeability of *Mycobacterium smegmatis*. J. Biol. Chem. **277** (2002) 37567–37572.

Mycobacteria protect themselves with an outer lipid bilayer, which is the thickest biological membrane hitherto known and has an exceptionally low permeability rendering mycobacteria intrinsically resistant against many antibiotics. Pore proteins mediate the diffusion of hydrophilic nutrients across this membrane. Electron microscopy revealed that the outer membrane of *Mycobacterium smegmatis* contained about 1000 protein pores per $m^2$, which are about 50-fold less pores per $m^2$ than in Gram-negative bacteria. The projection structure of the major porin MspA of *M. smegmatis* was determined at 17 resolution. MspA forms a cone-

like tetrameric complex of 10 nm length with a single central pore. Thus, MspA is drastically different from the trimeric porins of Gram-negative bacteria and represents a new class of channel proteins. The formation of MspA micelles indicated that the ends of MspA have different hydrophobicities. Oriented insertion of MspA into membranes was demonstrated in lipid bilayer experiments, which revealed a strongly asymmetrical voltage gating of MspA channels at 30 mV. The length of MspA is sufficient to span the outer membrane and contributes in combination with the tapering end of the pore and the low number of pores to the low permeability of the cell wall of *M. smegmatis* for hydrophilic compounds.—Authors' Abstract

**Gooding, R. M., Johnson, P. D., Smith, M., Kemp, A. S. and Robins-Browne, R. M.** Cytokine profiles of patients infected with *Mycobacterium ulcerans* and unaffected household contacts. Infect. Immun. **70** (2002) 5562–5567.

*Mycobacterium ulcerans,* the cause of Buruli ulcer, is an environmental mycobacterium with a distinct geographic distribution. The reasons why only some individuals who are exposed to *M. ulcerans* develop ulcers are not known but are likely to reflect individual differences in the immune response to infections with this bacterium. In this study, we investigated cytokine profiles of peripheral blood mononuclear cells (PBMC) from 23 Buruli ulcer patients and 25 household contacts in a region of Australia where Buruli ulcer is endemic. The results showed that following stimulation with *M. ulcerans* or *Mycobacterium bovis* BCG, PBMC from Buruli ulcer patients mounted a Th2-type response, which was manifested by the production of mRNA for interleukin 4 (IL-4), IL-5, IL-6, and IL-10, whereas unaffected contacts responded mainly with the Th1 cytokines gamma interferon (IFN-gamma) and IL-12. For example, mRNA for IL-4 was detected in 18 of 23 patients but in only 3 of 25 control subjects ($p < 0.0001$). By contrast, PBMC from 21 of 25 unaffected individuals produced IFN-gamma compared with 3 of 23 patients ($p < 0.0001$). IFN-gamma release following stimulation with mycobacteria was markedly reduced in affected subjects. Frequencies of antibodies to *M. ulcerans* in serum samples from affected and unaffected subjects were similar, indicating that many of the control subjects had been exposed to this bacterium. Together, these findings suggest that a Th1-type immune response to *M. ulcerans* may prevent the development of Buruli ulcer in people exposed to *M. ulcerans,* but a Th-2 response does not.—Authors' Abstract

**Marsollier, L., Robert, R., Aubry, J., Saint Andre, J. P., Kouakou, H., Legras, P., Manceau, A. L., Mahaza, C. and Carbonnelle, B.** Aquatic insects as a vector for *Mycobacterium ulcerans.* Appl. Environ. Microbiol. **68** (2002) 4623–4628.

*Mycobacterium ulcerans* is an emerging environmental pathogen which causes chronic skin ulcers (i.e., Buruli ulcer) in otherwise healthy humans living in tropical countries, particularly those in Africa. In spite of epidemiological and PCR data linking *M. ulcerans* to water, the mode of transmission of this organism remains elusive. To determine the role of aquatic insects in the transmission of *M. ulcerans,* we have set up an experimental model with aquariums that mimic aquatic microenvironments. We report that *M. ulcerans* may be transmitted to laboratory mice by the bite of aquatic bugs (*Naucoridae*) that are infected with this organism. In addition, *M. ulcerans* appears to be localized exclusively within salivary glands of these insects, where it can both survive and multiply without causing any observable damage in the insect tissues. Subsequently, we isolated *M. ulcerans* from wild aquatic insects collected from a zone in the Daloa region of Ivory Coast where Buruli ulcer is endemic. Taken together, these results point to aquatic insects as a possible vector of *M. ulcerans.*—Authors' Abstract

**Mustafa, A.** Development of new vaccines and diagnostic reagents against tuberculosis. Mol. Immunol. **39** (2002) 113.

Tuberculosis (TB) is a major infectious disease problem with one-third of the world population infected, 8 million people developing the active disease and 2 million dying of TB each year. The attenuated *Mycobacterium bovis* Bacillus Calmette Guerin (BCG) is the only available vaccine against TB. However, the trials conducted in different parts of the world have shown that this vaccine does not provide consistent protection against TB. The purified protein derivative (PPD) of *Mycobacterium tuberculosis* is the commonly used reagent for the diagnosis of TB. However, PPD lacks specificity because of the presence of antigens crossreactive with *M. bovis* BCG and other mycobacteria. The studies to identify *M. tuberculosis* antigens and epitopes as candidates for new protective vaccines and specific diagnostic reagents against TB have led to the identification and characterization of several major antigens of *M. tuberculosis* including heat shock proteins (hsp) and secreted antigens present in the culture filtrate (CF) of *M. tuberculosis.* Some of these antigens have shown promise as new candidate vaccines (hsp60, Ag85 and ESAT-6, etc.) and specific diagnostic reagents (ESAT-6 and CFP10, etc.) for TB. Moreover, in the mouse model of TB, vaccination with DNA-hsp60 has immunotherapeutic effects and helps in eradication of persisters. In addition, identification of proper adjuvant and delivery systems has shown the promise to overcome the problem of poor immunogenicity associated with subunit and peptide based vaccines. More recently, the comparison of the genome sequence of *M. tuberculosis* with *M. bovis* BCG and other mycobacteria has led to the identification of *M. tuberculosis*-specific genomic regions. Evaluation of these regions for encoding proteins with immunological reactivity can lead to the identification of additional antigens of *M. tuberculosis* useful as new vaccines and reagents for specific diagnosis of TB.—Author's Abstract

**Raynaud, C., Papavinasasundaram, K. G., Speight, R. A., Springer, B., Sander, P., Bottger, E. C., Colston, M. J. and Draper, P.** The functions of OmpATb, a pre-forming protein of *Mycobacterium tuberculosis.* Mol. Microbiol. **46** (2002) 191–201.

The functions of OmpATb, the product of the ompATb gene of *Mycobacterium tuberculosis* and a putative porin, were investigated by studying a mutant with a targeted deletion of the gene, and by observing expression of the gene in wild-type *M. tuberculosis* H37Rv by real-time polymerase chain reaction (PCR) and immunoblotting. The loss of ompATb had no effect on growth under normal conditions, but caused a major reduction in ability to grow at reduced pH. The gene was substantially upregulated in wild-type bacteria exposed to these conditions. The mutant was impaired in its ability to grow in macrophages and in normal mice, although it was as virulent as the wild type in mice that lack T cells. Deletion of the ompATb gene reduced permeability to several small water-soluble substances. This was particularly evident at pH 5.5; at this pH, uptake of serine was minimal, suggesting that, at this pH, OmpATb might be the only functioning porin. These data indicate that OmpATb has two functions: as a pore-forming protein with properties of a porin, and in enabling *M. tuberculosis* to respond to reduced environmental pH. It is not known whether this second function is related to the porin-like activity at low pH or involves a completely separate role for OmpATb. The involvement with pH is likely to contribute to the ability of *M. tuberculosis* to overcome host defense mechanisms and grow in a mammalian host.—Authors' Abstract

**Rossetti, M. L., Valim, A. R., Silva, M. S. and Rodrigues, V. S.** Resistant tuberculosis: a molecular review. Rev. Saude Publica. **36** (2002) 525–532.

Progress to understanding the basis of resistance to antituberculous drugs has allowed molecular tests for detection of drug-resistant tuberculosis to be developed. Drug-resistant tuberculosis poses a threat to tuberculosis control programs. It is necessary thus to know drug susceptibilities of individual patient's strain to provide the appropriate drug combinations. Molecular studies on the mechanism of action of antituberculous drugs have elucidated the genetic basis of drug resistance in *M. tuberculosis.* The mech-

anisms of drug resistance in tuberculosis are a result of chromosomal mutations in different genes of the bacteria. Upon drug exposure there is a selective pressure for such resistant mutants. Multidrug-resistant tuberculosis is a health problem of increasing significance for the whole global community. This paper reviews the molecular mechanisms associated with drug-resistance as well the new perspectives for detecting resistant isolates.—Authors' Abstract

**Sambandamurthy, V. K., Wang, X., Chen, B., Russell, R. G., Derrick, S., Collins, F. M., Morris, S. L. and Jacobs, W. R.** A pantothenate auxotroph of *Mycobacterium tuberculosis* is highly attenuated and protects mice against tuberculosis. Nat. Med. **8** (2002) 1171–1174.

With the advent of HIV and the widespread emergence of drug-resistant strains of *Mycobacterium tuberculosis,* newer control strategies in the form of a better vaccine could decrease the global incidence of tuberculosis. A desirable trait in an effective live attenuated vaccine strain is an ability to persist within the host in a limited fashion in order to produce important protective antigens *in vivo.* Attenuated *M. tuberculosis* vaccine candidates have been constructed by deleting genes required for growth in mice. These candidate vaccines did not elicit adequate protective immunity in animal models, due to their inability to persist sufficiently long within the host tissues. Here we report that an auxotrophic mutant of *M. tuberculosis* defective in the *de novo* biosynthesis of pantothenic acid (vitamin B5) is highly attenuated in immunocompromised SCID mice and in immunocompetent BALB/c mice. SCID mice infected with the pantothenate auxotroph survived significantly longer (250 days) than mice infected with either bacille Calmette-Guerin (BCG) vaccine or virulent *M. tuberculosis* (77 and 35 days, respectively). Subcutaneous immunization with this auxotroph conferred protection in C57BL/6J mice against an aerosol challenge with virulent *M. tuberculosis,* which was comparable with that afforded by BCG vaccination. Our findings highlight the importance of *de novo* pantothenate biosynthesis in limiting the intracellular survival and pathogenesis of *M. tuberculosis* without reducing its immunogenicity in vaccinated mice.—Authors' Abstract

**Wu, T. S., Chiu, C. H., Su, L. H., Chia, J. H., Lee, M. H., Chiang, P. C., Kuo, A. J., Wu, T. L. and Leu, H. S.** *Mycobacterium marinum* infection in Taiwan. J. Microbiol. Immunol. Infect. **35** (2002) 42–46.

*Mycobacterium marinum* often causes skin infections, tenosynovitis, arthritis and osteomyelitis, and occasionally results in severe disseminated infections in immunocompromised patients. In this study, the clinical features of 14 cases (8 males and 6 females, aged 9–72 years) of *M. marinum* infection were retrospectively analyzed. Clinical isolates from each of these patients were collected during June 1999–November 2000 from the clinical microbiology laboratory of the Chang Gung Memorial Hospital, Taoyuan, Taiwan. One patient had septic arthritis, the other 13 had skin infections and/or tenosynovitis. It usually took 2 months or longer for a definite diagnosis to be made in these patients. Three of the 14 patients were cured using clarithromycin alone or in combination with rifampin [rifampicin] plus ethambutol. Most patients did not respond to conventional antituberculosis agents. Pulsed-field gel electrophoresis and infrequent-restriction-site polymerase chain reaction are efficient tools for the molecular typing of *M. marinum.* Both methods yielded a concordant result, and 4 of 12 isolates were genetically closely related to each other based on their banding patterns. This study indicates that these isolates were derived from the same clone. Because *M. marinum* infection is curable, early diagnosis is important. Poor healing of wounds after exposure to aquatic animals appears to be the most important clinical clue indicating the need for culture and inclusion of *M. marinum* infection in the differential diagnosis.—Trop. Dis. Bull.