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# CURRENT LITERATURE

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

## General and Historical

**Andrade, V., Virmond, M., Gil Suarez, R., Moreira, T., Fernando, G. and Custodio, A.** New approach to accelerate the elimination of leprosy. Hansen. Int. **24** (1999) 49–54.

With the existing effective regimens for treatment (MDT/WHO), it is unbearable that, in the turn of the century, leprosy is still an important and worrying problem of public health in Brazil. Although some estimation could lead to the conclusion that the increasing rate of new cases detected in our country is linked to improved quality of health care, the size of the problem is expressive and calls for innovative actions to cope with.

In a country with the size and cultural variety of Brazil, it is essential to take advantage of the principles of the health system in Brazil (SUS) to effectively increase coverage of MDT. In this regard, CONASEMS, being the political representative of secretaries of health of the municipalities of Brazil, is the most appropriate locus to center a multi-participative taskforce with technical competence, financial flexibility and political support to introduce a carefully designed group of actions aiming at the acceleration of the elimination of leprosy as a public health problem. Partnership is also essential, mainly from the community and its organized representatives (MORHAM) as well as national and international nongovernmental organizations (ILEP) and intergovernmental organizations (PAHO/WHO).

CONASEMES is the organization with the power to guarantee the essence of the proposal—to increase extensively the coverage of MDT; taking into consideration this extensive expansion of care, simplification and dismystification of leprosy is essential and is another key point in the proposal; the new and positive image of leprosy is the framework of these initiatives, since we want leprosy diagnosis and treatment reaching every patient in every village.—Authors' Conclusion

**Bhatki, W. S. and Singh, M. G.** Modified leprosy elimination campaign in Mumbai (Bombay), India—a report. Lepr. Rev. **70** (1999) 459–464.

With appropriate planning and preparation, a modified leprosy elimination campaign (MLEC) was undertaken in Brihan Mumbai (Bombay), which has a population of around 11 million. For the campaign, 4879 nonleprosy paramedical and nonmedical personnel were trained and utilized as searchers. The MLEC revealed 1410 new leprosy cases, with a new case detection rate of 1.83/10,000. Over 80% of all cases detected were either single-lesion or paucibacillary (PB), and thus of limited significance with regard to transmission. Further efforts are required to detect and treat cases of consequence (those with more than five lesions and those with positive skin smears) and to identify reservoirs of infection.—Authors' Summary

**Dharmshaktu, N. S., Barkakaty, B. N., Patnaik, P. K. and Arif, M. A.** Progress towards elimination of leprosy as a public health problem in India and role of modified leprosy elimination campaign. Lepr. Rev. **70** (1999) 430–439.

India (population 943 million) has seen a highly significant decrease in the prevalence of leprosy since the introduction of

multidrug therapy (MDT) in 1981. From a prevalence rate of 57/10,000 of the population in March 1981, the figure has declined to 5.2/10,000 in March 1999. This was possible due to the creation of a completely vertical (specialized) infrastructure for leprosy control in the 218 endemic districts of the country and skeleton vertical staff in the remaining districts, coupled with the recruitment of additional staff on contract basis to provide MDT through vertical staff in endemic districts and mobile treatment units in the moderate and low-endemic districts. Despite all efforts, however, new case detection has not shown a decline over the last 14 years due to the presence of hidden (and undiagnosed) cases. Therefore, in order to intensify and hasten progress toward elimination (less than 1 case per 10,000 of the population) in the whole country, it was decided to implement a massive leprosy elimination campaign (LEC) in all the States/Union Territories (UTs). The reports of 22 States/UTs indicate that 415 out of the total of 490 districts in the country were covered by modified LEC (MLEC), with 85% coverage of the population. The campaign used in India was modified from the pattern previously described by the World Health Organization. The detection of hidden or suspected cases took place within a short, intensive period of 6–7 days and relied heavily on house-to-house searches by General Health Care staff trained in leprosy detection and confirmation was made by appropriately trained staff. This MLEC received widespread government and public support, resulting in the detection of 454,290 hidden cases of leprosy, while providing training to a large number of General Health Care staff and volunteers and creating widespread awareness about leprosy and the availability of treatment free of charge for all cases. This program proved to be one of the most successful health care interventions undertaken in India in recent years, particularly in the states of Bihar and Orissa. Although a few states in India are unlikely to reach the current WHO goal of elimination before end of the year 2000, the results of the MLEC strongly support the possibility that elimination levels will be achieved in the majority of states by the end of the year 2000 and at national level by the end of the year 2002.—Authors' Summary

**Patnaik, P. K. B.** Modified leprosy elimination campaign (MLEC) in the state of Orissa, India. Lepr. Rev. **70** (1999) 440–447.

As part of a country-wide, modified leprosy elimination campaign (MLEC) carried out in 21 selected states in India in 1998, the state of Orissa launched activities in early January of that year, during which 28.9 million people were examined, giving 85% coverage of the enumerated population. Using general health care staff and volunteers, 416,604 suspected cases were identified and 62,804 of these were confirmed as leprosy by experienced observers. The period of intensive search activity lasted 1 week only, but this was preceded by several months of community mobilization and involvement, health education, training of government and voluntary staff, media messages and the involvement of all relevant health departments, officials and politicians. Both this and the intensive search period were characterized by a high level of interest and cooperation by all concerned. The total of new cases detected and put on treatment (multidrug therapy; MDT) during the period of only 7 days was approximately equal to that which, on routine population survey by the leprosy services, would be recorded over a period of 2 years. The MLEC in Orissa is judged to have been not only an historic step forward in the control of leprosy in a state previously classified as highly endemic for leprosy, but also one of the most successful state health interventions ever mounted. In the 5 months after completion of the campaign, the voluntary reporting rate increased from 50% to 90%. As a direct result of the campaign, facilities for the diagnosis and treatment of leprosy are now available daily in an additional 1639 institutions, over and above those in existence before the campaign was launched. The achievements in terms of detecting hidden (and thus undiagnosed and untreated) cases exceeded the outset predictions, underlining the importance of continued vigilance and the need to maintain involvement of general health care staff. It is anticipated that the rise in prevalence due to the addition of 62,884 cases will be reduced by the implementation of MDT by 80% by about March 1999. Overall the results of

the MLEC in Orissa strongly support the likelihood that an elimination level of less than 1 case per 10,000 of the population will be reached in this state by the year 2000.—Author's Summary

**Smith, W. C. S.** Future scope and expectations: why, when, and how LECs should continue. Lepr. Rev. **70** (1999) 498–505.

There is a strong case to continue to use LEC approaches since they are a comprehensive and cost effective means of delivering the key elements of leprosy control. LECs should be conducted when there is evidence of large numbers of hidden cases. Probably a minimum of two LECs is required but where large numbers of new cases continue to be detected they could be run on an annual basis. The methodology of LECs needs to be improved through experience, evaluation and from LECs conducted elsewhere; feedback from the community is also important. There is room to improve all aspects of LECs: planning, training, education, diagnosis and treatment completion.—Author's Summary

**Sofola, O.** Leprosy elimination campaigns: the Nigerian experience. Lepr. Rev. **70** (1999) 465–471.

In Nigeria leprosy elimination campaigns (LEC) have been found to be a useful intervention to improve case detection and to facilitate the integration of leprosy services within the general health services. LEC has indeed strengthened our routine leprosy services and enables us to involve new partners—general health workers and volunteers in the fight against leprosy. Therefore, their continuation in other selected areas of the country is most justified to complete and sustain leprosy elimination activities at the subnational level.—Author's Conclusion

**Vijaykumaran, P., Prabhakar Rao, T. and Krishnamurthy, P.** Pace of leprosy elimination and support teams in Bihar State, India. Lepr. Rev. **70** (1999) 452–458.

Despite the extensive implementation of multiple drug therapy (MDT) in most leprosy-endemic countries worldwide since 1982, bringing about a remarkable reduction in prevalence, there are still regions at the subnational level where the implementation of MDT remains difficult. The state of Bihar (population 86.3 million) in India is a good example of such a region. Previously rated as one of the most highly endemic states, it still contributes about 21% of the total caseload in India and about 12% of the global caseload. For various reasons, case finding and drug treatment have lagged behind the progress made in most other states in the country and in 1996 the Damien Foundation India Trust (DFIT) volunteered technical support to increase the pace of elimination. Sixteen out of the 39 districts in the state were allocated, with a population of 41.8 million. Support teams, including a medical advisor and a nonmedical supervisor, both with over 10 years' experience of leprosy work and control programs, were provided to assist and work alongside government staff in case detection, treatment delivery, case holding and discharge in their respective areas of operation. New case detection by intensive survey increased by 394% and total new case detection by 226% during the year 1996–1997, with similar trends in the following year. Striking improvements were also observed in MDT coverage, treatment regularity, monitoring and discharge of patients and in the training of local staff. This collaboration between a nongovernment agency (DFIT) and the staff of the National Leprosy Eradication Programme in 16 out of 39 districts in the state of Bihar has clearly been extremely successful. Similar approaches in the remaining districts of Bihar, and in other parts of India, where the infrastructure is available but inadequate, may contribute significantly to achieving the elimination goal at national and subnational levels.—Authors' Summary

**Virmond, M.** Role of leprosy related research and training institutions in management and prevention of disabilities and rehabilitation. Hansen. Int. **24** (1999) 38–42.

As a conclusion, in the field of prevention of disability and rehabilitation, research and training institutions should be

regarded as specialized units responsible to maintain and to further develop knowledge. However, no matter the complexity of actions developed in these institutions, they should bear in mind that their most relevant role is to be ready to provide the control program in the field with adequate answers to their needs to achieve an effective control of leprosy as a public health problem, before and beyond the year 2000.—Author's Conclusion

## Chemotherapy

**Jacobs, M. R.** Activity of quinolones against mycobacteria. Drugs **58** Suppl. 2 (1999) 19–22.

The fluoroquinolones have been shown to be active *in vitro* against many mycobacterial species, including most strains of *Mycobacterium tuberculosis* complex and *M. fortuitum,* and some strains of *M. kansasii, M. avium-intracellulare* (MAI) complex and *M. leprae.* Ciprofloxacin, ofloxacin and sparfloxacin are the best studied of these agents to date, and are among the most active of this group against *M. tuberculosis* and other mycobacteria. Treatment of patients with multidrug-resistant pulmonary tuberculosis using ofloxacin has resulted in the selection of quinolone-resistant mutants in a few patients. Many strains of MAI, however, are resistant to fluoroquinolones, and structure-activity relationships and DNA gyrase studies have been undertaken to identify the moieties associated with activity and the lack thereof. The genetic and molecular basis of quinolone resistance in mycobacteria has revealed both the recent progress made in these areas and the limitations of the quinolones against this genus. Considerable progress will need to be made in resolving these issues in order for the quinolones to become clinically useful antimycobacterial agents.—Author's Abstract

**Katoch, K., Natarajan, M., Katoch, V. M., Singh, H. B. and Bhatia, A. S.** Chemotherapy trial in paucibacillary leprosy using clofazimine. Indian J. Lepr. **71** (1999) 311–324.

In a double blind study, 300 paucibacillary patients (smear negative, indeterminate, tuberculoid and borderline tuberculoid) were randomly allotted to two regimens: the control subjects (150 patients) received the standard WHO multidrug regimen of six doses of once a month rifampin with daily dapsone therapy for 6 months, while the study group (150 patients) received 50 mg of clofazimine daily for 6 months in addition to the WHO regimen. After stoppage of therapy all the patients were followed up on placebo. The regimens were well tolerated. In 7.5% of patients on the clofazimine-containing regimen, the lesions showed persisting activity at the time of stoppage of therapy, compared with 16% on the control regimen. This activity subsided spontaneously, more rapidly, in the study group (80% compared with 30% in the control group) in 6 months. Two patients in the control group and one patient in the study group developed late reactions. There were no relapses in the study group; whereas two patients have relapsed in the control group during a follow up of 2.5 to 3.5 years.—Authors' Abstract

**Prabhakaran, K., Harris, E. B. and Randhawa, B.** Bactericidal action of ampicillin/sulbactam against intracellular mycobacteria. Int. J. Antimicrob. Agents **13** (1999) 133–135.

The resistance of mycobacteria to β-lactam antibiotics is attributed to their ability to synthesize β-lactamase. In our previous studies, β-lactam/β-lactamase-inhibitor combinations suppressed the growth of several mycobacteria in axenic cultures and ampicillin/sulbactam was bactericidal to *Mycobacterium tuberculosis* H37Rv *in vitro* and to *M. leprae* multiplying in mouse footpads. Since both these organisms multiply in phagocytic cells in the host, it is important to know whether the drug combination is active against mycobacteria multiplying in macrophages. We tested the action of

ampicillin/sulbactam against four potentially pathogenic (to humans or to animals) mycobacteria, *M. simiae, M. haemophilum, M. avium, M. microti,* when phagocytosed by mouse macrophages. Bacteria were exposed to monolayers of peritoneal macrophages harvested from BALB/c mice. Unphagocytosed bacilli were removed and three concentrations of ampicillin/sulbactam were tested. Optimum activity was observed at 100 mg/l which killed 58%–97% of the mycobacteria within macrophages, as determined by the CFU. β-Lactam/β-lactamase-inhibitors, especially ampicillin/sulbactam, might provide an effective alternative therapy against infections caused by mycobacteria resistant to other drugs.—Authors' Abstract

**Sharma, A., Sharma, V. K., Rajwanshi, A., Das, A., Kaur, I. and Kumar, B.** Presence of *M. leprae* in tissues in slit skin smear negative multibacillary (MB) patients after WHO-MBR. Lepr. Rev. **70** (1999) 281–286.

This study looked for *M. leprae* in the lymph node, nerve and skin of multibacillary (MB) leprosy patients who become slit-skin-smear negative after the completion of WHO-MBR. Twenty-five WHO-MBR-treated MB leprosy patients were studied; borderline lepromatous (BL) leprosy (N = 11) and lepromatous (LL) leprosy (N = 14). Fifteen patients had reaction (erythema nodosum leprosum 11, upgrading reaction 4) either at presentation or during therapy. All patients attained slit-skin-smear negativity after WHO-MBR (range 24–39 months). Sixteen (64%) patients with MB leprosy showed fragmented bacilli in skin and nerve biopsy or lymph node aspirates after WHO-MBR. Lymph node aspirates alone revealed *M. leprae* in 7 patients, followed by nerve in 2 and skin in 1 patient. Four cases showed *M. leprae* at all sites followed by nerve and skin or lymph node in one case each. A pretreatment bacterial index (BI) of 4+ or more was significantly associated with the presence of *M. leprae* at the end of treatment. Also, significantly more lymph node aspirates contained *M. leprae* in comparison with nerve or skin biopsies. All 7 cases in whom treatment was extended beyond 24 months showed *M. leprae* in tissues even after attaining slit-smear negativity. In conclusion, *M. leprae* persist in tissues after 2 years of WHO-MBR and patients with an initial BI of 4+ or more need to be closely followed up after stopping MDT.—Authors' Summary

**Tomioka, H., Sato, K., Akaki, T., Kajitani, H., Kawahara, S. and Sakatani, M.** Comparative *in vitro* antimicrobial activities of the newly synthesized quinolone HSR-903, sitafloxacin (DU-6859a), gatifloxacin (AM-1155), and levofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **43** (1999) 3001–3004.

We compared the *in vitro* antimycobacterial activity of a new fluoroquinolone, HSR-903, with strong activity against gram-positive cocci with those of levofloxacin (LVFX), sitafloxacin (STFX), and gatifloxacin (GFLX). The MICs of the quinolones for *Mycobacterium tuberculosis* and *M. avium* complex were in the order STFX $\approx$ SGLX < LVFX $\leqq$ HSR-903 and STFX $\leqq$ GFLX $\leqq$ HSR-903 $\leqq$ LVFX, respectively. HSR-903 effectively eliminated intramacrophagial *M. tuberculosis,* as did LVFX, and exhibited bacteriostatic effects against *M. tuberculosis* replicating in type II alveolar cells.—Authors' Abstract

**van Rensburg, C. E. J., Joone, G. K., Siegel, F. A., Matlola, N. M. and O'Sullivan, J. F.** *In vitro* investigation of the antimicrobial activities of novel tetramethylpiperidine-substituted phenazines against *Mycobacterium tuberculosis.* Chemotherapy **46** (2000) 43–48.

The intra- and extracellular activities of five novel tetramethylpiperdine (TMP)-substituted phenazines against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) were determined and compared with those of clofazimine and rifampin. Two of these agents, together with clofazimine, were also tested for their activities against drug-resistant strains of *M. tuberculosis.* Three of the TMP-substituted phenazine compounds were significantly more active than clofazimine against *M. tuberculosis,* including multidrug-resistant clinical strains of this

microbial pathogen, demonstrating a lack of crossresistance between the riminophenazines and standard antituberculous drugs. Using *M. tuberculosis*-infected monocyte-derived macrophages, all of the TMP-substituted phenazines were found to possess intracellular activity which was superior to that of both clofazimine and rifampin. In this model of intracellular bioactivity, the experimental compounds inhibited bacterial growth at concentrations which were approximately 10-fold lower than the corresponding minimal inhibitory concentration values obtained using conventional *in vitro* sensitivity testing procedures. These results demonstrate that the novel TMP phenazines are active against multidrug-resistant *M. tuberculosis* strains, and particularly effective intracellularly.—Authors' Abstract

# Clinical Sciences

**Anderson, A. M. and Croft, R. P.** Reliability of Semmes Weinstein monofilament and ballpoint sensory testing, and voluntary muscle testing in Bangladesh. Lepr. Rev. **70** (1999) 305–313.

The reliability of methods of testing nerve function is important, since diagnostic decision making is a direct function of the quality of the test. Three methods of nerve function testing were investigated at the Danish Bangladesh Leprosy Mission (DBLM) in north Bangladesh, and assessed for inter-observed reliability. The three methods were 1) ballpoint pen test (BPT) for sensory function; 2) graded Semmes Weinstein monofilament test (SWM) for sensory function and 3) voluntary muscle testing (VMT) for motor function. The weighted kappa ($\kappa_w$) statistic was used to express inter-observer reliability. Using this statistic, 0 represents agreement no better than random, and 1.0 complete agreement. $\kappa_w$ values of $\geq 0.80$ are reckoned to be adequate for monitoring and research. Fifty-three patients were tested, a senior physiotechnician acting as "gold standard" against whom four other staff physiotechnicians were assessed. All three testing methods were found to have minimal inter-observer variation, with the $\kappa_w$ for inter- observer agreement using BPT being 0.86, the SWM 0.92, and VMT 0.94. It is concluded that in trained and experienced hands, all three methods are reliable and repeatable to a level allowing confident use of results obtained in monitoring and research.—Authors' Summary

**Braga, F. J. H. N., Foss, N. T., Ferriolli, E., Pagnano, C., Miranda, J. R. D. and deMoraes, R.** The use of bone scintigraphy to detect active Hansen's disease in mutilated patients. Eur. J. Nucl. Med. **26** (1999) 1497–1999.

Mutilation of extremities was very frequent in patients affected by leprosy in the past; although it is now much less common, it is still seen, mainly in patients with long-term disease. In general, mutilation of the nose and ears is caused by the bacillus and mutilation of the hands and feet a consequence of chronic trauma. Leprosy must be chronically treated and any decision to interrupt therapy is based on laboratory tests and biopsy. Scintigraphy is a noninvasive procedure which could be of great value in determining disease activity. We studied 8 patients (5 males and 3 females, aged 64–73 years) who presented with mutilation of the nose (2), ear (1), feet (3) or foot and hand (2). Conventional three-phase bone scintigraphy (750 MBq) and X-ray examinations of the affected areas were performed in all patients. Bone scintigraphy was abnormal in 4 patients (the presence of bacilli was confirmed by biopsy in 2 of them), and normal in the other 4. In all patients except for the one with ear mutilation, radiography only showed the absence of bone. We conclude that bone scintigraphy is very useful to determine disease activity in cases of mutilation caused by leprosy. It seems to be superior to conventional radiography and may enable bone biopsies to be avoided.—Authors' Abstract

**de Almeida, J. A., Vitti, M. and Garbino, J. A.** [Anatomic study of Martin-Gruber anastomosis.] Hansen Int. **24** (1999) 15–20. (in Portuguese)

Forty forearms of human cadavers were dissected and studied. Five (12.5%) were found to have Martin-Gruber anastomosis (MG.A), two on the right side and three on the left side. From the total of five, two cases of this anastomosis occurred among the branches reserved to the flexor digitorum profundus muscle, one from the branch of the anterior interosseous nerve, and two directed from the median to the ulnar nerve. In this study, MG.A was found in agreement with the percentages of the literature.—Authors' English Summary

**Lemieux, L., Cherian, T. A. and Richard, B.** The stapedial reflex as a topographical marker of proximal involvement of the facial nerve in leprosy; a pilot study. Lepr. Rev. **70** (1999) 324–332.

This study aimed to determine the parameters necessary for a study of stapedial reflexes in leprosy patients to ascertain if the facial nerve is involved more proximally than the stylomastoid foramen. It involved leprosy patients with and without facial nerve involvement and nonleprosy controls. Clinical examination of the patients' ears, a tympanogram and audiogram to exclude conductive and sensorineural deafness, followed by the measurement of a stapedial reflex and the acoustic reflex threshold, were carried out. The number of absent reflexes and the acoustic reflex thresholds did not differ between the three groups of subjects. A definitive study would be logistically impossible. Suggestions are made as to more exact patient selection in order to demonstrate any stapedial reflex changes due to leprosy. The findings of this study do not suggest that facial nerve pathology extends proximally to the stylomastoid foramen, unless such proximal involvement is subclinical to the detection methods used.—Authors' Summary

**Lockwood, D. N. J.** Nerve damage in leprosy: a problem for patients, doctors and scientists. (Kellersberger Memorial Lecture 1998). Ethiop. Med. J. **37** (1999) 133–140.

The pathology, detection and treatment of leprosy nerve damage are discussed. Present knowledge and future developments are considered.—Trop. Dis. Bull. **96** (1999) 1156

**Nadkarni, N. S. and Rege, V. L.** Significance of histopathological classification in leprosy. Indian J. Lepr. **71** (1999) 325–332.

A retrospective blind study was carried out on 2640 patients of leprosy to correlate the histopathological and clinical classification of leprosy using the criteria laid down by Ridley and Jopling. There was complete agreement between histopathological and clinical classification in 81.8% of the cases, with one-step deviation in 5.1% of the cases. Histopathological diagnosis of indeterminate leprosy in high percentage (15.9%) as against 3.3% of indeterminate leprosy clinically in our series was an interesting feature. Type-wise correlation between histopathological with clinical classification was very high, it being the highest in LL (98%) followed by TT (97%), BT, BB and BL (95%, 89% and 87%, respectively).—Authors' Abstract

**Richard, B. M. and Jacobs, J. M.** Facial nerve pathology in leprosy: searching for the proximal extent of the lesion in facial nerve biopsies. Lepr. Rev. **70** (1999) 333–344.

A light and electron microscope study was made of resin-embedded facial nerves in three cases of leprosy involving the facial nerve. The patients had irreversible facial nerve palsies and had requested facial reconstruction. No consistent pattern of nerve fiber damage was found. In one case the temporozygomatic was affected, but the cervical branch was normal, suggesting the damage begins distally. In two cases the loss of nerve fibers in the trunk and all branches was similar, and is likely to emanate from damage at a more proximal site. The presence of increased numbers of un-

myelinated axons, often in clusters, is evidence of regeneration. These axons probably have the potential to develop into functional myelinated fibers provided that they can innervate a viable distal target such as a muscle graft. These regenerating axons are distal to the stylomastoid foramen suggesting that the most proximal level of involvement of the facial nerve could be intracranial. The finding of a more proximal level of nerve involvement, implies that the misreinnvervation seen in partially recovered facial nerve palsies in leprosy could be due to some regenerating axons being misdirected at the level of the main trunk bifurcation.—Authors' Summary

**Selvasekar, A., Ebenezer, G. J. and Partheebarajan, M.** Lepromatous lymphadenopathy and concomitant tuberculous axillary lymphadenitis with sinus; a case report. Lepr. Rev. **70** (1999) 345–350.

A 25-year-old male patient with florid lepromatous leprosy presented with right axillary lymphadenopathy and a discharging sinus. He also had scabies with chronic right otitis media. Histopathological examination of the lymph node revealed lepromatous lymphadenitis coexisting with tuberculosis. This unusual combination of two different clinical entities is recorded in this case report.—Authors' Summary

**Sukpanichnant, S., Hargrove, N. S., Kachintorn, U., Manatsathit, S., Chanchairujira, T., Siritanaratkul, N., Akaraviputh, T. and Thakerngpol, K.** Clofazimine-induced crystal-storing histiocytosis producing chronic abdominal pain in a leprosy patient. Am. J. Surg. Pathol. **34** (2000) 129–135.

Clofazimine-induced, crystal-storing histiocytosis is a rare but well-recognized condition in the literature. Besides the common reddish discoloration of the skin, clofazimine produces gastrointestinal disturbances—sometimes severe abdominal pain, prompting exploratory laparotomy, because pathologic and radiologic findings can produce diagnostic difficulties if the pathologic changes caused by clofazimine are not recognized. The authors report such a case in a leprosy patient to emphasize the importance of history taking, the radiologic abnormalities of the small intestine, and the pathologic findings in small intestine and lymph node biopsies. Clofazimine crystals are red in the frozen section and exhibit bright-red birefringence. However, they are clear in routinely processed histologic sections because they dissolve in alcohol and organic solvents. They also appear as clear crystal spaces during electron microscopic study, but some osmiophilic bodies can be observed. Histiocytosis caused by clofazimine crystals produces infiltrative lesions in radiologic studies mimicking malignant lymphoma or other infiltrative disorders. Associated plasmacytosis in the histologic sections can simulate lymphoplasmacytic lymphoma or multiple myeloma with crystal-storing histiocytosis. With the knowledge of this rare condition caused by clofazimine, appropriate management to avoid an unnecessary laparotomy is possible.—Authors' Abstract

**Sundar Rao, P. S. S., Augustine, V. and Joseph, G. A.** Being a female leprosy patient in South India. Indian J. Lepr. **71** (1999) 279–284.

The problems of women patients as revealed by a case study of a woman patient and a questionnaire study of 100 leprosy patients (47 men and 53 women) are presented. These include, besides general ones like ignorance of facts about the disease, specific ones like lack of privacy during clinical examination, indifference toward women's feelings and difficulties in communicating with male workers. A greater sensibility toward the sentiments and problems of women patients on the part of the health service is required to amend the situation. Recruiting more women workers might help in this regard.—Authors' Abstract

**Vaquero, N. L., Ortiz, M. C., Soto, I., Bruni, M. E., Yonadi, V. and Weiberlen, H.** [Late reversal reactions in Hansen's disease.] Rev. Argent. Dermatol. **80** (1999) 152–156. (in Spanish)

Late reversal reactions are acute events that arise in leprosy patients after finishing

their treatment, when their CMI improves. The differential diagnosis between reversal reaction and relapses is often difficult and it involves a different treatment with a new course of multidrug therapy in the latter. Several guidelines may help to make the right diagnosis. Steroids may be a therapeutic proof when severe neural involvement exists as is usually seen in reversal reactions. Its early use allows one to avoid disabilities.—Authors' English Summary

## Immuno-Pathology

**Alcais, A., Sanchez, F. O., Thuc, N. V., Lap, V. D., Oberti, J., Lagrange, P. H., Schurr, E. and Abel, L.** Granulomatous reaction to intradermal injection of lepromin (Mitsuda reaction) is linked to the human NRAMP1 gene in Vietnamese leprosy sibships. J. Infect. Dis. **181** (2000) 302–308.

The Mitsuda test, which measures the specific immune response against intradermally injected lepromin, has a high prognostic value for susceptibility or resistance to the lepromatous form of leprosy. A sib-pair linkage analysis between the Mitsuda response and the NRAMP1 gene was done among 20 nuclear families with leprosy (totaling 188 sibs) from Ho Chi Minh City, Vietnam. All family subjects were genotyped for several intragenic and flanking NRAMP1 markers, leading to the definition of a fully informative NRAMP1 haplotype. Significant linkage was observed between NRAMP1 and Mitsuda reaction when considered either as a quantitative ($p = 0.002$) or as a categorical ($p = 0.001$) trait. Separate analyses among healthy and affected sibs showed evidence for linkage in both subsamples, indicating that linkage between the Mitsuda reaction and NRAMP1 is independent of leprosy status. These results support the view that NRAMP1 plays a regulatory role for the development of acquired antimycobacterial immune responses as determined by *in vivo* Mitsuda test reaction.—Authors' Abstract

**Andreu, D., Carreno, C., Linde, C., Boman, H. G. and Andersson, M.** Identification of an anti-mycobacterial domain in NK-lysin and granulysin. Biochem. J. **344** (1999) 845–849.

NK-lysin and granulysin are homologous cationic antibacterial peptides produced by pig and human cytolytic lymphocytes, respectively. The solution structures of NK-lysin comprises five amphipathic alpha-helices. To investigate the properties of a helix-loop-helix region postulated to be a membrane-docking part of NK-lysin, we synthesized 22- and 29-residue peptides reproducing this region for both NK-lysin and granulysin. CD spectroscopy of the synthetic peptides in a liposomal solution showed spectra typical of alpha-helical peptides. The peptides were active against gram-positive and gram-negative bacteria, with the two NK-lysin peptides showing higher antibacterial activities than the two from granulysin. One NK-lysin peptide was active against *Pseudomonas aeruginosa* and *Staphylococcus aureus,* two organisms against which NK-lysin is inactive. Granulysin peptides were inactive against these bacteria, in contrast with granulysin, which is known to be active against them. Both NK-lysin and all synthetic analogs killed *Mycobacterium tuberculosis* and K562 tumor cells, but did not display hemolytic activity. These results identify a potent anti-mycobacterial domain in NK-lysin and granulysin consisting of a 22-residue (helix 3) sequence plus a disulfide-constrained loop.—Authors' Abstract

**Batoni, G., Esin, S., Pardini, M., Bottai, D., Senesi, S., Wigzell, H. and Campa, M.** Identification of distinct lymphocyte subsets responding to subcellular fractions of *Mycobacterium bovis* bacille Calmette-Guerin (BCG). Clin. Exp. Immunol. **119** (2000) 270–279.

In order to investigate the ability of *Mycobacterium bovis* BCG vaccination to in-

duce immune responses toward different classes of mycobacterial antigens and the cell populations involved in such responses, proliferation of distinct human lymphocyte subsets from BCG-vaccinated donors in response to different subcellular fractions of BCG was analyzed and compared with that of not sensitized subjects. Proliferation of different cell subsets was evaluated by flow cytometric determination of bromodeoxyuridine incorporated into DNA of dividing cells and simultaneous identification of cell surface markers. Although a certain degree of variability was observed among different donors, after 6 days of *in vitro* stimulation BCG-vaccinated subjects displayed, as a mean, a stronger blastogenic response to all the classes of antigens compared with nonsensitized ones. PPD, culture filtrates and membrane antigens induced a predominant proliferation of CD4+ T cells. In contrast, preparations enriched in cytosolic antigens elicited strong proliferation of gamma delta+ T cells which, as a mean, represented 55% of the proliferating cells. Although to a lesser extent, proliferation of gamma delta+ T cells was also elicited by preparations enriched in membrane and cell wall antigens. In response to the latter preparation proliferation of CD4+ T cells and CD16+/CD3– [natural killer (NK)] cells was observed, as well. In particular, cell wall antigens were found to induce significantly higher levels of proliferation of NK cells compared with all the other classes of antigens.—Authors' Abstract

**Belman, C., Espinosa, E., Poupot, R., Peyrat, M. A., Guiraud, M., Poquet, Y., Bonneville, M. and Fournie, J. J.** 3-Formyl-1-butyl pyrophosphate; a novel mycobacterial metabolite activating human gamma delta T cells. J. Biol. Chem. **274** (1999) 32079–32084.

Most human blood gamma delta T cells react without major histocompatibility complex restriction to small phosphorylated nonpeptide antigens (phosphoantigens) that are abundantly produced by mycobacteria and several other microbial pathogens. Although isopentenyl pyrophosphate has been identified as a mycobacterial antigen for gamma delta T cells, the structure of several other stimulating compounds with bioactivities around 1000-fold higher than isopentenyl pyrophosphate remains to be elucidated. This paper describes the structural identification of 3-formyl-1-butyl-pyrophosphate as the core of several non-prenyl mycobacterial phosphoantigens bioactive at the nM range. Recognition of this molecule by gamma delta T cells is very selective and relies on its aldehyde and pyrophosphate groups. This novel pyrophosporylated aldehyde most probably corresponds to a metabolic intermediate of the non-mevalonate pathway of prenyl phosphate biosynthesis in eubacteria and algae. The reactivity to 3-formyl-1-butyl-pyrophosphate supports the view that human gamma delta T cells are physiologically devoted to antimicrobial surveillance.—Authors' Abstract

**Boomershine, C. S., Lafuse, W. P. and Zwilling, B. S.** Beta 2-adrenergic receptor stimulation inhibits nitric oxide generation by *Mycobacterium avium* infected macrophages. J. Neuroimmunol. **101** (1999) 68–75.

Catecholamine regulation of nitric oxide (NO) production by interferon gamma (IFN-γ)-primed macrophages infected with *Mycobacterium avium* was investigated. Epinephrine treatment of IFN-γ-primed macrophages at the time of *M. avium* infection inhibited the anti-mycobacterial activity of the cells. The anti-mycobacterial activity of macrophages correlated with NO production. Using specific adrenergic receptor agonists, the abrogation of mycobacterial killing and decreased NO production by catecholamines was shown to be mediated via the beta 2-adrenergic receptor. Elevation of intracellular cAMP levels mimicked the catecholamine-mediated inhibition of NO in both *M. avium*-infected and LPS-stimulated macrophages. Specific inhibitors of both adenylate cyclase and protein kinase A prevented the beta 2-adrenoceptor-mediated inhibition of nitric oxide production. P2-adrenoreceptor stimulation at the time of *M. avium* infection of IFN-γ-primed macrophages also inhibited expression of iNOS mRNA. These observations show that catecholamine hormones can affect the outcome of macrophage-pathogen interactions and suggest that one result of sympathetic nervous system activation is the sup-

pression of the capacity of macrophages to produce antimicrobial effector molecules.—Authors' Abstract

**Cuevas-Santos, J., Contreras, F. and McNutt, N. S.** Multibacillary leprosy: lesions with macrophages positive for S100 protein and dendritic cells positive for Factor 13a. J. Cutan. Pathol. **25** (1998) 530–537.

The cutaneous lesions of 69 patients with leprosy (42 lepromatous, 5 mid-borderline, and 22 tuberculoid) were evaluated by immunohistochemistry for the expression of S100 protein, CD1a, CD68, muramidase, HLA-DR, and Factor 13a. The macrophages from lesions of polar, subpolar and borderline lepromatous leprosy patients expressed S100 protein intensely and constantly. In contrast, the lesions of polar and subpolar tuberculoid leprosy had very few cells that were immunoreactive for S100 proteins ("S100+") in the granulomas in the dermis. The macrophages in all lesions were reactive for CD68 and muramidase. In paraffin sections, macrophages of lepromatous lesions failed to stain for HLA-DR; whereas in tuberculoid lesions, they were strongly positive for HLA-DR. Three patients with histoid leprosy (relapse lesions) had lesions that were strongly positive for Factor 13a and were negative for S100 protein ("S100–"). It is concluded that given the possible chemotactic and migration inhibition effects of the calcium-binding proteins of the S100 family, these data suggest a possibly important role for S100 protein in the accumulation of macrophages in lepromatous leprosy, and also reveal infection of Factor 13a + dermal dendritic cells in histoid leprosy.—Trop. Dis. Bull. **96** (1999) 1266

**Fafutis-Morris, M., Guillen-Vargas, C. M., Navarro-Fierros, S., Alfaro-Bustamante, F., Zaitzeva-Petrovna, G., Daneri-Navarro, A., Santoscoy-Tovar, L. and Armendariz-Borunda, J.** Addition of anti-CD28 antibodies restores PBMC proliferation and IFN-gamma production in lepromatous leprosy patients. J. Interferon Cytokine Res. **19** (1999) 1237–1243.

During antigen recognition, T lymphocytes are primed by a physical interaction with antigen-presenting cells (APC). At least two signals are needed to activate T cells. One is provided by T-cell receptor (TCR)/CD3 in the context of the major histocompatibility complex (MHC), and another signal is mediated by antigen-independent molecules, that is T-cell membrane-bound CD28 and its specific ligand B7-1 (CD80) present in APC. Both signals trigger a series of metabolic events initiating right at the cell membrane and ending with activation and proliferation of T cells as well as specific cytokine synthesis. Our main goal was to determine whether deficiency in interferon-gamma (IFN-$\gamma$) production shown by peripheral blood mononuclear cells (PBMC) from lepromatous leprosy (LL) patient could be overcome by reconstituting *in vitro* the appropriate signals (by means of addition of anti-CD28 and anti-CD80 monoclonal antibodies). We also determined the stimulation index (SI) in the same PBMC. Our results demonstrated no significant differences in CD80 expression of monocytes and B lymphocytes from LL patients when compared with healthy subjects. Nonetheless, CD28 expression significantly decreased in lymphocytes from LL patients ($p < 0.01$). Regarding IFN-$\gamma$ levels and SI, LL-PBMC failure before mitogenic stimuli could be reversed by further incubation with anti-CD28 antibody, but stimulation by specific antigen of *Mycobacterium leprae* was not changed. Addition of anti-CD80 antibody significantly increased IFN-$\gamma$ levels in phytohemagglutinin (PHA)-stimulated PBMC, although proliferation deficiency persisted. Cells stimulated with specific antigen did not modify either their proliferation or IFN-$\gamma$ levels. —Authors' Abstract

**Faldt, J., Dahlgren, C., Karlsson, A., Ahmed, A. M. S., Minnikin, D. E. and Ridell, M.** Activation of human neutrophils by mycobacterial phenolic glycolipids. Clin. Exp. Immunol. **118** (1999) 253–260.

The interaction between mycobacterial phenolic glycolipids (PGLs) and phagocytes was studied. Human neutrophils were allowed to interact with each of four puri-

fied mycobacterial PGLs and the neutrophil production of reactive oxygen metabolites was followed kinetically by luminol-/isoluminol-amplified chemiluminescence. The PGLs from *Mycobacterium tuberculosis* and *M. kansasii,* respectively, were shown to stimulate the production of oxygen metabolites, while PGLs from *M. marinum* and *M. bovis* BCC, respectively, were unable to induce an oxidative response. Periodate treatment of the *M. tuberculosis* PGL decreased the production of oxygen radicals, showing the importance of the PGL carbohydrate moiety for the interaction. The activation, however, could not be inhibited by rhamnose or fucose, indicating a complex interaction which probably involves more than one saccharide unit. This is in line with the fact that the activating PGLs from *M. tuberculosis* and *M. kansasii* contain tri- and tetrasaccharides, respectively, while the nonactivating PGLs from *M. marinum* and *M. bovis* BCG each contain a monosaccharide. The complement receptor 3 (CR3) has earlier been shown to be of importance for the phagocyte binding of mycobacteria, but did not appear to be involved in the activation of neutrophils by PGLs. The subcellular localization of the reactive oxygen metabolites formed was related to the way in which the glycolipids were presented to the cells.—Authors' Abstract

**Fietta, A., Francioli, C. and Grassi, G. G.** Mycobacterial lipoarabinomannan affects human polymorphonuclear and mononuclear phagocyte functions differently. Haematologica **85** (2000) 11–18.

Background and Objectives. The role of mycobacterial lipoarabinomannan (LAM) in regulating the granulomatous responses and its effects on cells involved in early responses to tuberculosis have not been clearly defined. The aim of this study was to acquire further evidence about the mechanisms by which LAM takes part in the host response to mycobacterial infections.

Design and Methods. We compared the *in vitro* ability of mannosylated LAM (ManLAM) and LAM lacking the terminal mannosyl units (AraLAM) to induce distinct responses in human polymorphonuclear (PMNs) and mononuclear phagocytes [both monocytes and 48-hr monocyte-derived macrophages (MDMs)]. The responses examined were chemotaxis, transient changes in free cytosolic calcium, phagocytosis and metabolic activation.

Results. AraLAM and ManLAM affected mononuclear, but not polymorphonuclear, phagocyte functions. Both forms of LAM were chemotactic for monocytes and MDMs. The LAM-induced chemotactic response required new protein synthesis, did not induce a rise in cytosolic free calcium levels and was partially inhibited (about 50%) by genistein, but not by calphostin C or PD 98059. Lastly, at physiologic doses ManLAM significantly reduced phagocytosis of *M. tuberculosis* and zymosan particles by MDMs.

Interpretation and Conclusions. Different phagocytic cells can exhibit variable responses to AraLAM and ManLAM. Moreover, LAMs affect cell functions through different mechanisms. Protein synthesis and activation of protein tyrosine kinases are important intermediates in the signal transduction pathway of the chemotactic response of mononuclear phagocytes to AraLAM and ManLAM; whereas ManLAM-induced inhibition of macrophage phagocytic ability could depend on the binding of macrophage mannose receptors and/or the insertion of this molecule into cellular plasma membrane. Together these data highlight the danger of making generalizations regarding the activity of LAMs on immune defenses.—Authors' Abstract

**Fratazzi, C., Arbeit, R. D., Carini, C., Balcewicz Sablinska, M. K., Keane, J., Kornfeld, H. and Remold, H. G.** Macrophage apoptosis in mycobacterial infections. J. Leuk. Biol. **66** (1999) 763–764.

Mycobacterial diseases are a major public health concern. In the case of tuberculosis, the problem has been acerbated due to the emergence of drug-resistant strains of *Mycobacterium tuberculosis*, and *M. avium* is the major opportunistic pathogen in HIV-1 infection in the United States. *M. tuberculosis* and *M. avium* replicate in human macrophages and induce apoptosis. Incubation of freshly added uninfected autologous macrophages with apoptotic *M. avium*-infected

macrophages results in 90% inhibition of bacterial growth. Apoptosis also prevents the release of intracellular components and the spread of mycobacterial infection by sequestering the pathogens within apoptotic bodies. Consistent with the model that host-cell apoptosis is a defense mechanism against mycobacteria is the finding that the virulent *M. tuberculosis* strain H37Rv induces substantially less macrophage apoptosis than the attenuated strain H37Ra. Evasion of apoptosis by this pathogen is achieved by enhanced release of sTNFR2 by H57Rv-infected macrophages and subsequent formation of inactive TNF-alpha-TNFR2 complexes. These observations contribute to the hypothesis that apoptosis of the host macrophage is an important defense mechanism in mycobacterial infections, which prevents the spread of the infection.—Authors' Abstract

**Gonzales, A. C. de O., Silva, T. C., Barbosa, A. de A., Jr. and Sadigursky, M.** Immunohistologic appraisal of infiltrating cells in skin biopsies from young patients clinically suspected of having various forms of leprosy. An. Bras. Dermatol. **74** (1999) 365–371.

Skin biopsies obtained from 28 untreated young patients from Brazil, with clinically supsected leprosy, during 1988–93, were used to study the surface phenotypes produced by infiltrating cells and demonstrated by immunohistochemistry using the monoclonal antibodies LCA; HAM-56; Pan-B; Pan-T; CD4; CD8; Leu7, and the polyclonal anti-S-100 protein and anti-BCG. A semiquantitative analysis of the stained cells was performed. In 24 cases the histopathological diagnosis of leprosy could be made: 9 indeterminate (IND), 5 tuberculoid (TT), 6 borderline tuberculoid (BT) and 4 lepromatous (LL). All cases were positive for LCA. In the LL group the more conspicuous cells were the macrophages, follwed by the T lymphocytes. The subpopulation TCD8 cells were more frequent than the TCD4. In the BT group the T cells were predominant, with CD4 slightly more frequent than CD8, followed by the macrophages. In the TT group, the T cell was also predominant; among these, the CD4 cells were more conspicuous, followed by the macrophages. The IND group was heterogeneous; T cells were the most frequent, with CD4 and CD8 cells having approximately the same frequency as the macrophages. It is concluded that the T cells were the most frequent cells in the TT/BT groups and there were more such cells in the TT and BT than in LL cases, indicating immunological reactivity consistent with T-cell presence and activity. Antibodies against T-cell subpopulations showed that in the TT/BT cases more CD4-positive cells and in LL patients more CD8-positive cells proliferated. Indeterminate forms disclosed gradation in the composition of the infiltrates. It is concluded that the skin lesions of young patients with early indeterminate leprosy and with various definite forms of leprosy differ with the regard to the infiltrating cells.—Trop. Dis. Bull. **96** (1999) 1267

**Leal, I. S., Smedegard, B., Andersen, P. and Appelberg, R.** Interleukin-6 and interleukin-12 participate in induction of a type 1 protective T-cell response during vaccination with a tuberculosis subunit vaccine. Infect. Immun. **67** (1999) 5747–5754.

We examined the role of cytokines in the development of gamma interferon (IFN-γ)-secreting protective T cells following immunization with a culture filtrate subunit vaccine against *Mycobacterium tuberculosis* containing the adjuvant dimethyldioctadecylammonium bromide (DDA). Depletion of either interleukin-6 (IL-6) or IL-12 with specific neutralizing antibodies during vaccination reduced the priming of T cells for antigen-specific proliferation and IFN-γ secretion. Such reduction was also observed in IL-6 gene-disrupted mice as compared to wild-type animals. IL-6 was found to play a role in the initial differentiation of Th1 cells but not in their expansion. The defect found after IL-6 depletion or in IL-6-knockout mice was compensated by the inclusion of recombinant mouse IL-12 in the vaccine. The induction of protective immunity against an intravenous or an aerosol challenge with live, virulent *M. tuberculosis* was markedly reduced by neutralizing either IL-6 or IL-12 during immunization with the vaccine. Likewise, the effects of

IL-6 neutralization were partially reversed by including IL-12 in the vaccine. Our data point to an important role of IL-6 and IL-12 in the generation of cell-mediated immunity to tuberculosis.—Authors' Abstract

**Mitra, D. K., DeRosa, S. C., Luke, A., Balamurugan, A., Khaitan, B. K., Tung, J., Mehra, N. K., Terr, A. I., O'Garra, A., Herzenberg, L. A. and Roederer, M.** Differential representations of memory T cell subsets are characteristic of polarized immunity in leprosy and atopic diseases. Int. Immunol. **11** (1999) 1801–1810.

We identified functionally polarized subsets of CD4 memory T cells on the basis of the expression of CD11a, CD45RA and CD62L. Within the several phenotypically distinct subsets of CD4 memory cells are two that, upon stimulation, produce primarily IL-4 [MT2, CD45RA(–)DC62L(+) CD11a(dim)] or primarily IFN-gamma [MT1, CD45RA(–)CD62L(–)CD11a(bright)]. In addition, four other phenotypically distinct subsets of CD4 cells have unique cytokine profiles. To determine the clinical relevance of the representation of these cell types, we analyzed blood from patients with the chronic diseases leprosy and atopy. These diseases are characterized as immunologically polarized, since T-cell responses in affected individuals are often strongly biased toward T(h)1 (dominated by IFN-gamma production) or T(h)2 (IL-4 production). We show here that this polarization reflects homeostatic or differentiation mechanisms affecting the representation of the functionally distinct subsets of memory CD4 T cells, MT1 and MT2. Significantly, the representation of the MT1 and MT2 subsets differs dramatically between subjects with tuberculoid leprosy (a T(h)1 disease), or lepromatous leprosy or atopic disease [T(h) 2 diseases]. However, there was no difference in the cytokine profiles of these or any of the other finely resolved CD4 subsets when compared between individuals across all disease states. Thus, it is the representation of these subsets in peripheral blood that is diagnostic of the polarized state of the immune system.—Authors' Abstract

**Moraes, M. O., Sarno, E. N., Almeida, A. S., Saraiva, B. C. C., Nery, J. A. C., Martins, R. C. L. and Sampaio, E. P.** Cytokine mRNA expression in leprosy: a possible role for interferon-gamma and interleukin-12 in reactions (RR and ENL). Scand. J. Immunol. **50** (1999) 541–549.

Leprosy patients during the natural course of the disease may develop reactional episodes, namely, reversal reaction (RR) and erythema nodosum leprosum (ENL). Immunological events described as occurring during RR indicate upregulation of the immune response; whereas in ENL the events are not fully understood. The aim of this study was to analyze the *in vivo* pattern of cytokine gene expression in the reactional states of leprosy. Peripheral blood mononuclear cells (PBMC, N = 14) and tissue samples (N = 17) obtained from patients with ENL and RR were obtained and assayed by RT-PCR. PBMC obtained from unreactional patients (N = 15) and normal individuals (N = 5) were also assessed. Expression of interferon (IFN) gamma, granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-2Rp55, perforin and IL-1 beta mRNA in PBMC were detected mostly in ENL/RR patients, but not in unreactional patients. Likewise, cytokines such as IL-6, IL-8, tumor necrosis factor (TNF)-alpha and TNF-beta were also present in reactional and tuberculoid patients as opposed to lepromatous leprosy (BL/LL). Interestingly, the majority of ENL/RR patients showed messages for IL-6, IL-10, IL-12 and TNF-alpha in the skin. IFN-gamma was detected in 84.6% (ENL) and 100% (RR) of the patients; whereas IL-4 was detected only in few individuals (38.5% and 25%, respectively). Although mRNA expression and protein levels may be different, the data reported in this study suggest a cytokine mRNA profile that seems to be indistinguishable for RR and ENL. In addition, it shows upregulation of immunoinflammatory cytokines in the blood and tissue of the same patient examined before and during reaction. Furthermore, it is suggested that this pattern of response results from an immunological reactivation that might lead to an acute in-

flammatory response in both reactional episodes.—Authors' Abstract

**Mustafa, A. S.** HLA-restricted immune response to mycobacterial antigens: relevance to vaccine design. Hum. Immunol. **61** Special Issue (2000) 166–171.

Identification of mycobacterial antigens that are recognized by CD4+ Th1 cells in an HLA-nonrestricted manner or in association with multiple allelic products is required to develop universally effective vaccines against mycobacterial diseases. Our studies in this direction have shown that several recombinant mycobacterial antigens of cytosolic and culture filtrate origin are recognized by CD4+ Th1 cells. Mapping of T-cell epitopes with overlapping synthetic peptides covering the entire sequence of these antigens identified peptide sequences stimulatory for Th1 cells. HLA-restriction analysis showed that in addition to HLA-DRB1 products (serologically defined HLA-DR1 to HLA-DR10), the HLA molecules encoded by HLA-DRB3 (HLA-DR52) and HLA-DRB4 (HLA-DR53) are important in presentation of mycobacterial antigens and epitopes to T cells. Depending on the T-cell donor, the presentation of a given antigen or peptide could be restricted by HLA-DRB1, HLA-DRB3, and/or HLA-DRB4 products. In addition stimulation of Th1 cells by some antigens and peptides in the presence of autologous and HLA-DR mismatched allogeneic APC suggested promiscuous presentation. These results taken together suggest that from HLA-restriction perspective, several mycobacterial antigens qualify as candidates for subunit or recombinant vaccine design against mycobacterial diseases.—Author's Abstract

**Mustafa, A. S., Lundin, K. E. A., Meloen, R. H., Shinnick, T. M. and Oftung, F.** Identification of promiscuous epitopes from the mycobacterial 65-kilodalton heat shock protein recognized by human CD4+ T cells of the *Mycobacterium leprae* memory repertoire. Infect. Immun. **67** (1999) 5683–5689.

By using a synthetic peptide approach, we mapped epitopes from the mycobacterial 65-kDa heat shock protein (HSP65) recognized by human T cells belonging to the *Mycobacterium leprae* memory repertoire. A panel of HSP65 reactive CD4+ T-cell lines and clones were established from healthy donors 8 years after immunization with heat-killed *M. leprae* and then tested for proliferative reactivity against overlapping peptides comprising both the *M. leprae* and *M. tuberculosis* HSP65 sequences. The results showed that the antigen-specific T-cell lines and clones established responded to 12 mycobacterial HSP65 peptides, of which 9 peptides represented epitopes crossreactive between the *M. tuberculosis* and *M. leprae* HSP65 (amino acids [aa] 61 to 75, 141 to 155, 151 to 165, 331 to 345, 371 to 385, 411 to 425, 431 to 445, 441 to 455, and 501 to 515) and 3 peptides (aa 343 to 355, 417 to 429, and 522 to 534) represented *M. leprae* HSP65-specific epitopes. Major histocompatibility complex restriction analysis showed that presentation of 9 of the 12 peptides to T cells were restricted by 1 of the 2 HLA-DR molecules expressed from self HLA-DRB1 genes; whereas 3 peptides with sequences completely identical between the *M. leprae* and *M. tuberculosis* HSP65 were presented to T cells by multiple HLA-DR molecules: peptide (aa 61 to 75) was presented by HLA-DR1, -DR2, and -DR7, peptide (aa 141 to 155) was presented by HLA-DR2, -DR7, and -DR53; whereas both HLA-DR2 and -DR4 (Dw4 and Dw14) were able to present peptide (aa 501 to 515) to T cells. In addition, the T-cell lines responding to these peptides in proliferation assays showed cytotoxic activity against autologous monocytes/macrophages pulsed with the same HSP65 peptides. In conclusion, we demonstrated that promiscuous peptide epitopes from the mycobacterial HSP65 antigen can serve as targets for cytotoxic CD4+ T cells which belong to the human memory T-cell repertoire against *M. leprae*. The results suggest that such epitopes might be used in the peptide-based design of subunit vaccines against mycobacterial diseases.—Authors' Abstract

**Naik, M., Matsuoka, M., Ohara, N., Nomaguchi, H. and Yamada, T.** The anti-

gen 85 complex vaccine against experimental *Mycobacterium leprae* infection in mice. Vaccine **18** (1999) 795–798.

The proteins in culture filtrate derived from Bacillus Calmette-Guerin (BCG) were examined for protection against infection by *Mycobacterium 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 when mice were immunized with Ag85A. A single immunization with Ag85 could induce antigen-specific interferon gamma synthesis and more effective protection than live BCG vaccine. This study demonstrates that Ag85 is an important immunoprotective molecule against leprosy infection.—Authors' Abstract

**Namer, I. J. and Steibel, J.** Antibody directed against mannan of the *Mycobacterium tuberculosis* cell envelope provokes blood-brain barrier breakdown. J. Neuroimmunol. **103** (2000) 63–68.

Previous studies demonstrated that blood-brain barrier (BBB) breakdown observed at the cerebral level during experimental allergic encephalomyelitis (EAE) arises from the presence of *Mycobacterium tuberculosis* in Freund's adjuvant and not only from the encephalitogenic antigens. The main objective of this study was to check if the mannan moiety of lipoarabinomannan present in the mycobacterial cell envelope is responsible for an immune response provoking a BBB breakdown. The results showed that: firstly, the complete Freund's adjuvant (CFA) contains a high release of polysaccharides; secondly, the rats immunized with the CFA present an important serum concentration of anti-mannan antibody; and finally, a single 2-mg dose of anti-mannan antibody injected intravenously in naive rats provokes an immediate and reversible BBB breakdown. These results suggest that mannan arising from the solubilization of the mycobacterial cell wall in Freund's adjuvant induces a high production of anti-mannan antibody which, in turn, provokes a BBB breakdown and possibly facilitates the induction of EAE.—Authors' Abstract

**Rook, G. A. W. and Barker, R.** Cortisol metabolism, cortisol sensitivity and the pathogenesis of leprosy reactions. Trop. Med. Int. Health **4** (1999) 493–498.

A description is given of how local regulation of effective cortisol concentrations is largely independent of circulating cortisol concentrations, and is itself regulated by local cytokine production. The ways in which cortisol affects the function of macrophages and T cells most relevant to mycobacterial disease is also outlined. It is suggested that the hypothesis that this mechanism underlies events triggering leprosy reactions is readily testable, and if correct could lead to new diagnostic tests and treatments.—Trop. Dis. Bull. **96** (1999) 1264

**Santos, A. R., Degrave, W. M. and Suffys, P. N.** Use of polymerase chain reaction (PCR) in leprosy research. Indian J. Lepr. **71** (1999) 101–110.

This conference paper considers the diagnosis, treatment, case finding and early detection of leprosy and describes experience of using nucleic acid-based tools for leprosy control. It is concluded that PCR could be a promising complementary diagnostic tool for leprosy and has a certain potential for the detection of subclinical infection and early stages of the disease. However, it is suggested that some important considerations on the use of the technique have to be made.—Trop. Dis. Bull. **96** (1999) 1163

**Shetty, V. P., Shetty, K. T., Save, M. P. and Anta, N. H.** *M. leprae*-induced alteration in the neurofilament phosphorylation leads to demyelination in leprous nerves: a hypothesis. Indian J. Lepr. **71** (1999) 121–135.

A preliminary study was conducted to investigate the mechanism of demyelination in leprosy. Nerves were obtained from 2 normal subjects (amputated limbs) and 64 leprosy patients (total of 64 leprous nerves) [in India]. SMI-31, a specific antibody known to bind to the phosphorylated epitope of the carboxy terminal region of neurofilaments, was used as primary antibody and stained sections were examined to record the pattern and intensity of staining.

The 2 normal nerves showed a linear pattern of SMI-31 staining in the longitudinal sections and uniform and intense staining was observed in all the axons in the transverse sections. In both tuberculoid and lepromatous nerves that showed mild-to-moderate pathology there was a severe loss of SMI staining. It is suggested that these observations made on peripheral nerves in leprosy indicate hypophosphorylation of neurofilament proteins on carboxy terminal regions.—Trop. Dis. Bull. **96** (1999) 1163

**Stanford, J. L., Thapa, N., Rafi, A. N., Torres, P. and Singh, M.** Antibodies against heat shock proteins in long-standing leprosy patients, with its probable association with deteriorating tissue autoimmunity and the results of the application of immunotherapy with inactivated *Mycobacterium vaccae.* Rev. Leprol. Fontilles **22** (1999) 265–274.

In this small study of chronic leprosy patients randomized to receive an injection of killed *Mycobacterium vaccae* in the past, IgG and IgA antibody titers have been measured to the 65 kDa and 70 kDa heat shock proteins (hsp) of BCG. It was found that, in common with other inflammatory diseases with disregulated cellular immunity, patients had higher levels of IgG to these antigens than did healthy staff members. Immunotherapy was associated with antibody titers reduced toward normal levels. For IgG to hsp 65 kDa this was statistically significant in the Iranian patients, receiving the immunotherapy a year or more before sera were taken. In the Spanish patients receiving immunotherapy 10 years before the sera were taken, although not reaching statistical significance, there were strong trends toward the same finding for hsp 65 kDa, also seen in IgA titers. The relationship of these findings to autoimmune disease and to a switch from Th2 to Th1 helper T-cell activity is discussed.—Authors' Summary

**Teitelbaum, R., Cammer, M., Maitland, M. L., Freitag, N. E., Condeelis, J. and Bloom, B. R.** Mycobacterial infection of macrophages results in membrane-permeable phagosomes. Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 15190–15195.

Cell-mediated immunity is critical for host resistance to tuberculosis. T lymphocytes recognizing antigens presented by the major histocompatibility complex (MHC) class I and class II molecules have been found to be necessary for control of mycobacterial infection. Mice genetically deficient in the generation of MHC class I and class 1a responses are susceptible to mycobacterial infection. Although soluble protein antigens are generally presented by macrophages to T cells through MHC class II molecules, macrophages infected with *Mycobacterium tuberculosis* or bacille Calmette-Guerin have been shown to facilitate presentation of ovalbumin through the MHC class I presentation pathway via a TAP-dependent mechanism. How mycobacteria, thought to reside within membrane-bound vacuoles, facilitate communication with the cytoplasm and enable MHC class I presentation presents a paradox. By using confocal microscopy to study the localization of fluorescent-tagged dextrans of varying size microinjected intracytoplasmically into macrophages infected with bacille Calmette-Guerin expressing the green fluorescent protein, molecules as large as 70 kilodaltons were shown to gain access to the mycobacterial phagosome. Possible biological consequences of the permeabilization of vacuolar membranes by mycobacteria would be pathogen access to host-cell nutrients within the cytoplasm, perhaps contributing to bacterial pathogenesis, and access of microbial antigens to the MHC class I presentation pathway, contributing to host protective immune responses.—Authors' Abstract

**Tilley, P. A. G. and Menon, J. N.** Detection of *Mycobacterium*-specific interferon-gamma-producing human T lymphocytes by flow cytometry. APMIS **108** (2000) 57–66.

Flow cytometry has proven to be a useful tool for the investigation of cytokine synthesis by selected cell subpopulations. while most reports have used mitogen stimulation or long-term cultures with antigen, we describe here a novel method to allow the detection of rare mycobacterial antigen-specific cytokine synthesizing cells within one day. The most important feature of this

method is the use of an FITC-conjugated isotype-matched control antibody to identify and exclude cells which fluoresce non-specifically. With this technique, we demonstrate interferon-gamma (IFN-γ) staining in 785 cells per $1 \times 10^5$ T cells counted in mycobacterial antigen-stimulated peripheral blood mononuclear cells from a BCG-vaccinated subject. In comparison, only 14 IFN-γ-staining T cells were seen in the cultures not stimulated by mycobacterial antigen. Less than 10 cells per $1 \times 10^5$ T cells are stained by an irrelevant control antibody. Specific responses are detectable after 12 hr of *in vitro* culture, and peak at 24 hr. In volunteer health care workers, IFN-γ staining correlated with IFN-γ production using a published ELISPOT assay ($r = 0.927$). IFN-γ staining was also higher in PBMC from Mantoux skin test-positive volunteers compared to cells from skin test-negative subjects ($p = 0.0045$). Flow cytometry following short-term culture can thus be used for enumeration of antigen-specific IFN-γ synthesizing cells.—Authors' Abstract

**Tomimori-Yamashita, J., Cruad, P., Rotta, O. and Lagrange, P. H.** Antibody-based enzyme-linked immunosorbent assay for determination of anti-PGL-I specific circulating immune complex in leprosy patients. Lepr. Rev. **70** (1999) 261–271.

A serological study was performed in 122 individuals: 75 leprosy patients and 47 healthy controls. The ELISA test was performed for IgG and IgM using the glycolipid PGL-I antigen from *Mycobacterium leprae*. Circulating immune complexes (CIC) were isolated by PEG 6000 precipitation method and after dissociation with an acid solution, the IgG and IgM specific against PGL-I were tested with the ELISA. The multibacillary patients had high levels of antibodies compared with paucibacillary patients and controls. The antibodies isolated from the CIC presented a similar spectrum spectral distribution as the serology. A positive correlation between the levels of free and CIC-bound antibodies was observed. In contrast with tuberculosis patients, specific antibodies present in CIC were not responsible for false-negative results found in some multibacillary patients' serology since no or very low levels of specific antibodies were found in PEG precipitated serum of these patients. No relation was observed with specific antibody levels detected in CIC during leprosy reactions.—Authors' Summary

**Tomimori-Yamashita, J., Nguyen, T. M., Maeda, S. M., Flageul, B., Rotta, O. and Cruaud, P.** Anti-phenolic glycolipid-I (PGL-I) determination using blood collection on filter paper in leprosy patients. Rev. Inst. Med. Trop. Sao Paulo **41** (1999) 239–242.

A total of 70 leprosy patients and 20 healthy individuals from Brazil were studied for specific antibodies against the native phenolic glycolipid-I (PGL-I) from *Mycobacterium leprae,* comparing the traditional sera collection method with the finger-prick blood and conservation on filter paper method. The finger-prick blood dried on filter paper was eluated in phosphate buffered saline (PBS) containing 0.5% gelatin. The classical method for native PGL-I was performed for these eluates and compared with antibody determination for sera. Results showed a straight correlation between these two methods, although the titers found for the eluates were lower than those obtained for serology. It is suggested that this blood collection method could be useful for investigation of new leprosy cases in the field.—Trop. Dis. Bull. **96** (1999) 1266–1267

**van den Bos, I. C., Khanolkar-Young, S., Das, P. K. and Lockwood, D. N. J.** Immunohistochemical detection of PGL-I, LAM, 30 kD and 65 kD antigens in leprosy infected paraffin preserved skin and nerve sections. Lepr. Rev. **70** (1999) 272–280.

A panel of lipid, carbohydrate and protein antibodies were optimized for use in detecting *M. leprae* antigens in paraffin embedded material. Skin and nerve biopsies from 13 patients across the leprosy spectrum were studied. All antibodies detected antigen in tissues with a BI >1. Phenolic-glycolipid was not detected in bacteriologically negative tissue but lipoarabinomannan (LAM) and protein antigens were detected.

Staining with LAM was strongest and gave least background. The transfer of this immunohistochemical technique to paraffin-embedded material will allow examination of tissue with better morphology and from clinics without access to tissue freezing facilities.—Authors' Summary

**Watson, V. E., Hill, L. L., Owen Schaub, L. B., Davis, D. W., McConkey, D. J., Jagannath, C., Hunter, R. L. and Actor, J. K.** Apoptosis in *Mycobacterium tuberculosis* infection in mice exhibiting varied immunopathology. J. Pathol. **190** (2000) 211–220.

This study examined mechanisms contributing to pulmonary immunopathology following acute *Mycobacterium tuberculosis* (MTB) infection *in vivo* in a murine model. A/J and C57BL/6 mice were intravenously infected with MTB (Erdman). Pathological differences were found between strains, unrelated to pulmonary load of bacilli. A/J mice developed progressive interstitial pneumonitis, while C57BL/6 mice maintained granuloma formation. The contribution of FAS and FAS ligand-mediated apoptosis was assessed via bioluminescent reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemical staining, and TUNEL assessment of DNA fragmentation. Cytokine messages for pulmonary tumor necrosis factor-alpha (TNF-$\alpha$) and interferon-gamma (IFN-$\gamma$), as well as for the lytic molecules perforin and granzyme B, were quantified. Immunohistochemical staining for CD3 receptor was performed to monitor lymphocytic lung infiltration. Soon after infection, A/J mice exhibited increased pulmonary IFN-$\gamma$ message, concurrent with the appearance of CD3+ lymphocytes distributed throughout the lung. C57BL/6 mice exhibited perivascular cuffing, with no accompanying increase in IFN-$\gamma$ message. A/J mice also had elevated levels of FAS and FAS ligand message and protein early after infection, while the C57BL/6 mice had no increased expression of these molecules. Both strains exhibited qualitatively similar numbers of TUNEL-positive cells throughout infection, with a marked increase on day 7. Apoptotic cells appeared to co-localize with acid-fast bacilli. It is therefore proposed that apoptosis during initial granuloma formation following MTB infection may occur through a FAS/FAS ligand-independent pathway. Moreover, a failure of completion of the FAS/FAS ligand-mediated apoptosis pathway in the A/J mice may contribute to inefficient elimination of lymphocytes, thus further aggravating pulmonary pathology.—Authors' Abstract

**Yang, X., Wang, S., Fan, Y. and Zhu, L.** Systemic mycobacterial infection inhibits antigen-specific immunoglobulin E production, bronchial mucus production and eosinophilic inflammation induced by allergen. Immunology **98** (1999) 329–337.

As the burden of infectious diseases becomes reduced in many countries, a remarkable increase in the incidence of allergies has occurred. The basis for the rise in atopic disorders as a correlate of the decline in infectious diseases has not been defined. In the present study, we tested experimentally whether prior systemic infection with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) had any effect on ovalbumin (OVA) AI-$(OH)_3$ (alum)-induced immunoglobulin E (IgE) production, airway mucus production and eosinophilic inflammation. The data showed that allergen-specific IgE production and OVA-induced eosinophilia and goblet cell development were significantly inhibited by prior infection with BCG. Correspondingly, following immunization with OVA alum, BCG-infected mice exhibited significantly higher levels of allergen-driven interferon-gamma (IFN-$\gamma$) production than the mice without infection. The ratio of IFN-$\gamma$ : interleukin (IL)-4 production was higher in OVA-sensitized mice with prior BCG infection than in those without infection. The abrogation of OVA-induced mucus production and pulmonary eosinophilia in BCG-infected mice correlated with significantly decreased IL-5 production and increased IFN-$\gamma$ and IL-12 production. These data provide direct evidence that intracellular bacterial infection (i.e., BCG) can inhibit antigen-specific IgE and airway reactivity induced by environmental allergen. Furthermore, the results suggest that changes in cytokine-producing patterns of T lymphocytes and other cells may be the mechanism by which infections influence allergies.—Authors' Abstract

# Microbiology

**Banerjee, S. K., Bhatt, K., Misra, P. and Chakraborti, P. K.** Involvement of a natural transport system in the process of efflux-mediated drug resistance in *Mycobacterium smegmatis*. Mol. Gen. Genet. **262** (2000) 949–956.

The phosphate-specific transporter I (Pst) in bacteria is a multi-subunit system which belongs to the ABC family of transporters. The gene forms part of an operon and it is involved in phosphate uptake in prokaryotes. Its import function is known to be operative only under conditions of phosphate starvation. However, we found overexpression of this transporter in a *Mycobacterium smegmatis* strain selected for ciprofloxacin resistance (CIPr) which was grown under conditions in which the phosphate-scavenging function of this operon was inoperative. In CIPr cells, active efflux of the drug plays a predominant role in conferring high levels of fluoroquinolone resistance. We therefore investigated the role of this transporter in the process of efflux-mediated drug resistance by inactivating the pst operon in the CIPr strain. Phenotypic characterization of the resulting strain, CIPrd, showed a striking reduction in the minimal inhibitory concentration (MIC) of ciprofloxacin and in the drug extrusion profile as well. Genotype analysis, on the other hand, revealed partial disruption of the pst operon in CIPrd as a consequence of transporter gene amplification. Furthermore, disruption of this operon in wild-type cells resulted in hypersensitivity to ciprofloxacin and other xenobiotics to which CIPr cells exhibited cross-resistance. Thus our results provide strong evidence that Pst is a natural membrane transport system that has the ability to promote drug efflux in addition to its phosphate-scavenging function in the CIPr strain.—Authors' Abstract

**Billman-Jacobe, H., Haites, R. E. and Coppel, R. L.** Characterization of a *Mycobacterium smegmatis* mutant lacking penicillin binding protein 1. Antimicrob. Agents Chemother. **43** (1999) 3011–3013.

The *ponA* gene of *Mycobacterium smegmatis* encodes a 95-kDa penicillin binding protein, PBP1, that is similar to PBP1s of *M. tuberculosis* and *M. leprae*. Transposon disruption of *ponA* in *M. smegmatis* resulted in a PBP1-deficient mutant that was sensitive to β-lactam antibiotics, was more permeable to glycine, and grew slowly in liquid culture.—Authors' Abstract

**Delogu, G. and Brennan, M. J.** Functional domains present in the mycobacterial hemagglutinin, HBHA. J. Bacteriol. **181** (1999) 7464–7469.

Identification and characterization of mycobacterial adhesins and complementary host receptors required for colonization and dissemination of mycobacteria in host tissues are needed for a more complete understanding of the pathogenesis of diseases caused by these bacteria and for the development of effective vaccines. Previous investigations have demonstrated that a 28-kDa heparin-binding mycobacterial surface protein, HBHA, can agglutinate erythrocytes and promote mycobacterial aggregation *in vitro*. In this study, further molecular and biochemical analysis of HBHA demonstrates that it has three functional domains: a transmembrane domain of 18 amino acids residing near the N terminus, a large domain of 81 amino acids consistent with an or-helical coiled-coil region, and a Lys-Pro-Ala-rich C-terminal domain that mediates binding to proteoglycans. Using His-tagged recombinant HBHA proteins and nickel chromatography, we demonstrate that HBHA polypeptides which contain the coiled-coil region form multimers. This tendency to oligomerize may be responsible for the induction of mycobacterial aggregation since a truncated N-terminal HBHA fragment containing the coiled-coil domain promotes mycobacterial aggregation. Conversely, a truncated C-terminal HBHA fragment which contains Lys-Pro-Ala-rich repeats binds to the proteoglycan decorin. These results indicate that HBHA contains at least three distinct domains which facilitate intercalation into surface membranes,

promote bacterium-bacterium interactions, and mediate the attachment to sulfated glycoconjugates found in host tissues.—Authors' Abstract

**De Smet, K. A. L., Weston A., Brown, I. N., Young, D. B. and Robertson, B. D.** Three pathways for trehalose biosynthesis in mycobacteria. Microbiology **146** (2000) 199–208.

Trehalose is present as a free disaccharide in the cytoplasm of mycobacteria and as a component of cell-wall glycolipids implicated in tissue damage associated with mycobacterial infection. To obtain an overview of trehalose metabolism, we analyzed data from the *Mycobacterium tuberculosis* genome project and identified ORFs with homology to genes encoding enzymes from three trehalose biosynthesis pathways previously characterized in other bacteria. Functional assays using mycobacterial extracts and recombinant enzymes derived from these ORFs demonstrated that mycobacteria can produce trehalose from glucose 6-phosphate and UDP-glucose (the OtsA-OtsB pathway) from glycogen-like alpha (1—>4)-linked glucose polymers (the TreY-TreZ pathway) and from maltose (the TreS pathway). Each of the pathways was found to be active in both rapid-growing *M. smegmatis* and slow-growing *M. bovis* BCG. The presence of a disrupted treZ gene in *M. leprae* suggests that this pathway is not functional in this organism. The presence of multiple biosynthetic pathways indicates that trehalose plays an important role in mycobacterial physiology.—Authors' Abstract

**Harboe, M., Christensen, A., Haile, Y., Ulvund, G., Ahmad, S., Mustafa, A. S. and Wiker, H. G.** Demonstration of expression of six proteins of the mammalian cell entry (mce1) operon of *Mycobacterium tuberculosis* by anti-peptide antibodies, enzyme-linked immunosorbent assay and reverse transcription-polymerase chain reaction. Scand. J. Immunol. **50** (1999) 519–527.

Polyclonal rabbit antibodies were generated to synthetic peptides corresponding to predicted B-cell epitopes of six proteins of the mce1 operon of *Mycobacterium tuberculosis*. Antipeptide antibodies reacted with Mce1A and Mce1E fusion proteins in sonicates of recombinant *Escherichia coli* as well as with distinct bands in sonicates, but not in culture fluids of *M. tuberculosis* and *M. bovis* bacillus Calmette-Guerin (BCG). Polyvalent rabbit antibodies generated by immunization with sonicates of BCG bacilli reacted with synthetic peptides from the six Mce1 proteins on the solid phase in enzyme-linked immunosorbent assay (ELISA), albeit with different frequencies. The Mce1A peptide (p124–140) reacted most frequently, with 7 of the 9 antibodies tested, while the Mce1F peptide (p329–343) reacted with 2. Used as a control, 20 polyclonal rabbit antibodies to 12 isolated proteins of *M. tuberculosis* and *M. bovis* BCG did not react with any of the six synthetic peptides, except in one case. mRNA expression of the six mce1A–mce2F genes of *M. tuberculosis* was demonstrated by reverse transcription-polymerase chain reaction (RT-PCR). These data indicate that all Mce1A–Mce1F proteins of the mce1 operon are expressed by *in vitro*-grown *M. tuberculosis* and *M. bovis* BCG.—Authors' Abstract

**Katoch, V. M.** Molecular techniques for leprosy: present applications and future perspectives. Indian J. Lepr. **71** (1999) 45–49.

This conference paper considers molecular techniques for detection of nucleic acids and their therapeutic application, molecular techniques for epidemiology, expression of *M. leprae* genes by recombinant DNA techniques and sequencing of the genome of *M. leprae*.—Trop. Dis. Bull. **96** (1999) 1162

**Leoni, E., Legnani, P., Mucci, M. T. and Pirani, R.** Prevalence of mycobacteria in a swimming pool environment. J. Appl. Microbiol. **87** (1999) 683–688.

A study was performed to evaluate the prevalence of nontubercular mycobacteria in swimming pool environments. The bacteria in question were found in 88.2% of pool water samples. The most frequent species were *Mycobacterium gordonae* (73.5% of samples; range 1–840 cfu 100 $ml^{-1}$;

*M. chelonei* (38.2% 2–360 cfu 100 $ml^{-1}$; and *M. fortuitum* (35.3% 2–250 cfu 100 $ml^{-1}$. The same species were also recovered from the water at the different phases of the treatment cycle, with relative percentages similar to those of the pool water. Shower floors and pool edges also presented high concentrations of the mycobacteria (100% of samples) and *M. marinum* was isolated from the surfaces of pool edges on two occasions (4.5% of samples). The swimming pool environment provides a suitable habitat for the survival and reproduction of mycobacteria. Although mycobacteria are common in swimming pools, human mycobacterial disease associated with their use is rare. Apart from superficial infections with *M. marinum,* the risk of more serious diseases in subjects with weakened immune systems should not be underestimated, given the widespread presence of mycobacteria that are possible opportunistic pathogens and the direct contact bathers have with the water and aerosol.—Authors' Abstract

**Matsumoto, S., Furugen, M., Yukitake, H. and Yamada, T.** The gene encoding mycobacterial DNA-binding protein I (MDPI) transformed rapidly growing bacteria to slowly growing bacteria. FEMS Microbiol. Lett. **182** (2000) 297–301.

Pathogenic species of *Mycobacterium* are slowly growing intracellular bacteria. Slow growth is important for the parasitism of these organisms and chronicity of the disease, but its precise mechanism has not been elucidated. Recently, we found that a novel DNA-binding protein (MDPI) was expressed (7%–10% in total protein) in mycobacteria, such as *M. bovis* bacillus Calmette-Guerin *M. tuberculosis,* and *M. leprae.* In this study, we observed that MDPI interfered with replication, transcription, and translation in the analysis in *in vitro Escherichia coli* cell-free macromolecular biosynthesizing systems. Furthermore, MDPI inhibited the rapid growth of both *E. coli* and *M. smegmatis,* and NH2-terminal second amino acid, asparagine, was observed to be important in terms of this function. These data suggest an important role of MDPI for suppression of growth rates of mycobacteria.—Authors' Abstract

**Matsumoto, S., Yukitake, H., Furugen, M., Matsuo, T., Mineta, T. and Yamada, T.** Identification of a novel DNA-binding protein from *Mycobacterium bovis* bacillus Calmette-Guerin. Microbiol. Immunol. **43** (1999) 1027–1036.

A novel DNA-binding protein expressing (8%–10% in total protein) in *Mycobacterium bovis* bacillus Calmette-Guerin was observed. This protein was designated mycobacterial DNA-binding protein 1 (MDP1), MDP1 recognized bases, sugar moieties, phosphate-backbone on DNA and preferentially bound to DNA guanine and cytosine. In the gel retardation assay, MDP1 preferentially bound to closed circular plasmid DNA than to open circular and linear form plasmid DNA and also bound to RNA. MDP1 formed a highly polymerized structure and localized not only in the nucleoid but also at the 50S ribosomal subunits and cell surface. MDP1 was conserved in *Mycobacterium* thus far examined and the expression was enhanced in stationary growth phases. These results will provide a reasonable basis for further study of the function of MDP1 in living mycobacteria.—Authors' Abstract

**Mistry, N. F. and Antia, N. H.** Incomplete killing of intracellular mycobacteria—speculation on the creation of persisters. Indian J. Lepr. **71** (1999) 69–74.

A brief overview is given from interdisciplinary viewpoints of the phenomenon of incomplete intracellular killing of distinct species of mycobacteria. *Mycobacterium leprae* and *M. tuberculosis* infection are used as examples of disease persistence during chemotherapy.—Trop. Dis. Bull. **96** (1999) 1151

**Miyamoto, M., Yamaguchi, Y. and Sasatsu, M.** Disinfectant effects of hot water, ultraviolet light, silver ions and chlorine on strains of *Legionella* and nontuberculous mycobacteria. Microbios **101** (2000) 7–13.

The disinfectant effects on *Legionella* and nontuberculous mycobacteria of hot water, ultraviolet light, silver ions and chlorine were evaluated. The bacterial strains

*Legionella pneumophila* ATCC33152 and *Mycobacterium avium* ATCC25291 and strains of *L. pneumophila* and *M. avium* which had been isolated from a 24 hr bath were examined for their resistance to treatments. All strains were killed within 3 min on exposure to hot water at 70°C and exposure to ultraviolet light at 90 mW.s/cm$^2$. The strains of *L. pneumophila* tested were killed within 6 hr on exposure to a solution of silver ions at 50 µg/l. The number of viable cells of strains of *M. avium* fell from $10^5$ CFU/ml to $10^3$ CFU/ml after exposure to an aqueous solution of silver ions at 100 µg/l for 24 hr. Chlorine effectively killed strains of *Legionella* which were exposed to an aqueous solution of chlorine at 2 mg/l within 3 min, but strains of *Mycobacterium* survived exposure to chlorine at 4 mg/l for more than 60 min.—Authors' Abstract

**Mollenkopf, H. J., Jungblut, P. R., Raupach, B., Mattow, J., Lamer, S., Zimny Arndt, U., Schaible, U. E. and Kaufmann, S. H. E.** A dynamic two-dimensional polyacrylamide gel electrophoresis database: the mycobacterial proteome via Internet. Electrophoresis **20** (1999) 2172–2180.

Proteome analysis by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and mass spectrometry, in combination with protein chemical methods, is a powerful approach for the analysis of the protein composition of complex biological samples. Data organization is imperative for efficient handling of the vast amount of information generated. Thus, we have constructed a 2-D PAGE database to store and compare protein patterns of cell-associated and culture-supernatant proteins of different mycobacterial strains. In accordance with the guidelines for federated 2-DE databases, we developed a program that generates a dynamic 2-D PAGE database for the World-Wide-Web to organize and publish, via the Internet, our results from proteome analysis of different *Mycobacterium tuberculosis* as well as *M.* bovis BCG strains. The uniform resource locator for the database is http://www.mpiib-berlin.mpg. de/2D-PAGE and can be read with a Java compatible browser. The interactive hypertext markup language documents displayed are generated dynamically in each individual session from a rational data file, a 2-D gel image file and a map file describing the protein spots as polygons. The program consists of common gateway interface scripts written in PERL, minimizing the administrative workload of the database. Furthermore, the database facilitates not only interactive use, but also worldwide active participation of other scientific groups with their own data, requiring only minimal computer hardware and knowledge of information technology.—Authors' Abstract

**Nakata, N.** The absence of catalase function in *Mycobacterium leprae.* Indian J. Lepr. **71** (1999) 87–92.

A study was conducted to prove the absence or presence of a catalase peroxidase gene *(katG)* in *M. leprae.* Using a PCR amplified *katG* fragment, DNA fragments were cloned covering the whole *katG* region and the complete nucleotide sequence was determined. Although the *M. leprae katG* sequence showed approximately 70% homology to the *M. tuberculosis katG* gene, several lesions were found in it. It is suggested that this finding indicates that the *katG* gene has been inactivated in *M. leprae.* The 2 deletions in the *M. laprae katG* sequence were found in all 7 different *M. leprae* isolates examined. To confirm that the *M. leprae katG* region was not functional, it was examined whether the *katG* region of *M. leprae* could produce a functional catalase-peroxidase protein by using an *in vitro* protein synthesis system. In the *in vitro* translation experiment, no specific protein was produced from the *M. leprae katG* fragments while a 75 kDa protein was obtained from the corresponding *M.* tuberculosis *katG* fragments.—Trop. Dis. Bull. **96** (1999) 1162–1163

**Narayanan, R. B.** Recombinant proteins from *Mycobacterium leprae:* current status. Indian J. Lepr. **71** (1999) 93–99.

In this overview it is concluded that recombinant DNA technology is a potential tool to generate proteins from *M. leprae* and other related organisms. Proteins of diagnostic, therapeutic and functional importance can be produced in large quantities

and easily purified for research use.—Trop. Dis. Bull. **96** (1999) 1163

**Nopponpunth, V., Sirawaraporn, W., Greene, P. J. and Santi, D. V.** Cloning and expression of *Mycobacterium tuberculosis* and *Mycobacterium leprae* dihydropteroate synthase in *Escherichia coli.* J. Bacteriol. **181** (1999) 6814–6821.

The genes for dihydropteroate synthase of *Mycobacterium tuberculosis* and *M. leprae* were isolated by hybridization with probes amplified from the genomic DNA libraries. DNA sequencing revealed an open reading frame of 840 bp encoding a protein of 280 amino acids for *M. tuberculosis* dihydropteroate synthase and an open reading frame of 852 bp encoding a protein of 284 amino acids for *M. leprae* dihydropteroate synthase. The dihydropteroate synthases were expressed under control of the T5 promoter in a dihydropteroate synthase-deficient strain of *Escherichia coli.* Using three chromatography steps, we purified both *M. tuberculosis* and *M. leprae* dihydropteroate synthases to >98% homogeneity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed molecular masses of 29 kDa for *M. tuberculosis* dihydropteroate synthase and 30 kDa for *M. leprae* dihydropteroate synthase. Gel filtration of both enzymes showed a molecular mass of ca. 60 kDa, indicating that the native enzymes exist as dimers of two identical subunits. Steady-state kinetic parameters for dihydropteroate synthases from both *M. tuberculosis* and *M. leprae* were determined. Representative sulfonamides and dapsone were potent inhibitors of the mycobacterial dihydropteroate synthases, but the antimycobacterial agent p-aminosalicylate, a putative dihydropteroate synthase inhibitor, was a poor inhibitor of the enzymes.—Authors' Abstract

**Oftung, F., Mustafa, A. S. and Wiker, H. G.** Extensive sequence homology between the *Mycobacterium leprae* LSR (12 kDa) antigen and its *Mycobacterium tuberculosis* counterpart. FEMS Immunol. Med. Microbiol. **27** (2000) 87–89.

The *Mycobacterium* leprae LSR (12 kDa) protein antigen has been reported to mimic whole-cell *M. leprae* in T-cell responses across the leprosy spectrum. In addition, B-cell responses to specific sequences within the LSR antigen have been shown to be associated with immunopathological responses in leprosy patients with erythema nodosum leprosum. We have in the present study applied the *M. leprae* LSR DNA sequence as query to search for the presence of homologous genes within the recently completed *M. tuberculosis* genome database (Sanger Centre, U.K.) By using the BLASTN search tool, a homologous *M. tuberculosis* open reading frame (336 bp), encoding a protein antigen of 12.1 kDa, was identified within the cosmid MTCY07H7B.25. The gene is designated Rv3597c within the *M. tuberculosis* H37Rv genome. Sequence alignment revealed 93% identity between the *M. leprae* and *M. tuberculosis* antigens at the amino acid sequence level. The finding that some B- and T-cell epitopes were localized to regions with amino acid substitutions may account for the putative differential responsiveness to this antigen in tuberculosis and leprosy. —Authors' Abstract

**Ronning, D. R., Klabunde, T., Besra, G. S., Vissa, V. D., Belisle, J. T. and Sacchettini, J. C.** Crystal structure of the secreted form of antigen 85C reveals potential targets for mycobacterial drugs and vaccines. Nat. Struc. Biol. **7** (2000) 141–146.

The antigen 85 (Ag85) complex, composed of three proteins (Ag85A, B and C), is a major protein component of the *Mycobacterium tuberculosis* cell wall. Each protein possesses a mycolyltransferase activity required for the biogenesis of trehalose dimycolate (cord factor), a dominant structure necessary for maintaining cell wall integrity. The crystal structure of recombinant Ag85C from *M. tuberculosis,* refined to a resolution of 1.5 Angstrom, reveals an alpha/beta-hydrolase polypeptide fold, and a catalytic triad formed by Ser 124, Glu 228 and His 260. Ag85C complexed with a covalent inhibitor implicates residues Leu 40 and Met 125 as components of the oxyanion hole. A hydrophobic pocket and tunnel extending 21 Angstrom into the core of the protein indicates the location of a probable trehalose monomyco-

late binding site. Also, a large region of conserved surface residues among Ag85A, B and C is a probable site for the interaction of Ag85 proteins with human fibronectin.—Authors' Abstract

**Schaeffer, M. L., Khoo, K. H., Besra, G. S., Chatterjee, D., Brennan, P. J., Belisle, J. T. and Inamine, J. M.** The pimB gene of *Mycobacterium tuberculosis* encodes a mannosyltransferase involved in lipoarabinomannan biosynthesis. J. Biol. Chem. **274** (1999) 31625– 31631.

The biosynthesis of lipoarabinomannan (LAM), a key mycobacterial lipoglycan that has been implicated in numerous immunoregulatory functions, was examined utilizing D-mannosamine (ManN) as a tool to identify mannosyltransferase genes involved in LAM synthesis. Cell-free reactions utilizing cellular membranes of mycobacteria as the enzyme source indicated that ManN inhibited the synthesis of phosphatidylinositol mannosides, early precursors to LAM. A selection strategy was devised to screen a *Mycobacterium tuberculosis* genomic library in *M. smegmatis* for clones conferring conditional resistance to ManN, with the rationale that overexpression of the gene(s) encoding a target of ManN would impart a ManN-resistant phenotype under these conditions. This strategy led to the identification of pimB, whose deduced amino acid sequence shows similarity to mannosyltransferases and other glycosyltransferases. Partially purified recombinant PimB protein from *Escherichia coli* or membranes from *M. smegmatis* overexpressing the pimB gene were used in cell-free assays to show that PimB catalyzes the formation of triacylphosphatidylinositol dimannoside from GDP-mannose and triacylphosphatidylinositol monomannoside.—Authors' Abstract

**Sengupta, U.** Recombinant antigens: current situation and future. Indian J. Lepr. **71** (1999) 111–120.

This conference paper describes how gene cloning techniques have enabled several *Mycobacterium leprae* genes to be fused to those of other organisms, particularly *Escherichia coli,* and has led to the isolation, purification and characterization of several cloned proteins. The cloned proteins, mostly heat shock proteins, are described and their role in T-cell stimulation is discussed.—Trop. Dis. Bull. **96** (1999) 1163

**Wei, J., Dahl, J. L., Moulder, J. W., Roberts, E. A., O'Gaora, P., Young, D. B. and Friedman, R. L.** Identification of a *Mycobacterium tuberculosis* gene that enhances mycobacterial survival in macrophages. J. Bacteriol. **182** (2000) 377–384.

Intracellular survival plays a central role in the pathogenesis of *Mycobacterium tuberculosis.* To identify *M. tuberculosis* genes required for intracellular survival within macrophages, an *M. tuberculosis* H37Rv plasmid library was constructed by using the shuttle vector pOLYG. This plasmid library was electroporated into *M. smegmatis* 1-2c, and the transformants were used to infect the human macrophage-like cell line U-937. Because *M. smegmatis* does not readily survive within macrophages, any increased intracellular survival is likely due to cloned *M. tuberculosis* H37Rv DNA. After six sequential passages of *M. smegmatis* transformants through U-937 cells, one clone (p69) was enriched more than 70% as determined by both restriction enzyme and PCR analyses. P69 demonstrated significantly enhanced survival compared to that of the vector control, ranging from 2.4- to 5.3-fold at both 24 and 48 hr after infection. DNA sequence analysis revealed three open reading frames (ORFs) in the insert of p69. ORF2 (1.2 kb) was the only one which contained a putative promoter region and a ribosome-binding site. Deletion analysis of the p69 insert DNA showed that disruption of ORF2 resulted in complete loss of the enhanced intracellular survival phenotype. This gene was named the enhanced intracellular survival (eis) gene. By using an internal region of eis as a probe for Southern analysis, eis was found in the genomic DNA of various *M. tuberculosis* strains and of *M. bovis* BCG but not in that of *M. smegmatis* or 10 other nonpathogenic mycobacterial species. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis showed that all *M. smegmatis* eis-containing constructs ex-

pressed a unique protein of 42 kDa, the predicted size of eis. The expression of this 42-kDa protein directly correlated to the enhanced survival of *M. smegmatis* p69 in U-937 cells. These results suggest a possible role for eis and its protein product in the intracellular survival of *M. tuberculosis.*—Authors' Abstract

**Weinstein, D. E., Freedman, V. H. and Kaplan, G.** Molecular mechanism of nerve infection in leprosy. Trends Microbiol. **7** (1999) 185–186.

This paper discusses research into the molecular mechanisms that facilitate the entry *of Mycobacterium leprae* into the peripheral nerves and subsequent infection of Schwann cells. It considers the binding of *M.* leprae to Schwann cells, *M. leprae*-infected blood monocytes as the likely vector *of* mycobacterium entry into the nerve and nerve degeneration.—Trop. Dis. Bull. **96** (1999) 1160

## Experimental Infections

**Williams, D. L. and Gillis, T. P.** Detection of drug-resistant *Mycobacterium leprae* using molecular methods. Indian J. Lepr. **71** (1999) 137–153.

This overview considers the development of antileprosy drugs and drug resistance in *M. leprae* and discusses techniques used to detect drug resistance.—Trop. Dis. Bull. **96** (1999) 1163–1164

**Franco, R., Fliess, E., Cecere, L., Llopis, L. and Baudino, R.** [Experimental leprosy in *Dasypus hybridus* in Argentina.] Rev. Hosp. Nac. Baldomero Sommer **2** (1999) 135–143. (in Spanish)

Leprosy bacilli could be obtained only from biopsy specimens of untreated patients in Argentina. In order to assure the continuous production of lepromin we decided to focus the research on the armadillo. In the light of the international experience, the multiplication of *Mycobacterium leprae* in armadillo is the least expensive and complicated *in vivo* culture method. The susceptibility to *M. leprae* has already been studied in *Dasypus hybridus.* Seven animals were infected with an inoculum prepared with a human leproma obtained from an untreated patient. One ml of this inoculum adjusted to $10^8$ bacilli/ml was inoculated to each animal. The inoculum was injected under anesthesia (sodium pentobarbital, intraperitoneal) in the external femoral or subclavian veins. Intracardiac inoculation was performed only if it was not possible to inject any of these veins. One armadillo showed disseminated leprosy 26 month after intravenous inoculation. Abundant solid bacilli appeared in skin ulcer (leproma), liver, spleen and lymph nodes. It was possible to purify bacilli from the infected tissues. The inoculation of bacilli into mouse foot pad and pyridine extraction were positive. The cultures in different medium were negative. Purification of *M. leprae* was performed as described by Draper. Only highly purified bacilli were employed to prepare 500 ml lepromin.—Authors' English Abstract

**Nogueira, M. E. S., Coelho, K. I. R., Fleury, R. N. and de Arruda, M. S. P.** [Inoculation of *Mycobacterium leprae* into the cheek pouch of hamsters.] Hansen. Int. **24** (1999) 5–14. (in Portuguese)

The use of the hamster cheek pouch in experimental leprosy was evaluated through the inoculation of $6.0 \times 10^8$ *M. leprae*/ml in its subepithelial tissue in 60 animals. A control group of 12 hamsters was inoculated in the foot pad. The animals were sacrificed 20 and 48 hours, 7, 14, 21 and 28 days after inoculation. Histological sections stained with hematoxylin-eosin and Faraco-Fite were used to evaluate the evolution of the

lesions. The evaluation of the bacilli viability in the cheek pouch was done by a bacilli recovery test using mice inoculated in the foot pad and sacrificed 6 months after inoculation. The results led us to the following conclusions: a) after the exudative phase, the lesions evolved into macrophagic granuloma formation similar to lepromatous leprosy in humans; b) there was a remarkable increase of the bacteriologic index, as opposed to the biological characteristics of *M. leprae,* a fact that was interpreted as a local concentration after reabsorption of edema and congestion; c) the lesion in the foot pad evoluted into epithelioid granulomas similar to borderline leprosy; d) the *M. leprae* recovery test from the foot pad of mice suggests that there was no multiplication of the bacilli—Authors' English Summary

## Epidemiology and Prevention

**Ohara, N., Matsuoka, M., Nomaguchi, H., Naito, M. and Yamada, T.** Inhibition of multiplication of *Mycobacterium leprae* in mouse foot pads by recombinant Bacillus Calmette-Guerin (BCG). Vaccine **18** (2000) 1294–1297.

Immunization of mice with recombinant *Mycobacterium bovis* Bacillus Calmette-Guerin (rBCG) which overproduces a putative protective antigen candidate, the A component of antigen 85 complex (Ag85A), reduced the multiplication of *M. leprae* in the foot pads of mice. The inhibition by this rBCG (rBCG/85A) was more evident than that with parental BCG. Repeated rBCG/85A immunization significantly could reduce *M. leprae* multiplication in mice. This is the first report of rBCG to control mycobacterial infection in an animal model. Therefore, the rBCG technique may be useful for the development of a more effective mycobacteria vaccine.—Authors' Abstract

**LeBlanc, S. B., Naik, E. G., Jacobson, L. and Kaslow, R. A.** Association of DRB1*1501 with disseminated *Mycobacterium avium* complex infection in North American AIDS patients. Tissue Antigens **55** (2000) 17–23.

The HLA class II allele, DR2 (DRB1*1501), has been repeatedly found to be associated with development of tuberculosis and leprosy. We searched for associations of these and other class II alleles with disseminated *Mycobacterium avium* complex (DMAC) infection in North American Caucasian homosexual AIDS patients. Molecular typing for HLA-DRB1 and -DQB1 alleles in 176 cases of DMAC and 176 matched controls showed an association of accelerated onset of disease with DRB1*1501 (and the closely linked DQB1*0602) that was stronger upon adjustment for the degree and duration of CD4+ cell deficiency ($p = 0.04$) and in multivariate analysis ($p = 0.02$) than in unadjusted analysis. A similar trend was seen with DRB1*0701, and no other allele showed a relationship of similar magnitude. *M. avium* complex organisms may more effectively evade host defenses in individuals carrying an HLA polymorphism identical to that associated with *M. tuberculosis* and *M. leprae.*—Authors' Abstract

**Lechat, M. F.** [The program for the elimination of leprosy: ambitious challenge, innovative approaches, questions in abeyance.] Bull. Mem. Acad. R. Med. Belg. **154** (1999) 201–207. (in French)

The WHO-sponsored program on the "elimination of leprosy as a public health problem by the year 2000" has been highly successful. Over 9 million patients were treated by multiple drug therapy. The number of patients worldwide has been reduced by more than 90% over the last 10 years, being at present less than 800,000. Transmission, however, the ultimate goal of the program, has not been interrupted. The

number of new cases detected per year is still high. This observation raises serious questions regarding the future of the program.—Author's English Summary

**Naik, S. S., Thakar, U. H., Phrande, A. M. and Ganapati, R.** Survey of leprosy in unapproachable and uncovered areas. Indian J. Lepr. **71** (1999) 333–335.

Leprosy surveys in tribal population, fishermen and laborers engaged in construction work revealed prevalence rates of 32/10,000, 109/10,000 and 20/10,000 respectively, suggesting that systematic surveys have to be carried out in such population groups to reach the goal of a "world without leprosy."—Authors' Abstract

**Ramos, J.-M. H.** [The Araguaia project in Brazil: some significant findings.] Rev. Leprol. Fontilles **22** (1999) 257–264. (in Portuguese)

The Araguaya Project for the eradication of leprosy, started in 1997 in the northern state of Mato Grosso (Brazil) an extensive and isolated part of the country, is described. The total area covered by the project is approximately 75.000 $km^2$ and a population estimated in 34,000. The work was carried out mainly in 3 villages: Sao Felix do Araguaia, Santa Terezinha and Porto Alegre do Norte. The number of multibacillary cases (59.8%) is greater than the paucibacillary (40.2%) with 109 and 73 patients, respectively, and 76 new cases detected this year and a detection rate of 19.8 cases/10,000 population. The prevalence rate is 53.8/10,000 population, an indication of a very endemic area. The incidence of leprosy in children less then 14 years is very high, with 11 cases/10,000 population. The project will continue under the management and supervision of the work by the Fontilles medical staff.—Author's English Summary

**Selvasekar, A., Geetha, J., Nisha, K., Natrajan, M., Jesudasan, K., Sundar Rao, P. S. S.** Childhood leprosy in an endemic area. Lepr. Rev. **70** (1999) 21–27.

A study was conducted of 794 new cases of leprosy among children (aged 0–14 years) detected and treated with multidrug therapy during 1990–1995 in Gudiyatham Taluk, Tamil Nadu, India. Incidence rates of leprosy and proportion of multibacillary cases increased with age, with the maximum incidence among children aged 10–14 years. Borderline tuberculoid was the most common type of leprosy encountered in the children; 83.1% had a single patch and most children were detected through surveys, 29.8% had history of household contacts with leprosy, mostly parents or grandparents. Reactions and relapses were not uncommon. The findings emphasize the need for more careful surveys for case detection and better follow up in case management.—Trop. Dis. Bull. **96** (1999) 1049

## Rehabilitation

**Zodpey, S. P., Bansod, B. S., Shrikhande, S. N., Maldhure, B. R. and Kulkarni, S. W.** Protective effect of Bacillus Calmett-Guerin (BCG) against leprosy: a population-based case-control study in Nagpur, India. Lepr. Rev. **70** (1999) 287–294.

A population-based, pair-matched, case-control study was carried out in an urban community, Nagpur, India, to estimate the effectiveness of BCG vaccination in the prevention of leprosy. The study included 212 cases of leprosy (diagnosed by WHO criteria), below the age of 35 years, detected during a leprosy survey conducted by the government of Maharashtra over a population of 2,003,325. Each case was pair-matched with one neighborhood control for age, sex and socioeconomic status. A significant protective association between BCG and leprosy was observed (OR = 0.40, 95% CI = 0.23–0.68). The overall vaccine effectiveness (VE) was estimated to be 60%

(95% CI = 32–77). The BCG effectiveness against multibacillary and paucibacillary leprosy was 72% (95% CI = 35–88) and 45% (95% CI = 3–73), respectively. Vaccine was more effective during the first decade of life, among females and in lower socioeconomic strata. The overall prevented fraction was 39% (95% CI = 16–58). In conclusion, this first-ever population-based case-control study performed in central India identified a beneficial role of BCG vaccination in prevention of leprosy in a study population.—Authors' Summary

**Andreotti, C. G. and Fantoni, R. D.** [Peripheral neuropathy in Hansen's disease.] Rev. Hosp. Nac. Baldomero Sommer **2** (1999) 153–157. (in Spanish)

The subject of the present paper is to describe the peripheral neuropathy in Hansen's disease across the clinical spectrum. Multiple mononeuritis is the usual presentation. A population of 400 leprosy patients was studied by clinic examination and electromyography. The patients were asked about: age, gender, time of disease, clinical aspects, reactions, treatments, associated diseases (diabetes, chronic renal insufficiency, alcoholism) nerve microsurgery. Electromyography was performed in all patients. No difference in peripheral nerve injury was found between tuberculoid and lepromatous leprosy patients. Neither was found a significant increase of the leprosy polyneuropathy in patients with another associated pathology (diabetes, chronic renal insufficiency, and alcoholism).—Authors' English Summary

**Beine, A. O.** Modification of the "wrap around" tendon anastomosis of fascia lata graft with a slim double tendon of palmaris longus. Indian J. Lepr. **71** (1999) 337–340.

A modification of Brand's "wrap around" technique of anastomosis is described, which allows joining a double tendon or split tendon of palmaris longus to fascia lata graft, when one of the slim tendons would not allow performance of the Brand tendon anastomosis. Four such cases have been done successfully.—Author's Abstract

**Dong, L., Li, F., Jiang, J. and Zhang, G.** Techniques for covering soft tissue defects resulting from plantar ulcers in leprosy: Part II—First toe web and dorsal foot flaps. Indian J. Lepr. **71** (1999) 297–309.

The first toe web flap consists of the skin and subcutaneous tissues of the contiguous sides between the great and second toes. It is based on the first dorsal metatarsal artery or the common plantar digital artery. This flap was used as an artery pedicled island graft to reconstruct losses of skin and soft tissue cushion in the ball of the foot in the first and second metatarsal head region in 16 cases. Follow-up examination revealed that ulceration had recurred in one case due to dehiscence of the flap margin 12 months post-operatively. The other 15 patients have done well without recurrence at 48 to 124 months follow-up examination.

The dorsal flap of the foot based on the dorsalis pedis artery, the corresponding veins and the deep peroneal nerve was designed in 1974 to resurface skin and soft tissue defects in the sole of the foot. This flap was used in 30 cases of leprosy with excellent results. During follow up 36 to 120 months after surgery the plantar ulcer had recurred in only one case. All the others have done well. The long-term curative effect has thus proved satisfactory.—Authors' Abstract

**Dong, L., Li, F., Wang, Z., Jiang, J., Zhang, G., Peng, J., Zhang, J. and Ye, Y.** Techniques for covering soft tissue defects resulting from plantar ulcers in leprosy: Part I—General considerations and summary of results. Indian J. Lepr. **71** (1999) 285–295.

Recurrent plantar ulceration is a common and serious complication occurring consequent to impairment of the tibial nerve in leprosy patients. In spite of many therapies and a long therapeutic course, it is extremely difficult to abolish this complication in many cases because of extensive skin and soft tissue cushion loss due to repeated infection. Since the early 1970s we have been using microscopic surgical techniques to reconstruct the ulcerated area using eight types of the flaps. In this series of

papers we review our experience (76 patients). Post-operatively, the flaps survived in all cases, the long-term results have proved satisfactory, and recurrent ulceration occurred in only three patients.—Authors' Abstract

**Duerksen, F., Opromolla, D. V. A., Virmond, M. and Garbino, J.** Teaching and training for surgical rehabilitation in hanseniasis: results of 20 years of activities of the Instituto Lauro de Souza Lima in South America. Hansen. Int. **24** (1999) 55–60.

In summary, we can say, based on 20 years of experience, that with education and information—waking up a motivation, training in areas of each team member, keeping continuity by regular supervision visits and allowing and furthering growth through courses and congresses, stable and effective rehabilitation teams for hanseniasis can be established and maintained at a very low cost. More than half of the programs listed have worked with the same team members for over 10 years.—From the Article

**Ogbeiwi, O. and Nash, J.** What would make your life better? A needs analysis of leprosy settlements in the middle belt region of Nigeria. Lepr. Rev. **70** (1999) 295–304.

A needs analysis using rural appraisal and matrix ranking techniques was done in six leprosy communities in the middle belt region of Nigeria. Asked "What would make their life better?" whole village groups were made to list, prioritize and rank their expressed needs by voting in a matrix table drawn on the ground. Out of a total of 504 votes, 31% was for health care or drugs for their general ailments, 23.6% for money and less than 10% for other needs that ranged from water, trade and housing to love and, least, mobility aids. Health care was prioritized in all communities but got the highest votes in three communities; money got the highest in the only two communities where it was prioritized and water in one. The need ranked the highest in each settlement seemed to be a reflection of its peculiar socioeconomic situation. Apart from the similar priorities of health care and money, men's differing priorities were water, housing, clothes and assistance with farming, and women's, school fees for children, family, trade and food. These reflect their different traditional roles. Considering the variety of needs, we think that there is no one solution to rehabilitation in the Nigerian context, but the situation and context of individual settlements should be considered, looking at general health care, income generation or loan schemes, schooling and water supply.—Authors' Summary

**Paschoal, V. D. and Soler, Z. A. S. G.** [A color system for the biopsychosocial characterization of leprosy patients in reaction.] Hansen. Int. **24** (1999) 21–31. (in Portuguese)

This study's objective is to analyze the issues related to nursing assistance Hansen's disease, especially when there is a reactional phenomenon. It was aimed at characterizing a sample of patients with reactional Hansen's disease, emphasizing aspects of the disease and the reaction and investigate, by means of a color system, the major biopsychosocial changes. It is a descriptive-investigatory study carried out at a university hospital developing a Program for Hansen's Disease Control, with 28 adult male and female patients with reactional Hansen's disease participating in the program. Two data collection instruments were used: an interview using a form as a guide, with questions to be orally answered by the patients and a list of the changes experienced by the patients resulting from the reactional crisis, where he/she made a summary, based on the color systems. The data collection period was from October 1977 to March 1998. We concluded that the reactional crisis occurs both in female and male patients with Hansen's disease, with no relation to their age and clinical manifestation of the disease, and were seen throughout up to 5 years of follow up after chemotherapy discharge. It was observed that the reactional crises significantly change the patients' life quality, signaled by the recordings in green, yellow and red, showing the difficulties found to deal with the issues in question, such as living with the pain, future expectations, feeding, and sleep, among

others, in their daily lives. The color system used facilitated the anamnesis and the analysis of the items, providing a basis for more effective nursing and aimed at the individual needs of the patients.—Authors' English Abstract

**van Brakel, W. H., Anderson, A. M., Worpel, F. C., Saiju, R., Bk, H. B., Sherpa, S., Sunwar, S. K., Gurung, J., de Boer, M. and Scholten, E.** A scale to assess activities of daily living in persons affected by leprosy. Lepr. Rev. **70** (1999) 314–323.

The aim of this study was to develop scale for identifying disability among people in the rural areas of developing countries. The studies were carried out in the Green Pastures Hospital and the leprosy field program of the Western Region of Nepal. With the help of staff experienced in working with people with disability, a 68-question questionnaire was made based on the International Classification of Impairments, Activities and Participation (ICIDH-2). A survey was carried out of 269 people affected by leprosy who had impairments, as well as a sample of those who were unimpaired. The survey results were used to develop the questionnaire into a scale, using standard scale development methods. This included checking of criterion validity, discrimination and reliability and stability using weighted kappa statistics. Of the 68 questions, 38 were included in the second draft of the instrument. Eight questions were added to identify difficulty in relationships, about the use of aids and about occupation and employment. The sum score of the scale against the expert score gave a Spearman correlation coefficient of 0.72. Intra- and inter-interviewer reliability coefficients were 0.77 (95% CI 0.73–0.81) and 0.61 (95% CI 0.56–0.67), respectively. The stability test gave an overall kappa of 0.76 (95% CI 0.70–0.82). Four questions with particularly poor results were omitted from the final draft of the instrument. An interview-based instrument was developed for identifying limitations in activities of daily living (disability) in people living in a rural setting in a developing country—the Green Pastures Activity Scale (GPAS). The scale performed well during validity and reliability testing. It consists of 34 activity questions, 5 relationship questions, and 3 questions on the use of aids, occupation and employment.—Authors' Summary

## Other Mycobacterial Diseases and Related Entities

**Ahmad, S., Akbar, P. K., Wiker, H. G., Harboe, M. and Mustafa, A. S.** Cloning, expression and immunological reactivity of two mammalian cell entry proteins encoded by the mce1 operon of *Mycobacterium tuberculosis*. Scand. J. Immunol. **50** (1999) 510–518.

The DNA segments corresponding to two members of the mammalian cell entry operon 1 (mce1) encoding Mce1A and Mce1E proteins were amplified from *Mycobacterium tuberculosis* genomic DNA by polymerase chain reaction, cloned and subcloned into pGEM-T and pGEX-4T-3 vectors, respectively, and expressed in *Escherichia coli* as fusion proteins with glutathione-S-transferase (GST) of *Schistosoma japonicum* as the fusion partner. The recombinant proteins appeared as major cellular proteins in SDS-PAGE gels at the expected molecular mass of 68 kDa and 64 kDa for GST-Mce1A and GST-Mce1E, respectively. The identity of each fusion protein was confirmed by reactivity with anti-GST antibodies in Western immunoblots. The fusion proteins were purified to near homogeneity by affinity chromatography, and purified Mce1A and Mce1E, free of the fusion partner, were recovered following specific proteolytic cleavage of the GST portion by thrombin protease. Purified Mce1E appeared as a single band of 38 kDa; whereas purified Mce1A tended to ex-

ist in degraded as well as aggregated forms of different sizes. The fusion proteins, free GST and monomeric Mce1A and Mce1E reacted in Western immunoblots with antibodies in pools of human sera from 6 to 11 tuberculosis patients. Similar analysis showed the presence of antibodies to GST and Mce1A in pools of human sera from *M. bovis* BCG-vaccinated healthy subjects. When pure Mce1E was blotted against individual sera, antibodies in 4 of 10 sera from tuberculosis patients reacted; whereas no reaction was seen with 10 individual sera from *M. bovis* BCG-vaccinated healthy subjects. However, when the same sera were tested for reactivity to the purified preparation of Mce1A, 8 of 10 sera from both tuberculosis patients and *M. bovis* BCG-vaccinated healthy subjects showed positive reactivity. These findings demonstrate that both Mce1A and Mce1E are expressed and immunogenic during natural infection with *M. tuberculosis.*—Authors' Abstract

**Alderson, M. R., Bement, T., Day, C. H., Zhu, L. Q., Molesh, D., Skeiky, Y. A. W., Coler, R., Lewinsohn, D. M., Reed, S. G. and Dillon, D. C.** Expression cloning of an immunodominant family of *Mycobacterium tuberculosis* antigens using human CD4+ T cells. J. Exp. Med. **191** (2000) 551–559.

Development of a subunit vaccine for *Mycobacterium tuberculosis* (Mtb) is likely to be dependent on the identification of T-cell antigens that induce strong proliferation and interferon gamma (IFN-γ) production from healthy purified protein derivative (PPD+) donors. We have developed a sensitive and rapid technique for screening an Mtb genomic library expressed in *Escherichia coli* using Mtb-specific CD4+ T cells. Using this technique, we identified a family of highly related Mtb antigens. The gene of one family member encodes a 9.9-kD antigen, termed Mtb9.9A. Recombinant Mtb9.9A protein, expressed and purified from *E. coli,* elicited strong T-cell proliferation and IFN-γ production by peripheral blood mononuclear cells from PPD+ but not PPD– individuals. Southern blot analysis and examination of the Mtb genome sequence revealed a family of highly related genes. A T-cell line from a PPD+ donor that failed to react with recombinant Mtb9.9A recognized one of the other family members, Mtb9.9C. Synthetic peptides were used to map the T-cell epitope recognized by this line, and revealed a single amino acid substitution in this region when compared with Mtb9.9A. The direct identification of antigens using T cells from immune donors will undoubtedly be critical for the development of vaccines to several intracellular pathogens.—Authors' Abstract

**Andersen, P. and Smedegaard, B.** CD4+ T-cell subsets that mediate immunological memory to *Mycobacterium tuberculosis* infection in mice. Infect. Immun. **68** (2000) 621–629.

We have studied CD4+ T cells that mediate immunological memory to an intravenous infection with *Mycobacterium tuberculosis.* The studies were conducted with a mouse model of memory immunity in which mice are rendered immune by a primary infection followed by antibiotic treatment and rest. Shortly after reinfection, tuberculosis-specific memory cells were recruited from the recirculating pool, leading to rapidly increasing precursor frequencies in the liver and a simultaneous decrease in the blood. A small subset of the infiltrating T cells was rapidly activated (<20 hr) and expressed high levels of intracellular gamma interferon and the T-cell activation markers CD69 and CD25. These memory effector T cells expressed intermediate levels of CD45RB and were heterogeneous with regard to the L-selectin and CD44 markers. By adoptive transfer into nude mice, the highest level of resistance to a challenge with *M. tuberculosis* was mediated by CD45RB(high), L-selectin(high), CD44(low) cells. Taken together, these two lines of evidence support an important role for memory cells which have reverted to a naive phenotype in the long-term protection against *M. tuberculosis.*—Authors' Abstract

**Appelberg, R., Leal, I. S., Pais, T. F., Pedrosa, J. and Florido, M.** Differences in resistance of C57BL/6 and C57BL/10 mice to infection by *Mycobacterium*

*avium* are independent of gamma interferon. Infect. Immun. **68** (2000) 19–23.

After infection with a low-virulence strain of *Mycobacterium avium,* C57BL/6 and C57BL/10 mice had clear differences in the-control of the infection in their livers and spleens. This difference in susceptibility was not associated with differences in the H-2 complex. It was dependent on the activity of CD4+ T cells but unrelated to the ability of these cells to secrete gamma interferon or to the development of delayed-type hypersensitivity responses at 3 weeks of infection. It was associated with lower total numbers of CD4+ cells present in infected spleens and was related to an earlier induction of protective T cells, as measured by adoptive-transfer assays. These data further strengthen the notion of gamma-interferon-independent mechanisms of protection against mycobacteria.—Authors' Abstract

**AvGay, Y., Jamil, S. and Drews, S. J.** Expression and characterization of the *Mycobacterium tuberculosis* serine/threonine protein kinase PKnB. Infect. Immun. **67** (1999) 5676–5682.

PknB is a member of the newly discovered eukaryotic-like protein serine/threonine kinase (PSTK) family of proteins. The pknB gene was cloned and expressed in *Escherichia coli.* The active recombinant protein was purified and shown to be reactive with antiphosphoserine antibodies, as well as with antibodies to the phosphorylated eukaryotic Ser/Thr kinases, mitogen-activated protein kinase, kinase 3 and 6, P38, and Creb. *In vitro* kinase assays demonstrated that PknB is a functional kinase that is autophosphorylated on serine/threonine residues and is also able to phosphorylate the peptide substrate myelin basic protein. Analysis of pknB expression in *Mycobacterium tuberculosis* indicates the presence of pknB mRNA in (a) organisms grown *in vitro* in bacteriological media, (b) a murine macrophage *in vitro* infection model, and (c) *in vivo* alveolar macrophages from a patient with tuberculosis.—Authors' Abstract

**Banales, J. L., Rivera Martinez, E., Perez Gonzalez, L., Selman, M., Raymond, Y. and Nava, A.** Evaluation of adenosine deaminase activity in the *Mycobacterium tuberculosis* culture supernatants. Arch. Med. Res. **30** (1999) 358–359.

Background. Adenosine deaminase (ADA) catalyzes hydrolytic and irreversible deamination of deoxyadenosine into deoxyinosine and of adenosine into inosine, and is related to lymphocytic proliferation and differentiation. The measurement of ADA activity in body fluids is a useful tool in the evaluation of mycobacterial infections. Elevated ADA activity has been found in pleural effusions of patients with pleural tuberculosis relative to those from patients with nontuberculous pleural diseases, and is mainly associated with cellular host factors such as monocyte-macrophages or lymphocytes. In contrast, there is little information about ADA activity measurement in mycobacteria culture supernatants.

Methods. We evaluated ADA activity as described by Giusti in the culture supernatants of eight *Mycobacterium tuberculosis* isolates.

Results. Mycobacteria culture supernatants did not display any ADA activity.

Conclusions. This result supports the notion that *M. tuberculosis* is not the source of ADA activity. However, increased ADA activity in biological fluids from tuberculosis patients might be due to the interaction of the mycobacterium with host factors.—Authors' Abstract

**Bodman, T., Miltner, E. and Bermudez, L. E.** *Mycobacterium avium* resists exposure to the acidic conditions of the stomach. FEMS Microbiol. Lett. **182** (2000) 45–49.

Organisms of the *Mycobacterium avium* complex are common pathogens immunosuppressed patients such as individuals with AIDS. There is evidence that in AIDS patients, the main route for *M. avium* infection is the gastrointestinal tract. The stomach is a formidable barrier to pathogens and the ability to resist exposure to pH lower than 3 has been shown to be a virulence determinant of enteric pathogens. Incubation of three clinical isolates of *M. avium* under acidic pH revealed resistance of *M. avium* grown both to the exponential and station-

ary phase at pH 2.2 for 2 hr. Inhibition of protein synthesis had no effect ort the acid tolerance. When the duration of the incubation at pH 2.2 was extended to 24 hr, bacteria grown to the stationary phase had a significantly greater tolerance to acid than exponential phase bacteria. *M. avium* incubated with acid in the presence of water was significantly more resistant to pH 2.2 than *M. avium* in the presence of buffer. Pre-adaptation in water prior to exposure to acidic conditions was also associated with increased resistance to pH 2.2. Isoosmolarity of Hanks balanced salt solution appears to be responsible for the impaired resistance to acid between 2 and 24 hr of incubation. These findings indicate that *M. avium* is naturally tolerant to pH <3 and that preadaptation under conditions similar to the conditions where *M. avium* is found in the environment results in increased acid resistance.—Authors' Abstract

**Brandt, L., Elhay, M., Rosenkrands, I., Lindblad, E. B. and Andersen, P.** ESAT-6 subunit vaccination against *Mycobacterium tuberculosis.* Infect. Immun. **68** (2000) 791–795.

The ESAT-6 antigen from *Mycobacterium tuberculosis* is a dominant target for cell-mediated immunity in the early phase of tuberculosis (TB) in TB patients as well as in various animal models. The purpose of our study was to evaluate the potential of ESAT-6 in an experimental TB vaccine. We started out using dimethyl dioctadecylammonium bromide (DDA), an adjuvant which has been demonstrated to be efficient for the induction of cellular immune responses and has been used successfully before as a delivery system for TB vaccines. Here we demonstrate that, whereas immune responses to both short-term-culture filtrate and Ag85B are efficiently induced with DDA, this adjuvant was inefficient for the induction of immune responses to ESAT-6. Therefore, we investigated the modulatory effect of monophosphoryl lipid A (MPL), an immunomodulator which in different combinations has demonstrated strong adjuvant activity for both cellular and humoral immune responses. We show in the present study that vaccination with ESAT-6 delivered in a combination of MPL and DDA elicited a strong ESAT-6-specific T-cell response and protective immunity comparable to that achieved with *M. bovis* BCG.—Authors' Abstract

**Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B. and Guilhot, C.** Identification of a virulence gene cluster of *Mycobacterium tuberculosis* by signature-tagged transposon mutagenesis. Mol. Microbiol. **34** (1999) 257–267.

Tuberculosis remains the greatest cause of death worldwide due to a single pathogen. In order to identify the genes required for the pathogenicity of *Mycobacterium tuberculosis,* a functional genomic approach was developed. A library of signature-tagged transposon mutants of this bacterium was constructed and screened for those affected in their multiplication within the lungs of mice. From 1927 mutants tested, 16 were attenuated for their virulence. The insertions harbored by the selected mutants were mapped on the *M. tuberculosis* genome and most of the mutated loci appeared to be involved in lipid metabolism or transport across the membrane. Four independent mutations identified a cluster of virulence genes located on a 50 kb chromosomal region. These genes might be involved in the production of phthiocerol and phenolphthiocerol derivatives, a group of molecules restricted to eight mycobacterial species, seven of them being either strict or opportunistic pathogens. The interaction of five mutant strains with mouse bone marrow macrophages was investigated. These five mutants were still able to multiply in this cell type. However, in three cases, there was a growth defect in comparison with the wild-type strain. The other two strains exhibited no clear difference from the virulent strain, MT103, in this model. This study, which is the first global research of virulence factors of *M. tuberculosis,* opens the way to a better understanding of the molecules that are key players in the interaction of this pathogen with its host.—Authors' Summary

**Chetchotisakd, P., Mootsikapun, P., Anunnatsiri, S., Jirarattanapochai, K., Choonhakarn, C., Chaiprasert, A.,**

**Ubol, P. N., Wheat, L. J. and Davis, T. E.** Dissemination 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. We report 16 cases of such infection with an unusual presentation seen at Srinagarind 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 disease, were found in these patients. However, 8 patients had 11 episodes of prior infection or coinfection 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.—Authors' Abstract

**Colangeli, R., Spencer, J. S., Bifani, P., Williams, A., Lyashchenko, K., Keen, M. A., Hill, P. J., Belisle, J. and Gennaro, M. L.** MTSA-10, the product of the Rv3874 gene of *Mycobacterium tuberculosis,* elicits tuberculosis-specific, delayed-type hypersensitivity in guinea pigs. Infect. Immun. **68** (2000) 990–993.

In a search for new skin-test reagents specific for tuberculosis, we found that the antigen encoded by gene Rv3874 of *Mycobacterium tuberculosis* elicited delayed-type hypersensitivity in *M. tuberculosis*-infected guinea pigs but not in control animals immunized with *M. bovis* bacillus Calmette-Guerin (BCG) or *M. avium.* The antigen, which was named MTSA-10 (for *M. tuberculosis*-specific antigen 10), is a prime candidate for a component of a new tuberculin that will allow discrimination by a skin test of latent *M. tuberculosis* infection from vaccination with BCG or from sensitization with environmental, nontuberculous mycobacteria.—Authors' Abstract

**Datta, M., Vallishayee, S. R. S., and Diwakara, S. A. M.** Fifteen year follow up of trial of BCG vaccines in South India for tuberculosis prevention. Indian J. Med. Res. **110** (1999) 56–69.

A large-scale, community-based, double-blind, randomized controlled trial was carried out in the Chingleput district of South India to evaluate the protective effect of BCG against bacillary forms of pulmonary tuberculosis. From among 366,625 individuals registered, 281,161 persons were vaccinated with BCG or placebo by random allocation. Two strains of BCG were used, the French and Danish, with a high dose (0.1 mg/0.1ml) and a low dose (0.01 mg/0.1 ml) in each strain. The entire population was followed up for 15 years by means of resurveys every 30 months, and selective follow up every 10 months and continuous passive case finding. There were 560 cases (189, 191 and 180 from the high dose, low dose and placebo groups, respectively) arising over 15 years among 109,873 persons who were tuberculin negative and had a normal chest X-ray at intake. This represents a small fraction of the total incidence of 2.6 per 1000 person-years, most of which came from those who were initially tuberculin positive. The incidence rates in the three "vaccination" groups were similar, confirming the complete lack of protective efficacy seen at the end of 7½ years. BCG offered no overall protection in adults and a low level of overall protection (27%; 95% C.I. –8% to 50%) in children. This lack of protection could not be explained by methodological flaws, or the influence of prior sensitization by nonspecific sensitivity, or because most of the cases arose as a result of exogenous re-infection. The findings at 15 years show that in this population with high infection rates and high nonspecific sensitivity, BCG did not offer any protection against adult forms of bacillary pulmonary tuberculosis.—Authors' Abstract

**Demissie, A., Ravn, P., Olobo, J., Doherty, T. M., Eguale, T., Geletu, M., Hailu, W., Andersen, P. and Britton, S.** T-cell recognition of *Mycobacterium tuberculosis* culture filtrate fractions in tuberculosis patients and their household contacts. Infect. Immun. **67** (1999) 5967–5971.

We examined the immune responses of patients with active pulmonary tuberculosis (TB) and their healthy household contacts to short-term culture filtrate (ST-CF) of *Mycobacterium tuberculosis* or molecular mass fractions derived from it. Our goal was to identify fractions strongly recognized by donors and differences among the donor groups of possible relevance for vaccine development. The study population consisted of 65 human immunodeficiency virus-negative donors from the Hossana Regional Hospital, Hossana, Ethiopia. Peripheral blood leukocytes from the donors were stimulated with different antigens and immune responses were determined. Household contacts produced significantly higher levels of gamma interferon (IFN-γ) than the TB patients in response to antigens present in ST-CF and the 10 narrow-molecular-mass fractions. A similar difference in leukocyte proliferative responses to the antigens between the two groups was also found. In general, while all fractions stimulated immune responses, the highest activity was seen with the low-molecular-mass fractions, which include well-defined TB antigens such as ESAT-6. Leukocytes from contacts of TB patients with severe disease produced higher levels of antigen-specific IFN-γ than those from contacts of patients with minimal disease. Both groups of contacts exhibited higher cell-mediated responses than the patients themselves. The enhanced immune response of healthy contacts, especially those of patients with severe disease, to secreted mycobacterial antigens is suggestive of an early stage of infection by *M. tuberculosis,* which could in time result in overt disease or containment of the infection. This possibility is currently being investigated by follow-up studies of the household contacts.—Authors' Abstract

**De Smet, K. A. L., Kempsell, K. E., Gallagher, A., Duncan, K. and Young, D. B.** Alteration of a single amino acid residue reverses fosfomycin resistance of recombinant MurA from *Mycobacterium tuberculosis.* Microbiology **145** (1999) 3177–3184.

*Mycobacterium tuberculosis* has innate resistance to a range of broad-spectrum antimicrobial agents. This may in part reflect the relative impermeability of the mycobacterial cell wall, but additional specific mechanisms may also be important. In the case of fosfomycin, it has been suggested that a key difference in the active site of the *M. tuberculosis* MurA enzyme might confer resistance. In *Escherichia coli,* fosfomycin covalently binds to a cysteine normally involved in the enzymic activity, while protein alignments predict an aspartate at this position in the *M. tuberculosis* MurA. In the present study, it is demonstrated that the wild-type *M. tuberculosis* MurA is indeed resistant to fosfomycin, and that it becomes sensitive following replacement of the aspartate residue in position 117 by a cysteine. In addition, the study illustrates the use of an inducible expression system in mycobacteria to allow functional characterization of an *M. tuberculosis* enzyme that is unstable during constitutive expression.—Authors' Abstract

**De Voss, J. J., Rutter, K., Schroeder, B. G., Su, H., Zhu, Y. Q. and Barry, C. E.** The salicylate-derived mycobactin siderophores of *Mycobacterium tuberculosis* are essential for growth in macrophages. Proc. Natl. Acad. Sci. U.S.A. **97** (2000) 1252–1257.

*Mycobacterium tuberculosis* is an important pathogen of mammals that relies on 2-hydroxyphenyloxazoline-containing siderophore molecules called mycobactins for the acquisition of iron in the restrictive environment of the mammalian macrophage. These compounds have been proposed to be biosynthesized through the action of a cluster of genes that include both nonribosomal peptide synthase and polyketide synthase components. One of these genes encodes a protein, MbtB, that putatively couples activated salicylic acid with serine or threonine and then cyclizes this precursor to the phenyloxazoline ring sys-

tem. We have used gene replacement through homologous recombination to delete the mbtB gene and replace this with a hygromycin-resistance cassette in the virulent strain of *M. tuberculosis* H37Rv. The resulting mutant is restricted for growth in iron-limited media but grows normally in iron-replete media. Analysis of siderophore production by this organism revealed that the biosynthesis of all salicylate-derived siderophores was interrupted. The mutant was found to be impaired for growth in macrophage-like THP-1 cells, suggesting that siderophore production is required for virulence of *M. tuberculosis.* These results provide conclusive evidence linking this genetic locus to siderophore production.—Authors' Abstract

**Ehlers, S., Kutsch, S., Benini, J., Cooper, A., Hahn, C., Gerdes, J., Orme, I., Martin, C. and Rietschel, E. T.** NOS2-derived nitric oxide regulates the size, quantity and quality of granuloma formation in *Mycobacterium avium*-infected mice without affecting bacterial loads. Immunology **98** (1999) 313–323.

Granuloma formation in response to mycobacterial infections is associated with increased expression of inducible nitric oxide synthase (NOS2) within granuloma macrophages and increased levels of nitrate/nitrite in the sera of infected mice. Continuous treatment with 5 mM or 10mM L-N-6-(1-imino-ethyl)-lysine (L-NIL), a selective NOS2-inhibitor, in acidified drinking water for up to 7 weeks consistently reduced infection-induced nitrate/nitrite to background levels in mycobacteria-infected BALB/c mice. Oral treatment with 5 mM L-NIL initiated at the time of infection significantly exacerbated growth of *Mycobacterium tuberculosis,* but had no effect on *M. avium* colony-forming unit development in the liver, spleen and lungs of intravenously infected mice. In order to examine the role of nitric oxide in mycobacteria-induced granulomatous inflammation in the absence of any effect on the bacterial load, *M. avium*-infected mice were treated with 5 mM L-NIL from day 1 through 38 and the development of granulomatous lesions in the liver was assessed by histology, immunohistology and reverse-transcription-polymerase chain reaction (RT-PCR). Computer- and video-assisted morphometry performed at 4 and 7 weeks post-infection showed that treatment with L-NIL led to markedly increased number, cellularity and size of granulomatous lesions in infected mice regardless of the virulence of the *M. avium* isolate used for infection. Immunohistology of the liver revealed that in mice treated with L-NIL, the numbers of CD3+ T cells, CD21/35+ B cells, CD11b+ macrophages and RB6-8C5+ granulocytes associated with granulomatous lesions were increased. RT-PCR of the liver showed that in L-NIL-treated mice infected with *M. avium,* mRNA levels of tumor necrosis factor, interleukin-12p40, interferon-gamma, interleukin-10 and interferon-gamma-inducible protein-10 (IP-IO) were upregulated, while mRNA levels of interleukin-11, monocyte chemotactic protein-1 (MCP-1) and MCP-5 were similar to those in untreated control infected mice. When *M. avium*-infected mice were treated with 5 mM L-NIL between the 5th and 12th weeks of infection, similar changes in granuloma number and size were found in the absence of any effect on the bacterial load. These findings demonstrate that nitric oxide regulates the number, size and cellular composition of *M. avium*-induced granulomas independently of antibacterial effects by modulating the cytokine profile within infected tissues.—Authors' Abstract

**Ehrenpreis, E. D., Kane, S. V., Cohen, L. B., Cohen, R. D. and Hanauer, S. B.** Thalidomide therapy for patients with refractory Crohn's disease: an open-label trial. Gastroenterology **117** (1999) 1271–1277.

Background & Aims: Inhibition of tumor necrosis factor is a proposed mechanism for the anti-inflammatory properties of thalidomide. We performed an open-label trial of thalidomide in refractory Crohn's disease.

Methods: Twenty-two patients with refractory Crohn's disease (Crohn's Disease Activity Index [CDAI] >200 and/or draining perianal disease) initiated therapy with thalidomide, 200 mg at bedtime (18 patients), or 300 mg at bedtime (4 patients). CDAI and goal interval scores (GIS) were assessed at weeks 0, 4, and 12. Clinical re-

sponse for patients with luminal disease was defined as reduction in CDAI score of >150 points and for fistula patients was two scores of greater than or equal to 1+ in three parameters of the GIS. Clinical remission was defined as a total CDAI <150 (luminal patients) or ≥2+ for all parameters of the GIS (fistula patients).

Results: Nine patients with luminal disease and 13 with fistulas (16 male, 6 female) were enrolled. The median CDAI score at entry was 371 (95–468). Sixteen patients completed 4 weeks of treatment (12 clinical responses, 4 clinical remissions). All 14 patients completing 12 weeks met criteria for clinical response. Nine achieved clinical remission (3 luminal, 6 fistula patients). The median CDAI score was 175 (30–468; $p < 0.001$ vs. baseline).

Conclusions: Thalidomide is efficacious in some patients with refractory Crohn's disease.—Authors' Abstract

**Eisen, T., Boshoff, C., Mak, I., Sapunar, F., Vaughan, M. M., Pyle, L., Johnston, S. R. D., Ahern, R., Smith, I. E. and Gore, M. E.** Continuous low dose thalidomide: a phase II study in advanced melanoma, renal cell, ovarian and breast cancer. Br. J. Cancer **82** (2000) 812–817.

To grow and metastasize, solid tumors must develop their own blood supply by neo-angiogenesis. Thalidomide inhibits the processing of mRNA encoding peptide molecules including tumor necrosis factor-alpha (TNF-γ) and the angiogenic factor vascular endothelial growth factor (VEGF). This study investigated the use of continuous low-dose thalidomide in patients with a variety of advanced malignancies. Sixty-six patients (37 women and 29 men; median age, 48 years; range 33–62 years) with advanced measurable cancer (19 ovarian, 18 renal, 17 melanoma, 12 breast cancer) received thalidomide 100 mg orally every night until disease progression or unacceptable toxicity was encountered. Three of 18 patients with renal cancer showed partial responses and a further 3 patients experienced stabilization of their disease for up to 6 months. Although no objective responses were seen in the other tumor types, there were significant improvements in patients' sleeping ($p < 0.05$) and maintained appetite ($p < 0.05$). Serum and urine concentrations of basic fibroblast growth factor (bFGF), TNF-γ and VEGF were measured during treatment and higher levels were associated with progressive disease. Thalidomide was well tolerated: 2 patients developed WHO grade 2 peripheral neuropathy and 8 patients developed WHO grade 2 lethargy. No patients developed WHO grade 3 or 4 toxicity. Further studies evaluating the use of thalidomide at higher doses as a single agent for advanced renal cancer and in combination with biochemotherapy regimens are warranted.—Authors' Abstract

**Fernandez Roblas, R., Esteban, J., Cabria, F., Lopez, J. C., Jimenez, M. S. and Soriano, F.** *In vitro* susceptibilities of rapidly growing mycobacteria to telithromycin (HMR 3647) and seven other antimicrobials. Antimicrob. Agents Chemother. **44** (2000) 181–182.

The antimicrobial activities of telithromycin (HMR 3647) and seven other antimicrobials against 94 strains of rapidly growing mycobacteria were determined. Telithromycin at a concentration of 1 μg/ml inhibited *Mycobacterium peregrinum* (100%), *M. chelonae* (80%), *M. abscessus-M. mucogenicum* (44.4%), and *M. fortuitum* (2.1%). All or most strains of *M. peregrinum, M. fortuitum,* and *M. mucogenicum* were inhibited by 2 μg of quinolones per ml.—Authors' Abstract

**George, K. M., Pascopella, L., Welty, D. M. and Small, P. L. C.** A *Mycobacterium ulcerans* toxin, mycolactone, causes apoptosis in guinea pig ulcers and tissue culture cells. Infect. Immun. **68** (2000) 877–883.

*Mycobacterium ulcerans* is the causative agent of Buruli ulcer, a tropical ulcerative skin disease. One of the most intriguing aspects of this disease is the presence of extensive tissue damage in the absence of an acute inflammatory response. We recently purified and characterized a macrolide toxin, mycolactone, from *M. ulcerans.* Injection of this molecule into guinea pig skin reproduced cell death and lack of acute inflammatory response similar to that seen

following the injection of viable bacteria. We also showed that mycolactone causes a cytopathic effect on mouse fibroblast L929 cells that is characterized by cytoskeletal rearrangements and growth arrest within 48 hr. However, these results could not account for the extensive cell death which occurs in Buruli ulcer. The results presented here demonstrate that L929 and J774 mouse macrophage cells die via apoptosis after 3 to 5 days of exposure to mycolactone. Treatment of cells with a pan-caspase inhibitor can inhibit mycolactone-induced apoptosis. We demonstrate that injection of mycolactone into guinea pig skin results in cell death via apoptosis and that the extent of apoptosis increases as the lesion progresses. These results may help to explain why tissue damage in Buruli ulcer is not accompanied by an acute inflammatory response.—Authors' Abstract

**Glatman Freedman, A., Mednick, A. J., Lendvai, N. and Casadevall, A.** Clearance and organ distribution of *Mycobacterium tuberculosis* lipoarabinomannan (LAM) in the presence and absence of LAM-binding immunoglobulin M. Infect. Immun. **68** (2000) 335–341.

Lipoarabinomannan (LAM) is a component of the mycobacterial surface which has been associated with a variety of deleterious effects on immune system function. Despite the importance of LAM to the pathogenesis of mycobacterial infection, there is no information available on its fate *in vivo*. In this study, we determined the pharmacokinetics and tissue distribution of exogenously administered LAM in mice. For measurements of serum and tissue LAM concentrations, we developed an enzyme-linked immunosorbent assay which used monoclonal antibodies of different isotypes to capture and detect LAM at concentrations of ≥0.4 μg/ml. Intravenous administration of LAM to mice resulted in transient serum levels with organ deposition in the spleen and in the liver. Immunohistochemical studies localized LAM to the spleen marginal zone macrophages and, to a lesser degree, to liver macrophages. When LAM was administered to mice previously given a LAM-binding immunoglobulin M (IgM), LAM was very rapidly cleared from circulation. In those mice, deposition of LAM in the spleen was significantly reduced while LAM deposition in the liver increased. Administration of LAM-binding IgM resulted in significant levels of IgM to LAM in bile consistent with an increased hepatobiliary excretion of LAM in the presence of specific antibody. Bile, liver extracts, and bile salts were found to rapidly inactivate the immunoreactivity of LAM. The results indicate that serum clearance and organ deposition of LAM in mice are affected by the presence of LAM-binding antibody and suggest a mechanism by which antibody could modify the course of mycobacterial infection.—Authors' Abstract

**Harth, G., Zamecnik, P. C., Tang, J. Y., Tabatadze, D. and Horwitz, M. A.** Treatment of *Mycobacterium tuberculosis* with antisense oligonucleotides to glutamine synthetase mRNA inhibits glutamine synthetase activity, formation of the poly-L-glutamate/glutamine cell wall structure, and bacterial replication. Proc. Nat. Acad. Sci. U.S.A. **97** (2000) 418–423.

New antibiotics to combat the emerging pandemic of drug-resistant strains of *Mycobacterium* tuberculosis are urgently needed. We have investigated the effects on *M. tuberculosis* of phosphorothioate-modified antisense oligodeoxyribonucleotides (PS-ODNs) against the mRNA of glutamine synthetase, an enzyme whose export is associated with pathogenicity and with the formation of a poly-L-glutamate/glutamine cell wall structure. Treatment of virulent *M. tuberculosis* with 10 μm antisense PS-ODNs reduced glutamine synthetase activity and expression by 25%–50% depending on whether one, two, or three different PS-ODNs were used and the PS-ODNs' specific target sites on the mRNA. Treatment with PS-ODNs of a recombinant strain of *M. smegmatis* expressing *M. tuberculosis* glutamine synthetase selectively inhibited the recombinant enzyme but not the endogenous enzyme for which the mRNA transcript was mismatched by 2–4 nt. Treatment of *M. tuberculosis* with the antisense PS-ODNs also reduced the amount of poly-L-glutamate/glutamine in the cell wall by

24%. Finally, treatment with antisense PS-ODNs reduced *M. tuberculosis* growth by 0.7 logs (1 PS-ODN) to 1.25 logs (3 PS-ODNs) but had no effect on the growth of *M. smegmatis,* which does not export glutamine synthetase nor possess the poly-L-glutamate/glutamine (P-L-glx) cell wall structure. The experiments indicate that the antisense PS-ODNs enter the cytoplasm of *M. tuberculosis* and bind to their cognate targets. Although more potent ODN technology is needed, this study demonstrates the feasibility of using antisense ODNs in the antibiotic armamentarium against *M. tuberculosis.*—Authors' Abstract

**Hess, J. and Kaufmann, S. H. E.** Development of novel tuberculosis vaccines. C. R. Acad. Sci. III **322** (1999) 953–958.

Efficacious control of tuberculosis (TB), one of the world's major health threats, is best achieved by a combination of chemotherapy and vaccination. The current vaccine, BCG, fails to prevent pulmonary TB in adults, which is the most prevalent form of this disease. Consequently, the design of novel vaccines against TB is urgently required. Because the acquired immune response is mediated by different T-cell sets, an optimal combination of these populations must be stimulated. Since one third of the world's population is already infected with *Mycobacterium tuberculosis,* two types of vaccine may be required: one for eradication of already established infection and the other for prompt combat of invading microbes. A rational judgment on the efficacy of the different types of vaccine currently under development needs to await further evaluation.—Authors' Abstract

**Hou, W., Wu, Q.-X., Yu, H., Yin, Y.-P., Zhang, Z.-P. and Cai, X.-L.** [Evaluation of Tb-NT-P-BSA antigen in the serodiagnosis of tuberculosis.] J. Zoonoses **15** (1999) 43–44. (in Chinese)

Sera from 40 tuberculous patients and 52 healthy controls [in China] were assayed by ELISA using Tb-NT-P-BSA (containing the terminal triglycosyl unit of the *M. tuberculosis* phenolic glycolipid) as antigen. The sensitivity and specificity were 94.2%, 20% for IgG and 79.6%, 45% for IgM antibody activity, respectively. The results show that the antigen is not sensitive, which will limit its use in the serodiagnosis of tuberculosis.—Trop. Dis. Bull. **96** (1999) 1154

**Huang, K. L., Chang, D. M. and Lu, J. J.** Tuberculosis of skeletal muscle in a case of polymyositis. Scand. J. Rheumatol. **28** (1999) 380–382.

We describe a patient with polymyositis receiving corticosteroid therapy, who presented with persistent fever and mass lesion at the left thigh. Surgical exploration and mycobacterial culture proved to be *Mycobacterium tuberculosis* infection involving the semitendinous muscle of the left thigh. Suitable surgical debridement, anti-TB medications, and sufficient corticosteroid administration resulted in a good control of both polymyositis and the tuberculous infection.—Authors' Abstract

**Keane, J., Remold, H. G. and Kornfeld, H.** Virulent *Mycobacterium tuberculosis* strains evade apoptosis of infected alveolar macrophages. J. Immunol. **164** (2000) 2016–2020.

Human alveolar macrophages (Amϕ) undergo apoptosis following infection with *Mycobacterium tuberculosis in vitro.* Apoptosis of cells infected with intracellular pathogens may benefit the host by eliminating a supportive environment for bacterial growth. The present study compared Amϕ apoptosis following infection by *M. tuberculosis* complex strains of differing virulence and by *M. kansasii.* Avirulent or attenuated bacilli (*M. tuberculosis* H37Ra, *M. bovis* bacillus Calmette-Guerin, and *M. kansassii*) induced significantly more AMϕ apoptosis than virulent strains (*M. tuberculosis* H37Rv, Erdman, *M. tuberculosis* clinical isolate BMC 96.1, and *M. bovis* wild type). Increased apoptosis was not due to greater intracellular bacterial replication because virulent strains grew more rapidly in AMϕ than attenuated strains despite causing less apoptosis. These findings suggests the existence of mycobacterial virulence determinants that modulate the apoptotic response of AMϕ to intracellular infection and support the hypothesis that macrophage

apoptosis contributes to innate host defense in tuberculosis.—Authors' Abstract

**Kim, T. S., Chung, S. W., Kang, B. Y., Choe, Y. K. and Hwang, S. Y.** Induction of *in vivo* persistent anti-mycobacterial activity by interferon-gamma-secreting fibroblasts. Vaccine **18** (2000) 1067–1073.

To determine whether the paracrine secretion of interferon-gamma (IFN-γ) can efficiently stimulate the resistance to *Mycobacterium avium* complex (MAC) infection, 3T3 fibroblasts were stably transduced to secrete IFN-γ (500 units/$10^6$ cells/48 hr) and their effects on MAC infection were investigated in genetically susceptible BALB/c mice, compared with that of free recombinant IFN-γ (rIFN-γ). Immunization with IFN-γ-secreting fibroblasts (3T3-IFN-γ) during intranasal infection with MAC resulted in a significant decrease in bacterial load of lung during the entire 8-week observation period, while rIFN-γ reduced the bacterial load at initial 1 week but not by 8 weeks postinfection. Furthermore, immunization with the 3T3-IFN-γ cells induced and maintained significantly higher levels of cytotoxic activity and nitric oxide production by lung cells than those of rIFN-γ immunization. This work suggests that IFN-γ-secreting fibroblasts may serve as a vehicle for paracrine secretion of IFN-γ in immunotherapy of MAC infection.—Authors' Abstract

**Kirschner, R. A., Parker, B. C. and Falkinham, J. O.** Humic and fulvic acids stimulate the growth of *Mycobacterium avium.* FEMS Microbiol. Ecol. **30** (1999) 327–332.

*Mycobacterium avium,* an environmental, opportunistic pathogenic mycobacterium, has been isolated frequently and in high numbers from waters in Finland and from acid, brown water swamps of the southeastern coastal U.S.A. *M. avium* has also been recovered in high numbers from Finnish drinking water and frequently isolated from Finnish AIDS patients. Boreal forests and brown water swamps are similar in that they are rich in humic and fulvic acids and of low pH and dissolved oxygen. Growth of representative isolates of *M. avium* in natural water was stimulated markedly by the addition of humic and fulvic acids. Further, the *M. avium* isolates grew at pH levels as low as 4.0 and at oxygen levels equal to 4% of atmospheric levels. The high numbers of *M. avium* in boreal waters and brown water swamps are likely due to their ability to proliferate in those humic- and fulvic-rich, acidic, microaerobic environments.—Authors' Abstract

**Kusner, D. J. and Adams, J.** ATP-induced killing of virulent *Mycobacterium tuberculosis* within human macrophages requires phospholipase D. J. Immunol. **164** (2000) 379–388.

The global dissemination of antibiotic-resistant *Mycobacterium tuberculosis* has underscored the urgent need to understand the molecular mechanisms of immunity to this pathogen. Use of biological immunomodulatory compounds to enhance antituberculous therapy has been hampered by the limited efficacy of these agents toward infected human macrophages and lack of information regarding their mechanisms of activity. We tested the hypotheses that extracellular ATP ($ATP^e$) promotes killing of virulent *M. tuberculosis* within human macrophages, and that activation of a specific macrophage enzyme, phospholipase D (PLD), functions in this response. $ATP^e$ treatment of infected monocyte-derived macrophages resulted in 3.5-log reduction in the viability of three different virulent strains of *M. tuberculosis.* Stimulation of macrophage P2X7 purinergic receptors was necessary, but not sufficient, for maximal killing by primary macrophages or human THP-1 promonocytes differentiated to a macrophage phenotype. Induction of tuberculocidal activity by $ATP^e$ was accompanied by marked stimulation of PLD activity, and two mechanistically distinct inhibitors of PLD produced dose-dependent reductions in $ATP^e$-induced killing of intracellular bacilli. Purified PLD restored control levels of mycobacterial killing to inhibitor-treated cells, and potentiated $ATP^e$-dependent tuberculocidal activity in control macrophages. These results demonstrate that $ATP^e$ promotes killing of virulent *M. tuberculosis* within infected human macrophages and strongly suggest that activation

of PLD plays a key role in this process.—Authors' Abstract

**Leuenberger, R. and Bodmer, T.** [Clinical presentation and treatment of *Mycobacterium marinum* infection as seen in 12 cases.] Dtsch. Med. Wochenschr. **125** (2000) 7–10. (in German)

Background and Objective: *Mycobacterium marinum* (M.m.) is the causative pathogen of skin infections that have been called "swimming pool granulomas." An increasing number of reports that deep structures are involved in these infections was the reason for studying the clinical presentation and response of the infection to different therapeutic regimens.

Patients and Methods: All patients (8 men, 4 women, age range 18–73 years) were included in whom, between January 1991 and February 1995, M.m. infection had been proven by culture. The clinical data of these patients were retrospectively obtained by standardized questionnaire.

Results: The infection was limited to the skin in 4 of the 12 patients, deep structures only were involved in 3, and 5 had both. Infections limited to the skin were successfully treated with sulfamethoxazole, and trimethoprim or with tetracylcines, while rifampin, alone or in combination with ethambutol, was efficacious when deep structures were involved. No surgical intervention was—or should be—performed.

Conclusions: Infections with M.m. often involve deep structures, even in the absence of the skin being involved. The term "swimming pool granuloma" is, therefore, misleading when the infection is limited to the skin. A history of a chronic and indolent course, frequent changes of doctor and striking polypharmacy in its treatment are pointers to this infection.—Authors' English Abstract

**Lim, J. H., Park, J. K., Jo, E. K., Song, C. H., Min, D. L., Song, Y. J. and Kim, H. J.** Purification and immunoreactivity of three components from the 30/32 kilodalton antigen 85 complex in *Mycobacterium tuberculosis.* Infect. Immun. **67** (1999) 6187–6190.

The three proteins of the antigen 85 complex (85A, 85B, and 85C), which are major secretory products of *Mycobacterium tuberculosis,* were purified to homogeneity in large amounts by a combination of chromatography on hydroxylapatite, DEAE-Sepharose, and DEAE-Sephacel and gel filtration from *M. tuberculosis* culture filtrate. Then we examined the immunological reactivity of the three proteins in tuberculosis patients and healthy controls. Antibody responses to the 85B and 85A proteins in patients were significantly greater than responses to the 85C protein. In contrast, all three antigens induced significant lymphoproliferation and gamma-interferon production in peripheral blood mononuclear cells from healthy tuberculin reactors.—Authors' Abstract

**Manabe, Y. C., Saviola, B. J., Sun, L., Murphy, J. R. and Bishai, W. R.** Attenuation of virulence in *Mycobacterium tuberculosis* expressing a constitutively active iron repressor. Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 12844–12848.

Iron is an essential nutrient for the survival of most organisms and has played a central role in the virulence of many infectious disease pathogens. Mycobacterial IdeR is an iron-dependent repressor that shows 80% identity in the functional domains with its corynebacterial homolog, DtxR (diphtheria toxin repressor). We have transformed *Mycobacterium tuberculosis* with a vector expressing an iron-independent positive dominant, corynebacterial dtxR hyperrepressor, DtxR (E175K). Western blots of whole-cell lysates of *M. tuberculosis* expressing the dtxR (E175K) gene revealed the stable expression of the mutant protein in mycobacteria. BALB/c mice were infected by tail vein injection with $2 \times 10^5$ organisms of wild type or *M. tuberculosis* transformed with the dtxR mutant. At 16 weeks, there was a 1.2 log reduction in bacterial survivors in both spleen ($p = 0.0002$) and lungs ($p = 0.006$) with *M. tuberculosis* DtxR (E175K). A phenotypic difference in colonial morphology between the two strains also was noted. A computerized search of the *M. tuberculosis* genome for the palindromic consensus sequence to which DtxR and IdeR bind revealed six putative "iron boxes" within 200 bp of an ORF. Using a gel-shift assay we showed

that purified DtxR binds to the operator region of five of these boxes. Attenuation of *M. tuberculosis* can be achieved by the insertion of a plasmid containing a constitutively active, iron-insensitive repressor, DtxR (E175K), which is a homolog of IdeR. Our results strongly suggest that IdeR controls genes essential for virulence in *M. tuberculosis*.—Authors' Abstract

**Martinez, A., Torello, S. and Kolter, R.** Sliding motility in mycobacteria. J. Bacteriol. **181** (1999) 7331–7338.

Mycobacteria are nonflagellated gram-positive microorganisms. Previously thought to be nonmotile, we saw here that *Mycobacterium smegmatis* can spread on the surface of growth medium by a sliding mechanism. *M. smegmatis* spreads as a monolayer of cells which are arranged in pseudofilaments by close cell-to-cell contacts, predominantly along their longitudinal axis. The monolayer moves away from the inoculation point as a unit with only minor rearrangements. No extracellular structures such as pili or fimbriae appear to be involved in this process. The ability to translocate over the surface correlates with the presence of glycopeptidolipids, a mycobacterium-specific class of amphiphilic molecules located in the outermost layer of the cell envelope. We present evidence that surface motility is not restricted to *M. smegmatis* but is also a property of the slow-growing opportunistic pathogen *M. avium*. This form of motility could play an important role in surface colonization by mycobacteria in the environment as well as in the host.—Authors' Abstract

**Miller, B. H. and Shinnick, T. M.** Evaluation of *Mycobacterium tuberculosis* genes involved in resistance to killing by human macrophages. Infect. Immun. **68** (2000) 387–390.

A coinfection assay was developed to examine *Mycobacterium tuberculosis* genes suspected to be involved in resistance to killing by human macrophages. THP-1 macrophages were infected with a mixture of equal numbers of recombinant *M. smegmatis* LR222 bacteria expressing an *M. tuberculosis* gene and wild-type *M. smegmatis* LR222 bacteria expressing the xylE gene. At various times after infection, the infected macrophages were lysed and the bacteria were plated. The resulting colonies were sprayed with catechol to determine the number of recombinant colonies and the number of xylE-expressing colonies. *M. smegmatis* bacteria expressing the *M. tuberculosis* glutamine synthetase A (glnA) gene or open reading frame Rv2962c or Rv2958c demonstrated significantly increased survival rates in THP-1 macrophages relative to those of xylE-expressing bacteria. *M. smegmatis* bacteria expressing *M. tuberculosis* genes for phospholipase C (plcA and plcB) or for high temperature requirement A (htrA) did not.—Authors' Abstract

**Monastirli, A., Georgiou, S., Bolsen, K., Pasmatzi, E., Papapanagiotou, A., Goerz, G., Kalofoutis, A., Merk, H. F. and Tsambaos, D.** Treatment of porphyria cutanea tarda with oral thalidomide. Skin Pharmacol. Appl. Skin Physiol. **12** (1999) 305–311.

Eight male patients with overt clinical and biochemical features of porphyria cutanea tarda (PCT) were orally treated with 300 mg/day thalidomide for 1 week and with 200 mg/day for 3 more weeks. Already after the first week of treatment no new vesicles and/or bullae could be observed. Spontaneous blisters completely disappeared, increased skin fragility subsided and skin hyperpigmentation receded about 2 months after completion of therapy; whereas hypertrichosis persisted. There was a rapid decrease in the urinary total porphyrin excretion which reached normal levels in all patients by the end of the fourth week of therapy; whereas the post-treatment chromatographic pattern of urinary porphyrins revealed a slight reduction of higher carboxylated porphyrin metabolites and an increase in the amount of the excreted coproporphyrin, as compared to the pretreatment period. Somnolence, intermittent constipation and dry mouth occurred in all patients; two patients additionally experienced dizziness. No evidence of peripheral neuropathy could be detected and laboratory investigations revealed no abnormalities, as compared to the pretreatment period. Dur-

ing the 16- to 28-month follow up of the patients, no clinical or biochemical relapse was observed. In view of the encouraging results of the present investigation, further studies are now warranted in order to definitely answer the question whether oral thalidomide may be regarded as an effective alternative approach to the treatment of PCT.—Authors' Abstract

**Mulder, N. J., Powles, R. E., Zappe, H. and Steyn, L. M.** The *Mycobacterium tuberculosis* mysB gene product is a functional equivalent of the *Escherichia coli* sigma factor, KatF. Gene **240** (1999) 361–370.

*Mycobacterium tuberculosis,* the causative agent of tuberculosis, may remain dormant within its host for many years. The nature of this dormant or latent state is not known, but it may be a specialized form of the stationary growth phase. In *Escherichia coli,* KatF (or RpoS) is the major stationary phase sigma factor regulating an array of genes expressed in this phase of growth. A potential *M. tuberculosis* katF homolog was cloned using a fragment of the *E. coli* katF gene as a probe. DNA sequence analysis of a resultant clone showed 100% identity to a fragment of DNA encoding the *M. tuberculosis* mysA and mysB genes. Overexpression of mysB in *M. bovis* BCG resulted in an increase in katG mRNA and catalase and peroxidase activity and an increase in sensitivity of the cells to isoniazid. An increase in katG promoter activity from a reporter vector was demonstrated when mysB was overexpressed from the same plasmid, indicating a direct relationship between MysB and katG expression.—Authors' Abstract

**Murugasu Oei, B., Tay, A. and Dick, T.** Upregulation of stress response genes and ABC transporters in anaerobic stationary-phase *Mycobacterium smegmatis.* Mol. Gen. Genet. **262** (1999) 677–682.

Oxygen starvation triggers an adaptive stationary-phase response in *Mycobacterium smegmatis.* During this anaerobic stationary phase, RNA synthesis continues at a low but significant level. Employing a modified expressed-sequence-tag (EST) approach in combination with the *M. tuberculosis* genome data and comparative Northern analysis, we have identified the first genes that show an increase in transcription in *M. smegmatis* cells that have entered the anaerobic stationary phase. One gene encodes the counterpart of the *M. tuberculosis* NifS-like protein Rv1464. Two genes are homologs of *M. tuberculosis* Rv1460 and Rv3368c, of unknown function. Strikingly, several genes induced by oxygen starvation encode putative stress protection proteins (counterparts of *M. tuberculosis* DnaK, Rv0350; betaine-aldehyde dehydrogenase, Rv768; thioredoxin reductase, Rv3913) and ABC transporters (counterparts of *M. tuberculosis* Rv1463, Rv1473, Rv3197). We conclude that development of general stress resistance and certain active transport processes might play a role in the survival of oxygen-starved *M. smegmatis.*—Authors' Abstract

**Mustafa, T., Phyu, S., Nilsen, R., Bjune, G. and Jonsson, R.** Increased expression of Fas ligand on *Mycobacterium tuberculosis*-infected macrophages: a potential novel mechanism of immune evasion by *Mycobacterium tuberculosis*? Inflammation **23** (1999) 501–521.

We have studied the location and mechanism of apoptosis within the granulomas in the lungs at various stages of slowly progressive primary murine *Mycobacterium tuberculosis* infection. Parallel sections were analyzed for detection of mycobacterial antigens, Fas, and Fas ligand (FasL) by immunohistochemistry, and for apoptotic cells by terminal deoxynucleotidyl-transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) method. The frequency of apoptosis was high in the macrophage aggregates as compared to the lymphocyte aggregates and at the interface between them. Five to seven percent of the vacuolated macrophages in the granulomas expressed FasL intensely. These cells contained large amounts of mycobacterial antigens. These findings suggest that *M. tuberculosis* infection can induce increased expression of FasL in a population of infected macrophages. As a consequence the infected macrophages will be protected from the attack of cytotoxic T cells and activa-

tion of bactericidal mechanisms by Th1 type lymphocytes. This constitutes a novel evasion mechanism for *M. tuberculosis,* possibly explaining the chronic course of infection.—Authors' Abstract

**Mve Obiang, A., Remacle, J., Palomino, J. C., Houbion, A. and Portaels, F.** Growth and cytotoxic activity by *Mycobacterium ulcerans* in protein-free media. FEMS Microbiol. Lett. **181** (1999) 153–157.

The pathogenic slow-growing *Mycobacterium ulcerans* has, until now, been cultured in liquid media containing albumin. Here, we report the first description of use of Sauton medium and modified Reid medium, two protein-free media in which *M. ulcerans* was able to grow and produce its toxin, a major virulence factor of this environmental organism which causes a skin disease commonly called Buruli ulcer. These results suggest that Sauton and modified Reid media may be useful for certain fields of *M. ulcerans* research requiring protein-free growth conditions.—Authors' Abstract

**Nasca, M. R., O'Toole, E. A., Palicharla, P., West, D. P. and Woodley, D. T.** Thalidomide increases human keratinocyte migration and proliferation. J. Invest. Dermatol. **113** (1999) 720–724.

Thalidomide is reported to have therapeutic utility in the treatment of pyoderma gangrenosum, Behcet's disease, aphthous ulcers, and skin wounds. We investigated the effect of thalidomide on human keratinocyte proliferation and migration, two early and critical events in the re-epithelialization of skin wounds. Thalidomide at concentrations less than 1 μm did not affect keratinocyte viability. Using a thymidine incorporation assay, we found that thalidomide, at therapeutic concentrations, induced more than a 2.5-fold increase in the proliferative potential of the cells. Keratinocyte migration was assessed by two independent motility assays: a colloidal gold assay and an *in vitro* scratch assay. At optimal concentrations, thalidomide increased keratinocyte migration on a collagen matrix more than 2-fold in the colloidal gold assay and more than 3-fold in the scratch assay over controls. Although pro-migratory, thalidomide did not alter the level of metalloproteinase-9 secreted into culture medium. Thalidomide did, however, induce a 2- to 4-fold increase in keratinocyte-derived interleukin-8, a pro-migratory cellular autocrine factor. Human keratinocyte migration and proliferation are essential for re-epithelialization of skin wounds. Interleukin-8 increases human keratinocyte migration and proliferation and is chemotactic for keratinocytes. Therefore, thalidomide may modulate keratinocyte proliferation and motility by a chemokine-dependent pathway.—Authors' Abstract

**Oliver, S. J., Freeman, S. L., Corral, L. G., Ocampo, C. J. and Kaplan, G.** Thalidomide analogue CC1069 inhibits development of rat adjuvant arthritis. Clin. Exp. Immunol. **118** (1999) 315–321.

The cytokine tumor necrosis factor-alpha (TNF-α) has been implicated in the etiology of rheumatoid arthritis in humans as well as of experimental arthritis in rodents. Thalidomide, and to a greater extent the new thalidomide analog CC1069, inhibits monocyte TNF-α production both *in vitro* and *in vivo.* The aim of the present study is to establish whether these drugs block production of TNF-α as well as IL-2 by rat leukocytes and whether this inhibition affects the development of rat adjuvant arthritis (AA). Cultured splenocytes were stimulated with either lipopolysaccharide (LPS) or concanavalin A (ConA) in the presence of thalidomide, CC1069, or solvent, and the production of TNF-α and IL-2 were compared. Next, adjuvant was injected into the base of the tail of rats without or with daily intraperitoneal injections with 100–200 mg/kg per day thalidomide or 50–200 mg/kg per day CC1069. Disease activity, including ankle swelling, hind limb radiographic and histological changes, weight gain, and ankle joint cytokine mRNA levels, were monitored. CC1069, but not the parent drug thalidomide, inhibited *in vitro* production of TNF-α and IL-2 by stimulated splenocytes in a dose-dependent manner. *In vivo,* a dose-dependent suppression of AA disease activity occurred in the CC1069-treated animals. In contrast,

thalidomide-treated rats experienced comparable arthritis severity to placebo-treated animals. There was also a reduction in TNF-α and IL-2 mRNA levels in the ankle joints of CC1069-treated rats compared with thalidomide- and placebo-treated arthritic rats. Early initiation of CC1069 treatment suppressed AA inflammation more efficiently than delayed treatment. We conclude that thalidomide, which did not suppress TNF-α or IL-2 production *in vitro* by Lewis rat cells, did not suppress development of rat AA. However, the development of rat AA can be blocked by the thalidomide analog CC1069, which is an efficient inhibitor of TNF-α production and IL-2 *in vitro.*—Authors' Abstract

**Pais, T. F. and Appelberg, R.** Macrophage control of mycobacterial growth induced by picolinic acid is dependent on host cell apoptosis. J. Immunol. **164** (2000) 389–397.

The effects of picolinic acid (PA) on the intramacrophagic growth of *Mycobacterium avium* were studied. PA reduced *M. avium* growth inside mouse macrophages and led to a complete control of mycobacterial growth when added together with IFN-gamma. The mechanism involved did not require TNF-alpha, NO, or the respiratory burst, and was not dependent on either iron or zinc withholding. The mycobacteriostatic activity of the macrophages was associated with the induction of morphological changes that culminated in apoptosis at day 4 of treatment. PA alone induced apoptosis in macrophages, and this effect was increased by IFN-gamma treatment. Apoptosis at day 4 of infection was reduced by inhibiting macrophage activation with the prostaglandin 15 deoxyprostaglandin J(2) or by treating the cells with the antioxidant N-acetylcysteine. Mycobacterial growth was partially restored in macrophages treated with PA and IFN-gamma when 15 deoxy-prostaglandin J(2) was added, concomitant with a delay in apoptosis. N-Acetylcysteine or glutathione could also completely revert the mycobacteriostatic effects of PA or PA plus IFN-gamma.—Authors' Abstract

**Pedrosa, J., Saunders, B. M., Appelberg, R., Orme, I. M., Silva, M. T. and Cooper, A. M.** Neutrophils play a protective nonphagocytic role in systemic *Mycobacterium tuberculosis* infection of mice. Infect. Immun. **68** (2000) 577–583.

Evidence showing that neutrophils play a protective role in the host response to infection by different intracellular parasites has been published in the past few years. We assessed this issue with regard to the infection of mice with *Mycobacterium tuberculosis.* We found a chronic recruitment of neutrophils to the infection foci, namely, to the peritoneal cavity after intraperitoneal infection and to the spleen and liver after intravenous inoculation of the mycobacteria. However, bacilli were never found associated with the recruited neutrophils but rather were found inside macrophages. The intravenous administration of the antineutrophil monoclonal antibody RB6-8C5 during the first week of infection led to selective and severe neutropenia associated with an enhancement of bacillary growth in the target organs of the mice infected by the intravenous route. The neutropenia-associated exacerbation of infection was most important in the liver, where a bacterial load 10-fold higher than that in nonneutropenic mice was found; the exacerbation in the liver occurred both during and after the neutropenic period. Early in infection by *M. tuberculosis,* neutropenic mice expressed lower levels of mRNAs for gamma interferon and inducible nitric oxide synthase in the liver compared to nondepleted mice. These results point to a protective role of neutrophils in the host defense mechanisms against *M. tuberculosis,* which occurs early in the infection and is not associated with the phagocytic activity of neutrophils but may be of an immunomodulatory nature.—Authors' Abstract

**Piatek, A. S., Telenti, A., Murray, M. R., El Hajj, H., Jacobs, W. R., Kramer, F. R. and Alland, D.** Genotypic analysis of *Mycobacterium tuberculosis* in two distinct populations using molecular beacons: implications for rapid susceptibility testing. Antimicrob. Agents Chemother. **44** (2000) 103–110.

Past genotypic studies of *Mycobacterium tuberculosis* may have incorrectly estimated the importance of specific drug resistance mutations due to a number of sampling biases including an overrepresentation of multidrug-resistant (MDR) isolates. An accurate assessment of resistance mutations is crucial for understanding basic resistance mechanisms and designing genotypic drug resistance assays. We developed a rapid, closed-tube PCR assay using fluorogenic reporter molecules called molecular beacons to detect reportedly common *M. tuberculosis* mutations associated with resistance to isoniazid and rifampin. The assay was used in a comparative genotypic investigation of two different study populations to determine whether these known mutations account for most cases of clinical drug resistance. We analyzed samples from a reference laboratory in Madrid, Spain, which receives an overrepresentation of MDR isolates similar to prior studies and from a community medical center in New York where almost all of the resistant isolates and an equal number of susceptible controls were available. The ability of the molecular beacon assay to predict resistance to isoniazid and rifampin was also assessed. The overall sensitivity and specificity of the assay for isoniazid resistance were 85% and 100%, respectively, and those for rifampin resistance were 98% and 100%, respectively. Rifampin resistance mutations were detected equally well in isolates from both study populations; however, isoniazid resistance mutations were detected in 94% of the isolates from Madrid but in only 76% of the isolates from New York ($p = 0.02$). In New York, isoniazid resistance mutations were significantly more common in the MDR isolates (94%) than in single-drug-resistant isolates (44%; $p < 0.001$). No association between previously described mutations in the kasA gene and isoniazid resistance was found. The first mutations that cause isoniazid resistance may often occur in sequences that have not been commonly associated with isoniazid resistance, possibly in other as yet uncharacterized genes. The molecular beacon assay was simple, rapid, and highly sensitive for the detection of rifampin-resistant *M. tuberculosis* isolates and for the detection of isoniazid resistance in MDR isolates.—Authors' Abstract

**Saunders, B. M., Frank, A. A. and Orme, I. M.** Granuloma formation is required to contain bacillus growth and delay mortality in mice chronically infected with *Mycobacterium tuberculosis.* Immunology **98** (1999) 324–328.

Previous studies in this laboratory have shown that mice with a gene disruption to the intracellular adhesion molecule-1 (ICAM-K/O) express normal cell-mediated immunity but cannot mount delayed-type hypersensitivity reactions following *Mycobacterium tuberculosis* infection. However, even in the absence of any appreciable granuloma formation, these mice control bacterial growth for at least 90 days. While not required to control the infection initially, we hypothesized that granuloma formation was required to control chronic infection, acting by surrounding infected cells to prevent bacterial dissemination. To test this, ICAM-1 KO mice were infected with a low dose aerosol of *M. tuberculosis* Erdman and were found to succumb to infection 136 ± 30 days later, displaying highly elevated bacterial loads compared to wild-type mice. Lung tissue from ICAM-K/O mice displayed extensive cellular infiltration and widespread tissue necrosis, but no organized granulomatous lesions were evident; whereas the control mice displayed organized compact granulomas. These data demonstrate that while a granulomatous response is not required initially to control *M. tuberculosis* infection, absence of granulomas during chronic infection leads to increased bacterial growth and host death. Thus, these data support the hypothesis that granuloma formation is required to control chronic infection, acting by surrounding and walling off sites of infection to prevent bacterial dissemination and maintain a state of chronic infection.—Authors' Abstract

**Schon, T., Gebre, N., Sundqvist, T., Aderaye, G. and Britton, S.** Effects of HIV co-infection and chemotherapy on the urinary levels of nitric oxide metabolites in patients with pulmonary tuberculosis. Scand. J. Infect. Dis. **31** (1999) 123–126.

The metabolites of nitric oxide NO (nitrite ($NO_2^-$) and nitrate ($NO_3^-$)) in urine from Ethiopian patients suffering from tuberculosis were measured. The urinary level of $NO_2^-/NO_3^-$ in a group of healthy Ethiopians was 1020 ± 471 μM (N = 22). Untreated HIV-negative patients with active pulmonary tuberculosis (1574 ± 588 μM, $p < 0.01$, N = 12) and household contacts to tuberculosis patients (1949 ± 812 μM, $p = 0.006$, N = 7) had significantly higher levels of urinary $NO_2^-/NO_3^-$ than the control group. Untreated HIV-positive patients with pulmonary tuberculosis did not have increased levels of urinary $NO_2^-/NO_3^-$ (1101 ± 614 μM, N = 6). Some of the HIV-negative untreated patients with pulmonary tuberculosis (1710 ± 519 μM, N = 6) were followed up after treatment and showed a reduction in the levels of urinary $NO_2^-/NO_3^-$ 1 week after treatment (945 ± 599 μM, $p < 0.05$). It is concluded that HIV-negative patients with active pulmonary tuberculosis have increased urinary levels of nitric oxide metabolites with a reduction following specific antituberculous chemotherapy.—Trop. Dis. Bull. **96** (1999) 1152

**Shannon, E. J., Aseffa, A., Pankey, G., Sandoval, F. and Lutz, B.** Thalidomide's ability to augment the synthesis of IL-2 *in vitro* in HIV-infected patients is associated with the percentage of CD4+ cells in their blood. Immunopharmacology **46** (2000) 175–179.

Thalidomide is used for treating erythema nodosum leprosum. It is also used to treat aphthous ulcers in HIV-infected patients. The mechanism of action of this drug is not yet fully understood, but modulation of inflammatory cytokines like IL-2 and TNF-alpha may play a role. We investigated the effect of thalidomide on the production of IL-2 and TNF-alpha by staphylococcal enterotoxin A (SEA) stimulated peripheral blood mononuclear cells (PBMC) from HIV-infected patients. The PBMC from 20 patients was incubated in the presence of 4.0 μg/ml of thalidomide and 50 ng/ml of SEA. After 18 hr, the culture supernatant was assayed for IL-2 and TNF-alpha. The PBMC incubated with thalidomide and SEA produced significantly more IL-2 than those incubated with SEA alone. The TNF-alpha secreted by the same cells incubated with thalidomide and SEA was not significantly different from that secreted by the cells incubated with SEA alone. The amount of IL-2 produced in the thalidomide and SEA treated cultures was directly correlated with the percentage of CD4+ cells in blood and inversely correlated with the percentage of CD8+ cells in blood. No statistically significant correlations were found when comparing the amount of TNF-alpha produced in the thalidomide and SEA treated cultures with the percentage of CD4+ or CD8+ cells in the blood. Thalidomide can act, *in vitro,* as an additional stimulant to augment the synthesis of IL-2 in HIV-infected patients. Increased production of IL-2 by activated T cells may be a mechanism through which it exerts its immunomodulatory effects.—Authors' Abstract

**Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., Munshi, N., Anaissie, E., Wilson, C., Dhodapkar, M., Zeldis, J., Barlogie, B., Siegel, D. and Crowley, J.** Antitumor activity of thalidomide in refractory multiple myeloma. N. Engl. J. Med. **341** (1999) 1565–1571.

Background: Patients with myeloma who relapse after high-dose chemotherapy have few therapeutic options. Since increased bone marrow vascularity imparts a poor prognosis in myeloma, we evaluated the efficacy of thalidomide, which has antiangiogenic properties, in patients with refractory disease.

Methods: Eighty-four previously treated patients with refractory myeloma (76 with a relapse after high-dose chemotherapy) received oral thalidomide as a single agent for a median of 80 days (range 2 to 465). The starting dose was 200 mg daily, and the dose was increased by 200 mg every 2 weeks until it reached 800 mg per day. Response was assessed on the basis of a reduction of the myeloma protein in serum or Bence Jones protein in urine that lasted for at least 6 weeks.

Results: The serum or urine levels of paraprotein were reduced by at least 90% in 8 patients (2 had a complete remission), at least 75% in 6 patients, at least 50% in 7 patients, and at least 25% in 6 patients, for a

total rate of response of 32%. Reductions in the paraprotein levels were apparent within 2 months in 78% of the patients with a response and were associated with decreased numbers of plasma cells in bone marrow and increased hemoglobin levels. The microvascular density of bone marrow did not change significantly in patients with a response. At least one third of the patients had mild or moderate constipation, weakness or fatigue, or somnolence. More severe adverse effects were infrequent (occurring in less than 10% of patients), and hematologic effects were rare. As of the most recent follow up, 36 patients had died (30 with no response and 6 with a response). After 12 months of follow up, Kaplan-Meier estimates of the mean (±S.E.) rates of event-free survival and overall survival for all patients were 22 ± 55 and 58 ± 5%, respectively.

Conclusions: Thalidomide is active against advanced myeloma. It can induce marked and durable responses in some patients with multiple myeloma, including those who relapse after high-dose chemotherapy.—Authors' Abstract

**South Africa, Durban Immunotherapy Trial Group.** Immunotherapy with *Mycobacterium vaccae* in patients with newly diagnosed pulmonary tuberculosis: a randomised controlled trial. Lancet (Br.) **354** (1999) 116–119.

The hypothesis that the addition of *M. vaccae* to standard short-course antituberculosis chemotherapy would decrease the time to achieve a negative sputum culture was tested in a South African study. Patients with newly diagnosed tuberculosis were enrolled between 1994 and 1996 and randomly assigned an injection of saline (placebo) or *M. vaccae* ($10^9$) on treatment day 8. All patients received antituberculosis chemotherapy with rifampin, isoniazid, pyrazinamide, and ethambutol. Sputum samples were checked by microscopy and culture every week for the first 8 weeks and monthly until the end of chemotherapy at 6 months. The primary outcome was the time to a negative sputum culture in the first 8 weeks. Intention-to-treat analysis was used and time to sputum clearance was assessed by log-rank test and Cox's proportional-hazards regression; 172 patients received *M. vaccae* and 175 patients received placebo. At 8 weeks, 70 patients in the *M. vaccae* group and 65 patients in the placebo group had a negative culture; there was no difference between groups in the time to a negative culture ($p = 0.83$). There was no interaction between HIV status and treatment. It is concluded that *M. vaccae* immunotherapy has no benefit when added to standard antituberculosis chemotherapy.—Trop. Dis. Bull. **96** (1999) 1262

**Sun, Z. H. and Zhang, Y.** Spent culture supernatant of *Mycobacterium tuberculosis* H37Ra improves viability of aged cultures of this strain and allows small inocula to initiate growth. J. Bacteriol. **181** (1999) 7626–7628.

Spent culture supernatant from early stationary-phase *Mycobacterium tuberculosis* H37Ra cultures increased the viability of bacilli from aged cultures of this strain and allowed small inocula to initiate growth in liquid culture. The resuscitation factor was acid labile and heat stable, with a mass of less than 1,375 Da.—Authors' Abstract

**Triccas, J. A., Berthet, F.-X., Pelicic, V. and Gicquel, B.** Use of fluorescence induction and sucrose counterselection to identify *Mycobacterium tuberculosis* genes expressed within host cells. Microbiology **145** (1999) 2923–2930.

The identification of *Mycobacterium tuberculosis* genes expressed within host cells would contribute greatly to the development of new strategies to combat tuberculosis. By combining the natural fluorescence of the *Aequoria victoria* green fluorescent protein (GFP) with the counterselectable property of the *Bacillus subtilis* SacB protein, *M. tuberculosis* promoters displaying enhanced *in vivo* activity have been isolated. Macrophages were infected with recombinant *M. bovis* bacille Calmette-Guérin containing a library of *M. tuberculosis* promoters controlling *gfp* and *sacB* expression, and fluorescent bacteria recovered by fluorescence-activated cell sorting. The expression of *sacB* was used to eliminate clones with strong promoter activity outside the macrophage, resulting in the

isolation of seven clones containing *M. tuberculosis* promoters with greater activity intracellularly. The gene products identified displayed similarity to proteins from other organisms whose functions include nutrient utilization, protection from oxidative stress and defense against xenobiotics. These proposed functions are consistent with conditions encountered within the host cell and, thus, suggest that the augmented activity of the isolated promoters/genes may represent strategies employed by *M. tuberculosis* to enhance intracellular survival and promote infection.—Authors' Abstract

**Underhill, D. M., Ozinsky, A., Smith, K. D. and Aderem, A.** Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 14459-14463.

The recognition of mycobacterial cell wall components causes macrophages to secreate tumor necrosis factor-alpha (TNF-α) and other cytokines that are essential for the development of a protective inflammatory response. We show that toll-like receptors are required for the induction of TNF-α in macrophages by *Mycobacterium tuberculosis.* Expression of a dominant negative form of MyD88 (9 signaling components required for toll-like receptor signaling) in a mouse macrophage cell line blocks TNF-α production induced by *M. tuberculosis.* We identify toll-like receptor-2 (TLR2) as the specific toll-like receptor required for this induction by showing that expression of an inhibitory TLR2 (TLR2-P681H) blocks TNF-α production induced by whole *M. tuberculosis.* Further, we show that TLR2-dependent signaling mediates responses to mycobacterial cell wall fractions enriched for lipoarabinomannan, mycolylarabinogalactan-peptidoglycan complex, or *M. tuberculosis* total lipids. Thus, although many mycobacterial cell wall fractions are identified to be inflammatory, all require TLR2 for induction of TNF-α in macrophages. These data suggest that TLR2 is essential for the induction of a protective immune response to mycobacteria.—Authors' Abstract

**Vasiliauskas, E. A., Kam, L. Y., Abreu Martin, M. T., Hassard, P. V., Papadakis, K. A., Yang, H. Y., Zeldis, J. B. and Targan, S. R.** An open-label pilot study of low-dose thalidomide in chronically active, steroid-dependent Crohn's disease. Gastroenterology **117** (1999) 1278–1287.

Background and Aims: Thalidomide decreases production of tumor necrosis factor-alpha, a proinflammatory cytokine associated with Crohn's disease (CD). In this study the safety, tolerance, and efficacy of low-dose thalidomide were evaluated for treatment of moderate-to-severe, steroid-dependent CD.

Methods: Twelve adult male patients with Crohn's Disease Activity Index (CDAI) scores of ≥250 and ≤500 despite ≥20 mg prednisone/day were enrolled. The first 6 patients received 50 mg thalidomide every night, the next 6 received 100 mg every night. Steroid doses were stable during the first 4 weeks of treatment, then tapered during weeks 5–12. CDAI was used to assess response.

Results: (1) Disease activity improved consistently in all patients during weeks 1–4: 58% response, 17% remission. (2) Clinical improvement was generally maintained despite steroid taper during weeks 5–12. All patients were able to reduce steroids by 50%. Forty-four percent discontinued steroids entirely. In weeks 5–12, 70% of patients responded and 20% achieved remission. (3) Side effects were mild and mostly transient, with the most common being drowsiness, peripheral neuropathy, edema, and dermatitis.

Conclusions: Low-dose thalidomide appears to be well tolerated and effective over a 12-week period. Results of this pilot study support the need for controlled multicenter trials of thalidomide for treatment of CD.—Authors' Abstract

**Wallis, R. S., Patil, S., Cheon, S.-H., Edmonds, K., Phillips, M., Perkins, M. D., Joloba, M., Namale, A., Johnson, J. L., Teixeira, L., Dietze, R., Siddiqi, S., Mugerwa, R. D., Eisenach, K. and Ellner, J. J.** Drug tolerance in *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **43** (1999) 2600–2606.

Although *Mycobacterium tuberculosis* is eradicated rapidly during therapy in some

patients with pulmonary tuberculosis, it can persist for many months in others. This study examined the relationship between mycobacterial drug tolerance (delayed killing *in vitro*), persistence, and relapse. It was performed with 39 fully drug-susceptible isolates from a prospective trial of standard short-course antituberculous therapy with sputum smear-positive, human immunodeficiency virus-uninfected subjects with pulmonary tuberculosis in Brazil and Uganda. The rate of killing *in vitro* was determined by monitoring the growth index (GI) in BACTEC 12B medium after addition of drug to established cultures and was measured as the number of days required for 99% sterilization. Drugs differed significantly in bactericidal activity, in the following order from greatest to least, rifampin > isoniazid-ethambutol > ethambutol ($p < 0.001$). Isolates from subjects who had relapses (N = 2) or in whom persistence was prolonged (N = 1) were significantly more tolerant of isoniazid-ethambutol and rifampin than isolates from other subjects ($p < 0.01$). More generally, the duration of persistence during therapy was predicted by strain tolerance to isoniazid and rifampin ($p = 0.012$ and 0.026, respectively). Tolerance to isoniazid-ethambutol and tolerance to rifampin were highly correlated ($p < 0.001$). Tolerant isolates did not differ from others with respect to the MIC of isoniazid; the rate of killing of a tolerant isolate by isoniazid-ethambutol was not increased at higher drug concentrations. These observations suggest that tolerance may not be due to drug-specific mechanisms. Tolerance was of the phenotypic type, although increased tolerance appeared to emerge after prolonged drug exposure *in vivo*. This study suggests that drug tolerance may be an important determinant of the outcome of therapy for tuberculosis.—Authors' Abstract

**Walsh, D. S., Meyers, W. M., Krieg, R. E. and Walsh, G. P.** Transmission of *Mycobacterium ulcerans* to the nine-banded armadillo. Am. J. Trop. Med. Hyg. **61** (1999) 694–697.

Animal models for *Mycobacterium ulcerans* infections (Buruli ulcer) include guinea pigs, rats, and mice, but each has limitations in replicating the spectrum of human disease. Here, 19 adult nine-banded armadillos were inoculated intradermally with *M. ulcerans*. Injection sites were examined and skin samples obtained for histologic and microbiology studies. Necropsies were conducted to assess systemic involvement. In group 1 (N = 4), 2 animals developed progressive skin ulcers with undermined borders at the injection sites within 6–10 weeks. Biopsies showed features similar to human disease including extensive necrosis in the deep dermis and subcutaneous fat, mixed cellular infiltrates, and acid-fast bacilli (AFB). In group 2 (N = 15), 5 animals developed progressive skin ulcers; 3 had evanescent papulo-nodules, 3 died shortly after inoculation of unknown causes, and 4 showed no signs of infection. Lesion samples from 3 animals with progressive ulcers were culture positive for AFB. Our findings indicate that nine-banded armadillos are susceptible to *M. ulcerans* and may develop cutaneous lesions that closely mimic Buruli ulcer.—Authors' Abstract

**Wichelhaus, T. A., Schafer, V., Brade, V. and Boddinghaus, B.** Molecular characterization of *rpoB* mutations conferring cross-resistance to rifamycins on methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **43** (1999) 2813–2816.

Mutations of the *rpoB* gene conferring resistance to rifampin were analyzed in 40 methicillin-resistant *Staphylococcus aureus* isolates obtained from six countries. Interestingly, the majority of clinical isolates showed multiple mutations within *rpoB*. The amino acid substitution 481His→Asn was the most prevalent one, capable of conferring low-level resistance on its own. Crossresistance to rifampin, rifabutin, and rifapentine was demonstrated for all mutants identified. The level of resistance to rifamycins correlated with both the mutation position and type of amino acid substitution.—Authors' Abstract

**Wilson, W., DeRisi, J., Kristensen, H. H., Imboden, P., Rane, S., Brown, P. O. and Schoolnik, G. K.** Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microar-

ray hybridization. Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 12833–12838.

Tuberculosis is a chronic infectious disease that is transmitted by cough-propelled droplets that carry the etiologic bacterium, *Mycobacterium tuberculosis.* Although currently available drugs kill most isolates of *M. tuberculosis,* strains resistant to each of these have emerged, and multiply resistant strains are increasingly widespread. The growing problem of drug resistance combined with a global incidence of seven million new cases per year underscore the urgent need for new antituberculosis therapies. The recent publication of the complete sequence of the *M. tuberculosis* genome has made possible, for the first time, a comprehensive genomic approach to the biology of this organism and to the drug discovery process. We used a DNA microarray containing 97% of the ORFs predicted from this sequence to monitor changes in *M. tuberculosis* gene expression in response to the antituberculous drug isoniazid. Here we show that isoniazid induced several genes that encode proteins physiologically relevant to the drug's mode of action, including an operonic cluster of five genes encoding type II fatty acid synthase enzymes and fbpC, which encodes trehalose dimycolyl transferase. Other genes, not apparently within directly affected biosynthetic pathways, also were induced. These genes, EfpA, fadE23, fadE24, and ahpC, likely mediate processes that are linked to the toxic consequences of the drug. Insights gained from this approach may define new drug targets and suggest new methods for identifying compounds that inhibit those targets.—Authors' Abstract