

CURRENT LITERATURE

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

General and Historical

Andayi, F. M., Ohenga, W. J. and Orege, P. A. Women and leprosy: time lag in seeking treatment. *Afr. J. Health Sci.* **5** (1998) 136–139.

A total of 50 female leprosy patients were interviewed in 1992 in a study to determine the magnitude of the problem of delay in seeking treatment in leprosy. The study was carried out in Busia District, Kenya. The possible sociocultural risk factors for the delay were also determined. Questionnaires were used in the collection of data. Results obtained indicated a high level of illiteracy (82%) and low socioeconomic status; 64% of the women were married, many had a family history of leprosy. Knowledge about the causative agent of leprosy was poor, although the majority knew the disease was disabling. A high proportion had problems getting to health units while many held misconceptions about the place to seek medical treatment and the mode of treatment. Many also believed family and community members and some health staff held negative attitudes about leprosy and hence leprosy patients; 64% sought treatment long after disease onset (2–10 years or more). It is concluded that the problem of delay in seeking medical treatment among female leprosy patients is high and is due to socioeconomic and sociocultural factors as well as lack of adequate knowledge of the disease.—*Trop. Dis. Bull.* **97** (2000) 181

Arneson, T. and Nord, E. The value of DALY life: problems with ethics and validity of disability adjusted life years. *Lepr. Rev.* **71** (2000) 123–127.

The DALY approach currently in use presupposes that life years of disabled

people are worth less than life years of people without disabilities. Through the imposition of consistency between substantially different questions, people participating in evaluation panels are forced to adopt discriminatory positions on the value of life of disabled people. Inasmuch as the disability weightings do not correspond to a clear preference but are the results of forced compromise, they must be seen basically as artifacts. Revision of the DALY protocol should deal with these problems appropriately. In particular, the use of disability weightings in the valuation of gained life years should be abandoned.—Authors' Conclusion

Beiguelman, B. [The Mitsuda reaction after eighty years.] *Hansen. Int.* **24** (1999) 144–161. (in Portuguese)

The present paper intends to be a brief review of the literature on Mitsuda reaction at its eightieth anniversary. Emphasized is the fundamental contributions of Brazilian researchers to the clinic-epidemiologic, histologic, genetic and animal experimentation studies of this skin test, which has important prognostic value for indeterminate and borderline leprosy, and is also sensitive for evaluating the resistance of leprosy contacts to lepromatous leprosy.—Author's English Summary

Bhattacharya, S. N. and Sehgal, V. N. Leprosy in India. In: *Special issue: Geographic dermatology* (edited by Imamura, S. and Diaz, L. J.) *Clinics Dermatol.* **17** (1999) 159–170.

The history, socio-epidemiological aspects, clinical aspects, diagnosis and ther-

apy of *Mycobacterium leprae* infections in India are reviewed, including leprosy in children, and the prevention of disability.—Trop. Dis. Bull. **97** (2000) 294

Chen, S., Han, C., Li, B., Zheng, R. and Zhang, L. A survey on knowledge and skills in the early diagnosis of leprosy in general health services at different levels in Shandong Province, People's Republic of China. *Lepr. Rev.* **71** (2000) 57–61.

In the late phase of a leprosy control program, problems arise with regard to the early detection and treatment of a small number of new incident cases. We describe a study in the province of Shandong, People's Republic of China, on the knowledge and skills regarding leprosy of general health service staff, including rural doctors, paramedical doctors at the township level, doctors from county general and provincial hospitals and dermatologists. The results showed that there is a continuing need for suitable training programs for medical staff in the general health services. Most dermatologists had good levels of knowledge and skills and more than 80% of new cases have been diagnosed in skin clinics in this province since 1990. Their participation in early diagnosis and training of staff should be strengthened.—Authors' Summary

Chen, X.-S., Li, W.-Z., Jiang, C., Zhu, Z.-L. and Ye, G. Computerization of leprosy records: national leprosy recording and reporting system in China. *Lepr. Rev.* **71** (2000) 47–56.

This paper describes the national system of leprosy recording and reporting in China and the computerization of records. The system was designed for data collection at the local level and data entry by optically scanned or manual mode as well as for sophisticated data analysis. The major functions include data entry, data check, sum-up, maintenance, communication, inquiry, statistics, graph and print. A total of 17 options for epidemiological and clinical data analysis are available. Through the implementation for about 10 years, the system has gained widespread acceptance. This ac-

ceptance would facilitate introduction of computer analysis to other leprosy projects and other disease control programs in China. Up to 1998, a database of more than 740,000 records covering all the leprosy patients detected since 1949 had been established by this system.—Authors' Summary

Morrison, A. A woman with leprosy is in double jeopardy. *Lepr. Rev.* **71** (2000) 128–143.

The double jeopardy associated with female leprosy patients is the central theme underpinning this essay. It constitutes a combination of biological factors unique to women and culturally defined bias, resulting in more stigmatization and isolation for women. Having examined the female immunological response and biological roles, the essay continues by focusing on the gender-culture perspective of leprosy. It draws upon an historical analysis of the experiences of Indian and African women to illustrate the ways in which gender roles impact upon health education and the utilization of health care services. Concluding comments suggest strategies that might improve female leprosy patient status, and views toward future research.—Author's Summary

Nash, J. Learning materials: their use and evaluation. *Lepr. Rev.* **70** (1999) 254–260.

This review considers the use of learning materials in leprosy programs. It is concluded that the principles of adult learning should be incorporated into their development and use, so that they are practical, problem-oriented and relevant to the learners' situations and learning needs.—Trop. Dis. Bull. **97** (2000) 175

Rao, B. R. and Krishnamurthy, P. A comparative study of the cost and effectiveness of a modified system of MDT drug delivery system in a high endemic district (Nalagonda) of South India. *Indian J. Lepr.* **70** Suppl. (1998) 63S–71S.

A modified system of drug delivery for leprosy treatment was developed and as-

sessed in terms of the cost and effectiveness, its overall effect on other activities in the program and its acceptability to the field staff. Four Leprosy Control Units (LCUs) in Nalgonda, Andhra Pradesh, India, were selected and were randomly assigned either to a study (Gudibanda, Suryapet) or a control (Nalgonda, Bhuvanagiri) group. In the study group the modified drug delivery system replaced the existing system. Under the modified system the paramedical worker was responsible for patients at all the drug delivery points (DDPs) in his subcenter. The clinics were managed alternately by medical officers and nonmedical supervisors every month. In the control group each clinic was managed by medical officers every month and it covered 2 subcenters and assistance was provided by a paramedical worker at each drug delivery point. The study revealed that the modified system resulted in a saving of 130 man-days a month, a 30% saving in use of a vehicle, a 30% saving in POL, and improvement in case detection. There was no change in the clinic attendance and drug consumption compliance in the units where the modified system was introduced.—*Trop. Dis. Bull.* **97** (2000) 66

Shale, M. J. H. Women with leprosy; a woman with leprosy is in double jeopardy. *Lepr. Rev.* **71** (2000) 5–17.

The female with leprosy does indeed face a double jeopardy; her socially inferior

status and her highly stigmatized disease result in greater social and mental problems, even if the disease is often less severe (physically) in women than in men. Many societies where leprosy occurs have such different views about the world and illness that greater attention should be paid to understanding those beliefs, including attitudes to women on drug treatment. While women are socially vulnerable, they are also more responsive if targeted correctly, and are responsible for the care of their family's health. Improved education of women about the disease can produce progress toward making leprosy the minor public health problem it should be. Further requirements for achieving disease control are raised levels of literacy for all and greater education of health workers about the disease, to allow greater dissemination of information within the community. Misconceptions and misunderstanding exist about the disease in many nonleprosy specialists, and this also needs to be addressed. In summary, if leprosy is to be conquered, consideration must be given to a detailed study of the varying social conditions in leprosy-endemic countries, including attitudes to the disease and to women. Education of all about the disease, in terms they understand and that do not dismiss or ridicule their beliefs or the teachings of their religions, is the key to success. While women are in double jeopardy, given time and the correct measures this need not be the case.—*Author's Conclusions*

Chemotherapy

Dhople, A. M. Search for newer antileprosy drugs. *Indian J. Lepr.* **72** (2000) 5–20.

In 1991, the World Health Organization proclaimed the goal of global elimination of leprosy as a public health problem by the year 2000 by implementing multidrug therapy (MDT). Since then the prevalence rate has declined by 85%. However, during the same period the incidence rate of leprosy

has remained constant or even has been increasing. This suggests that it will take a long time for the eradication of leprosy and that without *in-vitro* cultivation of *M. leprae* eradication of leprosy is not likely to be achieved. While *in-vitro* cultivation is a long-term goal, as an immediate measure there is an urgent need for the development of newer drugs and newer MDT regimens. Using the *in-vitro* system for screening potential antileprosy drugs and also using the

mouse foot-pad system, we have evaluated several compounds in four classes of drugs—dihydrofolate reductase inhibitors, fluoroquinolones, rifampin analogs and phenazines—and identified at least two compounds that appear to be more potent than dapsone, rifampin and clofazimine. Newer combinations of rifampin analogs and fluoroquinolones have also been identified that seem to be better than the combination of rifampin and ofloxacin.—Author's Abstract

Gallo, M. E. N., Nery, J. A. C., Albuquerque, E. C. A., Signorelli, M. and Silva Filho, V. F. [Multibacillary leprosy: bacilloscopic index and viability of *M. leprae* after 24 doses of World Health Organization multidrug therapy (WHO/MDT).] *An. Bras. Dermatol.* **75** (2000) 291–297. (in Portuguese)

Background—The inoculation technique in the foot pad of mice, Shepard's technique, enables the checking of resistance to chemotherapeutics, and the viability of *Mycobacterium leprae*.

Objectives—Assess the viability of *M. leprae* in skin biopsies taken from multibacillary (MB) leprosy patients by means of the Shepard technique.

Patients and methods—Skin biopsies and slit-skin smears were performed in 21 MB leprosy patients after 24 doses of the multidrug therapy (WHO/MDT).

Results—The results of the bacilloscopic and morphologic index that were determined from the slit-skin smears and the homogenate skin biopsies used in the inoculation were compared. The results of the two samples demonstrated values that were significantly higher in the bacilloscopic index of the homogenate of the skin biopsies.

Conclusions—The *M. leprae* concentration in the material collected 12 months after the inoculation presented values that were inferior to the "multiplication standard," suggesting that the bacillus population present in the skin biopsy of patients given 24 doses of the WHO/MDT may be considered nonviable.—Authors' English Summary

Girdhar, B. K., Girdhar, A. and Kumar, A. Relapses in multibacillary leprosy patients: effect of length of therapy. *Lepr. Rev.* **71** (2000) 144–153.

Two groups of MB leprosy patients, one treated to the point of smear negativity (TSN) and the other given therapy for fixed duration (24 doses of WHO MB regimen) (FDT), were compared for relapse rates during treatment and in the post-treatment period. During the follow up of 980.2 person-years in 260 patients treated with FDT, 20 relapses (2.04/100 patient-years) were observed. In the other group of 301 patients who received therapy until smear negativity, 12 relapses in 1085.46 person-years (1.10/100 patient-years) occurred. Comparison of survival rates (without relapse) has shown that although there is no difference up to 4 years, the risk of relapse was significantly higher on longer follow up in the FDT group. In addition, when patients were compared on the basis of initial bacterial load, it was found that the relapse rates in patients with a BI of ≥ 4 were significantly higher ($p < 0.01$) in the FDT group as compared to those receiving treatment until the point of smear negativity (4.29 versus 1.27/100 patient-years). All the relapsed patients responded to re-treatment with the same drug combination, indicating that the exacerbation in their conditions was because of insufficient treatment. It is suggested that to prevent or reduce relapses, treatment where feasible would be continued until smear negativity, at least in patients with a high BI.—Authors' Summary

Grosset, J. [Leprosy and tuberculosis: opposite results?] In: Proceedings: Sixièmes Actualités du Pharo, Marseille, 3 et 4 Septembre 1999. *Med. Trop.* **59** Suppl. 2 (1999) 25–30. (in French)

Global differences in the success of treatment of leprosy and tuberculosis, during 1981–1999, are discussed, including treatment and prevention measures, and the numbers of cases worldwide. Essential differences and similarities between treatment strategies for the two diseases are also considered.—Trop. Dis. Bull. **97** (2000) 181

Gupta, U. D. and Katoch, V. M. Drug resistance in leprosy: lessons from past and future perspective. *Indian J. Lepr.* **71** (1999) 451–463.

Some recent studies indicate that the problem of drug resistance in leprosy is very much there but the exact picture is not clear. In the emerging scenario with an increasing number of new cases with low bacterial load, the conventional *in-vivo* and most of current *in-vitro* methods for the determination of drug resistance may not help. It is pointed out that newer molecular approaches may be more useful and that it will be important to undertake studies to develop such tools.—Authors' Abstract

Kremer, L., Douglas, J. D., Baulard, A. R., Morehouse, C., Guy, M. R., Alland, D., Dover, L. G., Lakey, J. H., Jacobs, W. R., Brennan, P. J., Minnikin, D. E. and Besra, G. S. Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting KasA and KasB condensing enzymes in *Mycobacterium tuberculosis*. *J. Biol. Chem.* **275** (2000) 16857–16864.

Prevention efforts and control of tuberculosis are seriously hampered by the appearance of multidrug-resistant strains of *Mycobacterium tuberculosis*, dictating new approaches to the treatment of the disease.

Thiolactomycin (TLM) is a unique thiolactone that has been shown to exhibit anti-mycobacterial activity by specifically inhibiting fatty acid and mycolic acid biosynthesis. In this study, we present evidence that TLM targets two beta-ketoacyl-acyl-carrier protein synthases, KasA and KasB, consistent with the fact that both enzymes belong to the fatty-acid synthase type II system involved in fatty acid and mycolic acid biosynthesis. Overexpression of KasA, KasB, and KasAB in *M. bovis* BCG increased *in vivo* and *in vitro* resistance against TLM. In addition, a multidrug-resistant clinical isolate was also found to be highly sensitive to TLM, indicating promise in counteracting multidrug-resistant strains of *M. tuberculosis*. The design and synthesis of several TLM derivatives have led to

compounds more potent both *in vitro* against fatty acid and mycolic acid biosynthesis and *in vivo* against *M. tuberculosis*. Finally, a three-dimensional structural model of KasA has also been generated to improve understanding of the catalytic site of mycobacterial Kas proteins and to provide a more rational approach to the design of new drugs.—Authors' Abstract

Shover, C. K., Warren, P., van de Venter, D. R., Sherman, D. R., Arain, T. M., Langhorne, M. H., Anderson, S. W., Towell, J. A., Yuan, Y., McMurray, D. N., Kreiswirth, B. N., Barry, C. E. and Baker, W. R. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* **405** (2000) 962–966.

Mycobacterium tuberculosis, which causes tuberculosis, is the greatest single infectious cause of mortality worldwide, killing roughly two million people annually.

Estimates indicate that one-third of the world population is infected with latent *M. tuberculosis*. The synergy between tuberculosis and the AIDS epidemic and the surge of multidrug-resistant clinical isolates of *M. tuberculosis* have reaffirmed tuberculosis as a primary public health threat. However, new antitubercular drugs with new mechanisms of action have not been developed in over 30 years. Here we report a series of compounds containing a nitroimidazopyran nucleus that possess antitubercular activity. After activation by a mechanism dependent on *M. tuberculosis* F420 cofactor, nitroimidazopyrans inhibited the synthesis of protein and cell wall lipid. In contrast to current antitubercular drugs, nitroimidazopyrans exhibited bactericidal activity against both replicating and static *M. tuberculosis*. Lead compound PA-824 showed potent bactericidal activity against multidrug-resistant *M. tuberculosis* and promising oral activity in animal infection models. We conclude that nitroimidazopyrans offer the practical qualities of a small molecule with the potential for the treatment of tuberculosis.—Authors' Abstract

Sun, Z. and Zhang, Y. Antituberculosis activity of certain antifungal and antihelminthic drugs. *Tuberc. Lung Dis.* **79** (1999) 319–320.

A panel of antifungal and anthelmintic drugs was tested for activity against *Mycobacterium tuberculosis in vitro*. Antifungal drugs, miconazole, 2-nitroimidazole, clotrimazole, and the antihelminthic drug

niclosamide showed significant antituberculosis activity with minimal inhibitory concentrations between 1 and 10 µg/ml. Niclosamide and 2-nitroimidazole also had activity against stationary phase tubercle bacilli. Further testing of these drugs and their derivatives *in vitro* and *in vivo* appears to be warranted.—*Trop. Dis. Bull.* **97** (2000) 76

Clinical Sciences

Amenu, A. and Saunderson, P. Use of prednisolone blister packs in the field. *Lepr. Rev.* **71** (2000) 366–367.

This correspondence briefly describes the use of prednisolone blister strips in the All Africa Leprosy, Tuberculosis and Rehabilitation Training Center (ALERT) field program [in Ethiopia] in 1998. The advantages and disadvantages of this method of treating leprosy reactions are discussed.—*Trop. Dis. Bull.* **92** (2000) 178–179.

Bowen, J. R. C., McDougall, A. C., Morris, J. H., Lucas, S. B. and Donaghy, M. Vasculitic neuropathy in a patient with inactive treated lepromatous leprosy. *J. Neurol. Neurosurg. Psychiatry* **68** (2000) 496–500.

A 46-year-old Asian male with previously treated lepromatous leprosy developed a stepwise multifocal sensory disturbance 25 years later. Neurophysiology demonstrated marked deterioration from previous studies. Sural nerve biopsy disclosed a vasculitic process superimposed on inactive lepromatous leprosy. Immunocytochemical stains for mycobacterial antigen showed deposits within nerve and vessel walls. A delayed vasculitic neuropathy precipitated by persisting mycobacterial antigen is proposed.—Authors' Abstract

Croft, R. P., Nicholls, P. G., Richardus, J. H. and Smith, W. C. S. Incidence rates

of acute nerve function impairment in leprosy: a prospective cohort analysis after 24 months (The Bangladesh Acute Nerve Damage Study). *Lepr. Rev.* **71** (2000) 18–33.

In this paper, the incidence rates and cumulative incidence of nerve function impairment (NFI) and leprosy reactions over 24 months follow up of the prospective cohort of 2664 new leprosy cases are presented. Graphs showing the cumulative incidence of NFI relative to time since registration are presented. Hazard ratios (HRs) for the development of NFI for four variables are given. The majority of patients who developed NFI after registration did so in the first year (67% of multibacillary (MB) patients, and 91% of paucibacillary (PB) patients who developed NFI). Thirty-three percent of all MB patients who developed NFI after registration did so in the second year of follow up. No PB patients developed NFI for the first time in the last 6 months of follow up. However, seven NFI events occurred among PB patients in that period, among those who had already had one NFI event. The incidence rate (IR) of NFI among MB patients was 24/100 person-years at risk (PYAR), and among PB patients was 1.3/100 PYAR. The HR for the development of NFI among MB patients compared with PB patients was 16 using univariate analysis. Among patients who had long-standing NFI present at registration, the IR was 27/100 PYAR compared with 1.7/100 PYAR among those who did

not have long-standing NFI. The HR for developing acute NFI among those with long-standing NFI present at registration compared with those without was 14 using univariate analysis. When multivariate regression analysis is applied, the apparently significant univariate HRs for sex and age disappeared. The resultant multivariate HR for leprosy group is 8.8, and 6.1 for the presence/absence of long-standing NFI at registration. In all, 142/166 (86%) of all new NFI events were silent, underlining the need for regular nerve function testing. IRs are presented for the four 6-month periods of the 24-month follow up. They show a clear stepwise reduction over the total period. The IRs among MB patients and those with long-standing NFI present at registration are very high at 34 and 41/100 PYAR, respectively, for the first 6 months of follow up. Even during the final 6-month period, the IR is maintained at a moderately high level (18 and 15/100 PYAR, respectively).—Authors' Summary

Croft, R. P., Nicholls, P. G., Richardus, J. H. and Smith, W. C. S. The treatment of acute nerve function impairment in leprosy: results from a prospective cohort study in Bangladesh. *Lepr. Rev.* **71** (2000) 154–168.

In this paper, the outcome of 132 patients having acute nerve function impairment (NFI) is reported at 4 and 12 months after the start of prednisolone treatment. In all, 68% of sensory nerves and 67% of motor nerves showed improvement at 12 months, with no statistical difference in responsiveness of various nerves to prednisolone. Duration and severity of impairment were not found significant predictors of treatment outcome. A core of 32% of impaired nerves did not respond to prednisolone, and 12% of impaired nerves had functional deterioration despite treatment. The mean eye-hand-foot (EHF) score improved from 2.02 to 1.33 in the treatment group (median score improved from 2 to 1). Approximately one-third of all patients requiring prednisolone treatment did not receive it, an important reason being that some patients developed new NFI against a background of chronic impairment, and were thus overlooked. The

“unjustly untreated” group of patients had a spontaneous sensory nerve function improvement rate of 62% and a motor nerve function improvement rate of 33% at 12 months from onset of NFI. The EHF score showed no statistically significant improvement.—Authors' Summary

Croft, R. P., Nicholls, P. G., Steyerberg, E. W., Richardus, J. H. and Smith, W. C. S. A clinical prediction rule for nerve-function impairment in leprosy patients. *Lancet* **355** (2000) 1603–1606.

Background: Nerve-function impairment (NFI) commonly occurs during or after chemotherapy in leprosy and is the key pathological process leading to disability and handicap. We describe the development of a simple clinical prediction rule for estimating the risk of NFI occurrence.

Methods: New leprosy cases who presented to a center in Bangladesh were recruited and followed up for 2 years in a field setting. We used multivariable regression analysis by Cox's proportional hazards model to identify predictive variables for NFI. Discriminative ability was measured by a concordance statistic. Internal validity was assessed with bootstrap resampling techniques.

Findings: 2510 patients were followed up for 2 years, 166 developed NFI. A simple model was developed with a leprosy group (either paucibacillary [PB] leprosy or multibacillary [MB] leprosy) and the presence of any nerve-function loss at registration as predictive variables. Patients with PB leprosy and no nerve-function loss had a 1.3% (95% CI 0.8%–1.8%) risk of developing NFI within 2 years of registration; patients with PB leprosy and nerve-function loss, or patients with MB leprosy and no nerve-function loss had a 16.0% (12%–20%) risk; and patients with MB leprosy with nerve-function loss had a 65% (56%–73%) risk.

Interpretation: Our prediction rule can be used to plan surveillance of new leprosy patients. Patients at low risk of NFI may need no follow up beyond their course of chemotherapy (6 months); patients with intermediate risk need a minimum of 1 year of surveillance; and patients with high risk

should have at least 2 years of surveillance for new NFI. Current recommendations for surveillance of patients with leprosy (for the duration of chemotherapy only) exclude an important group of patients who are at risk of developing NFI after completion of treatment.—Authors' Abstract

D'Abreu, P. C., Duraes, S. M. B., Estrela, R. R., Baltazar, M. C. N. P. and Rochael, M. C. [Lepromatous leprosy diagnosed through the pathological examination of oral lesions in a patient having pemphigus foliaceus.] *An. Bras. Dermatol.* **75** (2000) 339–346. (in Portuguese)

The authors report a case of long-term pemphigus foliaceus under treatment with prednisone, which evolved with lesions on the palate, histopathologically diagnosed as lepromatous leprosy. There were no skin lesions suggestive of leprosy. The histopathology of the abdominal vesicle indicated the coincidence of the diseases. The authors also discuss the most common location of leprosy's oral manifestations.—Authors' English Summary

Ebenso, B. E. Genital nodules and testicular hydrocele in a case of relapsed lepromatous leprosy. *Lepr. Rev.* **71** (2000) 81–82.

A borderline lepromatous leprosy patient treated initially with dapsone monotherapy for 10 years followed by a combination of dapsone and clofazimine for 6 months stopped antileprosy treatment in 1991. He presented 6 years later due to new, widespread nodules and recurrent testicular hydrocele. He responded to WHO-MB-MDT and steroid therapy. His hydrocele was treated surgically. The co-existence of recurrent testicular hydrocele with genital nodules in relapsed LL led to this report.—Author's Summary

Facer, P., Mann, D., Mathur, R., Pandya, S., Ladiwala, U., Singhal, B., Hongo, J. A., Sinicropi, D. V., Terenghi, G. and Anand, P. Do nerve growth factor-

related mechanisms contribute to loss of cutaneous nociception in leprosy? *Pain* **85** (2000) 231–238.

While sensory loss in leprosy skin is the consequence of invasion by *M. leprae* of Schwann cells related to unmyelinated fibers, early loss of cutaneous pain sensation, even in the presence of nerve fibers and inflammation, is a hallmark of leprosy and requires explanation. In normal skin, nerve growth factor (NGF) is produced by basal keratinocytes, and acts via its high affinity receptor (trk A) on nociceptor nerve fibers to increase their sensitivity, particularly in inflammation. We have therefore studied NGF- and trk A-like immunoreactivity in affected skin and mirror-site clinically unaffected skin from patients with leprosy, and compared these with non-leprosy, control skin, following quantitative sensory testing at each site. Sensory tests were within normal limits in clinically unaffected leprosy skin, but markedly abnormal in affected skin. Subepidermal PGP 9.5- and trk A-positive nerve fibers were reduced only in affected leprosy skin, with fewer fibers contacting keratinocytes.

However, NGF-immunoreactivity in basal keratinocytes, and intra-epidermal PGP 9.5-positive fibers, were reduced in both sites compared to nonleprosy controls, as were nerve fibers positive for the sensory neurone specific sodium channel SNS/PN3, which is regulated by NGF, and may mediate inflammation-induced hypersensitivity. Keratinocyte trk A expression (which mediates an autocrine role for NGF) was increased in clinically affected and unaffected skin, suggesting a compensatory mechanism secondary to reduced NGF secretion at both sites. We conclude that decreased NGF- and SNS/PN3-immunoreactivity, and loss of intra-epidermal innervation, may be found without sensory loss on quantitative testing in clinically unaffected skin in leprosy; this appears to be a subclinical change, and may explain the lack of cutaneous pain with inflammation.

Sensory loss occurred with reduced subepidermal nerve fibers in affected skin, but these still showed trk A-staining, suggesting NGF treatment may restore pain sensation.—Authors' Abstract

Fleury, R. N., Opromolla, D. V. A., Taborda, P. R., Nakandakari, M. T. C. B. R., Soares, C. T. and Bonfim, A. C. [Mycosis fungoides succeeding long-term borderline leprosy with multiple episodes of type 1 reaction.] *Hansen. Int.* **24** (1999) 137–143. (in Portuguese)

An 86-year-old male was referred to the Instituto Lauro de Souza Lima (Brazil) 20 years ago with subpolar lepromatous leprosy, saddle nose and subtotal madarosis. Two months after beginning treatment for Hansen's disease, the patient presented a type 1 reaction with neuritis. A biopsy showed a tuberculoid granulomatous reaction in the skin and nerves. During a second episode of a type 1 reaction, he developed severe laryngitis with upper respiratory obstruction and was submitted to a tracheostomy. Several other episodes of type 1 reaction appeared until the patient became cured, 7 years ago, after he had been under several therapeutic regimens. Shortly after he had been released from treatment, new skin lesions developed as several squamous macules with well-limited edges. In 1997 some biopsies confirmed the diagnosis of mycosis fungoides. The authors discuss: a) the pathogenesis of the several episodes of a type 1 reaction and laryngeal involvement; b) possible relationship between multibacillary leprosy and mycosis fungoides, and c) difficulties found on diagnosis of mycosis fungoides in this case.—Authors' English Summary

Gomez Echevarria, J. R. and Hernandez Ramos, J. M. [Indeterminate leprosy.] *Rev. Leprol. Fontilles* **22** (2000) 357–370. (in Spanish)

Since 1991 and up to the present the Sanatorium of Fontilles (Spain) has had health workers collaborating in public health projects with a special dedication to leprosy and dermatological problems in the state of Mato Grosso (Brazil). The projects were carried out in the northeastern part of this Brazilian state (Santa Terezinha, Sao Felix do Araguaia, Porto Alegre do Norte, Luciara y Alto do Boa Vista), a region of high endemicity for leprosy.

From January 1991 to the present, 882 individuals were diagnosed and treated for leprosy (585 men, 297 women and 66 children under 14 years); 724 patients received treatment according to WHO-Fundacion Nacional de Saude and were taken off register; 158 patients remain active and on treatment. The prevalence for disease in the area is higher than 45/10,000 people. The two doctors who have worked in the field with this project reviewed personal experience with indeterminate leprosy.

The number of patients diagnosed as indeterminate leprosy were 141: 93 men (66%) and 48 women (34%), 21 children less than 14 years (15%); 129 cases were treated and taken off register and at present there are 22 cases of this type in treatment.—Authors' English Summary

Mitchell, P. D. and Mitchell, T. N. The age-dependent deterioration in light touch sensation on the plantar aspect of the foot in a rural community in India: implication when screening for sensory impairment. *Lepr. Rev.* **71** (2000) 169–178.

Regular testing for impaired sensation is important in the management of diseases that can cause progressive nerve damage, such as leprosy. It has been shown that light touch sensibility decreases with age in the hands of healthy individuals, but little research has been undertaken to assess possible changes in the feet in developing countries. This information is needed to allow an appropriate level of sensation to be chosen when screening for nerve damage in the foot. To clarify this, a cross-sectional study on male adults was carried out in the rural town of Salur, Andhra Pradesh, India. A range of Semmes-Weinstein monofilaments were employed at 12 locations on the foot to determine sensation to light touch stimuli in individuals from each decade of adult life. It was found that in this population, sensibility threshold in the foot increases with age and this was noted in both soft and callous skin. This shows the increase was due to neurological factors, not merely due to an increase in callous deposition with advancing age. In the majority of individuals

in their fifties and sixties, the callous skin at the forefoot and heel was unable to detect the 5.07 monofilament (equivalent to 8–12 g), previously recommended as a method to screen for plantar neuropathy. All areas of all feet were able to detect the 5.46 filament (approximately 30 g). The size of this study (54 individuals) prevents the determination of definitive normal ranges for each decade of life in this population. However, it does demonstrate the degree to which sensation deteriorates with age and could be used as an approximate guide when interpreting the results of sensory testing in similar rural areas of the developing world.—Authors' Summary

Moreira, D. and Alvarez, R. R. A. [Use of Semmes-Weinstein monofilaments to evaluate sensitivity of upper limbs of Hansen's disease patients attended in the Federal District.] *Hansen. Int.* **24** (1999) 121–128. (in Portuguese)

The present study has as its objective to identify the prevalence of sensitivity disturbance in upper limbs of patients with leprosy inscribed in the control program for Hansen's disease in the Distrito Federal. This research is a transversal descriptive study involving 80 patients who were submitted to Semmes-Weinstein monofilaments test, also known as extensometer; (pocket model—Sensikit), in order to collect the data. The extensometer constitutes an important tool in clinical routine when dealing with leprosy. The results showed that a great part (above 50%) of the patients in all clinical forms of Hansen's disease responded to the green monofilament stimulus, indicating normal sensitivity of the hands. The study is in agreement with the literature and concludes that the damaged sensitivity represents a main physiopathogenic cause of physical deficiencies in upper limbs of leprosy patients. The parameters utilized during the sensitivity examination should be standardized in order to entrust the results and to encourage others to reproduce them. It is recommended routinely to use Semmes-Weinstein monofilaments in clinical practice for the early diagnosis of neural damage in Hansen's disease patients. The test is essen-

tial, but it should not be performed solely during the physical examination.—Authors' English Summary

Nunez Marti, J. M. [Dental caries in leprosy patients.] *Rev. Leprol. Fontilles* **22** (2000) 371–376. (in Spanish)

Dental caries is a disease with a slow evolution, usually months and even years are needed for the production of cavitation. Dental caries do not have the same effect on all teeth and dental surfaces. They develop preferentially in areas most affected by plaque and of most difficult access. The factors that influence the production of cariogenic bacterial plaque are proliferation of certain types of flora, sugars with permeability and consequent acid pH production. All these conditions for the formation of dental caries are favored in Hansen's disease patients due to disabilities that hinder a normal oral hygiene together with their multidrug therapy that reduces considerably their lingual saliva pH. Therefore, in this type of patient we find basically three different types of caries: 1. Proximal superficial caries under or on areas of contact. 2. Radicular caries, located on the amelocementarial joint when the dental necks are exposed to the oral environment. 3. Caries on free surfaces that are the less frequent. Considering these three types of lesions, an early diagnosis and early treatment of damaged tissue are fundamental before a major complication and consequent tooth loss.—Author's English Summary

Opromolla, D. V. A. The question of the "12 doses" of the multidrug therapy for Hansen's disease. (Editorial) *Hansen. Int.* **24** (1999) 100–102.

In this thought-provoking editorial, the author questions the wisdom of shortening the recommended duration of treatment of multibacillary leprosy to 12 months. He concludes as follows:

"There is no way to change a politically defined situation. The only thing we can do is to hope everything turns out all right and the patients do not suffer with the chosen

measures. Anyway, any positive result will be more a result from the Holy Spirit than a consequence of decisions taken in an ethical and scientific manner."—RCH

Saunderson, P. and Groenen, G. Which physical signs help most in the diagnosis of leprosy? A proposal based on experience in the AMFES project, ALERT, Ethiopia. *Lepr. Rev.* **71** (2000) 34–42.

As integration of leprosy control programs proceeds, general health staff will have responsibility for the diagnosis of most new cases of leprosy. The training required by these workers has not yet been set out in detail. In this paper the criteria for making the diagnosis of leprosy in the AMFES cohort of 594 new cases are examined. Since this study does not include details of suspects in whom leprosy was excluded on clinical grounds, true sensitivity and specificity values cannot be calculated, but the positive predictive value of the diagnostic criteria can be measured. Sensory loss in a typical skin patch is the most important sign of early leprosy, but was not present in 132 (49%) of the 268 cases with a positive skin smear. Thickening of the ulnar nerve is a valuable sign of leprosy in Ethiopia. It can be taught to health workers, who can practice by examining their own ulnar nerves. It is more likely to be present than nerve function impairment, and is particularly important when skin smears are difficult to do or are unreliable. We recommend that five basic signs are used, the presence of any two being diagnostic of leprosy:

- Skin lesion(s) consistent with leprosy.
- Loss of sensation in such a lesion.
- Thickening of either ulnar nerve.
- Loss of sensation in the palm of the hand or the sole of the foot.
- Presence of acid-fast bacilli in skin smears.

Exact policies for the diagnosis of leprosy should be worked out and validated for each national program.—Authors' Summary

Sharma, P., Kar, H. K., Misra, R. S., Mukherjee, A., Kaur, H., Mukherjee, R. and Rani, R. Reactional states and neuritis in multibacillary leprosy patients

following MDT with/without immunotherapy with *Mycobacterium w* anti-leprosy vaccine. *Lepr. Rev.* **71** (2000) 193–205.

A vaccine based on autoclaved *Mycobacterium w* was administered, in addition to standard multidrug therapy (MDT), to 157 untreated, bacteriologically positive, lepromin-negative multibacillary leprosy patients, supported by a well-matched control group of 147 patients with similar type of disease who received a placebo injection in addition to MDT. The MDT was given for a minimum period of 2 years and continued until skin-smear negativity, while the vaccine/placebo was given at 3-month intervals up to a maximum of eight doses. The incidence of type 2 reaction and neuritis during treatment and follow up showed no statistically significant difference in the vaccine and placebo groups. The incidence of type 1 reaction (mild in most cases), however, was higher in the vaccine group ($p = 0.041$, relative risk ratio 1.79), considering LL, BL and BB leprosy types together, and considerably higher ($p = 0.009$) in LL type, probably because of confounding due to higher number of patients with previous history of reaction in this group. The occurrence of reactions and neuritis in terms of single or multiple episodes was similar in the vaccine and placebo groups. The association of neuritis and reactions, as well as their timing of occurrence (during MDT or follow up), was also similar in the two groups with more than 90% of occurrences taking place during MDT. The incidence of reversal reaction was significantly higher among the males in the vaccine group (34.5% versus 8.3%, $p = 0.019$). Patients with high initial BI (4.1–6.0) showed higher incidence of reactions (70.3%) as compared to those with medium (2.1–4.0) and low (0.3–2.0) BI, whose reactions were observed with a frequency of 56.1% and 38.8%, respectively. However, unlike reactions, neuritis incidence did not seem to be affected by the initial BI to the same extent in the vaccine group, with frequencies of 35.3%, 36.3% and 25.9% in the three mentioned BI ranges. Overall, the vaccine did not precipitate reactional states and neuritis over and above that observed with MDT alone.—Authors' Summary

Sharma, P., Misra, R. S., Kar, H. K., Mukherjee, A., Poricha, D., Kaur, H., Mukherjee, R. and Rani, R. *Mycobacterium w* vaccine, a useful adjuvant to multidrug therapy in multibacillary leprosy: a report on hospital-based immunotherapeutic clinical trials with a follow up of 1–7 years after treatment. *Lepr. Rev.* **71** (2000) 179–192.

A vaccine based on autoclaved *Mycobacterium w* was administered, in addition to standard multidrug therapy (MDT), to 156 bacteriologically positive, lepromin-negative, multibacillary leprosy patients compared to a well-matched control group of 145 patients with a similar type of disease who received a placebo injection in addition to MDT. The MDT was given for a minimum period of 2 years and continued until skin-smear negativity, while the vaccine was given at 3-month intervals up to a maximum of eight doses. The fall in clinical scores and the bacterial index (BI) was significantly more rapid in vaccinated patients, from 6 months onward until years 2 or 3 of therapy. However, no difference was observed in the fall in the BI in the two groups from year 4 onward. The number of LL and BL patients released from therapy (RFT) following attainment of skin-smear negativity after 24–29 months of treatment was 84/133 (63.1%) in vaccinated and 30/120 (25.0%) in the placebo group; the difference was highly statistically significant ($p < 0.0001$). In all, 90.2% patients (146/162) converted from lepromin negativity to positivity in the vaccine group as against 37.9% (56/148) in the placebo group. The average duration of lepromin positivity maintained following eight doses of vaccine administered over 2 years was 3.016 years in the vaccine and 0.920 years in the placebo group. Histological upgrading after 2 years of treatment in the LL type was observed in 34/84 (40.5%) cases in the vaccine and 5/85 (5.9%) cases in the placebo group, the difference being statisti-

cally significant ($p < 0.001$). The incidence of type 1 reactions was significantly higher (30.5%) in the vaccine group than (19.7%) in the placebo group ($p = 0.0413$); the difference was mainly observed in LL type ($p = 0.009$). The incidence of type 2 reactions was similar (31.8 and 34.6%) in the vaccine and placebo groups. The vaccine did not precipitate neuritis or impairments over and above that encountered with MDT alone. After 5 years of follow up following RFT, no incidence of bacteriological or clinical relapses was observed in either group.—Authors' Summary

Wilder-Smith, E. P. V., Wilder-Smith, A. J. and Nirkko, A. C. Skin and muscle vasomotor reflexes in detecting autonomic dysfunction in leprosy. *Muscle Nerve* **23** (2000) 1105–1112.

There are few tests to assess the function of small unmyelinated nerve fibers. One established test is the skin vasomotor reflex (SVMR), which uses laser doppler flow velocimetry. The SVMR has the disadvantages of being susceptible to interference (from change of temperature and alerting stimuli) and of requiring expensive equipment. An ultrasound doppler method, which is less expensive, can be used to detect muscle vasomotor reflex (MVMR) activity. We sought to compare the efficacy of these two methods in detecting dysfunction of small unmyelinated nerve fibers in patients with leprosy. SVMR was shown to be less sensitive ($p < 0.01$) and specific ($p < 0.001$) than MVMR. The favorable results of MVMR may be attributed to its lesser susceptibility to interfering sympathetic vasoconstriction from alerting stimuli. MVMR also reflects larger areas of blood vessel innervation than the laser doppler method. In leprosy, nerve damage is typically patchy and may be missed by the smaller sampling of the laser method.—Authors' Abstract

Immuno-Pathology

Aleman, M., Dela Barrera, S., Fink, S., Finiasz, M., Farina, M. H., Pizzariello, G. and Sasiain, M. D. Interleukin-12 amplifies the *M. leprae* hsp65-cytotoxic response in the presence of tumor necrosis factor-alpha and interferon-gamma generating CD56+ effector cells: interleukin-4 downregulates this effect. *Scand. J. Immunol.* **51** (2000) 262-270.

Interleukin-12 (IL-12) is a major immunomodulatory cytokine that represents a functional bridge between the early resistance and the subsequent antigen specific adaptive immunity. TNF-alpha and IFN-gamma have an important role in the generation of hsp65 specific cytotoxic T lymphocytes (CTL) that lyse hsp65-pulsed autologous macrophages (hsp65 CTL). Since a positive feedback mechanism between TNF-alpha, IFN-gamma and IL-12 has been described, we undertook to evaluate the role of IL-12 on the hsp65 CTL generation in leprosy patients. Our results show that the presence of IL-12 during the first 24 hr of the *in vitro* antigen stimulation amplifies the hsp65 cytotoxic response whenever both IFN-gamma and TNF-alpha are present. The addition of these three cytokines (CKs) was able to abrogate the inhibitory effect of IL-10 on hsp65 CTL in cells from paucibacillary (PB) patients but not that of IL-4 in PB and normal controls (N). Both IL-12 or anti-IL-4 enhanced the cytotoxic activity in cells from multibacillary (MB) patients. Anti-IL-4 upregulated the binding of IFN-gamma and did not modify that of TNF-alpha so the low CTL activity could be as a result of IL-4 by a decrease of the IFN-gamma binding on MB cells. Cells from those MB patients taking thalidomide (MB-T) did neither bind IFN-gamma nor TNF-alpha even when antigen or anti-IL-4 were added, demonstrating that thalidomide inhibits either the *in vitro* binding or receptor expression of both TNF-alpha and IFN-gamma. Development of CD56 effector cells during the hsp65 stimulation was observed in PB and N by the addition of IL-12 plus TNF-alpha and IFN-gamma, while in MB and MB-T anti-IL-4 was also required.

So, the inhibitory effect of IL-4 on either production of IFN-gamma, TNF-alpha and/or IL-12 or their receptors could be the mechanism underlying the lack of the hsp65 CTL generation in cells from MB.—Authors' Abstract

Beatty, W. L., Rhoades, E. R., Ullrich, H. J., Chatterjee, D., Heuser, J. E. and Russell, D. G. Trafficking and release of mycobacterial lipids from infected macrophages. *Traffic* **1** (2000) 235-247.

Analysis of infected macrophages revealed that lipid-containing moieties of the mycobacterial cell wall are actively trafficked out of the mycobacterial vacuole. To facilitate the analysis of vesicular trafficking from mycobacteria-containing phagosomes, surface-exposed carbohydrates were labeled with hydrazide-tagged markers. The distribution of labeled carbohydrate/lipid moieties and subsequent interaction with cellular compartments were analyzed by immunoelectron microscopy and by fluorescence microscopy of live cells. The released mycobacterial constituents were associated with several intracellular organelles and were enriched strikingly in tubular endocytic compartments. Subcellular fractionation of infected macrophages by density gradient electrophoresis showed temporal movement of labeled bacterial constituents through early and late endosomes. Thin-layer chromatography analysis of these subcellular fractions confirmed their lipid nature and revealed five dominant bacteria-derived species. These mycobacterial lipids were also found in extracellular vesicles isolated from the medium and could be observed in uninfected "bystander" cells. Their transfer to bystander cells could expand the bacteria's sphere of influence beyond the immediate confines of the host cell.—Authors' Abstract

Buchmeier, N., Blanc-Potard, A., Ehrt, S., Piddington, D., Riley, L. and Groisman, E. D. A parallel intraphagosomal survival strategy shared by *Mycobacterium tuber-*

culosis and *Salmonella enterica*. Mol. Microbiol. **35** (2000) 1375–1382.

Mycobacterium tuberculosis and *Salmonella enterica* cause very different diseases and are only distantly related. However, growth within macrophages is crucial for virulence in both of these intracellular pathogens. Here, we demonstrate that in spite of the phylogenetic distance, *M. tuberculosis* and *Salmonella* employ a parallel survival strategy for growth within macrophage phagosomes. Previous studies established that the *Salmonella mgtC* gene is required for growth within macrophages and for virulence *in vivo*. *M. tuberculosis* contains an open reading frame exhibiting 38% amino acid identity with the *Salmonella* MgtC protein. Upon inactivation of *mgtC*, the resulting *M. tuberculosis* mutant was attenuated for virulence in cultured human macrophages and impaired for growth in the lungs and spleens of mice. Replication of the *mgtC* mutant was inhibited *in vitro* by a combination of low magnesium and mildly acidic pH, suggesting that the *M. tuberculosis*-containing phagosome has these characteristics. The similar phenotypes displayed by the *mgtC* mutants of *M. tuberculosis* and *Salmonella* suggest that the ability to acquire magnesium is essential for virulence in intracellular pathogens that proliferate within macrophage phagosomes.—Authors' Summary

Dieli, F., Singh, M., Spallek, R., Romano, A., Titone, L., Sireci, G., Friscia, G., DiSano, C., Santini, D., Salerno, A. and Ivanyi, J. Change of Th0 to Th1 cell-cytokine profile following tuberculosis chemotherapy. Scand. J. Immunol. **52** (2000) 96–102.

T cells mediate protection against tuberculosis, but little is known about their role during chemotherapy of patients with active disease. Here we examined the cytokine profile of CD4 T cells before and after 4 months of chemotherapy in six initial skin test anergic cases. Purified protein derivative (PPD) and 16-kDa antigen-reactive CD4 T-cell clones prior to therapy resided mostly in disease-associated body fluids and were of the Th0 (interferon (IFN)-

gamma + interleukin (IL)-4) secreting profile. In contrast, the majority of post-chemotherapy CD4 T-cell clones originated from blood and were of the IFN- γ secreting Th1 type. However, the recognition of several peptides derived from the 16-kDa antigen was not significantly different between the Th1 and Th0 clones. We conclude that chemotherapy shifts CD4 T cells from the affected body fluids to the blood circulation, accompanied by a change from Th0 to Th1 cytokine profile.—Authors' Abstract

Ehlers, S., Kutsch, S., Ehlers, E. M., Benini, J. and Pfeffer, K. Lethal granuloma disintegration in mycobacteria-infected TNFRp55 (–/–) mice is dependent on T cells and IL-12. J. Immunol. **165** (2000) 483–492.

Genetically susceptible, TNFRp55 gene-deficient [TNFRp55 (–/–)] mice succumb to infection with *Mycobacterium avium*. Before their death, *M. avium*-infected TNFRp55 (–/–) mice develop granulomatous lesions that, in contrast to granulomas in wild-type syngeneic mice, undergo acute disintegration. To determine the factors involved in these events, we depleted T-cell subsets or neutralized the inflammatory cytokines IFN- γ , IL-12, or TNF in TNFRp55 (–/–) mice infected i.v. with *M. avium*. Infected TNFRp55 (–/–) mice treated with a control monoclonal antibody became moribund between days 26 and 34 postinfection, showing widespread inflammatory cell apoptosis within disintegrating granulomas. In contrast, TNFRp55 (–/–) mice depleted of either CD4+ or CD8+ cells after granuloma initiation stayed healthy until at least day 38 postinfection and showed no signs of granuloma destruction. Neutralization of IL-12, but not of IFN- γ or TNF, also protected *M. avium*-infected TNFRp55 (–/–) mice from granuloma decomposition and from premature death. Treatment with dexamethasone or with a specific inhibitor of inducible NO synthase did not prevent granuloma dissolution or death of TNFRp55 (–/–) mice. In conclusion, granuloma disintegration in TNFRp55 (–/–) mice is a lethal event that is dependent on IL-12 and that is mediated by an excess of T cells.—Authors' Abstract

Fleury, R. N., Ura, S., Borges, M. B., Ghidella, C. C. and Opromolla, D. V. A. Cutaneous panarthritis as a late manifestation of the erythema nodosum leprosum. *Hansen. Int.* **24** (1999) 109–114.

The authors studied 11 cases of multibacillary Hansen's disease (9 lepromatous and 2 borderline lepromatous) with late erythema nodosum leprosum manifestations. The systemic symptoms were mild and there was a low number of cutaneous lesions in lower limbs. Histologically, those lesions presented necrotizing and exudative segmental arteritis in the deep dermis, with discrete inflammatory reaction in the neighboring dermis and sub-cutis. There were discrete or absent evidences of previous involvement by Hansen's disease; however, in seven patients, acid-fast bacilli were found on the wall of involved vessels. Probably there are pathogenic similarities between these arteritis and the necrotizing vasculitis that occurs in severe and generalized episodes of erythema nodosum leprosum throughout specific treatment due to the persistence of mycobacterial antigen on the vessel wall, even after their elimination from other cutaneous sites. The eventual exposure of mycobacterial antigens would stimulate immune complex formation and acute inflammatory and necrotizing reaction. In addition, the histopathological and even clinical aspects of these arteritis are very similar to cutaneous polyarteritis nodosa. These clinical and structural similarities may correspond to similar pathogenic mechanisms between the two cutaneous vasculitis.—Authors' Summary

Gatfield, J. and Pieters, J. Essential role for cholesterol in entry of mycobacteria into macrophages. *Science* **288** (2000) 1647–1650.

Mycobacteria are intracellular pathogens that can invade and survive within host macrophages, thereby creating a major health problem worldwide. The molecular mechanisms involved in mycobacterial entry are still poorly characterized. Here we report that cholesterol is essential for uptake of mycobacteria by macrophages. Cholesterol accumulated at the site of my-

cobacterial entry, and depleting plasma membrane cholesterol specifically inhibited mycobacterial uptake. Cholesterol also mediated the phagosomal association of TACO, a coat protein that prevents degradation of mycobacteria in lysosomes. Thus, by entering host cells at cholesterol-rich domains of the plasma membrane, mycobacteria may ensure their subsequent intracellular survival in TACO-coated phagosomes.—Authors' Abstract

Gonzalez Amaro, R., Portales Perez, D. P., Baranda, L., Moncada, B., Toro, C., Lopez Briones, S., Espitia, C. and Mancilla, R. Co-stimulatory signals increase the reactivity of gamma delta T cells toward mycobacterial antigens. *Clin. Exp. Immunol.* **120** (2000) 468–475.

Although it has been shown that gamma delta T lymphocytes are able to react with different cell-associated or soluble antigens, the immune repertoire of these cells appears to be skewed to the recognition of mycobacterial antigens. We have studied the number and reactivity of gamma delta T cells toward several mycobacterial antigens in patients with tuberculosis and leprosy, as well as their healthy contacts and control individuals. We found an increased number of V delta 2+ cells in healthy contacts (PPD+ and lepromin+) and tuberculoid leprosy patients. The gamma delta T cells from lepromatous leprosy showed a decreased response to all antigens tested, but some of these patients exhibited a significant response to the 30-kDa glycoprotein of *Mycobacterium tuberculosis*. Interestingly, the reactivity of gamma delta T cells against mycobacterial antigens was significantly increased by costimulatory signals generated through CD7, LFA-1, CD50 and CD69 in all groups. However, signalling through CD69 did not enhance the responsiveness of gamma delta lymphocytes from lepromatous patients. On the other hand, the *in vitro* blockade of IL-10 with a specific antibody enhanced the cell proliferation of gamma delta lymphocytes from lepromatous leprosy patients; whereas exogenous IL-10 had an opposite effect in most individuals studied. These results suggest the potential role

of different cell membrane receptors in the regulation of gamma delta T-cell proliferation induced by mycobacteria, as well as the possible involvement of IL-10 in this phenomenon.—Authors' Abstract

Hess, J., Grode, L., Hellwig, J., Conradt, P., Gentschev, I., Goebel, W., Ladel, C. and Kaufmann, S. H. E. Protection against murine tuberculosis by an attenuated recombinant *Salmonella typhimurium* vaccine strain that secretes the 30-kDa antigen of *Mycobacterium bovis* BCG. *FEMS Immunol. Med. Microbiol.* **27** (2000) 283–289.

A recombinant (r-) *Salmonella typhimurium* aroA vaccine that secretes the naturally secreted protein of *Mycobacterium bovis* strain BCG, Ag85B, by means of the HlyB/HlyD/TolC export machinery (termed p30 in the following) was constructed. In contrast to r-*S. typhimurium* control, oral vaccination of mice with the r-*S. typhimurium* p30 construct induced partial protection against an intravenous challenge with the intracellular pathogen *M. tuberculosis*, resulting in similar vaccine efficacy comparable to that of the systemically administered attenuated *M. bovis* BCG strain.

The immune response induced by r-*S. typhimurium* p30 was accompanied by augmented interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α) levels produced by restimulated splenocytes. These data suggest that the HlyB/HlyD/TolC-based antigen delivery system with attenuated r-*S. typhimurium* as carrier is capable of inducing an immune response against mycobacterial antigens.—Authors' Abstract

Kamath, A. T., Groat, N. L., Bean, A. G. D. and Britton, W. J. Protective effect of DNA immunization against mycobacterial infection is associated with the early emergence of interferon-gamma (IFN- γ)-secreting lymphocytes. *Clin. Exp. Immunol.* **120** (2000) 476–482.

The development of more effective anti-tuberculosis (TB) vaccines would contribute to the global control of TB. Under-

standing the activated/memory T-cell response to mycobacterial infection and identifying immunological correlates of protective immunity will facilitate the design and assessment of new candidate vaccines. Therefore, we investigated the kinetics of the CD4+ T-cell response and IFN-gamma production in an intravenous challenge model of *Mycobacterium bovis* bacille Calmette-Guerin (BCG) before and after DNA immunization. Activated/memory CD4+ T cells, defined as CD44(hi) CD45RB(lo) expanded following infection, peaking at 3–4 weeks, and decreased as the bacterial load fell. Activated/memory CD4+ T cells were the major source of IFN-gamma and the level of antigen-specific IFN-gamma-secreting lymphocytes, detected by ELISPOT, paralleled the changes in bacterial load. To examine the effects of a DNA vaccine, we immunized mice with a plasmid expressing the mycobacterial secreted antigen 85B (Ag85B). This led to a significant reduction in mycobacteria in the liver, spleen and lung. This protective effect was associated with the rapid emergence of antigen-specific IFN-gamma-secreting lymphocytes which were detected earlier, at day 4, and at higher levels than in infected animals immunized with a control vector. This early and increased response of IFN-gamma-secreting T cells may serve as a correlate of protective immunity for anti-TB vaccines.—Authors' Abstract

Lima, M. C. B. S., Pereira, G. M. B., Rumjanek, F. D., Gomes, H. M., Duppre, N., Sampaio, E. P., Alvim, I. M. P., Nery, J. A. C., Sarno, E. N. and Pesolani, M. C. V. Immunological cytokine correlates of protective immunity and pathogenesis in leprosy. *Scand. J. Immunol.* **51** (2000) 419–428.

The *in vitro* production of interferon-gamma (IFN- γ), interleukin (IL)-5, tumor necrosis factor-alpha (TNF- α) and IL-10 by blood mononuclear cells in response to whole *Mycobacterium leprae* and polyclonal stimuli of 23 individuals, representing a variety of conditions in relation to exposure/susceptibility to *M. leprae*, was assayed. In most cases, healthy household contacts of newly diagnosed multibacillary

leprosy patients, designated exposed household contacts (EC), showed low-to-undetectable *in vitro* INF- γ production in addition to substantial TNF- α production in response to *M. leprae*. In contrast, peripheral blood mononuclear cells from previously exposed contacts (R) regarded as resistant-to-leprosy released low-to-moderate levels of INF- γ together with a mixed cytokine profile resembling a T helper (Th)0-type response. TNF- α /IL-10 ratios in response to *M. leprae* and concanavalin A were significantly higher in EC than in R contacts, suggesting a role for the TNF- α /IL-10 ratio in restraining mycobacteria proliferation and spreading early in infection. The cytokine profiles of leprosy patients were taken as reference points. Post-treatment leprous patients secreted relatively high levels of IL-10 in response to *M. leprae*; whereas one self-cured tuberculoid leprosy patient produced simultaneously high levels of INF- γ and TNF- α . In addition, the quantitative changes in the cytokines released by peripheral blood mononuclear cells in EC contacts after Bacille Calmette-Guerin (BCG) vaccination were investigated. Vaccination induced amplification of INF- γ production with a concomitant decrease in TNF- α /IL-10 ratios that resembled the cytokine pattern observed in R contacts. INF- γ production was observed in response to both a crossreactive antigen (Ag 85) and an *M. leprae*-specific protein (MMP-1), which attests to a BCG nonspecific stimulation of the immune system, thereby casting these antigens as likely candidates for inclusion in a subunit vaccine against leprosy. Finally, a model for protective pathologic response to mycobacteria is presented.—Authors' Abstract

Mustafa, A. S., Lundin, K. E. A., Meloen, R. H. and Oftung, F. Crossreactive epitopes and HLA-restriction elements in human T cell recognition of the *Mycobacterium leprae* 18-kDa heat shock protein. Clin. Exp. Immunol. **120** (2000) 85–92.

We have previously demonstrated that the *Mycobacterium leprae* 18-kD heat shock protein (hsp18) is represented among the antigenic targets of human T-cell re-

sponses induced by *M. leprae* immunization and that the peptide 38–50 serves as an immunodominant epitope recognized by CD4+ T-cell clones. By using peripheral blood mononuclear cells and T-cell lines from the same donor group, we have in this study shown that the *M. leprae* hsp18 and peptide 38–50 were recognized by memory T cells 8 years after immunization with *M. leprae*. The finding that *M. bovis* BCG-induced T-cell lines responded to *M. leprae* hsp18, but not to the peptide 38–50, suggested the existence of additional T-cell epitopes of a crossreactive nature. Consistent with this, testing of the T-cell lines for proliferative responses to the complete hsp18 molecule, truncated hsp18 [amino acid (aa) residues 38–148] and overlapping synthetic peptides, made it possible to identify two crossreactive epitope regions defined by aa residues 1–38 and 41–55. While peptide 38–50-reactive T-cell clones showed limited crossreactivity by responding to *M. leprae*, *M. avium* and *M. scrofulaceum*, the T-cell lines specific to the epitopes 1–38 and 41–55 were broadly crossreactive, as demonstrated by their response to *M. leprae*, *M. tuberculosis* complex, *M. avium* and other mycobacteria. MHC restriction analysis of the hsp18-responding T-cell lines showed that the epitopes 1–38 and 38–50 were presented by one of the two HLA-DR molecules expressed from self HLA-DRB1 genes; whereas the epitope 41–55 was recognized in the presence of autologous as well as HLA-DR and HLA-DQ mismatched allogeneic antigen-presenting cells. The results obtained in this study made it possible to identify crossreactive T-cell epitopes of the *M. leprae* hsp18, and provide an explanation for T-cell recognition of this antigen in individuals infected with species of the *M. tuberculosis* complex or environmental mycobacteria.—Authors' Abstract

Naito, M., Matsuoka, M., Ohara, N., Nomaguchi, H. and Yamada, T. The antigen 85 complex vaccine against experimental *Mycobacterium leprae* infection in mice. Vaccine **18** (1999) 795–798.

The proteins in culture filtrate derived from Bacillus Calmette-Guérin (BCG) were

examined for protection against infection by *M. leprae*. Immunization with the major secreted proteins, antigen 85 complex (Ag85) A, B and C, induced effective protective immunity against multiplication of *M. leprae* in the foot pads of mice. The most effective protection was observed with Ag85A. A single immunization with Ag85 could induce antigen-specific interferon-gamma (IFN- γ) synthesis and more effective protection than live BCG vaccine. It is concluded that Ag85 is an important immunoprotective molecule against leprosy infection.—Trop. Dis. Bull. **97** (2000) 293–294

Patil, S. A., Ramu, G. and Prasad, R. Detection of disease-related immune complexes in the serum of leprosy patients—a novel single-step method. *J. Neuroimmunol.* **105** (2000) 64–68.

Mycobacterium leprae antigen and antibody complexes could be detected in the serum of leprosy patients using monoclonal antibody ML34 and anti-BCG antibodies by enzyme-linked immunosorbent assay. This simplified system detects disease-related complexes without the need for isolating and purifying them from the serum. Immune complexes captured using monoclonal antibody ML34 revealed positivity in 7 out of 8 neuritic, 2 out of 9 tuberculoid (TT), 5 out of 10 borderline tuberculoid (BT), 4 out of 10 borderline lepromatous (BL) and 4 out of 10 lepromatous (LL) leprosy cases.

One of the controls also showed an immune complex of an IgM type. Anti-BCG-based IgG immune complexes assay revealed positivity in 6 out of 8 neuritic, 1 out of 9 TT, 4 out of 10 BT, 2 out of 10 BL, 4 out of 10 LL leprosy cases, and 2 out of 24 healthy controls. IgM type of mycobacterial immune complexes were almost negligible. Capture of complexes using monoclonal antibody ML34 which is against lipoarabinomannan of *M. leprae* seems to work better than polyclonal anti-BCG antibody. The probable role of the immune complexes in nerve damage needs to be evaluated since very high levels of immune complexes are found in neuritic leprosy by both assays. The above test would be useful in the im-

munodiagnosis of neuritic leprosy and also in cases where antibody response is not detectable because of the formation of immune complexes.—Authors' Abstract

Prabhakaran, K., Harris, E. B. and Randhawa, B. Regulation by protein kinase of phagocytosis of *Mycobacterium leprae* by macrophages. *J. Med. Microbiol.* **49** (2000) 339–342.

Mycobacterium leprae multiplies within host macrophages. The mechanism of internalization of the bacteria by the phagocytic cells is unknown. In this study, *M. leprae* was purified from the foot pads of experimentally infected nu/nu mice. Peritoneal macrophages were harvested from BALB/c mice or C57 beige (bg/bg) mice. The effect of protein kinase inhibitors (erbstatin, genistein or staurosporine for BALB/c and bg/bg mice, plus herbimycin for bg/bg mice) on phagocytosis of the mycobacteria by the macrophage monolayers was tested. The untreated (control) macrophages phagocytosed *M. leprae*. Phagocytosis by BALB/c macrophages was inhibited by erbstatin and staurosporine but not by genistein; all the protein kinase inhibitors prevented uptake of *M. leprae* by bg/bg cells. The results demonstrate that protein kinase regulates phagocytosis of *M. leprae* by macrophages. The mechanism might prove to be a rational drug target for mycobacteria that multiply intracellularly.—Authors' Abstract

Rojas-Espinosa, O., Luna-Herrera, J. and Arce-Paredes, P. Recognition of phenolic glycolipid-I (*Mycobacterium leprae*) and sulfolipid-I (*M. tuberculosis*) by serum from Mexican patients with leprosy and tuberculosis. *Int. J. Tuberc. Lung Dis.* **3** (2000) 1106–1112.

The recognition of phenolic glycolipid-I (PGL-I) of *M. leprae* and sulfolipid-I (SL-I) of *M. tuberculosis* by serum from patients from Mexico, with leprosy or pulmonary tuberculosis (PTB), was investigated. Purified PGL-I and SL-I were used as antigens in an ELISA to assess recognition of these lipids by serum from 43 leprosy patients, 44 PTB patients and 38 healthy individuals.

Leprosy patients had higher IgM than IgG responses to PGL-I and had comparable IgM and IgG responses to SL-I. A similar response was observed with PTB serum. Some healthy subjects were found to have significant levels of antibodies to both lipids. It is concluded that there is no specific recognition of either of the two lipid antigens tested by serum from both leprosy and tuberculosis patients, ruling out the possibility of using PGL-I and SL-I as tools for the differential diagnosis of the two mycobacterial diseases.—Trop. Dis. Bull. **97** (2000) 292–293

Scollard, D. M. Association of *Mycobacterium leprae* with human endothelial cells *in vitro*. Lab. Invest. **80** (2000) 663–669.

Endothelial cell infection by *Mycobacterium leprae* has long been described histologically in all types of leprosy and in some of the acute reactions occurring in this disease. Recent evidence from experimental lepromatous neuritis indicates that *M. leprae* colonizes endothelial cells of epineural blood vessels even in sites of minimal infection, suggesting that interaction between these cells and *M. leprae* may play an important role in the selective localization of this organism to peripheral nerve. To begin to study the mechanisms involved, we have examined the interaction between *M. leprae* and human umbilical vein endothelial cells (HUVEC) *in vitro* using light microscopy, scanning and transmission electron microscopy, and confocal laser scanning microscopy. When *M. leprae* were added to confluent monolayers of HUVEC, uptake increased slowly to a maximum at 24 hr. Maximal percentages of infected cells were similar at ratios of organisms: cell over a range of 25:1 to 100:1. The bacilli appeared to lie within membrane-bound vacuoles at all time points. The kinetics of association of *M. leprae* with HUVEC are much slower than has previously been observed with macrophages, possibly due to differences in the binding of *M. leprae*. Compared with other pathogens that infect endothelial cells, *M. leprae* also appear to be ingested more slowly and to a more limited degree. The receptors in-

involved in *M. leprae* binding to endothelial cells and the impact of intracellular infection by *M. leprae* on these cells remain to be determined.—Author's Abstract

Seiler, P., Aichele, P., Raupach, B., Odermatt, B., Steinhoff, U. and Kaufmann, S. H. E. Rapid neutrophil response controls fast-replicating intracellular bacteria but not slow-replicating *Mycobacterium tuberculosis*. J. Infect. Dis. **818** (2000) 671–680.

Being one of the first cells to invade the site of infection, neutrophils play an important role in the control of various bacterial and viral infections. In the present work, the contribution of neutrophils to the control of infection with different intracellular bacteria was investigated. Mice were treated with the neutrophil-depleting monoclonal antibody RB6-8C5, and the time course of infection in treated and untreated mice was compared by using intracellular bacterial species and strains varying in virulence and replication rate. The results indicate that neutrophils are crucial for the control of fast-replicating intracellular bacteria; whereas early neutrophil effector mechanisms are dispensable for the control of the slow-replicating *Mycobacterium tuberculosis*.—Authors' Abstract

Serbina, N. V., Lui, C. C., Scanga, C. A. and Flynn, J. L. CD8⁺ CTL from lungs of *Mycobacterium tuberculosis*-infected mice express perforin *in vivo* and lyse infected macrophages. J. Immunol. **165** (2000) 353–363.

CD8⁺ T lymphocytes have been implicated in the protective immune response against human and murine tuberculosis. However, the functional role that this cell subset plays during the resolution of infection remains controversial. In this study, we demonstrate the presence of *Mycobacterium tuberculosis*-specific CD8⁺ CTL in the lungs and lung-draining lymph nodes of mice infected with *M. tuberculosis* via the aerosol or i.v. route. These cells expressed perforin *in vivo* and specifically recognized and lysed *M. tuberculosis*-infected macro-

phages in a perforin-dependent manner after a short period of *in vitro* restimulation. The efficiency of lysis of infected macrophages was dependent upon the time allowed for interaction between macrophage and *M. tuberculosis* bacilli. Recognition of infected targets by CD8+ CTL was beta²-microglobulin and MHC class I-dependent and was not CD1d restricted. The presented data indicate that CD8+ T cells contribute to the protective immune response during *M. tuberculosis* infection by exerting cytotoxic function and lysing infected macrophages.—Authors' Abstract

Sieling, P. A., Ochoa, M. T., Jullien, D., Leslie, D. S., Sabet, S., Rosat, J. P., Burdick, A. E., Rea, T. H., Brenner, M. B., Porcelli, S. A. and Modlin, R. L. Evidence for human CD4+ T cells in the CD1-restricted repertoire: derivation of mycobacteria-reactive T cells from leprosy lesions. *J. Immunol.* **164** (2000) 4790–4796.

Both the CD4–CD8– (double negative) and CD4–CD8+ T-cell lineages have been

shown to contain T cells which recognize microbial lipid and glycolipid antigens (Ags) in the context of human CD1 molecules. To determine whether T cells expressing the CD4 coreceptor could recognize Ag in the context of CD1, we derived CD4+ T-cell lines from the lesions of leprosy patients. We identified three CD4+ *Mycobacterium leprae*-reactive, CD1-restricted T-cell lines: two CD1b restricted and one CD1c restricted. These T-cell lines recognize mycobacterial Ags, one of which has not been previously described for CD1-restricted T cells. The response of CD4+ CD1-restricted T cells, unlike MHC class II-restricted T cells, was not inhibited by anti-CD4 monoclonal antibody, suggesting that the CD4 coreceptor does not impact positive or negative selection of CD1-restricted T cells.

The CD4+ CD1-restricted T cell lines produced IFN-gamma and GM-CSF the Th1 pattern of cytokines required for cell-mediated immunity against intracellular pathogens, but no detectable IL-4. The existence of CD4+ CD1-restricted T cells that produce a Th1 cytokine pattern suggests a contributory role in immunity to mycobacterial infection.—Authors' Abstract

Microbiology

Adilakshmi, T., Ayling, P. D. and Ratledge, C. Polyadenylation in mycobacteria: evidence for oligo (dT)-primed cDNA synthesis. *Microbiology U.K.* **146** Part 3 (2000) 633–638.

The ability of mRNA to direct synthesis of cDNA in the presence of oligo(dT) was analyzed using a novel application of fluorescein-11-dUTP incorporation into cDNA by reverse transcriptase. Evidence is provided for the first time that a majority of the mycobacterial mRNA pool is polyadenylated. MRNA transcripts of hsp65 were also amplified with specific primers from the oligo(dT)-primed cDNA preparation in *Mycobacterium bovis* BCG, *M. smegmatis* and *M. vaccae*. Furthermore, PCR amplification of cDNAs for genes entD, entC and trpE2

from *M. bovis* BCG yielded the expected products when reverse transcription was primed with oligo(dT), suggesting that polyadenylation is a general phenomenon in mycobacteria.—Authors' Abstract

Bhatt, K., Banerjee, S. K. and Chakraborti, P. K. Evidence that phosphate specific transporter is amplified in a fluoroquinolone-resistant *Mycobacterium smegmatis*. *Eur. J. Biochem.* **267** (2000) 4028–4032.

We reported in an earlier study that active efflux of drug has a predominant role in conferring resistance in a laboratory-generated ciprofloxacin-resistant mutant of *Mycobacterium smegmatis*. This mutant exhib-

ited mRNA level overexpression, as well as chromosomal amplification, of the gene *pstB*, encoding the putative ATPase subunit of phosphate specific transport (Pst) system. We demonstrate here that this mutant shows enhanced phosphate uptake and that inactivation of *pstB* in the parental strain results in loss of high affinity phosphate uptake and hypersensitivity to fluoroquinolones. These findings suggest a novel role of the Pst system in active efflux, in addition to its involvement in phosphate transport.—Authors' Abstract

Borich, S. M., Murray, A. and Gormley, E. Genomic arrangement of a putative operon involved in maltose transport in the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. *Microbios* **102** (2000) 7–15.

A *Mycobacterium bovis* gene coding for a putative MalE maltose binding protein was cloned and its full-length sequence determined. Database searches revealed 99.9% identity with IpqY, encoding a putative sugar uptake protein from *Mycobacterium tuberculosis* strain H37Rv. The deduced protein product showed high sequence similarity to MalE-like proteins from a variety of bacterial species, including *M. leprae*. Analysis of flanking database sequences from *M. tuberculosis* and *M. leprae* revealed the presence of *malF*-, *malG*- and *malK*-like genes. Comparison of these mycobacterial sequences with other maltose operons has allowed us to deduce a unique genomic arrangement of the genes involved in the uptake of maltose in members of the *M. tuberculosis* complex and *M. leprae*.—Authors' Abstract

Brosch, R., Gordon, S. V., Eiglmeier, K., Garnier, T. and Cole, S. T. Comparative genomics of the leprosy and tubercle bacilli. *Res. Microbiol.* **151** (2000) 135–142.

To achieve the quantum leap in understanding required to overcome two major human diseases, leprosy and tuberculosis, systematic and comparative genome analysis has been undertaken. New insight into the biology of their causative agents has

been obtained and the principle findings are reported here.—Authors' Abstract

Brosch, R., Gordon, S. V., Pym, A., Eiglmeier, K., Garnier, T. and Cole, S. T. Comparative genomics of the mycobacteria. *Int. J. Med. Microbiol.* **290** (2000) 143–152.

The genus mycobacteria includes two important human pathogens *Mycobacterium tuberculosis* and *M. leprae*. The former is reputed to have the highest annual global mortality of all pathogens. Their slow growth, virulence for humans and particular physiology makes these organisms extremely difficult to work with. However the rapid development of mycobacterial genomics following the completion of the *M. tuberculosis* genome sequence provides the basis for a powerful new approach for the understanding of these organisms. Five further genome sequencing projects of closely related mycobacterial species with differing host range, virulence for humans and physiology are underway. A comparative genomic analysis of these species has the potential to define the genetic basis of these phenotypes which will be invaluable for the development of urgently needed new vaccines and drugs. This minireview summarizes the different techniques that have been employed to compare these genomes and gives an overview of the wealth of data that has already been generated by mycobacterial comparative genomics.—Authors' Abstract

Dhople, A. M. and Ryon, D. L. S. ATP content of *Mycobacterium tuberculosis* grown *in vivo* and *in vitro*. *Microbios* **101** (2000) 81–88.

In order to determine the reason for the slow growth of *Mycobacterium leprae* either in a host or *in vitro*, the growth characteristics of *M. tuberculosis* were studied. The ATP content of *in vitro*-grown *M. tuberculosis* was about 520 pg/10⁶ viable organisms. The ATP levels from *in vivo*-derived organisms obtained from the liver and spleen of mice was about 130 pg (in cases of chronic infection) and about 270 pg (in cases of acute infection). When the *in vivo*-

derived organisms were inoculated into culture medium, the growth rates for both types of organisms, acute as well as chronic infection, were the same and the maximum growth was reached during the fifth subculture. Although the maximum ATP content for both types of organism was the same, it was attained during the 4th subculture for organisms obtained during acute infection and during the 6th subculture for those obtained during chronic infection. The comparison between the ATP content of *M. leprae* and of *M. tuberculosis* indicates the reason for the slow growth of *M. leprae*.—Authors' Abstract

Frischkorn, K., Springer, B., Bottger, E. C., Davis, E. O., Colston, M. J. and Sander, P. *In vivo* splicing and functional characterization of *Mycobacterium leprae* RecA. J. Bacteriol. **182** (2000) 3590–3592.

The RecA proteins from *Mycobacterium tuberculosis* and *M. leprae* contain inteins. In contrast to the *M. tuberculosis* RecA, the *M. leprae* RecA is not spliced in *Escherichia coli*. We demonstrate here that *M. leprae* RecA is functionally spliced in *M. smegmatis* and produces resistance toward DNA-damaging agents and homologous recombination.—Authors' Abstract

Guo, M., Sun, Z. H. and Zhang, Y. *Mycobacterium smegmatis* has two pyrazinamidase enzymes, PncA and PzaA. J. Bacteriol. **182** (2000) 3881–3884.

The *Mycobacterium smegmatis* pncA gene, encoding nicotinamidase/pyrazinamidase, was identified. While it was similar to counterparts from other mycobacteria, the *M. smegmatis* PncA had little homology to the other *M. smegmatis* pyrazinamidase/nicotinamidase, encoded by the pzaA gene. Transformation of *M. bovis* strain BCG with *M. smegmatis* pncA or pzaA conferred susceptibility to pyrazinamide.—Authors' Abstract

Hillas, P. J., del Alba, F. S., Oyarzabal, J., Wilks, A. and de Montellano, P. R. O.

The AhpC and AhpD antioxidant defense system of *Mycobacterium tuberculosis*. J. Biol. Chem. **275** (2000) 18801–18809.

The peroxiredoxin AhpC from *Mycobacterium tuberculosis* has been expressed, purified, and characterized. It differs from other well-characterized AhpC proteins in that it has three rather than one or two cysteine residues. Mutagenesis studies show that all three cysteine residues are important for catalytic activity. Analysis of the *M. tuberculosis* genome identified a second protein, AhpD, which has no sequence identity with AhpC but is under the control of the same promoter. This protein has also been cloned, expressed, purified, and characterized. AhpD, which has only been identified in the genomes of mycobacteria and *Streptomyces viridosporus*, is shown here to also be an alkylhydroperoxidase. The endogenous electron donor for catalytic turnover of the two proteins is not known, but both can be turned over with AhpF from *Salmonella typhimurium* or, particularly in the case of AhpC, with dithiothreitol. AhpC and AhpD reduce alkylhydroperoxides more effectively than H_2O_2 , but do not appear to interact with each other. These two proteins appear to be critical elements of the antioxidant defense system of *M. tuberculosis* and may be suitable targets for the development of novel anti-tuberculosis strategies.—Authors' Abstract

Lefevre, P., Denis, O., De Wit, L., Tanghe, A., Vandenbussche, P., Content, J. and Huygen, K. Cloning of the gene encoding a 22-kilodalton cell surface antigen of *Mycobacterium bovis* BCG and analysis of its potential for DNA vaccination against tuberculosis. Infect. Immun. **68** (2000) 1040–1047.

Using spleen cells from mice vaccinated with live *Mycobacterium bovis* BCG, we previously generated three monoclonal antibodies reactive against a 22-kDa protein present in mycobacterial culture filtrate (CF) (K. Huygen, *et al.*, Infect. Immun. **61**:2687–2693, 1993). These monoclonal

antibodies were used to screen an *M. bovis* BCG genomic library made in phage lambda gt11. The gene encoding a 233-amino-acid (aa) protein, including a putative 26-aa signal sequence, was isolated, and sequence analysis indicated that the protein was 98% identical with the *M. tuberculosis* Lppx protein and that it contained a sequence 94% identical with the *M. leprae* 38-mer polypeptide 13B3 recognized by T cells from killed *M. leprae*-immunized subjects. Flow cytometry and cell fractionation demonstrated that the 22-kDa CF protein is also highly expressed in the bacterial cell wall and membrane compartment but not in the cytosol. C57BL/6, C3H, and BALB/c mice were vaccinated with plasmid DNA encoding the 22-kDa protein and analyzed for immune response and protection against intravenous *M. tuberculosis* challenge. Whereas DNA vaccination induced elevated antibody responses in C57BL/6 and particularly in C3H mice, Th1-type cytokine response, as measured by interleukin-2 and gamma interferon secretion, was only modest, and no protection against intravenous *M. tuberculosis* challenge was observed in any of the three mouse strains tested. Therefore, the 22-kDa antigen seems to have little potential for a DNA vaccine against tuberculosis, but it may be a good candidate for a mycobacterial antigen detection test.—Authors' Abstract

Naito, M., Fukuda, T., Sekiguchi, K. and Yamada, T. The domains of human fibronectin mediating the binding of alpha antigen, the most immunopotent antigen of mycobacteria that induces protective immunity against mycobacterial infection. *Biochem. J.* **347** Part 3 (2000) 725–731.

We have recently shown that alpha antigen (alpha-Ag), the immunodominant antigen of mycobacteria, has a novel fibronectin (FN)-binding motif that is unique among mycobacteria [Naito, Ohara, Matsumoto and Yamada (1998) *J. Biol. Chem.* **273**, 2905–2909]. In this study, we examined the domains of human FN that interacted with alpha-Ag. Fragments of FN generated by either proteolysis or recombinant

DNA techniques were compared for their ability to bind to alpha-Ag. Fragments containing either the C-terminal heparin-binding domain or the central cell-binding domain consistently bound to alpha-Ag. The fragment of the C-terminal heparin-binding domain, upon mutation that resulted in the loss of its heparin-binding activity, could not bind with alpha-Ag. These findings suggested that the mutated site, i.e., the main heparin-binding site of FN, was also the principal site for binding to alpha-Ag. The alpha-Ag-binding domains of FN could bind whole mycobacterial bacilli, suggesting that these two domains are important contributors to mycobacterial infection.—Authors' Abstract

Ojha, A. K., Mukherjee, T. K. and Chatterji, D. High intracellular level of quanosine tetraphosphate in *Mycobacterium smegmatis* changes the morphology of the bacterium. *Infect. Immun.* **68** (2000) 4084–4091.

Almost one-third of the world population today harbors the tubercle bacillus asymptotically. It is postulated that the morphology and staining pattern of the long-term persistors are different from those of actively growing culture. Interestingly, it has been found that the morphology and staining pattern of the starved *in vitro* population of mycobacteria is similar to the persistors obtained from the lung lesions. In order to delineate the biochemical characteristics of starved mycobacteria, *Mycobacterium smegmatis* was grown in 9.2% glucose as a sole carbon source along with an enriched culture in 2% glucose. Accumulation of the stringent factor guanosine tetraphosphate (ppGpp) with a concomitant change in morphology was observed for *M. smegmatis* under carbon-deprived conditions. In addition, *M. smegmatis* assumed a coccoid morphology when ppGpp was ectopically produced by overexpressing *Escherichia coli* relA, even in an enriched medium. The *M. tuberculosis* relA and spoT homolog, when induced in *M. smegmatis*, also resulted in the overproduction of ppGpp with a change in the bacterium's growth characteristics.—Authors' Abstract

Reddy, V. M. and Kumar, B. Interaction of *Mycobacterium avium* complex with human respiratory epithelial cells. *J. Infect. Dis.* **181** (2000) 1189–1193.

Adherence of *Mycobacterium avium* complex (MAC) to human respiratory epithelial cells (HEp-2) induced two distinct modes of internalization. In the first, MAC induced ruffling of HEp-2 cell membrane and formation of surface projections securing the bacilli on the surface, and concurrent membrane depressions, beneath the sites of attachment of bacilli, resulted in internalization of the organisms. The second mode involved formation of membrane folds wrapping around the bacilli, followed by internalization. Two MAC proteins similar to 31-kDa and similar to 25-kDa, respectively, were identified that mediated these interactions specific for HEp-2 cells. The N-terminal amino acid sequence of the 31-kDa MAC protein displayed homology with the 21-kDa hypothetical protein of *M. tuberculosis*, and the 25-kDa MAC protein showed homology with Mn-superoxide dismutase of MAC and *M. leprae*. These two HEp-2 cell-specific MAC proteins may be involved in the interaction of MAC with epithelial cells.—Authors' Abstract

Weber, I., Fritz, C., Rutkowski, S., Kreft, A. and Bange, F. C. Anaerobic nitrate reductase (narGHJI) activity of *Mycobacterium bovis* BCG *in vitro* and its contribution to virulence in immunodeficient mice. *Mol. Microbiol.* **35** (2000) 1017–1025.

Mycobacterium tuberculosis and *M. bovis* cause tuberculosis, which is responsible for the deaths of more people each year than any other bacterial infectious disease. Disseminated disease with *M. bovis* BCG, the only currently available vaccine against tuberculosis, occurs in immunocompetent and immunodeficient individuals. Although mycobacteria are obligate aerobes, they are thought to face an anaerobic environment during infection, notably inside abscesses and granulomas. The purpose of this study was to define a metabolic pathway that could allow mycobacteria to exist under these conditions. Recently, the complete

genome of *M. tuberculosis* has been sequenced, and genes homologous to an anaerobic nitrate reductase (narGHJI), an enzyme allowing nitrate respiration when oxygen is absent, were found. Here, we show that the narGHJI cluster of *M. tuberculosis* is functional as it conferred anaerobic nitrate reductase activity to *M. smegmatis*. A narG mutant of *M. bovis* BCG was generated by targeted gene deletion. The mutant lacked the ability to reduce nitrate under anaerobic conditions. Both mutant and *M. bovis* BCG wild type grew equally well under aerobic conditions *in vitro*. Histology of immunodeficient mice (SCID) infected with *M. bovis* BCG wild type revealed large granulomas teeming with acid-fast bacilli; all mice showed signs of clinical disease after 50 days and succumbed after 80 days. In contrast, mice infected with the mutant had smaller granulomas containing fewer bacteria; these mice showed no signs of clinical disease after more than 200 days. Thus, it seems that nitrate respiration contributes significantly to the virulence of *M. bovis* BCG in immunodeficient SCID mice.—Authors' Abstract

Williams, D. L., Spring, L., Harris, E., Roche, P. and Gillis, T. P. Dihydropterolate synthase of *Mycobacterium leprae* and dapsone resistance. *Antimicrob. Agents Chemother.* **44** (2000) 1530–1537.

Two *Mycobacterium leprae* genes, folP1 and folP2, encoding putative dihydropterolate synthases (DHPS), were studied for enzymatic activity and for the presence of mutations associated with dapsone resistance. Each gene was cloned and expressed in a folP knockout mutant of *Escherichia coli* [C600 Delta folP::Km(r)]. Expression of *M. leprae* folP1 in C600 Delta folP::Km(r) conferred growth on a folate-deficient medium, and bacterial lysates exhibited DHPS activity. This recombinant displayed a 256-fold greater sensitivity to dapsone (measured by the MIG) than wild-type *E. coli* C600, and 50-fold less dapsone was required to block [expressed as the 50% inhibitory concentration (IC₅₀)] the DHPS activity of this recombinant. When the folP1 genes of several dap-

sone-resistant *M. leprae* clinical isolates were sequenced, two missense mutations were identified. One mutation occurred at codon 53, substituting an isoleucine for a threonine residue (T53I) in the DHPS-1, and a second mutation occurred in codon 55, substituting an arginine for a proline residue (P55R). Transformation of the C600 Delta folP::Km(r) knockout with plasmids carrying either the T53I or the P55R mutant allele did not substantially alter the DHPS activity compared to levels produced by recombinants containing wild-type *M. leprae* folP1. However, both mutations increased dapsone resistance, with P55R having the greatest effect on dapsone

resistance by increasing the MIC 64-fold and the IC50 68-fold. These results prove that the folP1 of *M. leprae* encodes a functional DHPS and that mutations within this gene are associated with the development of dapsone resistance in clinical isolates of *M. leprae*. Transformants created with *M. leprae* folP2 did not confer growth on the C600 Delta folP::Km(r) knockout strain, and DNA sequences of folP2 from dapsone-susceptible and -resistant *M. leprae* strains were identical, indicating that this gene does not encode a functional DHPS and is not involved in dapsone resistance in *M. leprae*.—Authors' Abstract

Experimental Infections

Vilani-Moreno, F. R., de Arruda, M. S. P., Nogueira, M. E. S. and Baptista, I. M. F. D. [Murine experimental Hansen's disease: intranasal inoculation of *Mycobacterium leprae*.] *Hansen. Int.* **4** (1999) 115–120. (in Portuguese)

Considering the upper airways the most likely infective route for *M. leprae*, the present study evaluated the behavior of the bacilli experimentally inoculated intranasally. Thus, Swiss mice were inoculated with *M. leprae* by nasal instillation and then sacrificed at different periods of time. Lungs were removed and submitted to bronchoalveolar lavage (BAL) and histopathological exam in each sacrifice. The number of bacilli recovered and the total and differential number of inflammatory cells were evaluated in the BAL. The BAL was also cultured in Lowenstein-Jensen and

Middlebrook 7H9 and 7H11 media. Bacilli were found in the BAL until month 6 after inoculation. Cultures for mycobacteria were all negative. The total number of cells, lymphocytes and neutrophils in the BAL of infected mice was higher than in the control group until month 6 of experimentation. The histopathological analysis showed small cellular infiltrates distributed throughout the pulmonary parenchyma and around vessels, up to the 6th month of sacrifice. Lesions of dissemination were not found. The results obtained revealed that inoculation of *M. leprae* intranasally in Swiss mice did not result in development of disease and neither in multiplication of the bacilli. Therefore, additional studies involving other strains of mice would be useful to determine the importance of the intranasal route in leprosy infection.—Authors' English Summary

Epidemiology and Prevention

Bagade, P. L. and Baliram, B. Community participation in case detection of leprosy in Nagpur District of Maharashtra. *Indian J. Lepr.* **71** (1999) 465–469.

Involving special community groups for new case detection is of great importance for achieving the target of elimination of leprosy. During 1998–1999, 30 village-

level Mahila mandals (women's groups), 6950 teachers and students and 34,548 heads of families were co-opted to participate in case detection. They examined 56,113 persons, including 378,959 school students and 184,940 family members. Of the examined population, 275 were suspected to be cases of leprosy by mahila mandals, 411 by teachers and students, and 747 by heads of families. Subsequent examination of the suspected cases by trained medical officers and paramedical workers confirmed 203 of them to be cases of leprosy. This exercise showed that when proper attempts were made to involve the community, case detection activity became easier, besides helping to disseminate knowledge about leprosy in the community.—Authors' Abstract

Chen, X.-S., Li, W.-Z., Jiang, C. and Ye, G. Leprosy in China: delay in the detection of cases. *Ann. Trop. Med. Parasitol.* **94** (2000) 181–188.

In a national survey in China, 27,928 cases of leprosy detected by the health authorities between 1984 and 1998 were investigated. The delay between onset of symptoms (estimated from each case's recall) and confirmed diagnosis was ≤ 2 years for 55.1% of the new patients but >10 years for 7.0%, with a median value, overall, of 22.0 months. The median delay was longer: (1) for the multibacillary cases than the paucibacillary; (2) among farmers than among factory workers; (3) among some nationalities than among others (being longest among the Tu and shortest among the Wei); and (4) for some methods of case detection than for others. Over the study period, the mean delay decreased with time. The delay was greatest in the areas where leprosy was endemic and/or where access to health services was poor. The later the cases were detected the more likely they were to show disability.

Leprosy cases are still going undetected in China, although over the last 14 years case finding has significantly improved. Age, occupation, nationality, leprosy type and detection method all appear to affect the delay.—Authors' Abstract

de Carsalade, G. Y., Achirafi, A. and Flageul, B. [Leprosy in the territorial collective of Mayotte (Indian Ocean): retrospective study during 1990–1998.] *Bull. Epidemiol. Hebdomadaire* **44** (1999) 186–187. (in French)

In a retrospective study in Mayotte during 1990–1998, 254 cases of leprosy were diagnosed (19–40 new cases per year), giving a prevalence of 31.2 per 100,000. Three peaks in detection were noted, in 1992, 1995, and 1997, during which the numbers of cases diagnosed were 34, 40 and 35, respectively. The affected patients comprised 44.8% males and 55.2% females, with a predominance of cases in the 15 to 45-year age group; 37% of cases had the multibacillary form and 63% had the paucibacillary form of leprosy. All patients diagnosed since 1992 were treated with multiple drug therapy (comprising dapsone, rifampin and clofazimine).—*Trop. Dis. Bull.* **97** (2000) 294

de Matos, H. J., Duppre, N., Alvim, M. F. S., Vieira, L. M. M., Sarno, E. N. and Struchiner, C. J. [Leprosy epidemiology in a cohort of household contacts in Rio de Janeiro (1987–1991).] *Cad. Saude Publica* **15** (1999) 533–542. (in Portuguese)

Factors influencing the development of leprosy in household contacts were investigated. A dynamic cohort ($N = 670$) of contacts of leprosy patients was analyzed from 1987 to 1991 at the Hansens' Disease Department of the Oswaldo Cruz Foundation in Rio de Janeiro, Brazil. The incidence rate was 0.01694 per person-year of follow up. For subjects at the end of the first year of follow up the incidence rate was 0.06385 (end of second year, 0.03299; end of third year, 0.02370; end of fourth year, 0.018622; and end of observation period, 0.01694). A stepwise multivariate logistic regression model was proposed to study the risk of developing leprosy. Subjects were the 670 cohort members and 88 co-prevalent cases. In the final model, the risk was associated with a negative Mitsuda skin test ($OR = 3.093$; $CI\ 95\% = 1.735-5.514$), prior BCG vaccination ($OR = 0.3802$; $CI\ 95\% = 0.2151-$

0.66719), and multibacillary primary clinical form (OR = 2.547; CI 95% = 1.249–5.192). The results showed that both multibacillary leprosy and specific immune status are significant indicators for developing the disease in a cohort of household contacts.—*Trop. Dis. Bull.* **97** (2000) 403

Gil Suarez, R. E. and Lombardi, C. Leprosy elimination at sub-national level. *Lepr. Rev.* **71** (2000) 206–211.

New strategies for the countries that have already achieved the elimination goal, which includes the great majority of the endemic countries, are needed. There is current concern in these countries about the reduction in the political-technical commitment when the goal is achieved and the possibility of the re-emergence of the disease. A review of the literature on the leprosy post-elimination strategy was done. The proposal to estimate the true prevalence using hidden prevalence based on late diagnosis of the new cases was made. Suggestions are explored for strategies of the work after elimination at the national level is attained, such as the stratification at the first sub-national level, using estimated true prevalence. It is considered necessary to define strategies for the post-elimination phase with the aim of continuing the long-term objective of the interruption of transmission and the consequent leprosy eradication.—Authors' Summary

Gole, D., Bawalekar, V., Dongre, V. V. and Naik, S. S. "Outside project area" leprosy cases in "A" Ward of Mumbai. *Indian J. Lepr.* **71** (1999) 471–475.

Analysis of newly registered smear-positive cases in a ward of the metropolitan city of Mumbai, India, which has a railway terminus, during 1990–1997 revealed that 72% of the patients came from outside the project area, most of them from the states of Uttar Pradesh, Bihar and Orissa. They had unstable and temporary residences in the area and were employed in low-income hard-labor jobs. Nevertheless, it was found that their treatment completion rate was

high. Using different approaches, e.g., through the community leaders of footpath dwellers and railway platform dwellers and those of different state language groups' colonies, the new entrants were examined periodically and simultaneously proper rapport was maintained with the medical practitioners of the ward for more referrals to leprosy clinic. Such a special approach may have to be developed to tackle such a situation in other metropolises in the country.—Authors' Abstract

Kulkarni, H. R. and Zodpey, S. P. Differential protective effect of bacillus Calmette-Guérin vaccine against multibacillary and paucibacillary leprosy in Nagpur, India. *Public Health* **113** (1999) 311–313.

A secondary data analysis was conducted to test the hypothesis that a linear trend exists in the protective effect of bacille Calmette-Guérin (BCG) vaccine against types of leprosy. Data from two previous case-control studies were used to perform an unmatched test for linear trend. Both studies revealed a significant linear trend ($p < 0.00001$). One study that estimated an insignificant protective effect of BCG against paucibacillary leprosy showed a significant departure from linearity. It is concluded that the protective effect of BCG vaccination is differential across severity of leprosy as it brings about a shift in the immune response to a higher level of cell-mediated immunity. It is recommended that future studies dealing with the protective effect of BCG against leprosy should also conduct an analysis for trend.—*Trop. Dis. Bull.* **97** (2000) 518–519

Mandal, M. G., Pal, D., Majumdar, V., Biswas, P. C., Biswas, S. and Saha, B. Recent trends in leprosy in a large district of West Bengal, India, revealed by a modified leprosy elimination campaign (MLEC), 1998. *Lepr. Rev.* **71** (2000) 71–76.

A Modified Leprosy Elimination Campaign (MLEC) in September 1998 in the

District of Midnapore, West Bengal, India, covered a population of 8.1 million people and detected 8181 new cases. Available data from 7328 cases were studied to observe the trend for leprosy in this area. Data are presented on sex and age distribution, classification and the proportions of multibacillary (MB), paucibacillary (PB) and single skin lesion (SSL) cases discovered in a period of only 8 days. The large numbers of people examined in this district and the high total of new cases revealed are in keeping with experience in other parts of the state and in other parts of India. However, many cases were found in endemic areas and these will receive special attention in a second MLEC planned for January 2000.—Authors' Summary

Ogbeiwi, O. Are there "ghost" leprosy patients in Nigeria? An audit of the point prevalence of leprosy at 30 September 1996 in seven states. (Letter) *Lepr. Rev.* **70** (1999) 361–362.

A situational analysis of the leprosy control programs in 7 states of Nigeria in April 1996 suggested possible over-reporting. A prevalence audit was then conducted at the end of the third quarter of 1996 to verify the actual prevalence and determine the level of discrepancy between the reported and the actual registered figures. The states audited included Akwa Ibom in the southeast; Kwara, Kogi and Abuja (FCT) in the middle belt; and Niger, Kebbi and Sokoto in the northwest. The audit was a descriptive cross-sectional review of records of all leprosy clinics in the 7 states at the end of September 1996. Individual patient charts and clinic registers were examined. The actual prevalence figures in each state were compared with the figures reported in the quarterly statistical reports for the end of the third quarter of 1996. Compared with the reported prevalence of 3586 [paucibacillary (PB) 950, multibacillary (MB) 2636], there was a total discrepancy of 1310 cases (actual total = 2276; PB 358, MB 1918). The 1310 discrepant cases included 1411 non-existent but reported cases and 101 unreported cases. The 1411 cases (39.3%) of the total reported cases were therefore the "ghost cases" not found in the clinic registers but

reported in the statistical reports of the projects.—*Trop. Dis. Bull.* **97** (2000) 178

Pereira, E. S., Cunha, M. G. S., Maroja, M. F., Tavares, T. M., Ibanez, L. S., Berbegal, M. P. R. and Torrecilla, M. A. A. Leprosy elimination campaign, Amazonas, Brazil 1997. *Lepr. Rev.* **71** (2000) 77–80.

A leprosy elimination campaign (LEC) was carried out in 15 endemic areas of Amazonas state, Brazil, in 1997. The LEC concentrated effort to detect leprosy cases during a multi-vaccination national campaign for serious public health problems other than leprosy, such as polio, diphtheria, hepatitis, measles, etc. The national campaign involved intensive population mobilization, giving a valuable opportunity to examine people for leprosy. The LEC personnel included 2964 individuals (municipal and state health workers and community volunteers) distributed in 688 health units and 53 reference health centers. As a result of the LEC, 74,814 person-to-person communications in the community were given; 10,297 clinical skin examinations were conducted, and 40 new leprosy cases were detected on the day of the campaign in urban areas of the municipalities. This total was low, compared to results in other states of Brazil, possibly due to the development of health education activities and regular community services in the state of Amazonas since 1987 and to the early implementation of WHO multiple drug therapy (MDT) from 1982 onward. Despite the fact that the LEC was carried out only in the urban areas of the municipalities, the finding of no cases of leprosy in 7 out of 15 of them was surprising, and may indicate that the prevalence of hidden cases of leprosy is not all that high, at least in these areas of Amazonas state.—Authors' Summary

Pinto Neto, J. M. and Scatena Villa, T. C. [Epidemiological features of Hansen's disease contacts who developed the disease, notified in the Health Center 1 of Fernandópolis (Brazil) (1993 to 1997).] *Hansen. Int.* **24** (1999) 129–136. (in Portuguese)

This is a descriptive study in which data were compiled from the epidemiological records of Hansen's disease cases notified in the Health Center 1 of Fernandópolis (Brazil) between 1993 and 1997. The patients informed to live or have lived with Hansen's disease patients. The sample is composed of 57 patients that correspond to 42.2% of the 135 Hansen's disease patients who live in Fernandópolis, and were notified in this health unit in the same period. The objective of the study is to characterize the epidemiological profile of the studied population. Data showed that most of the patients were male, adult or young adult, with polar clinical forms, resident in the urban area, with low schooling grade. The majority of cases were detected through contact examination and had no BCG-id vaccine scar. Analysis of the data revealed operational problems related to development of actions of the Hansen's disease control program in reference to control of contacts, which can impair the goals of the National Hansen's Disease Elimination Plan as a public health problem.—Authors' English Summary

Soares, L. S., Moreira, R. O., Vilela, V. V., Alves, M. J. M., Pimentel, A. F. M., Ferreira, A. P. and Teixeira, H. C. The impact of multidrug therapy on the epidemiological pattern of leprosy in Juiz de Fora, Brazil. *Cad. Saude Publica Rio de Janeiro* **16** (2000) 343–350.

We investigated the impact of multidrug therapy (MDT) on the epidemiological pattern of leprosy in Juiz de Fora, Brazil, from 1978 to 1995. Evaluation of 1283 medical charts was performed according to the treatment regimen used in two different periods. Following the introduction of MDT in 1987, prevalence of leprosy decreased from 22 patients/10,000 inhabitants to 5.2 patients/10,000 inhabitants in 1995. Incidence rate of leprosy was lower in period II (1987–1995) than in period I (1978–1986). Decreasing prevalence and incidence ap-

pear to be related to drug efficacy rather than decreased case identification, since both self-referred and professionally referred treatment increased markedly from period I to period II. For both periods, multibacillary leprosy was the most frequent clinical form of the disease ($\pm 68\%$), and the main infection risk factor identified was household contact. Leprosy is predominantly manifested in adults, but an increase in the number of very old and very young patients was observed in period II. The MDT program has been effective both in combating leprosy and in promoting awareness of the disease.—Authors' Abstract

World Health Organization. Global leprosy situation, September 1999. *Wkly Epidemiol. Rec.* **74** (1999) 313–316.

At the beginning of 1999, 731,369 leprosy cases were registered for treatment in the world (as reported by 82 countries), the registered global prevalence of leprosy being around 1.4 per 10,000 population. In 1998, 755,305 new cases were detected (as reported by 82 countries). Leprosy remains a public health problem in 24 countries mainly situated in the intertropical belt, but around 735,000 registered cases and 750,000 new cases were found in 1999 in the top 11 endemic countries, representing 90% of worldwide prevalence and detection. The aggregate leprosy prevalence rate in the top endemic countries was 4.5 per 10,000. Four tables are included providing data on the following: prevalence and detection of leprosy by WHO region in countries having reported to WHO in 1999; latest available information on prevalence and detection of leprosy by WHO region; registered prevalence of leprosy and detection rate in the top endemic countries in 1999; and 1999 or latest available information on registered prevalence of leprosy, detection and cumulative number of persons cured with multidrug therapy by WHO region and by countries with more than 100 registered cases.—*Trop. Dis. Bull.* **97** (2000) 294–295

Rehabilitation

Dong, L., Li, F., Jiang, J. and Zhang, G.

Techniques for covering soft tissue defects resulting from plantar ulcers in leprosy: Part IV Use of medial leg flap and medial knee flap. *Indian J. Lepr.* **71** (1999) 437–450.

The medial leg flap, based on the cutaneous branches of the posterior tibial artery, is raised from the middle and lower regions of the medial aspect of the leg. It has a long pedicle, and it can be used as a free flap to reconstruct the distant soft tissue defects and also as an island flap. We have used this retrograde island flap for surfacing ulcerated areas in six leprosy patients. The flap survived in all cases. At 24 to 60 months follow-up examination, ulceration had not recurred in any of them.

The medial knee flap consisting of the skin and subcutaneous tissue of the lower part of the medial side of the thigh and upper part of the leg is suitable for covering soft tissue cushion defects of the extremities because of the constant vessels, long pedicle, wide diameter, well-recognizable sensory nerves and less subcutaneous fat. We have used the medial knee flap for the resurfacing of sizeable raw areas due to ulceration in three leprosy patients. The flap survived in all cases and there was no recurrence of ulceration during the 70–148 months follow up period.—Authors' Abstract

Dong, L., Li, F., Zhang, J., Ye, Y., Jiang, J. and Zhang, G.

Techniques for covering soft tissue defects resulting from plantar ulcers in leprosy: Part III Use of plantar skin or musculocutaneous flaps and anterior leg flap. *Indian J. Lepr.* **71** (1999) 423–436.

Anatomical studies suggest that five types of plantar flaps, namely, the lateral and medial plantar flaps, the abductor hallucis, the flexor digitorum brevis, and the abductor digiti minimi-myocutaneous flaps, can be incised from the central section of the sole. The advantages of a plantar flap are recognizable neurovascular bundles of

the sole, wide caliber of constantly located blood vessels, identical histological structure of the donor and the recipient sites, hidden donor site and absence of functional deficit. We have used the plantar flaps in seven cases. There has been no recurrence of ulceration in any of them during the follow-up period of 12 to 108 months.

An anterior leg flap based on the cutaneous branches of the anterior tibial artery, with firmly anchored vessels, a long pedicle with wide vessels, may be used not only as a free flap graft for reconstruction of moderate degree distant defects but also as a retrograde island flap graft for the reconstruction of adjacent tissue defect. We have used the retrograde island flap graft based on the anterior tibial artery in five cases of plantar ulceration with satisfactory results. There was no recurrence of ulceration during the follow-up period of 48 to 72 months.—Authors' Abstract

Patil, K. M. and Jacob, S. Mechanics of tarsal disintegration and plantar ulcers in leprosy by stress analysis in three-dimensional foot models. *Indian J. Lepr.* **72** (2000) 71–86.

This paper describes three-dimensional two-arch models of feet of a normal subject and two leprosy subjects, one in the early stage and the other in the advanced stage of tarsal disintegration, used for analysis of skeletal and plantar soft tissue stresses by the finite element technique using a NISA software package. The model considered the foot geometry (obtained from X-rays), foot bone, cartilage, ligaments, important muscle forces and sole soft tissue. The stress analysis is carried out for the foot models simulating quasi-static walking phases of heel-strike, mid-stance and push-off. The analysis of the normal foot model shows that highest stresses occur at push-off over the dorsal central part of the lateral and medial metatarsals and the dorsal junction of calcaneus and cuboid and neck of the talus. The skeletal stresses, in early stage leprosy with muscle paralysis and in

the advanced stage of tarsal disintegration (TD), are higher than those for the normal foot model, by 24% to 65% and 30% to 400%, respectively. The vertical stresses in the soft tissue at the foot-ground interface match well with experimentally measured foot pressures, and for the normal and leprosy subjects they are the highest in the push-off phase. In the leprosy subject with advanced TD, the highest soft tissue stresses and shear stresses (about three times the normal value) occur in the push-off phase in the scar tissue region. The difference in shear stresses between the sole and the adjacent soft tissue layer in the scar tissue for the same subject is about three times the normal value. It is concluded that the high bone stresses in leprosy may be responsible for tarsal disintegration when the bone's mechanical strength decreases due to osteoporosis and the combined effect of the high value of foot-sole vertical stresses, shear stresses and the relative shear stresses between two adjacent soft tissue layers may be responsible for plantar ulcers in the neuropathic leprosy feet.—Authors' Abstract

Ruchal, S. P., Butlin, C. R., Khadka, N. and Kumar, M. Extra depth shoes made on special last. (Letter) *Lepr. Rev.* **70** (1999) 363–365.

A brief description is given of the use in Nepal of a special wooden last designed to make deepened footwear for leprosy patients with badly deformed feet. Sufficient space is provided to accommodate various foot orthoses into the shoe, which protect the feet while being aesthetically acceptable among the users.—*Trop. Dis. Bull.* **97** (2000) 178

Sane, S. Surgery of less common conditions in leprosy. *Lepr. Rev.* **70** (1999) 351–359.

The causes and surgical treatment of primary deformities on the face (megalobules, nasal perforation, depression of nose and loss of eyebrows) and in the extremities (contractures of fingers and toes) together with secondary deformities (lagophthalmos, facial paralysis, wrist-drop, triple paralysis of the hand, neuro-arthropathies,

and gynaecomastia) are discussed.—*Trop. Dis. Bull.* **97** (2000) 294

Saylan, T., Karadeniz, A., Iyier, N., Soydan, M. and Pamuk, D. A scholarship project for the children of leprosy patients in Turkey. *Lepr. Rev.* **71** (2000) 212–216.

Most of the leprosy patients in Turkey live in the rural areas of Eastern and South-Eastern Anatolia. Those living in the suburbs of big cities of the Western parts of the country have come there by immigration. Nearly all patients are very poor; they have no land, or only a small amount of soil for cultivation. The incidence of deformities in our patients is high, excluding them from regular employment and a source of income. In Turkey, it is obligatory to attend primary school but after that education has to be paid for, and the poor families of leprosy patients find it difficult to continue the education of their children. As the "Society for the Struggle Against Leprosy," based in the Istanbul Leprosy Hospital at Bakirköy, we have developed a project to enable patients to continue sending their children to school while at the same time asking the mothers to seek advice and guidance on family planning. The outset objective of this project was to enable children and young people, who otherwise have almost no chance of continuing education, to pursue education at secondary, high school and university levels. It was envisaged that in the long term educated children would be able to find a job and provide effective care and support for parents and other members of the family. This paper describes the administrative and other measures adopted and the results of the project from 1995 to 1998, during which a total of 545 children have been supported at an overall cost of US\$107,378. The scholarship project has so far been remarkably successful in Turkey, and it is hoped that it may provide a model for similar approaches in other countries. An unexpected and extremely encouraging finding has been that females now exceed males in this project and are increasing at all levels, including university entrance.—Authors' Summary

Srinivasan, H. Guideline for implementing a disability prevention programme in the field. *Indian J. Lepr.* **71** Suppl. (1999) 539–612.

This 74-page supplement is a remarkable and masterful coverage of virtually all aspects of leprosy rehabilitation. No unit providing care to patients should be without it.—RCH

Other Mycobacterial Diseases and Related Entities

Aboulafia, D. M. Inflammatory pseudotumor causing small bowel obstruction and mimicking lymphoma in a patient with AIDS: clinical improvement after initiation of thalidomide treatment. *Clin. Infect. Dis.* **20** (2000) 826–831.

A patient with AIDS was diagnosed with inflammatory pseudotumor with small bowel involvement. After receiving thalidomide treatment, serum tumor necrosis factor (TNF) and soluble TNF receptor II levels normalized, his constitutional and gastrointestinal symptoms diminished, and the mass lesion shrunk.—Author's Abstract

Amaral, L. and Kristiansen, J. E. Phenothiazines: an alternative to conventional therapy for initial management of suspected multidrug resistant tuberculosis: a call for studies. *Int. J. Antimicrob. Agents* **14** (2000) 173–176.

Increased frequency of multidrug resistant strains of *Mycobacterium tuberculosis* results from inappropriate treatment and lack of patient compliance. The Centers for Disease Control/American Thoracic Society (CDC/ATS) guidelines issued for the management of newly diagnosed cases of tuberculosis (TB) will not be totally effective regardless of adherence to the guidelines and patient cooperation. The long interim period between the diagnosis of TB and confirmation of antibiotic susceptibility contributes to the infection rate. Consequently, the use of an adjuvant that is known to inhibit all encountered multidrug resistant strains of *M. tuberculosis* may be helpful until antibiotic susceptibility is known. Phenothiazines such as chlorpromazine, methdilazine and thioridazine are effective against strains of *M. tuberculosis*

in vitro and *in vivo*. It is recommended that studies be designed and conducted for the purpose of managing new cases of TB that emanate from areas known to harbor multidrug resistant strains of *M. tuberculosis*, with phenothiazines as adjuvants to the regimen recommended by the CDC/ATS guidelines until antibiotic susceptibility is defined. Because the normal maximum period for obtaining conventional antibiotic susceptibility results is less than 7 or 8 weeks, the probability of serious side effects from the use of a phenothiazine is remote.—Authors' Abstract

Arend, S. M., Andersen, P., van Meijgaarden, K. E., Skjot, R. L. V., Subronto, Y. W., van Dissel, J. T. and Ottenhoff, T. H. M. Detection of active tuberculosis infection by T cell responses to early secreted antigenic target 6-kDa protein and culture filtrate protein 10. *J. Infect. Dis.* **181** (2000) 1850–1854.

The purified protein derivative (PPD) skin test has no predictive value for tuberculosis (TB) in *Mycobacterium bovis* bacillus Calmette-Guerin (BCG)-vaccinated individuals because of crossreactive responses to nonspecific constituents of PPD. T-cell responses to early-secreted antigenic target 6-kDa protein (ESAT-6) and the newly identified culture filtrate protein 10 (CFP-10), two proteins specifically expressed by *M. tuberculosis* (MTB) but not by BCG strains, were evaluated. Most TB patients responded to ESAT-6 (92%) or CFP-10 (89%). A minority of BCG-vaccinated individuals responded to both ESAT-6 and CFP-10, their history being consistent with latent infection with MTB in the presence of protective immunity. No responses were found in PPD-negative controls. The

sensitivity and specificity of the assay were 84% and 100%, respectively, at a cutoff of 300 pg of interferon-gamma/ml. These data indicate that ESAT-6 and CFP-10 are promising antigens for highly specific immunodiagnosis of TB, even in BCG-vaccinated individuals.—Authors' Abstract

Arora, S. K., Kumar, B. and Sehgal, S.
Development of a polymerase chain reaction dot-blotting system for detecting cutaneous tuberculosis. *Br. J. Dermatol.* **142** (2000) 72–76.

For a definitive diagnosis of cutaneous tuberculosis the demonstration of mycobacteria is essential, but this is generally not possible in skin lesions. Routinely available techniques have poor sensitivity and are time consuming, therefore delaying the institution of timely therapy. The high sensitivity and speed of polymerase chain reaction (PCR) for the detection of infectious agents has prompted investigators to use this technique for the detection of *Mycobacterium tuberculosis* in body fluids such as cerebrospinal fluid or pleural fluid. In the present study, PCR was used to examine punch-biopsy specimens from the affected skin of 10 patients with clinical diagnoses of tuberculosis verrucosa cutis, lupus vulgaris, scrofuloderma, papulonecrotic tuberculide and erythema induratum. A control group of 20 patients included individuals having skin manifestations with definite clinical diagnoses other than cutaneous tuberculosis, such as leprosy, fungal mycetoma, chronic bullous disease of childhood and pemphigus vulgaris. The PCR amplified products were dot hybridized with a probe which was random prime labeled with ³²P. The results were compared with routine microbiological and histological findings. Among the test group, 6 of 10 (60%) were positive for *M. tuberculosis* by PCR, although their histopathology showed nonspecific chronic inflammation with no definite diagnosis. Microbiological investigations, including acid-fast bacillus smear and culture, were positive in a single case of scrofuloderma. All patients in the control group were negative by PCR for *M. tuberculosis*. The data indicate that the combination of dot hybridization with PCR

markedly increased the sensitivity and specificity of PCR. This may be a useful tool in the diagnosis of tuberculosis when conventional methods fail.—Authors' Abstract

**Bachrach, G., Colston, M. J., Berco-
vier, H., Bar-Nir, D., Anderson, C.
and Papavinasasundaram, K. G.** A new single-copy mycobacterial plasmid, pMF1, from *Mycobacterium fortuitum* which is compatible with the pAL5000 replicon. *Microbiology* **146** (2000) 297–303.

A 9.2 kb cryptic *Mycobacterium fortuitum* plasmid, pMF1, was isolated from strain 110 and its restriction map constructed. A 4.2 kb *Hind*III fragment of pMF1 was found to support replication in mycobacteria, and this fragment was cloned and sequenced to characterize the replication elements of the plasmid. Computer analysis identified a putative Rep protein (362 amino acids) with high homology to the putative Rep protein of the *M. celatum* plasmid pCLP and limited homology, mostly in the N-terminal region, to the Rep proteins of *M. avium* pLR7, *M. fortuitum* pJAZ38 and *M. scrofulaceum* pMSC262. A region containing a putative *ori* site was located upstream of the *rep* gene; this region displayed high homology at the nucleotide level with the predicted *ori* of pCLP and pJAZ38. A plasmid carrying the 4.2 kb *Hind*III fragment and a kanamycin resistance marker, designated pBP4, was maintained as a single-copy plasmid in *M. smegmatis* and was stably inherited in the absence of antibiotic selection. Plasmid pBP4 was incompatible with the pJAZ38 replicon but was compatible with the widely used pAL5000 replicon, indicating that among the mycobacterial vectors now available there are two incompatibility groups. Significantly, the plasmid was able to replicate in the pathogen *M. tuberculosis*, making it a useful tool for gene expression studies. To provide a choice of restriction sites and easy manipulation, a 2.1 kb fragment containing the minimal replication region was cloned to make the mycobacterial shuttle vector pBP10, which showed similar stability to pBP4.—Authors' Abstract

Bekker, L. G., Haslett, P., Maartens, G., Steyn, L. and Kaplan, G. Thalidomide-induced antigen-specific stimulation in patients with human immunodeficiency virus type 1 and tuberculosis. *J. Infect. Dis.* **181** (2000) 954–965.

Thalidomide, which inhibits monocyte tumor necrosis factor (TNF)-alpha production and costimulates T cells, was tested for immune modulation in patients with human immunodeficiency virus (HIV) infection and tuberculosis (TB) in a placebo-controlled study. Thalidomide therapy resulted in increased levels of plasma interleukin (IL)-2 receptor, soluble CD8, interferon-gamma, and IL-12, indicating immune stimulation. TNF-alpha levels were not reduced. Thalidomide treatment increased CD4+ and CD8+ T-cell counts and lymphocyte proliferation to purified protein derivative. Immune stimulation was not associated with an increase in plasma HIV levels. *In vivo*, a thalidomide dose-dependent costimulatory effect on T-cell proliferation and HIV replication was observed after stimulation with antigens or antf-CD3, respectively. Thalidomide-induced increased viral replication in CD4+ T cells was abrogated by adding back autologous CD8+ T cells. Thus, in the presence of thalidomide, antigen-specific immune responses *in vitro* and in patients with HTV/TB were enhanced.—Authors' Abstract

Beltan, E., Horgen, L. and Rastogi, N. Secretion of cytokines by human macrophages upon infection by pathogenic and nonpathogenic mycobacteria. *Microb. Pathogen.* **28** (2000) 313–318.

The interaction of various pathogenic (*Mycobacterium tuberculosis*, *M. avium*, *M. kansasii*, *M. xenopi*), and nonpathogenic mycobacteria (*M. smegmatis*, *M. phlei*) with human macrophages at the level of macrophage cytokine expression (TNF-alpha, IL-1, IL-6 and GM-CSF) was investigated. Both for TNF-alpha and GM-CSF, the lowest levels were obtained with pathogenic mycobacterial species; whereas about 2–8 times higher levels were observed for nonpathogenic species. Contrary to the above, the differences for IL-6 and IL-1

were not marked, although IL-6 appeared to be more elevated for nonpathogenic species. Heat-killed bacteria induced a lower level of the cytokines for all the three cytokines assayed (TNF-alpha, IL-6 and IL-1), except for *M. tuberculosis* for whom a significantly higher proportion of TNF-alpha was induced by killed bacilli. The RT-PCR experiments performed on *M. avium* (as a low inducer of the cytokines) and *M. smegmatis* (as a high inducer of the cytokines) showed that the differences observed among pathogenic versus nonpathogenic strains were also reflected at the transcriptional level for TNF-alpha and to a lesser extent for IL-6, but not for IL-1. This investigation underlined important differences existing between the pathogenic and nonpathogenic species, particularly as regards TNF-alpha and GM-CSF.—Authors' Abstract

Blackwell, V. C., Hamilton, A. J. and Hay, R. Analysis by enzyme-linked immunosorbent assay and immunoblot of the antibody response of patients infected with *Mycobacterium marinum*. *FEMS Immunol. Med. Microbiol.* **27** (2000) 185–190.

The serum antibody responses of a total of 14 patients with active or recently cured *Mycobacterium marinum* infections were analyzed via a combination of enzyme-linked immunosorbent assay (ELISA) and the immunodevelopment of Western blots of *M. marinum* antigen. Normal human sera and sera from patients with active pulmonary tuberculosis were also analyzed as controls. The detectable IgG response of *M. marinum* patients, as demonstrated by ELISA, was highly variable and did not differ significantly from normal controls. IgA and IgM levels were generally low in the *M. marinum* patients and were not significantly different from normal controls. Immunodevelopment of Western blots of *M. marinum* antigen with the sera of patients with *M. marinum* infections revealed that a number of antigens were recognized. Of particular note was an 18-kDa species that was recognized by 11 out of 14 patients (and by none of the normal controls). The 18-kDa antigen may be a useful serodiag-

nostic marker in the identification of *M. marinum* infections.—Authors' Abstract

Braunstein, M., Griffin, T. J., Kriakov, J. I., Friedman, S. T., Grindley, N. D. F. and Jacobs, W. R. Identification of genes encoding exported *Mycobacterium tuberculosis* proteins using a Tn552' *phoA* *in vitro* transposition system. *J. Bacteriol.* **182** (2000) 2732–2740.

Secreted and cell envelope-associated proteins are important to both *Mycobacterium tuberculosis* pathogenesis and the generation of protective immunity to *M. tuberculosis*. We used an *in vitro* Tn552' *phoA* transposition system to identify exported proteins of *M. tuberculosis*. The system is simple and efficient, and the transposon inserts randomly into target DNA. *M. tuberculosis* genomic libraries were targeted with Tn552' *phoA* transposons, and these libraries were screened in *M. smegmatis* for active *PhoA* translational fusions. Thirty-two different *M. tuberculosis* open reading frames were identified; 8 contain standard signal peptides, 6 contain lipoprotein signal peptides, and 17 contain one or more transmembrane domains. Four of these proteins had not yet been assigned as exported proteins in the *M. tuberculosis* databases. This collection of exported proteins includes factors that are known to participate in the immune response of *M. tuberculosis* and proteins with homologies, suggesting a role in pathogenesis. Nine of the proteins appear to be unique to mycobacteria and represent promising candidates for factors that participate in protective immunity and virulence. This technology of creating comprehensive fusion libraries should be applicable to other organisms.—Authors' Abstract

Calabrese, L. and Fleischer, A. B. Thalidomide: current and potential clinical applications. *Am. J. Med.* **108** (2000) 487–495.

More than three decades after its withdrawal from the world marketplace, thalidomide is attracting growing interest because of its reported immunomodulatory

and anti-inflammatory properties. Current evidence indicates that thalidomide reduces the activity of the inflammatory cytokine tumor necrosis factor (TNF)-alpha by accelerating the degradation of its messenger RNA. Thalidomide also inhibits angiogenesis. Recently, the drug was approved for sale in the United States for the treatment of erythema nodosum leprosum, an inflammatory complication of Hansen's disease. However, it has long been used successfully in several other dermatologic disorders, including aphthous stomatitis, Behcet's syndrome, chronic cutaneous systemic lupus erythematosus, and graft-versus-host disease, the apparent shared characteristic of which is immune dysregulation. Many recent studies have evaluated thalidomide in patients with human immunodeficiency virus (HIV) infection; the drug is efficacious against oral aphthous ulcers, HIV-associated wasting syndrome, HIV-related diarrhea, and Kaposi's sarcoma. To prevent teratogenicity, a comprehensive program has been established to control access to the drug, including registration of prescribing physicians, dispensing pharmacies, and patients; mandatory informed consent and education procedures; and limitation on the quantity of drug dispensed. Clinical and, in some patients, electrophysiologic monitoring for peripheral neuropathy is indicated with thalidomide therapy. Other adverse effects include sedation and constipation. With appropriate safeguards, thalidomide may benefit patients with a broad variety of disorders for which existing treatments are inadequate.—Authors' Abstract

Chan, E. D., Reves, R., Belisle, J. T., Brennan, P. J. and Hahn, W. E. Diagnosis of tuberculosis by a visually detectable immunoassay for lipoarabinomannan. *Am. J. Respir. Crit. Care Med.* **161** (2000) 1713–1719.

Recovery of tubercle bacilli from sputum, tissue, or body fluid is the standard for the diagnosis of tuberculosis (TB) although this process is technically demanding and relatively insensitive. We have developed a simplified, visually detectable, colloidal gold-based serological assay to qualitatively detect IgG directed against the my-

cobacterial cell wall component lipoarabinomannan (LAM). The objective of this investigation is to determine the accuracy of this assay in patients with active pulmonary TB and in control patients with or without latent infection. In patients with active TB, the sensitivity of anti-LAM IgG was 85% to 93%. In five patients with active TB who were smear-negative, all tested positive for anti-LAM IgG. The specificity of the test depended on the presence of tuberculous infection. In U.S. citizens comprised of young healthy adults and rheumatology patients, the specificity was 100%. In an at-risk population for tuberculous infection who were either tuberculin skin test negative or positive, the specificity was 89%. The negative and positive predictive values of the test were 98% and 52%, respectively. We conclude that anti-LAM IgG immunoassay is relatively sensitive and specific for active TB and, thus, a potentially useful screening test for active TB.—Authors' Abstract

Cheng, S. J., Thibert, L., Sanchez, T., Heifets, L. and Zhang, Y. *pncA* Mutations as a major mechanism of pyrazinamide resistance in *Mycobacterium tuberculosis*: spread of a monoresistant strain in Quebec, Canada. *Antimicrob. Agents Chemother.* **44** (2000) 528–532.

Pyrazinamide (PZA) is an important first-line tuberculosis drug that is part of the currently used short-course tuberculosis chemotherapy. PZA is a prodrug that has to be converted to the active form pyrazinoic acid by pyrazinamidase (PZase) activity, encoded by the *pncA* gene of *Mycobacterium tuberculosis*, and loss of PZase activity is associated with PZA resistance. To further define the genetic basis of PZA resistance and determine the frequency of PZA-resistant strains having *pncA* mutations, we sequenced the *pncA* gene from a panel of 59 PZA-resistant clinical isolates from Canada, the United States, and Korea. Two strains that did not contain *pncA* mutations and had positive PZase turned out to be falsely resistant. Three PZase-negative strains (MIC, >900 µg of PZA per ml) and one PZase-positive strain (strain 9739) (MIC, >300 µg of PZA per ml) did not have

pncA mutations. The remaining 53 of the 57 PZA-resistant isolates had *pncA* mutations, confirming that *pncA* mutation is the major mechanism of PZA resistance. Various new and diverse mutations were found in the *pncA* gene. Interestingly, 20 PZA-monoresistant strains and 1 multidrug-resistant isolate from Quebec, Canada, all had the same *pncA* mutation profile, consisting of an 8-nucleotide deletion and an amino-acid substitution of Arg140 → Ser. Strain typing indicated that these strains are highly related and share almost identical IS6110 patterns. These data strongly suggest the spread of a PZA-monoresistant strain, which has not previously been described.—Authors' Abstract

Chetchotisakd, P., Mootsikapun, P., Anunnatsiri, S., Jirattanapochai, K., Choonhakarn, C., Chaiprasert, A., Ubol, P. N., Wheat, L. J and Davis, T. E. Disseminated infection due to rapidly growing mycobacteria in immunocompetent hosts presenting with chronic lymphadenopathy: a previously unrecognized clinical entity. *Clin. Infect. Dis.* **30** (2000) 29–34.

Disseminated infection due to rapidly growing mycobacteria is uncommon and occurs mostly in immunocompromised patients. Sixteen cases of such infection with an unusual presentation were seen at Srirangar Hospital, a university hospital in northeastern Thailand. The clinical features were different from those in previous reports. All of the patients presented with chronic bilateral cervical lymphadenopathy. Twelve had mycobacterial involvement of other organs (sinuses, 6 patients; lungs, 4; liver, 4; spleen, 3; skin, 3; bone and joint, 2; and tonsils, 2). An interesting occurrence in 11 patients was 14 episodes of reactive skin manifestations (Sweet's syndrome, 9; generalized pustulosis and erythema nodosum, 2 each; and pustular psoriasis, 1). No identifiable predisposing factors, including human immunodeficiency virus disease, were found in these patients. However, 8 patients had 11 episodes of prior infection or co-infection with other opportunistic pathogens (salmonellosis, 4; penicilliosis, 3; pulmonary tuberculosis, 2; and melioidosis

and cryptococcosis, 1 each). These findings suggest that cell-mediated immunity is defective in these patients.—Trop. Dis. Bull. **97** (2000) 643

Chittasobhaktra, T., Vibhagool, A. and Prachaktam, R. Clinical manifestation and drug susceptibility of rapidly growing mycobacteria. J. Infect. Dis. Antimicrob. Agents **16** (1999) 109–114.

Thirty-seven patients infected with rapidly growing mycobacteria attending Ramathibodi Hospital, Bangkok, Thailand, during 1990–1997 were studied. Clinical spectrum included skin and soft tissue infection (43.2%), eye and ear infection (21.6%), localized lymphadenitis (5.4%), chronic pneumonia with empyema (2.7%), subphrenic abscess (2.7%) and disseminated disease (24.3%). One third of these patients were immunocompromised and HIV patients accounted for 50% of this subgroup. MICs of amikacin, erythromycin, clarithromycin, trimethoprim/sulfamethoxazole, imipenem, cefalotin, cefoxitin, cefpirome, ofloxacin, ciprofloxacin, levofloxacin were determined by E-test on 19 isolates. For 7 strains of *Mycobacterium fortuitum*, amikacin, clarithromycin and quinolone group were the most effective agents with 100%, 71.4% and 85.7% sensitivity, respectively. For *M. chelonae* (12 strains), only amikacin and clarithromycin were effective with 91.7% sensitivity for both drugs. *In vitro* susceptibility testing is recommended before treatment of rapidly growing mycobacteria.—Trop. Dis. Bull. **97** (2000) 170

Clemens, D. L., Lee, B. Y. and Horwitz, M. A. Deviant expression of Rab5 on phagosomes containing the intracellular pathogens *Mycobacterium tuberculosis* and *Legionella pneumophila* is associated with altered phagosomal fate. Infect. Immun. **68** (2000) 2671–2684.

The intracellular human pathogens *Legionella pneumophila* and *Mycobacterium tuberculosis* reside in altered phagosomes that do not fuse with lysosomes and are only mildly acidified. The *L. pneumophila*

phagosome exists completely outside the endolysosomal pathway, and the *M. tuberculosis* phagosome displays a maturational arrest at an early endosomal stage along this pathway. Rab5 plays a critical role in regulating membrane trafficking involving endosomes and phagosomes. To determine whether an alteration in the function or delivery of Rab5 could play a role in the aberrant development of *L. pneumophila* and *M. tuberculosis* phagosomes, we have examined the distribution of the small GTPase, Rab5c, in infected HeLa cells overexpressing Rab5c. Both pathogens formed phagosomes in HeLa cells with molecular characteristics similar to their phagosomes in human macrophages and multiplied in these host cells. Phagosomes containing virulent wild-type *L. pneumophila* never acquired immunogold staining for Rab5c; whereas phagosomes containing an avirulent mutant *L. pneumophila* (which ultimately fused with lysosomes) transiently acquired staining for Rab5c after phagocytosis. In contrast, *M. tuberculosis* phagosomes exhibited abundant staining for Rab5c throughout its life cycle. To verify that the overexpressed, recombinant Rab5c observed on the bacterial phagosomes was biologically active, we examined the phagosomes in HeLa cells expressing Rab5c Q79L, a fusion-promoting mutant. Such HeLa cells formed giant vacuoles, and after incubation with various particles, the giant vacuoles acquired large numbers of latex beads, *M. tuberculosis*, and avirulent *L. pneumophila* but not wild-type *L. pneumophila*, which consistently remained in tight phagosomes that did not fuse with the giant vacuoles. These results indicate that whereas Rab5 is absent from wild-type *L. pneumophila* phagosomes, functional Rab5 persists on *M. tuberculosis* phagosomes. The absence of Rab5 on the *L. pneumophila* phagosome may underlie its lack of interaction with endocytic compartments. The persistence of functional Rab5 on the *M. tuberculosis* phagosomes may enable the phagosome to retard its own maturation at an early endosomal stage.—Authors' Abstract

Delogu, G., Howard, A., Collins, F. M. and Morris, S. L. DNA vaccination

against tuberculosis: expression of a ubiquitin-conjugated tuberculosis protein enhances antimycobacterial immunity. *Infect. Immun.* **68** (2000) 3097–3102.

Genetic immunization is a promising new technology for developing vaccines against tuberculosis that are more effective. In the present study, we evaluated the effects of intracellular turnover of antigens expressed by DNA vaccines on the immune response induced by these vaccines in a mouse model of pulmonary tuberculosis. The mycobacterial culture filtrate protein MPT64 was expressed as a chimeric protein fused to one of three variants of the ubiquitin protein (UbG, UbA, and UbGR) known to differentially affect the intracellular processing of the coexpressed antigens. Immunoblot analysis of cell lysates of *in vitro*-transfected cells showed substantial differences in the degradation rate of ubiquitinated MPT64 (i.e., UbG64 < UbA64 < UbGR64). The specific immune response generated in mice correlated with the stability of the ubiquitin-conjugated antigen. The UbA64 DNA vaccine induced a weak humoral response compared to UbG64, and a mixed population of interleukin-4 (IL-4) and gamma interferon (IFN- γ)-secreting cells. Vaccination with the UbGR64 plasmid generated a strong Th1 cell response (high IFN- γ , low IL-4) in the absence of a detectable humoral response. Aerogenic challenge of vaccinated mice with *Mycobacterium tuberculosis* indicated that immunization with both the UbA64- and UbGR64-expressing plasmids evoked an enhanced protective response compared to the vector control. The expression of mycobacterial antigens from DNA vaccines as fusion proteins with a destabilizing ubiquitin molecule (UbA or UbGR) shifted the host response toward a stronger Th1-type immunity which was characterized by low specific antibody levels, high numbers of IFN- γ -secreting cells, and significant resistance to a tuberculous challenge.—Authors' Abstract

Dieli, F., Troye Blomberg, M., Ivanyi, J., Fournie, J. J., Bonneville, M., Peyrat, M. A., Sireci, G. and Salerno, A. V

gamma 90/V delta 2 T lymphocytes reduce the viability of intracellular *Mycobacterium tuberculosis*. *Eur. J. Immunol.* **30** (2000) 1512–1519.

An effective immune response against the intracellular pathogen *Mycobacterium tuberculosis* is strictly dependent on T cell activation. Although this protective response mainly depends on local release of pro-inflammatory cytokines by Th1 CD4+ T cells, contribution of V gamma 9/V delta 2 T lymphocytes to immune protection against this pathogen is suggested by the antimycobacterial reactivity of this subset and its ability to produce large amounts of Th1 cytokines. Here we show that V gamma 9/V delta 2 T lymphocytes kill macrophages harboring live *M. tuberculosis*. The cytotoxic activity of V gamma 9/V delta 2 T lymphocytes was not MHC class I or class II restricted but was blocked by anti-TCR monoclonal antibodies, thus indicating that it involved specific interaction between the TCR and the target cell. The cytotoxicity of V gamma 9/V delta 2 T lymphocytes was not mediated by TNF-alpha or Fas-Fas ligand, but was shown to occur through a granule-dependent mechanism that resulted in reduction of the viability of intracellular bacilli. Perforin was shown to play an important role in killing of both infected macrophages and intracellular mycobacteria. These data strongly suggest that V gamma 9/V delta 2 T lymphocytes contribute to the host defense against *M. tuberculosis* infection.—Authors' Abstract

DiFabio, S., Trabattini, D., Geraci, A., Ruzzante, S., Panzini, G., Fusi, M. L., Chiarotti, F., Corrias, F., Belli, R., Verani, P., Dalgleish, A., Clerici, M. and Titti, F. Study of immunological and virological parameters during thalidomide treatment of SIV-infected cynomolgus monkeys. *J. Med. Primatol.* **29** (2000) 1–10.

The potential therapeutic utility of thalidomide (Thd), an effective inhibitor of tumor necrosis factor (TNF)-alpha *in vitro*, was investigated in cynomolgus monkeys (*Macaca fascicularis*) at 10 months after infection with simian immunodeficiency

virus (SIV). Thd-treated macaques (N = 8) received an oral dose (10 mg) daily for 7 days, followed by a wash-out period of 5 weeks. A second cycle of treatment was performed on the same animals at higher doses (20 mg/Thd/day) for 14 days. The control monkeys (N = 7) received a placebo for the same period of time. In the present study, we show that Thd, in addition to inhibiting TNF- α production after *in vitro* mitogen stimulation of peripheral blood mononuclear cells (PBMCs), was able to restore the proliferative responses to SIV peptides in monkeys that were infected with SIV. Interestingly, we found that such effects are associated with an increased expression of CD28 cell surface receptors on CD4+ T cells paralleled by a decrease on CD8+ T cells. At the same time, significant reduction in either cell-associated viral load or plasma viral RNA was not observed among the SIV-infected monkeys during the two treatment cycles when compared with the placebo group.—Author's Abstract

Dobos, K. M., Spotts, E. A., Marston, B. J., Horsburgh, C. R., Jr., and King, C. H. Serologic response to culture filtrate antigens of *Mycobacterium ulcerans* during Buruli ulcer disease. *Emerging Infect. Dis.* **6** (2000) 158–164.

Buruli ulcer (BU) is an emerging necrotic skin disease caused by *Mycobacterium ulcerans*. To assess the potential for a serodiagnostic test, we measured the humoral immune response of BU patients to *M. ulcerans* antigens and compared this response with delayed-type hypersensitivity (DTH) responses to both Burulin and PPD. The DTH response generally supported the diagnosis of BU, with overall reactivity to Burulin in 28 (71.8%) of 39 patients tested, compared with 3 (14%) of 21 healthy controls. However, this positive skin-test response was observed primarily in patients with healed or active disease, and rarely in patients with early disease ($p = 0.009$). When tested for a serologic response to *M. ulcerans* culture filtrate, 43 (70.5%) of 61 BU patients had antibodies to these antigens, compared with 10 (37.0%) of 27 controls and 4 (30.8%) of 13 tuberculosis pa-

tients. There was no correlation between disease stage and the onset of this serum antibody response. Our findings suggest that serologic testing may be useful in the diagnosis and surveillance of BU.—Authors' Abstract

El Amin, N. M., Hanson, H. S., Pettersson, B., Petrini, B. and Von Stedingk, L. V. Identification of non-tuberculous mycobacteria: 16S rRNA gene sequence analysis versus conventional methods. *Scand. J. Infect. Dis.* **32** (2000) 47–50.

In a retrospective study 45 clinical isolates of nontuberculous mycobacteria were identified to the species level by biochemical profile, gas liquid chromatography and partial sequence analysis of 16S rRNA, and were found to represent 13 different species. The results of sequence analysis showed 100% identity with conventional tests for 34 isolates (76%) and could identify species such as *M. bohemium* which are difficult to characterize with conventional methods. Most of the discrepant results for the remaining 11 isolates resulted in species of the same group of mycobacteria. Based on these findings, we concluded that direct sequence analysis of amplified 16S rRNA gene is a promising rapid and accurate method for species determination of nontuberculous mycobacteria.—Authors' Abstract

Feng, C. G. and Britton, W. J. CD4+ and CD8+ T cells mediate adoptive immunity to aerosol infection of *Mycobacterium bovis* bacillus Calmette-Guerin. *J. Infect. Dis.* **181** (2000) 1846–1849.

An adoptive-transfer model using recombinase activation gene-deficient [RAG-1(–/–)] mice was developed to evaluate CD4+ and CD8+ T-cell responses to infection with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). After receiving immune, unfractionated T cells or T-cell subsets isolated by fluorescence-activated cell sorter, the RAG-1(–/–) mice were exposed to aerosol BCG, and the bacteria load in the infected organs was examined 4 weeks later. Adoptive immunity was ex-

pressed more effectively in the spleens than in the lungs. Although CD4+ or unfractionated T cells protected both lungs and spleens, CD8+ T cells conferred significant protection only in the spleens and not in the lungs. The results confirm that in addition to CD4+, CD8+ T cells also play a role in the prevention of bacterial dissemination. This transfer model may be useful for detecting T-cell responses to mycobacterial infection.—Authors' Abstract

Fine, P. E. M., Bruce, J., Ponnighaus, J. M., Nkhosha, P., Harawa, A., Vynnycky, E. Tuberculin sensitivity: conversions and reversions in a rural African population. *Int. J. Tuberc. Lung Dis.* **3** (1999) 962–975.

A study was conducted to measure and interpret incidence and prevalence of tuberculin sensitivity in Karonga District, northern Malawi, during 1980–1989. An initial survey comprising the entire population of this rural district had been conducted during 1979–1984. Tuberculin “positivity” and “conversion” were defined using criteria recommended by the Tuberculosis Surveillance Research Unit and the American Thoracic Society, respectively. Data on 64,225 tests were available for analysis, including paired results on 6991 individuals tested in both surveys. Frequency distributions of in-duration varied by age, sex, BCG scar status and zone within the district. The prevalence of “positivity” was similar in males and females until age 15 years, then higher among males, and was consistently higher among individuals with a BCG scar than among those without. Tuberculin “conversion” rates estimated from cross-sectional data ranged from 0.34% to 1.15% per annum. Conversion rates derived from longitudinal data increased linearly with age, and the reversion rates declined rapidly with age among younger individuals. Such trends were shown here to arise as an artefact of test instability. Prospective follow up of observed converters showed greatly increased risks of tuberculosis, in particular during the 2 years following the second (“converted”) test (relative risk >10). It is concluded that estimation of a convincing “true” annual risk of infection from tuber-

culin survey data is not possible from either cross-sectional or longitudinal data due to misclassifications and the instability of delayed-type hypersensitivity over time. An apparent increase in infection risk with age can arise as an artefact of test instability. It is suggested that it is necessary to consider tuberculin reversions, whether real or apparent, when interpreting tuberculin data on individuals or populations.—Trop. Dis. Bull. **97** (2000) 303

Frosch, M., Roth, J., Ullrich, K. and Harms, E. Successful treatment of *Mycobacterium avium* osteomyelitis and arthritis in a non-immunocompromised child. *Scand. J. Infect. Dis.* **32** (2000) 328–329.

In non-immunocompromised children, infections with *Mycobacterium avium* complex (MAC) are rare, except for cervical lymphadenitis. We report here a 34-month-old boy who developed osteomyelitis and septic arthritis due to MAC. No findings could be revealed for immunodeficiency. He was treated successfully for 12 months with combined therapy consisting of clarithromycin, rifabutin, and prothionamide.—Authors' Abstract

Fulton, S. A., Martin, T. D., Redline, R. W. and Boom, W. H. Pulmonary immune responses during primary *Mycobacterium bovis* Calmette-Guerin bacillus infection in C57BL/6 mice. *Am. J. Respir. Cell Mol. Biol.* **22** (2000) 333–343.

Mechanisms of protective immunity to mycobacterial infection in the lung remain poorly defined. In this study, T-cell subset expansion and cytokine expression in bronchoalveolar spaces, lung parenchyma, and mediastinal lymph nodes of mice infected intratracheally with *Mycobacterium bovis*-Calmette-Guerin bacillus (BCG) were analyzed in parallel with histopathology and bacterial burden. *M. bovis*-BCG was cleared rapidly from bronchoalveolar spaces without evidence for persistence. In lung parenchyma bacteria grew during the first 4 wk followed by gradual clearance with less than 0.1% of the original inocu-

lum persisting for more than 8 mo. Clearance of *M. bovis*-BCG from bronchoalveolar lavage was associated with recruitment of both neutrophils and lymphocytes. Lung CD4+, CD8+, and gamma delta T-cell receptor-positive T cells expanded maximally by week 3 and declined by week 8 to control values despite bacterial persistence. Both CD4+ and CD8+ lung T cells produced interferon (IFN)-gamma in response to *M. bovis*-BCG. Four distinct pathologic states of lung parenchymal infection were noted. Early focal sub-bronchial inflammation with transmigration of cells into airways was followed by diffuse peribronchitis, perivascularitis, and alveolitis with activated macrophages, lymphoblasts, and occasional giant cells. The latter stage corresponded to maximal *M. bovis*-BCG growth. Resolving infection consisted of small lymphocytes and foamy macrophages, which coincided with decreasing *M. bovis*-BCG colony-forming units, T-cell infiltration, and IFN-gamma expression. A final quiescent phase consisted of residual lymphoid aggregates and perivascularitis associated with persistent spontaneous IFN-gamma production. Bacterial dissemination to lymph node and spleen occurred by week 4 and declined in parallel to lung. In contrast to lung, IFN-gamma secretion was detected only late despite early expansion of CD4+ and CD8+ T cells. By reverse transcriptase/polymerase chain reaction, IFN-gamma and interleukin (IL)-12 p40 messenger RNA (mRNA) in lung paralleled IFN-gamma protein production. Tumor necrosis factor-alpha, IL-4 and IL-10 mRNA expression was not increased during *M. bovis*-BCG lung infection. Thus, protective immunity to *M. bovis*-BCG in the lung evolved differently in air space, lung, and lymph node.—Authors' Abstract

Garcia Quintanilla, A., Garcia, L., Tudo, G., Navarro, M., Gonzalez, J. and de Anta, M. T. J. Single-tube balanced heminested PCR for detecting *Mycobacterium tuberculosis* in smear-negative samples. *J. Clin. Microbiol.* **38** (2000) 1166–1169.

In order to achieve more sensitive and specific results for the rapid diagnosis of tu-

berculosis, we have developed a new method, named balanced heminested PCR, which avoids the inconvenience of asymmetric amplification and has the advantages of single-tube heminested PCR. This was achieved by replacing the outer primer that participates in both rounds of amplification in the standard heminested technique by another primer containing the sequence of the inner primer attached at its 5' end. When both techniques were tested for the IS6110 target of *Mycobacterium tuberculosis* complex in 80 smear-negative culture positive sputum samples and 60 control samples, the results showed 100% specificity for both techniques and sensitivities of 60% and 75% for heminested PCR and balanced heminested PCR, respectively ($p = 0.02$). In conclusion, the balanced heminested technique shows a higher sensitivity than that of the standard heminested, and it could be applied to any PCR by attaching the inner primer at the 5' end of the opposite outer primer. Thus, the balanced heminested technique provides a target for the inner primer in both strands, avoiding asymmetric amplification and thereby resulting in a more efficient amplification and, in practice, a higher sensitivity without loss of specificity and with a minimum risk of cross-contamination.—Authors' Abstract

Garnier, T., Brosch, R., Eiglmeier, K., Gordon, S. V. and Cole, S. T. Lesions from the *Mycobacterium tuberculosis* genome. In: *Tuberculosis in Human and Veterinary Medicine*. Rajnchapel Messai, J., Salikou, P., Antoine, H. M. and Dodet, B., eds. Paris: Edition Scientifiques et Medicales Elsevier, 1999, pp. 47–49. (in French)

The genome of the reference strain H37Rv of *Mycobacterium tuberculosis* is composed of 4,411,529 base pairs. A function has been attributed to 1,570 of the 3,924 genes identified, while a further 1,570 share certain similarities. This genome has a coding capacity greater than 90% and many repetitions. Gene sequencing has revealed the presence of 32 insertion sequences in addition to the previously described IS6110 and IS1081 sequences. It has also been shown that the MPTR and

PGRS repeat sequences belong to the PE and PPE families and represent 10% of the coding capacity of the *M. tuberculosis* genome.—Authors' English Abstract

Glickman, M. S., Cox, J. S. and Jacobs, W. R. A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of *Mycobacterium tuberculosis*. *Mol. Cell* **5** (2000) 717–727.

Colonial morphology of pathogenic bacteria is often associated with virulence. For *M. tuberculosis*, the causative agent of tuberculosis (TB), virulence is correlated with the formation of serpentine cords, a morphology that was first noted by Koch. We identified a mycobacterial gene, *pcaA*, that we show is required for cording and mycolic acid cyclopropane ring synthesis in the cell wall of both BCG and *M. tuberculosis*. Furthermore, we show that mutants of *pcaA* fail to persist within and kill infected mice despite normal initial replication. These results indicate that a novel member of a family of cyclopropane synthetases is necessary for lethal chronic persistent *M. tuberculosis* infection and define a role for cyclopropanated lipids in bacterial pathogenesis.—Authors' Abstract

Gomez, A., Mve Obiang, A., Vray, B., Remacle, J., Chemlal, K., Meyers, W. M., Portaels, F. and Fonteyne, P. A. Biochemical and genetic evidence for phospholipase C activity in *Mycobacterium ulcerans*. *Infect. Immun.* **68** (2000) 2995–2997.

This study reports the existence of phospholipase C and D enzymatic activities in *Mycobacterium ulcerans* cultures as determined by use of thin-layer chromatography to detect diglycerides in hydrolases of radiolabeled phosphatidylcholine. *M. ulcerans* DNA sequences homologous to the genes encoding phospholipase C in *M. tuberculosis* and *Pseudomonas aeruginosa* were identified by sequence analysis and DNA-DNA hybridization. Whether or not the phospholipase C and D enzymes of *M. ulcerans* play a role in the pathogenesis of

the disease needs further investigation.—Authors' Abstract

Gomez, L., Johnson, S. and Gennaro, M. L. Identification of secreted proteins of *Mycobacterium tuberculosis* by a bioinformatic approach. *Infect. Immun.* **68** (2000) 2323–2327.

Proteins secreted by *Mycobacterium tuberculosis* are usually targets of immune responses in the infected host. Here we describe a search for secreted proteins that combined the use of bioinformatics and *phoA* fusion technology. The 3,924 proteins deduced from the *M. tuberculosis* genome were analyzed with several computer programs. We identified 52 proteins carrying an NH₂-terminal secretory signal peptide but lacking additional membrane-anchoring moieties. Of these 52 proteins—the TM1 subgroup—only 7 had been previously reported to be secreted proteins. Our predictions were confirmed in 9 of 10 TM1 genes that were fused to *Escherichia coli phoA*, a marker of subcellular localization. These findings demonstrate that the systematic computer search described in this work identified secreted proteins of *M. tuberculosis* with high efficiency and 90% accuracy.—Authors' Abstract

Hondalus, M. K., Bardarov, S., Russell, R., Chan, J., Jacobs, W. R. and Bloom, B. R. Attenuation of and protection induced by a leucine auxotroph of *Mycobacterium tuberculosis*. *Infect. Immun.* **68** (2000) 2888–2898.

Attenuated mutants of *Mycobacterium tuberculosis* represent potential vaccine candidates for the prevention of tuberculosis. It is known that auxotrophs of a variety of bacteria are attenuated *in vivo* and yet provide protection against challenge with wild-type organisms. A leucine auxotroph of *M. tuberculosis* was created by allelic exchange, replacing wild-type *leuD* (Rv2987c), encoding isopropyl malate isomerase, with a mutant copy of the gene in which 359 bp had been deleted, creating a strain requiring exogenous leucine supplementation for growth *in vitro*. The fre-

quency of reversion to prototrophy was $<10^{-11}$. In contrast to wild-type *M. tuberculosis*, the Delta leuD mutant was unable to replicate in macrophages *in vitro*. Its attenuation *in vivo* and safety as a vaccine were established by the fact that it caused no deaths in immunodeficient SCID mice. Complementation of the mutant with wild-type leuD abolished the requirement for leucine supplementation and restored the ability of the strain to grow both in macrophages and in SCID mice, thus confirming that the attenuated phenotype was due to the Delta leuD mutation. As a test of the vaccine potential of the leucine auxotroph, immunocompetent BALB/c mice, susceptible to fatal infection with wild-type *M. tuberculosis*, were immunized with the Delta leuD mutant and subsequently challenged with virulent *M. tuberculosis* by both the intravenous and aerosol routes. A comparison group of mice was immunized with conventional *M. bovis* BCG vaccine. Whereas all unvaccinated mice succumbed to intravenous infection within 15 weeks, mice immunized with either BCG or the Delta leuD mutant of *M. tuberculosis* exhibited enhanced and statistically equivalent survival curves. However, the leuD auxotroph was less effective than live BCG in reducing organ burdens and tissue pathology of mice challenged by either route. We conclude that attenuation and protection against *M. tuberculosis* challenge can be achieved with a leucine auxotroph and suggest that to induce optimal protection, attenuated strains of *M. tuberculosis* should persist long enough and be sufficiently metabolically active to synthesize relevant antigens for an extended period of time.—Authors' Abstract

Hughes, M. S., James, G., Ball, N., Scally, M., Malik, R., Wigney, D. I., Martin, P., Chen, S., Mitchell, D. and Love, D. N. Identification by 16S rRNA gene analyses of a potential novel mycobacterial species as an etiological agent of canine leproid granuloma syndrome. *J. Clin. Microbiol.* **38** (2000) 953–959.

PCR amplifications of the 16S rRNA gene were performed on 46 specimens obtained from 43 dogs with canine leproid

granuloma syndrome to help determine its etiology. Sequence capture PCR was applied to 37 paraffin-embedded specimens from 37 dogs, and nested PCR was attempted on DNA from 9 fresh tissue specimens derived from 3 of the 37 aforementioned dogs and from an additional 6 dogs. Molecular analyses of the paraffin-embedded tissues and fresh tissue specimen analyses were performed at separate institutions. PCR products with identical sequences over a 350-bp region encompassing variable regions 2 and 3 of the 16S rRNA gene were obtained from 4 of 37 paraffin-embedded specimens and from all 9 specimens of fresh tissue originating from 12 of the 43 dogs. Identical sequences were determined from apicons obtained from paraffin-embedded and fresh specimens from one dog. The consensus DNA sequence, amplified from paraffin-embedded tissue and represented by GenBank accession no. AF144747, shared highest nucleotide identity (99.4% over 519 bp) with mycobacterial strain IWGMT 90413 but did not correspond exactly to any EMBL or GenBank database sequence. With a probe derived from the V2 region of the novel canine sequence, reverse cross blot hybridization identified an additional four paraffin-embedded specimens containing the same novel sequence. In total, molecular methodologies identified the proposed novel mycobacterial sequence in 16 of 43 dogs with canine leproid granuloma syndrome, indicating that the species represented by this sequence may be the principal etiological agent of canine leproid granuloma syndrome.—Authors' Abstract

Jacobs, M., Brown, N., Allie, N. and Ryffel, B. Fatal *Mycobacterium bovis* BCG infection in TNF-LT-alpha-deficient mice. *Clin. Immunol.* **94** (2000) 192–199.

Neutralization of TNF or disruption of TNF-R1 leads to fatal *Mycobacterium bovis* BCG infection. Here we used TNF-LT-alpha-deficient mice to test whether a complete disruption of TNF and LT-alpha reduces further host resistance to BCG infection. The bacterial burden, especially in the

lungs of TNF-LT-alpha-deficient mice, was significantly increased and the mice succumbed to infection between 8 and 10 weeks. In the absence of TNF-LT-alpha the granulomatous response was severely impaired and delayed. The cells in the granulomas of TNF-LT-alpha-deficient mice expressed low levels of MHC class II and ICAM-1. They contained a few T cells and F4/80-positive macrophages expressing little iNOS and acid phosphatase activity. By contrast, the lethal action of endotoxin was dramatically reduced in BCG-infected TNF-LT-alpha-deficient mice. In summary, in the absence of TNF-LT-alpha the recruitment and activation of mononuclear cells in response to BCG infection were significantly delayed and reduced resulting in immature granulomas allowing uncontrolled fatal infection.—Authors' Abstract

Jacobs, M., Marino, M. W., Brown, N., Abel, B., Bekker, L. G., Quesniaux, V. J. F., Fick, L. and Ryffel, B. Correction of defective host response to *Mycobacterium bovis* BCG infection in TNF-deficient mice by bone marrow transplantation. *Lab. Invest.* **80** (2000) 901–914.

Tumor necrosis factor-alpha (TNF) plays a central role in the recruitment and activation of mononuclear cells in mycobacterial infection. In the absence of type 1 TNF receptor, *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) infection of mice is not contained, leading to fatal disease. Because type 1 TNF receptor binds both TNF and lymphotoxin-alpha, we used TNF-deficient mice to determine the specific role of TNF in the host resistance to BCG infection. The bacterial burden of the lungs of TNF-deficient mice was substantially increased and the mice succumbed to pneumonia between 8 and 12 weeks with a defective granuloma response. Atypical granulomas developed by 4 weeks expressing low levels of MHC class II, intracellular adhesion molecule (ICAM-1), CD11b and CD11c. Macrophages showed little signs of activation and had low levels of acid phosphatase activity and inducible nitric oxide synthase (iNOS) expression. Despite the defective cellular recruitment, the chemokines, monocyte chemoattractant pro-

tein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1 alpha), were increased in broncho-alveolar lavage fluid of TNF-deficient mice. The defective host response was corrected by the transplantation of normal bone marrow cells into irradiated TNF-deficient mice. These results demonstrate that TNF derived from hemopoietic cells rather than from mesenchymal origin are essential for a normal host response to BCG infection. Furthermore, TNF dependent expression of adhesion molecules may be essential for the recruitment of mononuclear cells for the formation of bactericidal BCG granulomas.—Authors' Abstract

Jagannath, C., Emanuele, M. R. and Hunter, R. L. Activity of poloxamer CRL-1072 against drug-sensitive and resistant strains of *Mycobacterium tuberculosis* in macrophages and in mice. *Int. J. Antimicrob. Agents* **15** (2000) 55–63.

The present experiments evaluated a new, highly refined poloxamer, CRL-1072, alone and in combination with antibiotics against drug-sensitive and -resistant organisms. In macrophage culture, CRL-1072 reduced the drug concentration inhibiting 99% of control growth of isoniazid (INH) from 10 to 0.15 mg/l (fractional inhibitory concentration = 0.07) for a drug-resistant strain. CRL-1072 also increased the susceptibility of drug-resistant strains of *Mycobacterium tuberculosis* to INH, streptomycin, rifampin, pyrazinamide, ethambutol, PAS, thiacetazone and ethionamide. Fractional inhibitory concentration values of <0.5 indicated significant synergistic activity. In studies of acute infection in mice, CRL-1072 was only weakly bacteriostatic when used as a single agent but increased the bactericidal activity of INH, streptomycin, rifampin, pyrazinamide and clindamycin, but not that of ethambutol. CRL-1072 enhanced the bactericidal activity of streptomycin against a streptomycin resistant strain of *M. tuberculosis* in a murine infection.—Authors' Abstract

James, B. W., Williams, A. and Marsh, P. D. The physiology and pathogenicity of *Mycobacterium tuberculosis* grown un-

der controlled conditions in a defined medium. *J. Appl. Microbiol.* **88** (2000) 669–677.

A chemically defined culture medium was developed which supported batch growth of *Mycobacterium tuberculosis*, strain H37Rv, at a minimum doubling time of 14.7 hr. This medium also facilitated chemostat culture of *M. tuberculosis* at a constant doubling time of 24 hr. Chemostat growth was optimized at a dissolved oxygen tension of 20% (v/v) and 0.2% (v/v) Tween-80. Chemostat cultures were dispersed suspensions of single bacilli (1.5–3 μm long), or small aggregates, at a mean density of \log_{10} 8.3 cfu ml^{-1} . A limited number of amino acids was utilized (alanine, asparagine, aspartate and serine were depleted by >50%; glycine, arginine, isoleucine, leucine and phenylalanine, by approximately 40%). Chemostat-grown cells were pathogenic in aerosol-infected guinea pigs, producing disseminated infection similar to that caused by plate-grown cells. Cells from chemostat culture were significantly more invasive for J774A.1 mouse macrophages than agar- or batch-grown cells. This study demonstrates the suitability of chemostat culture for the growth of pathogenic mycobacteria in a defined physiological state with potential applications for the controlled production of mycobacterial components for therapeutic and vaccine applications.—Authors' Abstract

Jo, E. K., Kim, H. J., Lim, J. H., Min, D., Song, Y., Song, C. H., Paik, T. H., Suhr, J. W. and Park, J. K. Dysregulated production of interferon-gamma, interleukin-4 and interleukin-6 in early tuberculosis patients in response to antigen 85B of *Mycobacterium tuberculosis*. *Scand. J. Immunol.* **51** (2000) 209–217.

Both interferon-gamma (IFN- γ) and interleukin (IL)-4 expression in T cells and IL-6 expression in cells of the monocyte/macrophage lineage were monitored using antigen 85B (Ag85B) protein and purified protein derivative (PPD) antigen in the early stages of tuberculosis (TB). We showed that the levels of cell-associated IFN- γ and IL-4 (mRNA and intracellular

cytokine) in Ag85B-stimulated T cells were significantly depressed in TB patients compared with those in healthy tuberculin reactors. On the other hand, the capacity of peripheral blood mononuclear cells (PBMC) to produce IL-6 spontaneously *ex vivo* was enhanced in patients ($p < 0.001$), but their corresponding capacities to respond to Ag85B were not significantly different from those of normal donors. After 2 months of antituberculosis therapy, the mean blastogenic responses of Ag85B-stimulated PBMC from seven TB patients were increased 6.1-fold ($p = 0.011$). Furthermore, the proportions of both IFN- γ ($p < 0.01$) and IL-4 ($p = 0.05$) producing T cells were significantly increased. However, those of IL-6-producing cells were diminished in response to Ag85B ($p = 0.05$). Our results suggest that there may be an altered regulation of IFN- γ , IL-4 and IL-6 to Ag85B in the early stages of TB.—Authors' Abstract

Johnson, J. L., Kanya, R. M., Okwera, A., Loughlin, A. M., Nyole, S., Hom, D. L., Wallis, R. S., Hirsch, C. S., Wolski, K., Foulds, J., Mugerwa, R. D. and Ellner, J. J. Randomized controlled trial of *Mycobacterium vaccae* immunotherapy in nonhuman immunodeficiency virus-infected Ugandan adults with newly diagnosed pulmonary tuberculosis. *J. Infect. Dis.* **181** (2000) 1304–1312.

Adjunctive immunotherapy with heat-killed *Mycobacterium vaccae* was studied in a randomized, placebo-controlled trial of 120 nonhuman immunodeficiency virus-infected adults with newly diagnosed pulmonary tuberculosis. Patients were randomized to a single dose of *M. vaccae* or placebo 1 week after beginning chemotherapy and were followed up for 1 year. *M. vaccae* was safe and well tolerated. The rate of sputum culture conversion after 1 month of tuberculosis treatment was 35% in the *M. vaccae* group and only 14% in the placebo group ($p = 0.01$) but was comparable at 2 months and thereafter. Patients receiving *M. vaccae* had greater improvement on chest radiography at 6 months (91% vs 77% for placebo recipients; $p = 0.04$) and 12 months

(94% vs 80%; $p = 0.04$) after initiation of tuberculosis treatment. These data provide evidence of an early increase in sputum culture conversion and greater radiographic improvement among patients who received *M. vaccae*. Further studies are warranted.—Authors' Abstract

Juffermans, N. P., Verbon, A., Olszyna, D. P., van Deventer, S. J. H., Speelman, P. and van der Poll, T. Thalidomide suppresses upregulation of human immunodeficiency virus co-receptors CXCR4 and CCR5 on CD4+ T cells in humans. *J. Infect. Dis.* **181** (2000) 1813–1816.

Concurrent infection in patients with human immunodeficiency virus (HIV) infection increases the expression of HIV coreceptors CXCR4 and CCR5. Thalidomide has beneficial effects in a number of HIV-associated diseases. The effect of thalidomide on CXCR4 and CCR5 expression on CD4+ T cells was determined. Thalidomide produced a dose-dependent inhibition of lipopolysaccharide (LPS)-induced upregulation of CXCR4 and CCR5 *in vitro*. Antibody to tumor necrosis factor- α (TNF- α) also attenuated the LPS-induced HIV coreceptor upregulation, which was not further reduced by thalidomide. Thalidomide (400 mg) was orally administered to 6 men, and their blood was stimulated *ex vivo* with LPS, staphylococcal or mycobacterial antigens, or antibody to CD3 or CD28 cells. All stimuli induced upregulation of HIV coreceptors, which was reduced after ingestion of thalidomide. Thalidomide may be beneficial in the treatment of intercurrent infections during HIV infection by reducing the upregulation of CXCR4 and CCR5 expression on CD4+ T cells induced by bacterial and mycobacterial antigens, by a mechanism that involves inhibition of TNF- α .—Authors' Abstract

Juliussen, G., Celsing, F., Turesson, I., Lenhoff, S., Adriansson, M. and Malm, C. Frequent good partial remissions from thalidomide including best response ever in patients with advanced refractory and relapsed myeloma. *Br. J. Haematol.* **109** (2000) 89–96.

Twenty-three patients with advanced and heavily pretreated myeloma were treated with thalidomide. Starting dose was 200 mg/d, and 20 patients had dose escalations up to 400 (N = 5), 600 (N = 12) or 800 mg/d (N = 3), usually in divided doses. Nineteen patients were refractory to recent chemotherapy, and 4 had untreated relapse after prior intensive therapy. Ten out of 23 patients (43%) achieved partial response (PR; 9 with refractory and 1 with relapsed disease), 6 patients had minor response or stabilization of the disease and 4 had disease progression. Another 3 patients died early from advanced myeloma at less than 3 weeks of thalidomide therapy. Of the 10 patients with PR, 7 had a better response than after any prior therapy, despite vincristine-doxorubicin-dexamethasone (VAD)-based treatment in all but 1 and high-dose melphalan with autologous stem cell support in 4. Time to achieve PR was rapid in patients receiving thalidomide in divided doses (median 31 days). Responses also included reduced bone marrow plasma cell infiltration and improved general status. Normalized polyclonal gammaglobulin levels were seen in 4 cases. Six out of 10 patients with PR remained in remission with a median time on treatment of 23 weeks (range 15–50 weeks). Sedation was common but usually tolerable, and some patients continued full- or part-time work. Four patients had skin problems, 3 patients had pneumonia, 1 hypothyroidism, 1 sinus bradycardia and 1 minor sensory neuropathy. Thalidomide may induce good partial remissions in advanced refractory myeloma with tolerable toxicity, and should be evaluated in other settings for myeloma patients. Divided thalidomide doses seem to reduce time to achieve remission and may improve response rate.—Authors' Abstract

Karrow, N. A., McCay, J. A., Brown, R. D., Musgrove, D. L., Pettit, D. A., Munson, A. E., Germolec, D. R. and White, K. L. Thalidomide stimulates splenic IgM antibody response and cytotoxic T lymphocyte activity and alters leukocyte subpopulation numbers in female B6C3F1 mice. *Toxicol. Appl. Pharmacol.* **165** (2000) 237–244.

Thalidomide has been shown to have anti-inflammatory and, more recently, immunomodulating properties, which are beneficial for the treatment of an ever-increasing list of immune-related diseases. Although considerable knowledge regarding thalidomide's anti-inflammatory properties has been acquired, relatively little is known about its immunomodulating properties *in vivo*. In this paper, a panel of immune assays was used to evaluate immunomodulation in female B6C3F1 mice treated intraperitoneally for 28 days with thalidomide (30, 100, or 150 mg/kg/day). Spleen antibody forming cell response was significantly enhanced by 37% in mice treated with 150 mg/kg/day, despite an 8% decrease in the percentage of Ig+ B cells. A significant stimulatory trend was observed for the cytotoxic T-cell response across thalidomide treatment groups. An evaluation of the spleen leukocyte subpopulations revealed a 23% increase in the absolute number of CD8+ T cells in the 150 mg/kg treatment group and a 9% and 11% decrease in the absolute number of NK cells in both the 100 and 150 mg/kg thalidomide treatment groups, respectively. These findings demonstrate that, in addition to modulating spleen leukocyte numbers, thalidomide also stimulates murine humoral and cellular immune responses *in vivo*.—Authors' Abstract

Kneller, A., Raanani, P., Hardan, I. Avigdor, A., Levi, I., Berkowicz, M. and Ben-Bassat, I. Therapy with thalidomide in refractory multiple myeloma patients—the revival of an old drug. *Br. J. Haematol.* **108** (2000) 391–393.

We have treated 17 refractory or relapsed multiple myeloma patients resistant to chemotherapy with thalidomide at a dose of 200–800 mg/day. Eleven patients responded, 5 of whom had a very good partial response (>75% decline in M protein) and another 5 exhibited a partial response (>50% decline in M protein). Except for one patient, treatment was well tolerated with only mild side effects. Thalidomide should be included in the therapeutic options for refractory myeloma.—Authors' Abstract

Kremer, L., Estaquier, J., Wolowczuk, I., Biet, F., Ameisen, J. C. and Loch, C. Ineffective cellular immune response associated with T-cell apoptosis in susceptible *Mycobacterium bovis* BCG-infected mice. *Infect. Immun.* **68** (2000) 4264–4273.

It has previously been reported that inhibition of delayed-type hypersensitivity-mediated functions of T cells during mycobacterial infection in mice is haplotype dependent. In the present study, we show that *Mycobacterium bovis* BCG infection induced, in susceptible C57BL/6 and BALB/c mice but not in resistant C3H/HeJ and DBA/2 mice, an important splenomegaly. An *in vitro* defect in T-cell proliferation in response to T-cell receptor (TCR) stimulation with mitogens or anti-CD3 antibodies was associated with enhanced levels of CD4+ and CD8+ T-cell apoptosis in susceptible but not in resistant mice 2 weeks after infection. Further investigations of C57BL/6 and C3H/HeJ mice revealed that *in vivo* splenomegaly was associated with destruction of the lymphoid tissue architecture, liver cellular infiltrates, and increased numbers of apoptotic cells in both spleen and liver tissue sections. Infection of C57BL/6 mice but not of C3H/HeJ mice induced massive production of tumor necrosis factor alpha (TNF- α) in serum, as well as an increase in Fas and Fas ligand (FasL) expression in T cells. *In vitro* addition of neutralizing anti-TNF- α antibodies led to a significant reduction in CD3-induced T-cell apoptosis of both CD4+ and CD8+ T cells of C57BL/6 mice, while the blockade of Fas-FasL interactions reduced apoptosis only in CD4+ but not in CD8+ T cells. Together, these results suggest that TNF- α and Fas-FasL interactions play a role in the activation-induced cell death (AICD) process associated with a defect in T-cell proliferation of the susceptible C57BL/6 mice. T-cell death by apoptosis may represent one of the important components of the ineffective immune response against mycobacterium-induced immunopathology in susceptible hosts.—Authors' Abstract

Lauzardo, M. and Askin, D. Phthisiology at the dawn of the new century—a re-

view of tuberculosis and the prospects for its elimination. *Chest* **117** (2000) 1455–1473.

Tuberculosis (TB) has been and continues to be one of the most significant pathogens in terms of human morbidity and mortality. Although the resurgence of TB has been held in check in most developed countries, the epidemic rages on in most developing countries of the world. The specter of drug resistance is becoming a more credible challenge in many parts of the world, dimming the prospects of eventual elimination. However, great opportunities are arising as well, with an unprecedented focus on the global aspects of TB control. This article will review the status of TB today and put into perspective the prospects for its elimination in the coming century.—Authors' Abstract

Li, J. Y. W., Lo, S. T. H. and Ng, C. S. Molecular detection of *Mycobacterium tuberculosis* in tissues showing granulomatous inflammation without demonstrable acid-fast bacilli. *Diagn. Mol. Pathol.* **9** (2000) 67–74.

Early diagnosis of tuberculosis (TB) is important for early medical intervention and prevention of spread of the bacteria. It is not uncommon to observe granulomatous inflammation but without demonstrable acid-fast bacilli (AFB) on Ziehl-Neelsen (ZN) staining in tissues sent for histologic examination, and the definitive diagnosis of TB cannot be made because no concurrent tissue is sent for TB culture. In this study, the authors explored the feasibility of using polymerase chain reaction (PCR) for early detection of *Mycobacterium tuberculosis* (Mtb) in formalin-fixed, paraffin-embedded tissues where a definite diagnosis of TB cannot be made. One-hundred-fifteen patients (131 paraffin blocks of biopsy specimens) with granulomatous inflammation but ZN-negative for AFB were studied. DNA was extracted from paraffin sections and amplified by PCR with the IS6110 primers (specific for the Mtb complex) and the specific 122-base pairs (bp) PCR product was detected by agarose gel electrophoresis. Sixty-eight of the 115 (59%)

patients were TB-PCR positive, thus enabling definite diagnosis of TB in significant numbers of these patients in 3 days. The authors conclude that molecular diagnosis by PCR is useful for early detection of TB in histologic material where morphologic features are suggestive but not confirmatory because of negative staining for AFB.—Authors' Abstract

Little, R. F., Wyvill, K. M., Pluda, J. M., Welles, L., Marshall, V., Figg, W. D., Newcomb, F. M., Tosato, G., Feigal, E., Steinberg, S. M., Whitby, D., Goedert, J. J. and Yarchoan, R. Activity of thalidomide in AIDS-related Kaposi's sarcoma. *J. Clin. Oncol.* **18** (2000) 2593–2602.

Purpose: To assess the toxicity and activity of oral thalidomide in Kaposi's sarcoma (KS) in a phase II dose-escalation study.

Patients and Methods: Human immunodeficiency virus (HIV)-seropositive patients with biopsy-confirmed KS that progressed over the 2 months before enrollment received an initial dose of 200 mg/day of oral thalidomide in a phase II study. The dose was increased to a maximum of 1000 mg/day for up to 1 year. Anti-HIV therapy was maintained during the study period. Toxicity, tumor response, immunologic and angiogenic factors, and virologic parameters were assessed.

Results: Twenty patients aged 29 to 49 years with a median CD4 count of 246 cells/mm³ (range 14 to 646 cells/mm³) were enrolled. All patients were assessable for toxicity, and 17 for response. Drowsiness in 9 and depression in 7 patients were the most frequent toxicities observed. Eight (47%; 95% confidence interval [CI] 23% to 72%) of the 17 assessable patients achieved a partial response, and an additional 2 patients had stable disease. Based on all 20 patients treated, the response rate was 40% (95% CI 19% to 64%). The median thalidomide dose at the time of response was 500 mg/day (range 400 to 1000 mg/day). The median duration of drug treatment was 6.3 months, and the median time to progression was 7.3 months.

Conclusion: Oral thalidomide was tolerated in this population at doses up to 1000

mg/day for as long as 12 months and was found to induce clinically meaningful anti-KS responses in a sizable subset of the patients. Additional studies of this agent in KS are warranted.—Authors' Abstract

Majumdar, S., Kaur, H., Vohra, H. and Varshney, G. C. Membrane surface of *Mycobacterium microti*-infected macrophages antigenically differs from that of uninfected macrophages. *FEMS Immunol. Med. Microbiol.* **28** (2000) 71–77.

Identification of the antigenic changes in mycobacteria-infected macrophages may be important in understanding the mechanisms responsible for the intracellular survival of the bacteria. In the present study, *Mycobacterium microti*-infected macrophages were utilized to investigate the possibility of differentiating the infected cells from normal cells, based on the antigenic changes occurring in the membranes. Antisera were generated against bacterial extract, heat-killed bacteria and crude preparation of *M. microti*-infected homologous macrophage membrane. The reactivity of these antisera, towards *in vitro* infected macrophages, was compared by flow cytometry. Unlike antibacterial extract antiserum or anti-heat-killed bacterial antiserum, anti-infected macrophage membrane antiserum reacted with the infected macrophage surface. This reactivity increased with the increase in postinfection time. However, it was not observed with uninfected macrophages, PMA- or lipopolysaccharide-activated macrophages and those harboring *M. tuberculosis* H37Ra, heat-killed *M. microti* and *Leishmania donovani*. Interestingly, anti-infected macrophage membrane antiserum identified a 63-kDa antigen in *M. microti*-infected macrophage membranes which was not present in the membranes of normal macrophages, activated macrophages and of those infected with *M. tuberculosis* H37Ra, heat-killed *M. microti* and *L. donovani*. Thus, membranes of *M. microti*-infected macrophages differ antigenically from those of the normal macrophages and infected homologous macrophage membrane antiserum provides a useful tool in studying such changes.—Authors' Abstract

Martin, E., Kamath, A. T., Triccas, J. A. and Britton, W. J. Protection against virulent *Mycobacterium avium* infection following DNA vaccination with the 35-kilodalton antigen is accompanied by induction of gamma interferon-secreting CD4+ T cells. *Infect. Immun.* **68** (2000) 3090–3096.

Mycobacterium avium is an opportunistic pathogen that primarily infects immunocompromised individuals, although the frequency of *M. avium* infection is also increasing in the immunocompetent population. The antigen repertoire of *M. avium* varies from that of *M. tuberculosis*, with the immunodominant 35-kDa protein being present in *M. avium* and *M. leprae* but not in members of the *M. tuberculosis* complex.

Here we show that a DNA vector encoding this *M. avium* 35-kDa antigen (DNA-35) induces protective immunity against virulent *M. avium* infection, and this protective effect persists over 13 weeks of infection. In C57BL/6 mice, DNA vaccines expressing the 35-kDa protein as a cytoplasmic or secreted protein, both induced strong T-cell gamma interferon (IFN- γ) and humoral immune responses. Furthermore, the antibody response was to conformational determinants, confirming that the vector-encoded protein had adopted the native conformation. DNA-35 immunization resulted in an increased activated/memory CD4+ T-cell response, with an accumulation of CD4+ CD44(hi) CD45RB(lo) T cells and an increase in antigen-specific IFN- γ production.

The protective effect of the DNA-35 vectors against *M. avium* infection was comparable to that of vaccination with *M. bovis* BCG and significantly greater than that for previous treated infection with *M. avium*. These results illustrate the importance of the 35-kDa protein in the protective response to *M. avium* infection and indicate that DNA vaccination successfully promotes a sustained level of protection during chronic *M. avium* infection.—Authors' Abstract

Mendum, T. A., Chilima, B. Z. and Hirsch, P. R. The PCR amplification of nontuberculous mycobacterial 16S rRNA

sequences from soil. FEMS Microbiol. Lett. **185** (2000) 189–192.

Non-tuberculous mycobacteria are free living saprophytic organisms commonly found in soil and water. Some are major causes of opportunistic infection, particularly in immune-compromised patients, and may influence the efficacy of bacille Calmette-Guerin vaccinations. Many of these organisms are not amenable to culture, so information about their distribution is limited. PCR primers designed to amplify part of the mycobacterial 16S rRNA gene were applied to DNA extracted from cultured organisms and soil. The PCR products from soil contained sequences with similarity to slow-growing mycobacteria similar to *Mycobacterium lentiflavum*, and to fast-growing mycobacteria such as the xenobiotic degraders PYR-I and RJGII.—Authors' Abstract

Moody, D. B., Ulrichs, T., Muhlecker, W., Young, D. C., Gurcha, S. S., Grant, E., Rosat, J. P., Brenner, M. B., Costello, C. E., Besra, G. S. and Porcelli, S. A. Cd1c-mediated T-cell recognition of isoprenoid glycolipids in *Mycobacterium tuberculosis* infection. Nature **404** (2000) 884–888.

The discovery of the CD1 antigen presentation pathway has expanded the spectrum of T-cell antigens to include lipids(1–4), but the range of natural lipid antigens and functions of CD1-restricted T cells *in vivo* remain poorly understood. Here we show that the T-cell antigen receptor and the CD1c protein mediate recognition of an evolutionarily conserved family of isoprenoid glycolipids whose members include essential components of protein glycosylation and cell-wall synthesis pathways. A CD1c-restricted, mycobacteria-specific T-cell line recognized two previously unknown mycobacterial hexosyl-1-phosphoisoprenoids and structurally related mannosyl-beta 1-phosphodolichols. Responses to mannosyl-b1-phosphodolichols were common among CD1c-restricted T-cell lines and peripheral blood T lymphocytes of human subjects recently infected with *M. tuberculosis*, but were not

seen in naive control subjects. These results define a new class of broadly distributed lipid antigens presented by the CD1 system during infection *in vivo* and suggest an immune mechanism for recognition of senescent or transformed cells that are known to have altered dolichol lipids.—Authors' Abstract

Morris, S., Kelley, C., Howard, A., Li, Z. M. and Collins, F. The immunogenicity of single and combination DNA vaccines against tuberculosis. Vaccine **18** (2000) 2155–2163.

DNA immunization is a promising new approach for the development of novel tuberculosis vaccines. In this study, the immune responses following the administration of single and combination tuberculosis DNA vaccines were evaluated. Single DNA vaccines encoding the MPT-63 and MPT-83 tuberculosis antigens evoked partial protection against an aerogenic challenge with *M. tuberculosis* Erdman in the mouse model of pulmonary tuberculosis. Immunization with a multivalent combination DNA vaccine (containing the ESAT-6, MPT-64, MPT-63, and KatG constructs) generated immune responses that indicated an absence of antigenic competition since antigen-specific cell-mediated and humoral responses were detected to each component of the mixture. More importantly, the combination vaccine elicited a strong protective response relative to the protection evoked by live BCG vaccine.—Authors' Abstract

Mustafa, A. S., Shaban, F. A., Abal, A. T., Al Attiyah, R., Wiker, H. G., Lundin, K. E. A., Oftung, F. and Huygen, K. Identification and HLA restriction of naturally derived Th1-cell epitopes from the secreted *Mycobacterium tuberculosis* antigen 85B recognized by antigen-specific human CD4+ T-cell lines. Infect. Immun. **68** (2000) 3933–3940.

Antigen 85B (Ag85B/MPT59) is a major secreted protein from *Mycobacterium tuberculosis* which is a promising candidate antigen for inclusion in novel subunit vaccines against tuberculosis (TB). The present

study was undertaken to map naturally derived T-cell epitopes from *M. tuberculosis* Ag85B in relation to major histocompatibility complex (MHC) class II restriction. Antigen-specific CD4+ T-cell lines were established from HLA-typed TB patients and *M. bovis* BCG vaccinees by stimulation of peripheral blood mononuclear cells with purified Ag85B *in vitro*. The established T-cell lines were then tested for proliferation and gamma interferon (IFN- γ) secretion in response to 31 overlapping synthetic peptides (18-mers) covering the entire sequence of the mature protein. The results showed that the epitopes recognized by T-cell lines from TB patients were scattered throughout the Ag85B sequence; whereas the epitopes recognized by T-cell lines from BCG vaccinees were located toward the N-terminal part of the antigen. The T-cell epitopes represented by peptides p2 [amino acids (aa) 10 to 27], p3 (aa 19 to 36), and p11 (aa 91 to 108) were frequently recognized by antigen-specific T-cell lines from BCG vaccinees in both proliferation and IFN- γ assays. MHC restriction analysis demonstrated that individual T-cell lines specifically recognized the complete Ag85B either in association with one of the self HLA-DRB1, DRB3, or DRB4 gene products or nonspecifically in a promiscuous manner.

At the epitope level, panel studies showed that peptides p2, p3, and p11 were presented to T cells by HLA-DR-matched as well as mismatched allogeneic antigen-presenting cells, thus representing promiscuous epitopes.

The identification of naturally derived peptide epitopes from the *M. tuberculosis* Ag85B presented to Th1 cells in the context of multiple HLA-DR molecules strongly supports the relevance of this antigen to subunit vaccine design.—Authors' Abstract

Ordi Ros, J., Cortes, F., Cucurull, E., Mauri, M., Bujan, S. and Vilardell, M. Thalidomide in the treatment of cutaneous lupus refractory to conventional therapy. *J. Rheumatol.* **27** (2000) 1429–1433.

Objective. We describe a prospective treatment study of thalidomide in a series of

22 patients with cutaneous lupus refractory to other treatments.

Methods. From 1992 to 1998, 22 patients with cutaneous lupus (9 with discoid lupus erythematosus, 7 with subacute cutaneous lupus, 4 with profundus lupus, 2 with nonspecific rash) were treated with thalidomide. Initial treatment was started at 100 mg daily. If the cutaneous lesions vanished, the dose was lowered to 50–25 mg daily as a maintenance therapy, and it was considered a complete remission. If the lesions improved but remained, this was considered a partial response and treatment was continued until the lesions were not further modified. Periodically, adverse effects were evaluated.

Results. Three patients discontinued treatment because of side effects such as vertigo, persistent drowsiness, or paresthesia. Rash improved in 16/19 patients (84%). Complete remission occurred in 12/16 (75%). In 9 (65%) the rash resolved, but recurred 4–16 weeks after withdrawal of thalidomide; when it was used again, they improved. Partial response was achieved in 4/16 (25%) patients. No response occurred in 3/19 (16%). Many patients noted improvement within 2 weeks after starting thalidomide and maximum benefit was achieved within 3 months. Five of the 14 women had amenorrhea during the treatment with thalidomide.

Conclusion. Thalidomide is effective in the treatment of cutaneous lupus refractory to other treatments. However, only some patients had a remission; the remainder relapsed when treatment was withdrawn, or required low doses of thalidomide to preserve inactive lesions. Amenorrhea was observed as a new secondary effect of thalidomide.—Authors' Abstract

Pai, S. R., Actor, J. K., Sepulveda, E., Hunter, R. L. and Jagannath, C. Identification of viable and nonviable *Mycobacterium tuberculosis* in mouse organs by directed RT-PCR for antigen 85B mRNA. *Microb. Pathogen.* **28** (2000) 335–342.

Mycobacterium tuberculosis (MTB), the causative organism of tuberculosis, can remain dormant as a nonculturable organism,

reactivate and cause disease in man and animals. There is need for proof of viability of such organisms in order to understand the process of reactivation. PCR for bacterial DNA cannot distinguish between viable and nonviable bacilli. We have tested a previously described two tube directed reverse transcriptase polymerase chain reaction (RT-PCR) for the detection of mRNA of antigen 85B (Ag85B) of MTB that can distinguish between viable and nonviable organisms. Using a set of external and internal primers for Ag85B, a cDNA amplified product (216 bp) was seen among simulated samples containing only viable cfus at a sensitivity of >10 and <100 cfu/ml. Eucaryotic DNA rich normal mouse lung homogenate did not interfere among these samples. The method amplified the 216 bp product also among cfu positive tissues of naturally infected mice. Finally, in a mouse model of dormancy, direct RT-PCR detected a signal among multiple tissues that were negative for cfus and, hence, nonculturable. Ag85B is abundantly secreted by MTB and hyperexpressed under stress conditions. Thus, the method to identify its mRNA message may be useful to detect viable but dormant bacteria.—Authors' Abstract

Phyu, S., Tadesse, A., Mustafa, T., Tadesse, S., Jonsson, R. and Bjune, G. Diversity of lung and spleen immune responses in mice with slowly progressive primary tuberculosis. *Scand. J. Immunol.* **51** (2000) 147–154.

The aim of the present study was to assess the compartmentalized immune response, in terms of cytokine secretion and cell activation, in lungs and spleens of mice with slowly progressive primary tuberculosis. Immunocyte populations from both organs were isolated and stimulated with concanavalin A, purified protein derivatives and MPT 59. Production of interferon- γ (IFN- γ) and interleukin-4 (IL-4) was measured using an enzyme-linked immunosorbent assay, and cell activation was measured using a tetrazolium colorimetric assay. The IFN- γ and IL-4 levels in the supernatants of *Mycobacterium tuberculosis* antigen (Ag)-stimulated lung immunocytes

from infected mice were higher than the levels from uninfected mice. However, only IL-4 levels were raised in the supernatants of Ag-stimulated spleen immunocytes from infected mice. Spontaneous and Ag-stimulated immunocyte activation was lower only in the lungs of infected mice. The level of lung immunocyte activation was inversely associated with the extent of gross pulmonary pathology. In conclusion, cytokine secretion and cell activation were different between lungs and spleens in slowly progressive, primary murine tuberculosis. Cytokine diversity may explain the confinement of tuberculous lesions in the lungs and the absence of lesions in the spleens of mice with slowly progressive tuberculosis.—Authors' Abstract

Piddock, L. J. V., Williams, K. J. and Ricci, V. Accumulation of rifampicin by *Mycobacterium aurum*, *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*. *J. Antimicrob. Chemother.* **45** (2000) 159–165.

The characteristics of the accumulation of 2 mg/L [C-14]rifampin by wild-type strains of *Mycobacterium aurum* [A(+)], *M. smegmatis* (mc²155) and *M. tuberculosis* (H37Rv) were determined. After 10-min exposure, *M. aurum* had accumulated 200 ng rifampin/mg cells, *M. smegmatis* had accumulated 120 ng rifampin/mg cells and *M. tuberculosis* had accumulated 154 ng rifampin/mg cells. A steady-state concentration (SSC) of rifampin was accumulated rapidly by *M. aurum* and *M. tuberculosis* within minutes of drug exposure, unlike *M. smegmatis*, which accumulated rifampin more slowly. With an increase in the concentration of rifampin from 0.12 mg/L to 2 mg/L there was an increase in the concentration of rifampin accumulated by *M. tuberculosis*, with no detectable loss of viability over the 20 min of the accumulation experiment. With an increase in temperature there was also an increase in the concentration of rifampin accumulated by *M. tuberculosis*; between 15°C and 30°C the increase was linear. For all three species sub-inhibitory concentrations of ethambutol increased the concentration of rifampin accumulated. However, both growth and ac-

cumulation of rifampin were lower in the presence of 0.05% Tween 80. Accumulation of rifampin by *M. smegmatis* was unaffected by the presence of the proton motive force inhibitor, 2,4-dinitrophenol (1 mM), whether added before or after the addition of rifampin to the mycobacterial culture. For all three species, the gram-positive bacterial efflux inhibitor reserpine (20 mg/L) slightly increased the SSC of rifampin, but the increase was not statistically significant. Addition of glucose to energize a putative efflux pump had little effect on the accumulation of rifampin in the presence or absence of reserpine for *M. tuberculosis*; however, for *M. aurum* and *M. smegmatis* the reserpine effect was abolished by the addition of glucose. These data suggest that rifampin may be removed from wild-type mycobacteria by efflux, but that the pump(s) is expressed at low level.—Authors' Abstract

Rosenblatt, M. N., Burns, J. R., Duncan, V. E. and Hughes, J. A. Infection of the macrophage cell line NR8383 with *Mycobacterium tuberculosis* (H37Ra) leads to an increase in oligodeoxynucleotide accumulation. *Antisense Nucl. Acid Drug Dev.* **10** (2000) 1–9.

Mycobacterium tuberculosis infection continues to be a daunting clinical challenge. Although it may well be one of the most studied bacteria in history, several aspects of its pathology remain a mystery. The resurgence of drug-resistant *M. tuberculosis* strains and with unusual pathology have promoted a renewed basic and clinical research interest in developing new therapies to combat this pathogen. The primary localization site for *M. tuberculosis* is within alveolar macrophages. Drug delivery strategies and novel therapeutic agents designed to target alveolar macrophages may lead to efficient destruction of *M. tuberculosis*. Oligodeoxynucleotides (ODN) are short segments of nucleic acids that can interfere with transcription and translation processes. In this report, a monocytic-macrophage cell line was characterized in regard to ODN transport in the presence or absence of *M. tuberculosis* infection. The cells accumulated ODN in a time-depen-

dent and concentration-dependent manner, regardless of the presence of serum. After 4 hr of incubation with *M. tuberculosis* [multiplicity of infection (MOI) 10:1], infected NR8383 cells demonstrated 1.5–7-fold increase in fluorescein isothiocyanate (FITC)-labeled phosphorothioate ODN accumulation as measured by flow cytometry. The increase in uptake was associated only with fluorescent-labeled ODN and not labeled markers of fluid phase endocytosis [e.g., tetramethylrhodamine isothiocyanate (TRITC), FITC-labeled dextran]. NR8383 cells activated by phytohemagglutinin (PHA) did not demonstrate a significant increase in the uptake of either FITC-labeled dextran or FITC-labeled ODN. These studies demonstrate that NR8383 cells that have been infected with *M. tuberculosis* can specifically accumulate ODN, and this route of accumulation may lead to a means of drug targeting to mycobacteria-containing cells.—Authors' Abstract

Rosenkrands, I., Welding, K., Jacobsen, S., Hansen, C. V., Florio, W., Gianetri, I. and Andersen, P. Mapping and identification of *Mycobacterium tuberculosis* proteins by two-dimensional gel electrophoresis, microsequencing and immunodetection. *Electrophoresis* **21** (2000) 935–948.

Mycobacterium tuberculosis is the infectious agent giving rise to human tuberculosis. The entire genome of *M. tuberculosis*, comprising approximately 4000 open reading frames, has been sequenced. The huge amount of information released from this project has facilitated proteome analysis of *M. tuberculosis*. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was applied to fractions derived from *M. tuberculosis* culture filtrate, cell wall, and cytosol, resulting in the resolution of 376, 413, and 395 spots, respectively, in silver-stained gels. By microsequencing and immunodetection, 38 culture filtrate proteins were identified and mapped, of which 12 were identified for the first time. In the same manner, 23 cell-wall proteins and 19 cytosol proteins were identified and mapped, with 9 and 10, respectively, being novel proteins. One of the novel proteins

was not predicted in the genome project, and for four of the identified proteins alternative start codons were suggested. Fourteen of the culture filtrate proteins were proposed to possess signal sequences. Seven of these proteins were microsequenced and the N-terminal sequences obtained confirmed the prediction. The data presented here are an important complement to the genetic information, and the established 2-D PAGE maps (also available at: www.ssi.dk/publichealth/tbimmun) provide a basis for comparative studies of protein expression.—Authors' Abstract

Santucci, M. B., Amicosante, M., Cicconi, R., Montesano, C., Casarini, M., Giosue, S., Bisetti, A., Colizzi, V. and Fraziano, M. *Mycobacterium tuberculosis*-induced apoptosis in monocytes/macrophages: early modification and intracellular mycobacterial viability. *J. Infect. Dis.* **181** (2000) 1506–1509.

Apoptosis has been observed in monocytes/macrophages in the course of *in vivo* and *in vitro* *Mycobacterium tuberculosis* (MTB) infection. In order to define the early events of MTB-induced apoptosis, membrane CD14 expression and the exposure of Annexin V-binding sites in MTB-infected monocytes/macrophages have been monitored. Moreover, the role of MTB-induced apoptosis was further analyzed *in vitro* in terms of mycobacterial viability. Results show that monocyte/macrophage apoptosis is a very early event that is strictly dependent on the MTB amount, and this apoptosis is associated with a selective down-regulation of surface CD14 expression. Furthermore, no statistically significant decrease in mycobacterial viability was observed, which indicates that the apoptotic pathway triggered by high doses of MTB is associated with parasite survival rather than with killing of the parasite.—Authors' Abstract

Silva, C. L. and Lowrie, D. B. Identification and characterization of murine cytotoxic T cells that kill *Mycobacterium tuberculosis*. *Infect. Immun.* **68** (2000) 3269–3274.

As we seek to develop and evaluate new vaccines against tuberculosis, it is desirable that we understand the mechanisms of protective immunity in our models. Adoptive transfer of protection with hsp65-specific T-cell clones from infected or vaccinated mice into naive mice had indicated the cytotoxic T cells can make a major contribution to protection. We characterized 28 CD4⁺ CD8[–] and 28 CD4[–] CD8⁺ hsp65-specific T-cell clones derived from infected or vaccinated mice. Half of the CD4⁺ CD8[–] and 64% of the CD4[–] CD8⁺ clones were cytotoxic. Cytotoxicity was associated with high expression of CD44 and gamma interferon production. Most (86%) of the cytotoxic CD4⁺ CD8[–] clones lysed target cells via the Fas-FasL pathway, and most (83%) of the cytotoxic CD4[–] CD8⁺ clones lysed target cells via cytotoxic granules. Only the clones using the granule-mediated pathway caused substantial loss of viability of virulent *Mycobacterium tuberculosis* during lysis of infected macrophages, and the degree of killing closely correlated with the availability of granule marker enzyme activity. Granule-mediated cytotoxicity thus may have a key role in protection against tuberculosis by delivering mycobactericidal granule contents.—Authors' Abstract

Sousa, A. O., Mazzaccaro, R. J., Russell, R. G., Lee, F. K., Turner, O. C., Hong, S., Van Kaer, L. and Bloom, B. R. Relative contributions of distinct MHC class I-dependent cell populations in protection to tuberculosis infection in mice. *Proc. Natl. Acad. Sci. U.S.A.* **97** (2000) 4204–4208.

A necessary role for cytotoxic T lymphocytes in protection against *Mycobacterium tuberculosis* (MTB) has been suggested by studies of the beta 2-microglobulin-deficient mouse, which is unable to present antigens through MHC class I and class I-like molecules and invariably succumbs early after infection. To identify the relative contributions of distinct putative MHC class I-dependent cell populations in protection against tuberculosis, we compared a variety of gene-disrupted mouse strains for susceptibility to MTB infection. Among the strains tested, the most suscep-

tible mice, as measured by survival time and bacterial loads, were the beta 2-microglobulin (-/-), followed by transporter associated with antigen processing deficient [TAPI (-/-)], CD8 alpha (-/-), perforin (-/-), and CD1d (-/-) mice. These findings indicated that a) CD8+ T cells contribute to protection against MTB, and their protective activity is only partially dependent on perforin; b) beta 2-microglobulin-dependent T-cell populations distinct from CD8+ T cells also contribute to anti-MTB immunity; and c) protective immune mechanisms are predominantly TAP-dependent, although TAP-independent mechanisms also contribute to protection. Because CD1d-deficient animals were fully resistant to MTB, other TAP-independent mechanisms must contribute to protection. We suggest here that both classical and nonclassical MHC class I-restricted T cells, distinct from CD1d-restricted cells, may be involved in protective immune responses against tuberculosis.—Authors' Abstract

Stender, H., Lund, K., Petersen, K. H., Rasmussen, O. F., Hongmanee, P., Miorner, H. and Godtfredsen, S. E. Fluorescence *in situ* hybridization assay using peptide nucleic acid probes for differentiation between tuberculous and nontuberculous *Mycobacterium* species in smears of *Mycobacterium* cultures. *J. Clin. Microbiol.* **37** (1999) 2760–2765.

The TB PNA FISH, a new fluorescence *in situ* hybridization (FISH) method using peptide nucleic acid (PNA) probes for differentiation between species of the *M. tuberculosis* complex (MTC) and nontuberculous mycobacteria (NTM) in acid-fast bacillus-positive (AFB+) cultures, is described. The test is based on fluorescein-labeled PNA probes that target the ribosomal RNA (rRNA) of MTC or NTM species applied to smears of AFB+ cultures for microscopic examination. Parallel testing with the two probes serves as an internal control for each sample such that a valid test result is based on one positive and one negative reaction. TB PNA FISH was evaluated with 30 AFB+ cultures from Denmark and 42 AFB+ cultures from Thailand. The MTC-specific PNA probe showed diagnostic sen-

sitivities of 84% and 97%, respectively, and a diagnostic specificity of 100% in both studies; whereas the NTM-specific PNA probe showed diagnostic sensitivities of 91% and 64%, respectively, and a diagnostic specificity of 100% in both studies. The low sensitivity of the NTM-specific PNA probe in the Thai study was due to a relatively high prevalence of *M. fortuitum*, which is not identified by the probe. In total, 63 (87%) of the cultures were correctly identified as MTC (N = 46) or NTM (N = 17); whereas the remaining 9 were negative with both probes and, thus, the results were inconclusive. None of the samples were incorrectly identified as MTC or NTM; thus, the predictive value of a valid test result obtained with TB PNA FISH was 100%.—*Trop. Dis. Bull.* **97** (2000) 518

Stinear, T., Davies, J. K., Jenkin, G. A., Portaels, F., Ross, B. C., Oppedisano, F., Purcell, M., Hayman, J. A. and Johnson, P. D. R. A simple PCR method for rapid genotype analysis of *Mycobacterium ulcerans*. *J. Clin. Microbiol.* **38** (2000) 1482–1487.

The high-copy-number insertion sequences, IS2404 and IS2606, were recently identified in *Mycobacterium ulcerans* and were shown by Southern hybridization to possess restriction fragment length polymorphism between strains from different geographic origins. We have designed a simple genotyping method that captures these differences by PCR amplification of the region between adjacent copies of IS2404 and IS2606. We have called this system 2426 PCR. The method is rapid, reproducible, sensitive, and specific for *M. ulcerans*, and it has confirmed previous studies suggesting a clonal population structure of *M. ulcerans* within a geographic region. *M. ulcerans* isolates from Australia, Papua New Guinea, Malaysia, Surinam, Mexico, Japan, China, and several countries in Africa were easily differentiated based on an array of 4 to 14 PCR products ranging in size from 200 to 900 bp. Numerical analysis of the banding patterns suggested a close evolutionary link between *M. ulcerans* isolates from Africa and southeast Asia. The application of 2426 PCR to total DNA, ex-

tracted directly from *M. ulcerans*-infected tissue specimens without culture, demonstrated the sensitivity and specificity of this method and confirmed for the first time that both animal and human isolates from areas of endemicity in southeast Australia have the same genotype.—Authors' Abstract

Tanghe, A., Denis, O., Lambrecht, B., Motte, V., van den Berg, T. and Huygen, K. Tuberculosis DNA vaccine encoding Ag85A is immunogenic and protective when administered by intramuscular needle injection but not by epidermal gene gun bombardment. *Infect. Immun.* **68** (2000) 3854–3860.

Immunogenicity and protective efficacy of a DNA vaccine encoding Ag85A from *Mycobacterium tuberculosis* were compared in BALB/c and C57BL (B6 and B10) mice immunized by intramuscular (i.m.) needle injection or epidermal gene gun (gg) bombardment. In BALB/c mice, gg immunization could induce elevated antibody and cytotoxic T lymphocyte responses with plasmid doses 50-fold lower than those required for i.m. immunization. Interleukin-2 (IL-2) and gamma interferon (IFN- γ) secretion, however, was much lower in gg-immunized than in i.m.-immunized BALB/c mice. On the other hand, C57BL mice reacted only very weakly to gg immunization; whereas elevated Ag85A-specific antibody, IL-2, and IFN- γ responses (significantly higher than in BALB/c mice) were detected following vaccination by the i.m. route. Antibody isotypes were indicative of Th2 activation following gg injection of BALB/c and of Th1 activation following i.m. injection of C57BL mice. Finally, C57BL but not BALB/c mice were protected by i.m.

Ag85A DNA immunization against intravenous *M. tuberculosis* challenge, as measured by reduced numbers of CFU in spleen and lungs compared to animals vaccinated with control DNA. Gene gun immunization was not effective in either BALB/c or C57BL mice. These results indicate that i.m. DNA vaccination is the method of choice for the induction of protective Th1-type immune responses with the Ag85a tuberculosis DNA vaccine.—Authors' Abstract

Tascon, R. E., Soares, C. S., Ragno, S., Stavropoulos, E., Hirst, E. M. A. and Colston, M. J. *Mycobacterium tuberculosis*-activated dendritic cells induce protective immunity in mice. *Immunology* **99** (2000) 473–480.

Activated dendritic cells are critically important in the priming of T-cell responses. In this report we show that the infection of a conditionally immortalized dendritic cell line (tsDC) with *Mycobacterium tuberculosis* resulted in the up-regulation of B7-1 and B7-2 co-stimulatory molecules and the induction of several inflammatory cytokines, including tumor necrosis factor-alpha and interleukin-6, -1 beta and -12. In addition, we show that these activated dendritic cells were capable of eliciting antigen-specific T-cell responses and potent anti-mycobacterial protective immunity in a murine model of experimental tuberculosis infection.—Authors' Abstract

Thangaraj, H. S., Adjei, O., Allen, B. W., Portaels, F., Evans, M. R. W., Banerjee, D. K. and Wansbrough Jones, M. H. *In vitro* activity of ciprofloxacin, sparfloxacin, ofloxacin, amikacin and rifampicin against Ghanaian isolates of *Mycobacterium ulcerans*. *J. Antimicrob. Chemother.* **45** (2000) 231–233.

MICs of ciprofloxacin, sparfloxacin, ofloxacin, amikacin and rifampin were determined for 14 primary clinical isolates and three reference isolates of *Mycobacterium ulcerans* by modifying a standard agar dilution method for testing *M. tuberculosis* sensitivity. All these antimicrobials were active against every isolate of *M. ulcerans*. Sparfloxacin exhibited the highest activity and ofloxacin was the least effective. Rifampin exhibited the broadest range of activity.—Authors' Abstract

Thiele, A., Thormann, M., Hofmann, H. J., Naumann, W. W., Eger, K. and Hauschildt, S. A possible role of N-cadherin in thalidomide teratogenicity. *Life Sci.* **67** (2000) 457–461.

Several experimental findings indicate that the adhesion molecule N-cadherin

participates in distinct processes of embryogenesis that spatiotemporally correlate with high sensitivity to thalidomide. Therefore, we suppose that thalidomide might interfere with N-cadherin-mediated interactions. This hypothesis is supported by protein-ligand docking studies simulating and characterizing the binding of thalidomide to N-cadherin molecules. Thalidomide was found to bind at the N-terminal domain of N-cadherin, mimicking a tryptophan residue which is critical for the homodimerization of the adhesion molecule. Based on these results, we suggest that thalidomide might disturb cellular recognition and migration processes in morphogenesis by interaction with N-cadherin.—Authors' Abstract

Torrelles, J. B., Chatterjee, D., Lonca, J. G., Manterola, J. M., Ausina, V. R. and Brennan, P. J. Serovars of *Mycobacterium avium* complex isolated from AIDS and non-AIDS patients in Spain. *J. Appl. Microbiol.* **88** (2000) 266–279.

Antigen fingerprinting based on surface glycolipid antigens was applied to the epidemiology of clinical isolates of the *Mycobacterium avium* complex from 128 acquired immunodeficiency syndrome (AIDS) and 31 non-AIDS patients from several different regions of Spain. The application of thin-layer chromatography, gas chromatography-mass spectrometry and monoclonal antibodies, combined with ELISA, allowed a facile identification, differentiation and classification of the isolates. The cumulative results demonstrate that, among the clinical isolates, serovar 4 was predominant in both AIDS (33.6%) and non-AIDS (22.6%) isolates. In general, the results demonstrate geographical as well as disease-related differences in the distribution of *M. avium* complex serovars of clinical importance.—Authors' Abstract

Turner, O. C., Roberts, A. D., Frank, A. A., Phalen, S. W., McMurray, D. M., Content, J., Denis, O., D'Souza, S., Tanghe, A., Huygen, K. and Orme, I. M. Lack of protection in mice and necrotizing bronchointerstitial pneumonia with bronchiolitis in guinea pigs immunized with vaccines directed against the

hsp60 molecule of *Mycobacterium tuberculosis*. *Infect. Immun.* **68** (2000) 3674–3679.

In this study, the hsp60 and hsp70 heat-shock protein antigens of *Mycobacterium tuberculosis* were tested as potential vaccine candidates using purified recombinant protein antigens or antigens encoded in the form of a DNA plasmid vaccine. Guinea pigs vaccinated with a mixture of the two proteins showed no evidence of resistance to two-dose aerosol challenge infection and quickly developed severe lung damage characterized by necrotizing bronchointerstitial pneumonia and bronchiolitis. As a result, we turned instead to a DNA vaccination approach using a plasmid encoding the hsp60 antigen of *M. tuberculosis*. Although immunogenic in mice, vaccination with plasmid DNA encoding hsp60 was not protective in that model or in the guinea pig model and again gave rise to similar severe lung damage. This study seriously questions the safety of vaccines against tuberculosis that target highly conserved heat-shock proteins.—Authors' Abstract

van Crevel, R., Karyadi, E., Preyers, F., Leenders, M., Kullberg, B. J., Nelwan, R. H. H. and van der Meer, J. W. M. Increased production of interleukin-4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. *J. Infect. Dis.* **181** (2000) 1194–1197.

In tuberculosis, cellular immunity is considered to be responsible for the eradication of infection but also for damage of host tissues. In animal models, the balance between Th1-type cytokines, especially interferon (IFN)-gamma, and Th2-type cytokines, primarily interleukin (IL)-4, seems crucial for these effects. Reports on Th1-type and Th2-type cytokines in human tuberculosis are conflicting, and little is known about their role in tissue damage. Flow cytometric assessment of cytokine responses was performed in human immunodeficiency virus (HIV)-seronegative patients with active tuberculosis and in healthy controls. Patients and controls

showed no significant difference in expression of IFN- γ . However, patients showed a striking increase in production of IL-4 in CD4+ as well as CD8+ T cells. Most remarkably, the expression of IL-4 was especially elevated in patients with cavitary tuberculosis. The Th2-type response with increased production of IL-4 in patients with tuberculosis may antagonize host defense and lead to tissue necrosis.—Authors' Abstract

Wang, L., Slayden, R. A., Barry, C. E. and Liu, J. Cell wall structure of a mutant of *Mycobacterium smegmatis* defective in the biosynthesis of mycolic acids. *J. Biol. Chem.* **275** (2000) 7224–7229.

A mutant strain of *Mycobacterium smegmatis* defective in the biosynthesis of mycolic acids was recently isolated [Liu, J., and Nikaido, H. (1999) *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4011–4016]. This mutant failed to synthesize full-length mycolic acids and accumulated a series of long chain beta-hydroxymycolates. In this work, we provide a detailed characterization of the localization of meromycolates and of the cell-wall structure of the mutant. Thin-layer chromatography showed that the insoluble cell-wall matrix remaining after

extraction with chloroform/methanol and SDS still contained a large portion of the total meromycolates. Matrix-assisted laser desorption/ionization and electrospray ionization mass spectroscopy analysis of fragments arising from Smith degradation of the insoluble cell-wall matrix revealed that the meromycolates were covalently attached to arabinogalactan at the 5-OH positions of the terminal arabinofuranosyl residues. The arabinogalactan appeared to be normal in the mutant strain, as analyzed by NMR. Analysis of organic phase lipids showed that the mutant cell wall contained some of the extractable lipids but lacked glycopeptidolipids and lipooligosaccharides. Differential scanning calorimetry of the mutant cell wall failed to show the large cooperative thermal transitions typical of intact mycobacterial cell walls. Transmission electron microscopy showed that the mutant cell wall had an abnormal ultrastructure (without the electron-transparent zone associated with the asymmetric mycolate lipid layer). Taken together, these results demonstrate the importance of mycolic acids for the structural and functional integrity of the mycobacterial cell wall. The lack of highly organized lipid domains in the mutant cell wall explains the drug-sensitive and temperature-sensitive phenotypes of the mutant.—Authors' Abstract