CURRENT LITERATURE

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

General and Historical

Croft, R. A. and Croft, R. P. Tuberculosis control is good for established leprosy programmes. Lepr. Rev. **68** (1997) 139–146.

Tuberculosis (TB) control was introduced into part of the Danish Bangladesh Leprosy Mission's large leprosy control program in 1994. This was in line with the government's policy of combining leprosy and TB control. We report our experience with integration. Leprosy case finding has increased during the period, and staff satisfaction and morale has also risen despite the larger workload. We observed that the field work skills of leprosy workers was brought to bear in a very positive way on TB control. TB patients suffer considerable impoverishment as a result of their illness, paralleling the social dehabilitation often seen in leprosy sufferers. TB control is good for established leprosy programs.—Authors' Summary

Chemotherapy

Chen, X.-S., Ye, G.-Y., Jiang, C., Li, W.-Z., Bian, J., Wang, H. and Chen, W. An investigation of attitudes, beliefs, and behaviour of leprosy patients, family members and PHC workers towards multidrug therapy in Yangzhou and Dongtai Districts of China. Lepr. Rev. **68** (1997) 155–161.

To improve the operational efficiency of multidrug therapy (MDT) implementation in rural areas, an investigation into the attitudes, beliefs and behavior of leprosy patients and their family members as well as primary health care (PHC) workers toward MDT was carried out in the Yangzhou and Dongtai Districts of China. A sample of 370 leprosy patients, 594 family members and 730 PHC workers was interviewed or investigated individually using questionnaires. The results showed that: 1) the presently used MDT is acceptable to a wide range of patients although a small number of patients have various problems in their treatment; 2) the patients' habits in daily drug administration, their awareness of the risk of default and confidence in MDT have a positive influence in increasing drug compliance; and 3) the supervision and encouragement of family members to patients' treatment which is associated with their knowledge on MDT is also beneficial to patients' drug compliance. However, only half of the PHC workers had a basic knowledge of MDT and a desire to participate in MDT implementation, a finding which clearly calls for urgent attention and improvement. In order to ensure the effective implementation of MDT, there is a need to educate leprosy patients and their family members as well as PHC workers to establish the patients' correct awareness of MDT, obtain family support and motivate the PHC workers.—Authors' Summary

Lardo, M. M., Diaz, N. B., Artaza, J. R., Carbia, C. D., Nazer, R. and Valdez, R. [Vitamin E as protective agent against hemolysis in leprosy patients under dapsone treatment.] Medicina (Buenos Aires) **57** (1997) 150–154. (in Spanish)

Dapsone (4,4′diaminodiphenyl-sulfone) commonly used in the treatment of patients

who suffer from leprosy, is a strongly oxidative drug, producing damage to the red cell membrane. This study investigated whether vitamin E would have a protective effect on the red cell membrane from oxidant damage caused by dapsone in patients with leprosy. We have studied 16 patients for 4 months, divided into two groups. Group 1 (n = 7) dapsone (DDS): 100 mg/day; Group 2 (n = 9) dapsone: 100 mg/day plus vitamin E: 800 U/day. We did not include patients with low levels of glucose-6-phosphate dehydrogenase (G-6-PD) because of their sensitivity to this drug. At the beginning of the treatment we determined the level of G-6-PD.

All patients showed a normocytic normochromic anemia with a decrease in haptoglobin levels (below 5 mg/dl). Statistical analyses showed that reticulocyte counts did not show significant differences between the two groups.

As for methemoglobin, we observed in Group 1 an increase between the first and the fourth month, which was not seen in group 2.

Statistical analyses of the results suggest that oral vitamin E confers partial protective effect and does not correct the hemolysis parameters produced by dapsone treatment except for methemoglobin levels which were more sensitive to the oxidant damage.—Authors' English Abstract

Long, C., *et al.* [Efficacy of the drugs made in China used in WHO-MDT regimen for leprosy.] China Lepr. J. **13** (1977) 23–24. (in Chinese)

Since the year 1986, 58 cases of MB leprosy had been treated with the drugs made in China, including RFP, B663 and DDS, according to the WHO-MDT regimen for MB leprosy and have been followed up for 7 years after completion of MDT. The results showed that the effectiveness of the drugs made in China are equal to those of imported ones clinically and bacteriologically.—Authors' English Abstract

Mochizuki, Y., Oishi, M., Nishiyama, C. and Iida, T. Active leprosy treated effectively with ofloxacin. Intern. Med. (Tokyo) **35** (1996) 749–751.

A 25-year-old-Filipino who had been in Japan since December 1992 presented with polymorphous eruptions over the whole body, right ulnar nerve paresis, polyneuropathy and hypalgesia in the area of eruptions. Because the biopsy specimen showed foam cells, histiocytes, epithelioid cells, many *Mycobacterium leprae* and no giant cells, the diagnosis of borderline-lepromatous (BL) leprosy was made. The symptoms were improved by the administration of ofloxacin 300 mg/day.—Authors' Abstract

Shetty, V. P. and Antia, N. H. Relapse in a borderline-tuberculoid case of leprosy 5 years after the release from rifampicin monotherapy. (Case report) Lepr. Rev. **68** (1997) 162–166.

Paucibacillary cases of leprosy are now routinely being treated with two drugs, namely, rifampin (RFP) and diamino diphenyl sulfone (DDS). We report here a paucibacillary case of borderline-tuberculoid (BT) leprosy, who had taken a 1-year course of daily RFP monotherapy, and relapsed as BT 3 years after the release from treatment. *Mycobacterium leprae* derived from this case was sensitive to both 0.01 g% DDS and 0.03 g% RFP.—From the article

Shetty, V. P., Suchitra, K., Uplekar, M. W. and Antia, N. H. Higher incidence of viable *Mycobacterium leprae* within the nerve as compared to skin among multibacillary leprosy patients released from multidrug therapy. Lepr. Rev. **68** (1997) 131–138.

As identified by a significant growth in the foot pads of immunosuppressed mice, the incidence of viable bacteria in a group of 26 multibacillary (BL-LL) patients released from multidrug (MDT) treatment was found to be two times more in the nerves (46%) as compared to skin (23%). Evidently there was a positive correlation between the overall bacterial load and the

incidence of viable organisms. Bacterial growth was also observed in 2 out of 5 cases where neither the skin nor the nerve homogenate had shown any presence of acid-fast bacilli. Histopathology of biopsies, skin as well as nerve, including those having viable bacteria did not show any features of active disease.—Authors' Summary

Clinical Sciences

Hua, K. [A survey of vision defect among residents in Shanghai leprosary.] China Lepr. J. **13** (1997) 28–29. (in Chinese)

Among 200 residents in Shanghai Leprosarium, including 145 men and 55 women with a mean age of 61.8 years, a survey showed that there were 118 cases of lagophthalmos (54%) and that out of 180 eyes with lagophthalmos 69 (3.3%) were blind, 56 (31.1%) had low vision, 55 had some vision and 109 have keratitis (60.6%); 82 persons have no lagophthalmos. Of 220 eyes without lagophthalmos, 20.5% were blind, 24.1% had low vision and 55.5% have some vision. Twenty-three of 62 persons with bilateral lagophthalmos (37.1%) and 13 of 82 without lagophthalmos (15.8%) were bilaterally blind. Fifty-three persons have or had iridocyclitis (26.5%), of which six were bilaterally blind (11.3%).—Author's English Abstract

Husain, S., Mishra, B. and Malaviya, G. N. Rifampicin and isoniazid in the treatment of leprous nerve abscesses. Acta Leprol. **10** (1997) 147–150.

Thirty-nine cases of borderline tuberculoid leprosy having nerve abscesses (15 with sinuses) were treated with daily dose of rifampin and isoniazid for 6 months along with standard multidrug therapy. The patients were followed up for 3 to 5 years. No recurrence of abscess or sinus was observed. Observations indicate that a medical approach is required at times to supplement surgical intervention for management of these cases.—Authors' Summary

Lewallen, S. Prevention of blindness in leprosy: an overview of the relevant clinical and programme-planning issues. (Review) Ann. Trop. Med. Parasitol. **91** (1997) 341–348.

Visual disability continues to be a significant problem in leprosy patients due to cataract, chronic iridocyclitis, and corneal disease. Clinical and epidemiological aspects of these problems are described and the current status of eye care in leprosy programs is discussed.—Author's Abstract

Namisato, M., Kakuta, M., Kawatsu, K., Obara, A., Izumi, S. and Ogawa, H. Transepidermal elimination of lepromatous granuloma: a mechanism for mass transport of viable bacilli. (Case report) Lepr. Rev. **68** (1997) 167–172.

A 35-year-old male with lepromatous leprosy showed significant progression of the disease on initial examination. Along with typical lepromatous skin lesions, many scar-forming lesions were present, mainly on his extremities. Some lesions showed erosive surfaces. From clinicopathological findings, these lesions were suspected to be due to the partial excretion of intradermal lepromatous granulomata by "transepidermal elimination." Increased local volume, which might be due mainly to rapidly growing lepromatous infiltration before chemotherapy, is suspected of triggering this phenomenon. There is no doubt that many fresh *Mycobacterium leprae* were included in these excretions. After the initiation of chemotherapy, no new scar-forming lesions were observed.—Authors' Summary

Opromolla, D. V. A. A problem in leprosy classification. Hansen. Int. **21** (1996) 37–42.

Type 1 reactions are one of the greatest problems in the classification of leprosy,

and the evolution of its concept is analyzed since the pre-sulfone era. These reactions occur in established tuberculoid and borderline cases and can suddenly occur, being the only manifestation of the disease. On the other hand they can occur before, during and after treatment. In the opinion of the author, all of them are the expression of the same phenomenon, that is, a delayed type of hypersensitivity to antigens released by destruction of multiplying *M. leprae*. Based in discussion of these facts it is proposed a new classification of the disease, in which each clinical form is immutable and there is no shift in the immunity.—Author's Summary

Wang, D., *et al.* [The case of leprosy newly detected in the last seven years in Zhenjiang Province.] China Lepr. J. **13** (1997) 25–26. (in Chinese)

In the period of 1989 to 1995, 225 new cases of leprosy have been detected in Zhenjiang Province. Of 224 cases with complete records, 90 were found in 1989 and 1990, and then some 25 yearly. Among them there were 156 men and 68 women, 10 children with age less than 14 years, with the disease duration of 1 to 20 years, including 151 less than 2 years, and 180 peasants and 191 marrieds; 67 cases were found by leprosy workers, 62 by self- or others report and 80 by dermatological or other medical services. The position and characteristics of their first skin lesion were discussed in detail.—Authors' English Abstract

Wu, X., *et al.* [Disabilities of leprosy patients in Sichuan Province.] China Lepr. J. **13** (1997) 16–18. (in Chinese)

A disability survey has been done in 1990 to 1992 in Sichuan Province, involving 20,559 people who have or had leprosy, and showed that the disability rate was 63.08%, being 62.15% in MB and 64.83% in PB, and the disability of grade II and III was in 53.05%. Among active cases under MDT, those completed MDT and cures, the rates were 38.24%, 49.69% and 71.52% and the degree increased in turn, respectively. In a randomly sampled group of 626 people a similar condition was seen. It is proved that the disability might still develop or worsen even after completion of MDT. The authors emphasize the importance of self-care for the patients.—Authors' English Abstract

Immuno-Pathology

Antunes, S. L. G., Alves, S. L. S., Corte-Real, S., Meireles, M. N., Nery, J. A. daC. and Sarno, E. N. DOPA-stained melanocytes in the macular lesions of early leprosy. Hansen. Int. **21** (1996) 22–28.

The mechanism of association of hypopigmentation and sensory loss in leprosy macular lesions has not been clarified yet. The biopsy of a macular lesion on the medial face of the right forearm of a 14-year-old male leprosy patient was submitted to DOPA-staining for melanocytes, which is specific for the melanocytic tyrosinase enzyme and is a proper method for identifying and counting these cells in the skin. A contralateral specimen of the same patient went through the same procedure as a control experiment. The specimen from the macular lesion showed a higher number of DOPA-stained melanocytes than did the control fragment. Dermal melanocytes were present in high amounts in the abnormal specimen. Increased expression of tyrosinase by melanocytes in the macular lesions may reflect a positive feedback stimulus represented by the lack of substrate tyrosine, which may in turn be utilized by the mycobacterial agent. Ultrastructural study of the normal and pathological specimens showed no significant differences in the morphological appearance of melanocytes and their melanosomes. These results suggest that the utilization of phenolic compound by *Mycobacterium leprae* may be involved in the mechanism of hypopigmentation. A higher number of cases will be necessary to confirm this hypothesis.—Authors' Abstract

Antunes, S. L. G., Noviski, M. E. G., Nery, J. A. daC., Rocha de Almeida, S. M. and Sarno, E. N. Ultrastructural study on the dermal nerves in the cutaneous macular lesions of patients with early leprosy. Hansen. Int. **21** (1996) 14–21.

Thirteen biopsies of macular lesions of early leprosy patients were studied ultrastructurally with transmission electron microscopy (TEM). All of the biopsies displayed at least one dermal nerve partially or completely encircled by mononuclear cells in the conventional histopathological study with light microscopy. The patients' diagnoses varied from indeterminate leprosy to borderline tuberculoid (BT). In the ultrastructural study, 27 dermal nerve branches were found in the 13 biopsies. Twenty dermal nerve branches in 11 biopsies were found to display no inflammatory involvement. Seven nerves in seven biopsies were morphologically associated with mononuclear leukocytic cells. Four biopsies exhibited nerves with and without inflammatory involvement concomitantly. Three nerves showed morphological evidence of endoneurial fibrosis, not morphologically associated with the inflammatory process at least in the sections examined. No detectable axonal and Schwann cell ultrastructural changes even in the 27 nerves were found. The sensory loss exhibited by the patients before the institution of treatment was completely reversed in eight patients after the end of multidrug therapy regimen. These findings suggest that sensory loss in the early stages of leprosy may be caused by reversible pathological mechanisms, rather than by anatomical damage. It is also possible, concerning the mechanisms of nerve damage in leprosy, to speculate on the existence of a pathological process which may precede the inflammation.—Authors' Abstract

Castells Rodellas, A., Bartralot Soler, R., Pascual Valdes, C., Jauregui Pallares, L. and Terencio de las Aguas, J. [The immunology of leprosy, 1996.] Rev. Leprol. Fontilles **21** (1997) 11–81. (in Spanish)

The macrophage immune response, humoral and cellular immunity against *Mycobacterium leprae* and its different antigens are studied. Total and partial defects of the cellular immunity exist with disturbed secretion patterns of cytokines while the immediate immunity is unaltered. The immunodeficiency isn't satisfactorily explained (313 references).—Authors' English Summary

Chimelli, L., Freitas, M. and Nascimento, O. Value of nerve biopsy in the diagnosis and follow up of leprosy: the role of vascular lesions and usefulness of nerve studies in the detection of persistent bacilli. J. Neurol. **244** (1997) 318–323.

Nerve biopsy specimens from 53 patients with leprosy and neuropathy were taken from the sural, the dorsal branch of the ulnar, or the superficial radial nerves and processed for light and electron microscopy. There was inflammation in 40 cases (75%), 7 with a granulomatous reaction, various stages of fibrosis in 35 (66%), and endoneurial vascular neoformation in 7. In two cases, small focal infarcts were associated with marked endoneurial inflammation compressing the vessels, in addition to endoneurial lymphocytic vasculitis. Most had an axonal neuropathy of varying degree, some with total fiber loss, others with predominant small myelinated and unmyelinated fiber loss. Signs of demyelination and remyelination were the main findings in 9 cases (17%). Bacilli were present in endothelial, perineurial, Schwann cells and in macrophages. On two occasions, they lost their alcohol acid resistance, were suspected in semithin sections, and confirmed ultrastructurally. The biopsy was decisive for the diagnosis of leprosy in 15 cases (28%), most without skin lesions. We evaluated the effectiveness of the treatment in 20 (37.7%), 12 without and 8 with bacilli, despite negativity in the skin. The diagnosis of leprosy based on skin lesions was confirmed with the nerve biopsy in 9 cases, 6 had an inflammatory neuropathy suggestive of leprosy in the absence of bacilli, and 3 had nonspecific changes in the sural nerve since the neuropathy was in the upper limbs. We conclude that nerve biopsy is indicated for the diagnosis of leprosy in cases without clinically visible skin lesions and to

evaluate the effectiveness of the treatment. In these cases the ultrastructural studies are important for recognition of the bacilli. Vascular lesions may play an important role in the progression of the nerve damage, including the occurrence of focal nerve infarcts which, to our knowledge, have not been previously reported in association with leprosy.—Authors' Abstract

Cooper, A. M., Saunders, B. M., D'Souza, C. D., Frank, A. A. and Orme, I. M. *Mycobacterium tuberculosis*-driven processes in gene-disrupted mice. Bull. Inst. Pasteur **95** (1997) 85–96.

Mice which have disrupted genes for important components of the immune system have been used to study the role of these components in the immune response to infection with *Mycobacterium tuberculosis.* This has resulted in the identification of interleukin-12 (IL-12), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α) and inducible nitric oxide synthase (iNOS) as being essential to the protective response. Less crucial but perhaps more intriguing roles for other molecules such as intercellular adhesion molecule (ICAM) and IL-6 have also been suggested by this kind of analysis.—Authors' Abstract

Daniel, E., Ebenezer, G. J. and Job, C. K. Pathology of iris in leprosy. Br. J. Ophthalmol. **81** (1997) 490–492.

Aim—The histopathological features of the iris in leprosy were studied by light microscopy.

Method—Formalin-fixed and paraffin-embedded iris tissue excised during cataract surgery from 20 leprosy patients were sectioned and studied with hematoxylin and eosin stain and modified Fite Faraco's stain for acid-fast bacilli (AFB).

Results—Chronic inflammatory reactions were seen in the iris of 11 patients, seven of whom did not have any clinically demonstrable evidence of iridocyclitis. Smooth muscle disruption and destruction were seen in two specimens. AFB were found in the iris tissue of a polar lepromatous patient whose skin smears were negative for AFB and who had completed the WHO recommended antileprosy multidrug therapy (MDT).

Conclusion—Histopathology discloses far more silent chronic iridocyclitis in leprosy patients than are diagnosed clinically. AFB can persist in the iris tissue even after completion of MDT. Smooth muscle disruption and destruction, a cause of the miotic pupil in leprosy has been conclusively demonstrated histopathologically.—Authors' Abstract

de Jong, R., Janson, A. A. M., Faber, W. R., Naafs, B. and Ottenhoff, T. H. M. IL-2 and IL-12 act in synergy to overcome antigen-specific T cell unresponsiveness in mycobacterial disease. J. Immunol. **159** (1997) 786–793.

IL-2 secretion by APC is critical for the development of protective Th1-type responses in mycobacterial (*Mycobacterium avium* and *M. tuberculosis*) infections in mice. We have studied the role of IL-12 and IL-2 in the generation of *M. leprae*-specific T-cell responses in humans. Leprosy patients were defined as low/nonresponders or high responders based on the level of T-cell proliferation in *M. leprae*-stimulated PBMC. In high responders, *M. leprae*-induced proliferation was markedly suppressed by neutralizing anti-IL-12 mAb (inhibition 55% ± 6%). Neutralization of IL-2 activity resulted in an inhibition of 77% ± 4%. Given the importance of endogenous IL-2 and IL-12 in *M. leprae*-induced responses, we investigated the ability of rIL-2 and rIL-12 to reverse T-cell unresponsiveness in low/nonresponder patients. Interestingly, rIL-12 and rIL-2 strongly synergized in restoring both *M. leprae*-specific T-cell proliferation and IFN-gamma secretion almost completely to the level of responder patients. A similar synergy between rIL-2 and rIL-12 was also observed in high responders when suboptimal *M. leprae* concentrations were used for T-cell stimulation. Our data demonstrate a crucial role for endogenous IL-12 and IL-2 in *M. leprae*-induced T-cell activation. Most importantly, we show that rIL-2 and rIL-12 act in synergy to overcome Ag-specific Th1 cell unresponsiveness. These findings may be applicable to the design of an-

timicrobial and antitumor vaccines.—Authors' Abstract

Dhiman, N. and Khuller, G. K. Immunoprophylactic properties of 71-kDa cell wall-associated protein antigen of *Mycobacterium tuberculosis* H37Ra. Med. Microbiol. Immunol. **186** (1997) 45–51.

Proteins associated with the cell-wall peptidoglycan (CW-Pr) of *Mycobacterium tuberculosis* H37Ra were isolated to evaluate their immunoreactivity and immunoprophylactic properties against experimental tuberculosis. Chemical treatment of the cell wall with trifluoromethanesulfonic acid : anisole (2:1) resulted in the release of three proteins of 71, 60 and 45 kDa as reserved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A comparative study of immune responses elicited to individual proteins in mice immunized with CW-Pr emulsified in incomplete Freund's adjuvant showed the 71-kDa protein to be the most immunoreactive antigen. This 71-kDa protein was found to crossreact with the 70-kDa heat-shock protein from *M. leprae* and possessed ATPase activity. Mice immunized with the 71-kDa protein exhibited significantly higher immune responses, on the basis of T- and B-cell reactivity, as compared to a *M. bovis* bacillus Calmette Guerin (BCG)-vaccinated group. The culture supernatants collected from 71-kDa-stimulated lymphocytes exhibited increased interferon-gamma and interleukin-2 production. The protective efficacy of the 71-kDa protein in comparison to BCG was determined by challenging the mice with a virulent strain *M. tuberculosis* H37Rv. The 71-kDa protein was found to be more protective in animals challenged at 8 and 16 weeks post-immunization, shown by increased survival rates and decreased viable bacilli counts in the target organs as compared to BCG-vaccinated animals.—Authors' Abstract

Fleury, R. N., Taborda, P. R. O. and Opromolla, D. V. A. [Wagner's granulomatosis and hanseniasis; hardly a fortuitous association?] Hansen. Int. **21** (1996) 43–53. (in Spanish)

The authors review a case of a male patient presenting with borderline lepromatous leprosy for 28 years. After release from treatment (with negative skin smears) he was under irregular dapsone monotherapy. Ten years later he showed again active skin lesions with positive skin smears (2+ with some solid-stained bacilli).

Suspecting dapsone resistance, he was put under clofazimine (CFZ) for 12 months, showing partial regression of the skin lesions. Although he continued to use dapsone after this period, the skin lesions continued to show regression and skin smears presented no solid-stained bacilli. Episodes of ENL were noted in this period.

Four years before death, he began to present signs of chronic renal failure which was related to a probable secondary amyloidosis due to leprosy.

Autopsy revealed (1) intense impregnation of CFZ in the mucosa of the upper air pathway, stomach and bowels, as well as in lymph nodes; (2) regressive lepromatous infiltrate with positive bacilloscopy with granular bacilli in various sites and some organs such as liver, spleen and lymph nodes; (3) amyloid deposits in several organs and (4) a necrotizing nodular lesion close to the principal bronchi of the right lung, showing palisaded granuloma and granulomatous vasculitis, which was interpreted as a localized pulmonary manifestation of Wegener's granulomatosis (WG). The evidence showed that it was indeed a case of advanced borderline leprosy associated, fortuitously or not, to a WG, in the initial phase or an asymptomatic limited form. There is no mention of such association in the literature.

It is assumed that the WG could be related to some unidentified bacteria which exposes neutrophilic proteases producing antibodies to neutrophil cytoplasmic antigens (ANCAs). The authors suggest the possibility of leprosy to be the underlying bacterial disease in this case. The determination of these antibodies in leprosy and the search for WG may suggest a real association between these two intriguing clinical entities.—Authors' English Summary

Hasan, Z., Schlax, C., Kuhn, L., Lefkovits, I., Young, D., Thole, J. and

Pieters, J. Isolation and characterization of the mycobacterial phagosome: segregation from the endosomal/lysosomal pathway. Mol. Microbiol. **24** (1997) 545–553.

Mycobacteria have the ability to persist within host phagocytes, and their success as intracellular pathogens is thought to be related to the ability to modify their intracellular environment. After entry into phagocytes, mycobacteria-containing phagosomes acquire markers for the endosomal pathway, but do not fuse with lysosomes. The molecular machinery that is involved in the entry and survival of mycobacteria in host cells is poorly characterized. Here we describe the use of organelle electrophoresis to study the uptake of *Mycobacterium bovis* bacille Calmette Guerin (BCG) into murine macrophages. We demonstrate that live, but not dead, mycobacteria occupy a phagosome that can be physically separated from endosomal/lysosomal compartments. Biochemical analysis of purified mycobacterial phagosomes revealed the absence of endosomal/lysosomal markers LAMP-1 and β-hexosaminidase. Combining subcellular fractionation with two-dimensional gel electrophoresis, we found that a set of host proteins was present in phagosomes that were absent from endosomal/lysosomal compartments. The residence of mycobacteria in compartments outside the endosomal/lysosomal system may explain their persistence inside host cells and their sequestration from immune recognition. Furthermore, the approach described here may contribute to an improved understanding of the molecular mechanisms that determine the intracellular fate of mycobacteria during infection.—Authors' Summary

Kaur, G., Sachdeva, G., Bhutani, L. K. and Bamezai, R. Association of polymorphism at COL3A and CTLA4 loci on chromosome 2q31-33 with the clinical phenotype and *in vitro* CMI status in healthy and leprosy subjects: a preliminary study. Hum. Genet. **100** (1997) 43–50.

Two genetic loci, vit. COL3A and CTLA4, located within the chromosome 2q31-33 region in the vicinity of the proposed syntenic site of the mouse "Bcg" locus were genotyped by the polymerase chain reaction in leprosy patients and healthy individuals. All the subjects studied were assessed as *in-vitro* responders/nonresponders to mycobacterial antigens. Simple sequence length polymorphism analysis revealed five (236 to 312 bp) and eight (84 to 120 bp) allelomorphs for COL3A and CTLA4, respectively. Our preliminary analysis showed a significant association between the 250-bp COL3A allelomorph in the homozygous condition and the multibacillary form of leprosy ($p < 0.05$; relative risk = 5.5). Another allelic (312 bp) variant of COL3A was significantly correlated with nonresponsiveness to *M. leprae* antigens *in vitro* ($p < 0.01$). The 104-bp allelomorph of CTLA4 was not observed in any of the 25 cases of leprosy. This absence was statistically significant ($p < 0.05$) when compared with normal healthy controls and depicted a high relative risk (RR = 25.83). An additional observation of the predominance of a unique 84-bp CTLA4/CTLA4-like allelomorph was observed in the Indian subjects studied.—Authors' Abstract

Khare, S., Bhutani, L. K. and Rao, D. N. Quantitiave assessment of tuftsin receptor expression and second messenger during *in vitro* differentiation of peripheral blood derived monocytes of leprosy patients. Mol. Cell. Biochem. **171** (1997) 1–10.

Tuftsin, a tetrapeptide (Thr-Lys-Pro-Arg), is known to potentiate the immunogenic activity of antigen-fed macrophages. The present study describes the mechanism of action of tuftsin in leprosy patients throughout the spectrum of the disease *in vitro* as a function of culture age in terms of a) involvement of second messengers cAMP, cGMP and [Ca2+] and b) number of tuftsin binding sites/and their relative affinities on the monocytes/macrophages. There is apparently no direct involvement of either cAMP or cGMP while comparing the stimulated and unstimulated cultures during *in vitro* differentiation of monocytes (days 1, 3 and 7) or with the spectrum of the disease. Inhibition of superoxide anion release

either by verapamil or with Quin 2 clearly demonstrated the involvement of [Ca2+] as a second messenger during activation of monocytes/macrophages with tuftsin. Scatchard analysis of radiolabelled tuftsin binding data showed only one type of tuftsin receptor (low affinity) on BL/LL monocytes/macrophages and normal and BT/TT cultures showed a gradual change in receptor number and affinities (low to high) with the maturation of monocytes to macrophages in contrast to BL/LL groups which displayed a significantly less number of receptors. This study elicits a model which depicts that the biological responses/metabolic functions of early monocytes of normal and BT/TT gradually increase with the age of the culture until day 3 and tapers off thereafter in the older (day 7) cultures; whereas the monocytes/macrophages of BL/LL group are metabolically active only on day 1. The present study thereby implies that the clearance of leprosy bacilli from lepromatous leprosy lesions as a consequence of local or systemic immunotherapy (in the present study, the macrophage modulation by tuftsin) depends on the influx of new competent macrophages, rather than the local activation of resident lepromatous macrophages.—Authors' Abstract

Kim, J., Sette, A., Rodda, S., Southwood, S., Sieling, P. A., Mehra, V., Ohmen, J. D., Oliveros, J., Appella, E., Higashimoto, Y., Rea, T. H., Bloom, B. R. and Modlin, R. L. Determinants of T cell reactivity to the *Mycobacterium leprae* GroES homologue. J. Immunol. **159** (1997) 335–343.

The 10-kDa protein antigen of *Mycobacterium leprae,* a human GroES hsp10 cognate, is a major T-cell antigen in human leprosy infection. We investigated the mechanism for T-cell responsiveness to this antigen according to the trimolecular interaction between T cell, peptide, and antigen-presenting element. This research was accomplished by mapping T-cell epitopes in leprosy patients and correlating these responses with peptide-MHC binding affinities. We found that the majority of tuberculoid leprosy patients responded to peptides corresponding to residues 25–39 and 28–42. Truncation analysis of these peptides mapped the exact epitope to be within the overlapping region comprising residues 28–39. Responsiveness was correlated with the HLA-DRB5*0101 allele, which bound the peptides with moderate affinity. This allele is linked to HLA-DR2, which is associated with the resistant form of leprosy. Therefore, T-cell responsiveness in tuberculoid leprosy may be mediated by the ability of HLA-DRB5*0101 to bind and present peptides of the immunodominant 10-kDa antigen.—Authors' Abstract

Lowrie, D. B., Silva, C. L., Colston, M. J., Ragno, S. and Tascon, R. E. Protection against tuberculosis by a plasmid DNA vaccine. Vaccine **15** (1997) 834–838.

Past attempts to use fractions of mycobacteria as an alternative to BCG have given disappointing results. The availability of cloned genes and suitable vectors has now opened a new avenue in which individual mycobacterial protein antigens are synthesized within transfected mammalian cells. In an *ex vivo* transfection approach with a retroviral vector we found that even a single antigen (hsp65) could evoke strong protection when expressed as a transgene and that expression of protection was largely a function of antigen specific cytotoxic T cells. We now find that intramuscular injection of plasmid DNA expressing the antigen from either a viral or a murine promoter can also give protection equivalent to bacillus Calmette-Guerin (BCG). Plasmids expressing some other mycobacterial antigens, hsp70, 36 kDa and 6 kDa, are also effective, suggesting that this approach may lead to a new vaccine.—Authors' Abstract

Lozes, E., Huygen, K., Content, J., Denis, O., Montgomery, D. L., Yawman, A. M., Vandenbussche, P., Van Vooren, J. P., Drowart, A., Ulmer, J. B. and Liu, M. A. Immunogenicity and efficacy of a tuberculosis DNA vaccine encoding the components of the secreted antigen 85 complex. Vaccine **15** (1997) 830–833.

BALB/c and C57BL/6 mice were injected intramuscularly with plasmid DNA

encoding the three components of the immunodominant 30–32 kDa antigen 85 complex (Ag85A, Ag85B, and Ag85C) from *Mycobacterium tuberculosis* culture filtrate, in order to investigate the utility of nucleic acid vaccination for induction of immune responses against mycobacterial antigens. Ag85A and Ag85B encoding plasmids induced a robust Th1-like response toward native Ag85, characterized by elevated levels of interleukin (IL)-2, interferon-gamma, and TNF-alpha. Levels of IL-4, IL-6, and IL-10 were low or undetectable. Plasmid encoding Ag85C was not effective. Cytotoxic T-cell activity was also generated in *in vitro* restimulated splenocyte cultures from Ag85A and Ag85B DNA vaccinated mice. Finally, Ag85A and Ag85B DNA vaccination conferred significant protection against mycobacterial replication in lungs from B6 mice, subsequently challenged. Therefore, this technique may be useful for the definition of protective antigens of *M. tuberculosis* and the development of a more effective tuberculosis vaccine.—Authors' Abstract

Montgomery, D. K., Huygen, K., Yawman, A. M., Deck, R. R., Dewitt, C. M., Content, J., Liu, M. A. and Ulmer, J. B. Induction of humoral and cellular immune responses by vaccination with *M. tuberculosis* antigen 85 DNA. Cell. Mol. Biol. **43** (1997) 285–292.

DNA vaccines have been demonstrated to be effective in inducing protective cell-mediated immune responses in animal models of infectious disease. In order to investigate this approach for potential use as a vaccine for tuberculosis, DNA constructs encoding *Mycobacterium tuberculosis* antigen 85A (Ag85A) were prepared. Expression of Ag85A in mammalian cells was demonstrated by transient transfection of cells *in vitro*. Intramuscular injection of Ag85A DNA vaccines resulted in the generation of anti-Ag85A antibodies and robust cell-mediated immune responses, as measured by lymphoproliferation of spleen cells *in vitro* upon specific antigen restimulation, leading to protection in animal challenge models. Therefore, the technique of DNA vaccination is effective in inducing relevant immune responses for protection against tuberculosis and may be used to identify the protective antigens of *M. tuberculosis*.—Authors' Abstract

Narayan, R., Maheshwari, P. K., Desikan, K. V. and Harinath, B. C. Detection of S-100 protein and anticeramide antibodies in leprosy patients by ELISA. Lepr. Rev. **68** (1997) 117–124.

The status of assay for S-100 antigen protein and anticeramide antibodies in serum in understanding nerve damage in different forms of leprosy were evaluated by the enzyme immunoassay. Based on the clinical and smear examination, patients were classified as indeterminate (Ind), tuberculoid (TT), borderline tuberculoid (BT), borderline lepromatous (BL) and lepromatous.

Antibody levels against ceramide were observed in sera of leprosy patients with 37.5% of Ind, 28% of TT, 66% BT, 78% BL and 62% LL patients positive as against 8% endemic normal sera. The mean OD ranged from 0.141 to 0.275 in different groups of leprosy. In contrast, S-100 was detected in 71.4% Ind, 88.8% TT, 76.4% BT, 100% BL and 95.8% LL, while 5% of ENL samples were positive for S-100 antigen. Mean S-100 levels in these different categories of patients were significantly higher Ind—0.45 ng/ml, TT—0.32 ng/ml, BT—0.23 ng/ml, BL—0.23 ng/ml, LL—0.19 ng/ml as compared to that of normal 0.07 ng/ml.

In general S-100 seems to be a more sensitive and reliable marker than anticeramide antibodies for nerve damage. Five out of 7 indeterminate cases show increased levels of S-100, showing an extent of nerve damage similar to that of TT and could be a useful marker for assessing nerve damage in indeterminate patients for better management.—Authors' Summary

Nau, G. J., Guilfoile, P., Chupp, G. L., Berman, J. S., Kim, S. J., Kornfeld, H. and Young, R. A. A chemoattractant cytokine associated with granulomas in tuberculosis and silicosis. Proc. Natl. Acad. Sci. U.S.A. **94** (1997) 6414–6419.

Chronic inflammation and granuloma formation are associated with mononuclear

cell infiltrates and are characteristic pathologic responses in tuberculosis. To identify host cell genes involved in tuberculous pathology, we screened macrophage cDNA libraries for genes induced by mycobacterial infection. One gene isolated in this screen, osteopontin (also known as early T lymphocyte activation protein 1 or Eta-1), was of particular interest because it is a cytokine and macrophage chemoattractant. Further study revealed that *Mycobacterium tuberculosis* infection of primary human alveolar macrophages causes a substantial increase in osteopontin gene expression. Osteopontin protein was identified by immunohistochemistry in macrophages, lymphocytes, and the extracellular matrix of pathologic tissue sections of patients with tuberculosis. Increased osteopontin expression also was found to be associated with silicosis, another granulomatous disease. The association of osteopontin with granulomatous pathology, together with the known properties of the protein, suggest that osteoprotein may participate in granuloma formation. The strategy of identifying host genes whose expression is altered by infection thus can provide valuable clues to disease mechanisms and will be increasingly valuable as additional human genome sequences become available.—Authors' Abstract

Sachdeva, G., Kaur, G., Bhutani, L. K. and Bamezai, R. Genetic variations at the T cell receptor gamma locus in circulating peripheral blood mononuclear cells of clinically categorised leprosy patients. Hum. Genet. **100** (1997) 30–34.

The allelic polymorphisms at exon 3 and exon 2 of the T-cell receptor (TCR) C gamma 2 (TRGC2) gene, generating 18-kb and 5.4-kb HindIII fragments, respectively, were found to be more frequent in multibacillary (MB) leprosy patients than in the controls ($p < 0.005$ and $p < 0.001$, respectively) when screened with the IDP2.11 probe. The frequencies of heterozygotes for the 18-kb allele and homozygotes for the 5.4-kb allele were found to be significantly higher in the MB patients than in the controls ($p < 0.001$). Interestingly, the 8.0-kb allele, originating from the triplication of exon 2 of C gamma 2, was observed exclusively in the paucibacillary (PB) leprosy patients. Further, when DNA samples were screened with the pH60 probe for the HindIII RFLP at the TCR J gamma 2 (TRGJ2) gene segment, the 2.1-kb allele was again more prevalent in leprosy patients with the MB form of the disease than in the PB patients and the controls ($p < 0.025$). The frequency of homozygotes for the 2.1-kb allele was also significantly higher in the MB patients than in the PB patients ($p < 0.010$) and the controls ($p < 0.025$). A significant difference was observed in the frequencies of detectable rearrangements involving the V gamma 7/8 and V gamma 9 gene segments at the gamma locus between circulating peripheral blood mononuclear cells of the MB leprosy patients and the controls. These rearrangements were detected less frequently in the MB patients ($p < 0.001$ for V gamma 7/8 and $p < 0.005$ for V gamma 9).—Authors' Abstract

Singh, N. B., Gupta, H. P., Srivastava, A., Kandpal, H. and Srivastava, U. M. L. Lymphostimulatory and delayed-type hypersensitivity responses to a candidate leprosy vaccine strain: *Mycobacterium habana.* Lepr. Rev. **68** (1997) 125–130.

Lymphostimulatory and delayed-type hypersensitivity (DTH) immune responses to a candidate antileprosy vaccine *Mycobacterium habana* have been quantified in inbred AKR mice. *M. habana* vaccine in three physical states, live, heat-killed and γ-irradiated, was given intradermally to separate groups of mice and after 28 days these mice were given subcutaneous challenge with heat-killed *M. leprae* and heat-killed *M. habana* in the left hind foot pad. Live BCG vaccine alone and in combination with γ-irradiated *M. habana* were also compared similarly. A sufficient degree of DTH response was generated in mice by *M. habana* vaccine in all physical forms against two challenge antigens (lepromin and habanin). The BCG combination with *M. habana* did not increase the DTH response indicating internal adjuvanticity endowed in *M. habana.* The active hypersensitivity of immunized mice was transferable

to syngeneic mice by the transfer of sensitized cells from the donor to the recipient mice intravenously. *M. leprae*-infected rhesus monkey PBMC have shown comparable stimulatory response with *M. habana* (sonicate), and *M. leprae* (sonicate) antigens. The possibility of developing *M. habana* as a candidate antileprosy vaccine is discussed.—Authors' Summary

Ulmer, J. B., Liu, M. A., Montgomery, D. L., Yawman, A. M., Deck, R. R., DeWitt, C. M., Content, J. and Huygen, K. Expression and immunogenicity of *Mycobacterium tuberculosis* antigen 85 by DNA vaccination. Vaccine **15** (1997) 792–794.

Plasmid DNA expression vectors encoding *Mycobacterium tuberculosis* antigen 85 (Ag85) were tested as vaccines in preclinical animal models. Expression of secreted and nonsecreted forms of Ag85 was observed after transient transfection of cells *in vitro*. In mice, both types of Ag85 DNA constructs induced strong humoral and cell-mediated immune responses, as measured by ELISA of sera and recall responses of spleen cells restimulated *in vitro*, respectively. Therefore, DNA vaccination is an effective means of expressing mycobacterial proteins in eukaryotic cells leading to the induction of potent immune responses.—Authors' Abstract

Via, L. E., Deretic, D., Ulmer, R. J., Hibler, N. S., Huber, L. A. and Deretic, V. Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. J. Biol. Chem. **272** (1997) 13326–13331.

Mycobacterium tuberculosis and the closely related organism *M. bovis* can survive and replicate inside macrophages. Intracellular survival is at least in part attributed to the failure of mycobacterial phagosomes to undergo fusion with lysosomes. The transformation of phagosomes into phagolysosomes involves gradual acquisition of markers from the endosomal compartment. Members of the rab family of small GTPases which confer fusion competence in the endocytic pathway are exchanged sequentially onto the phagosomal membranes in the course of their maturation. To identify the step at which the fusion capability of phagosomes containing mycobacteria is compromised, we purified green fluorescent protein-labeled *M. bovis* BCG phagosomal compartments (MPC) and compared GTP-binding protein profiles of these vesicles with latex bead phagosomal compartments (LBC). We report that the MPC do not acquire rab7, specific for late endosomes, even 7 days postinfection; whereas this GTP-binding protein is present on the LBC within hours after phagocytosis. By contrast, rab5 is retained and enriched with time on the MPC, suggesting fusion competence with an early endosomal compartment. Prior infection of macrophages with *M. bovis* BCG also affected the dynamics of rab5 and rab7 acquisition by subsequently formed LBC. Selective exclusion of rab7, coupled with the retention of rab5 on the mycobacterial phagosome, may allow organisms from the *M. tuberculosis* complex to avert the usual physiological destination of phagocytosed material.—Authors' Abstract

Zhu, X. J., Venkataprasad, N., Thangaraj, H. S., Hill, M., Singh, M., Ivanyi, J. and Vordermeier, H. M. Functions and specificity of T cells following nucleic acid vaccination of mice against *Mycobacterium tuberculosis* infection. J. Immunol. **158** (1997) 5921–5926.

The 38-kDa glyolipoprotein of *Mycobacterium tuberculosis* has been known to evoke prominent T-cell and antibody responses in patients with active tuberculosis. In this study, we investigated its protective capacity using plasmid DNA immunization in a mouse experimental model. Prior knowledge of several antigenic determinants has been beneficial for analyzing the phenotype and specificity of T cells, which determine the efficacy of this vaccination procedure. C57BL/6 mice responded to the 38-kDa gene-pcDNA3 plasmid with strong CD4+ Th1 and CD8+ cytotoxic T-cell responses of the IFN-gamma-producing Tc1

phenotype. After challenge with virulent tubercle bacilli, the bacterial load in the spleens and lungs of vaccinated mice was reduced to a level similar to that imparted by *Mycobacterium bovis* bacille Calmette-Guerin vaccination. Notably, the specificity of CD4+ and CD8+ T cells from DNA-vaccinated and tubercle-infected mice was found to be strikingly different in respect of several peptide epitopes. The identified peptides recognized by T cells from protected mice are of further interest for the development of subunit-based vaccines against tuberculosis.—Authors' Abstract

Microbiology

Ainsa, J. A., Perez, E., Pelicic, V., Berthet, F. X., Gicquel, B. and Martin, C. Aminoglycoside 2′-N-acetyltransferase genes are universally present in mycobacteria: characterization of the aac(2′)-lc gene from *Mycobacterium tuberculosis* and the aac(2′)-lc gene from *Mycobacterium smegmatis.* Mol. Microbiol. **24** (1997) 431–441.

The genus *Mycobacterium* comprises clinically important pathogens such as *M. tuberculosis,* which has reemerged as a major cause of morbidity and mortality worldwide especially with the emergence of multidrug-resistant strains. The use of fast-growing species such as *M. smegmatis* has allowed important advances to be made in the field of mycobacterial genetics and in the study of the mechanisms of resistance in mycobacteria. The isolation of an aminoglycoside-resistance gene from *M. fortuitum* has recently been described. The aac(2′)-lb gene is chromosomally encoded and is present in all isolates of *M. fortuitum.* The presence of this gene in other mycobacterial species is studied here, and genes homologous to that of *M. fortuitum* have been found in all mycobacterial species studied. In this report, the cloning of the aac(2′)-lc gene from *M. tuberculosis* H37Rv and the aac(2′)-ld gene from *M. smegmatis* mc(2)155 is described. Southern blot hybridizations have shown that both genes are present in all strains of this species studied to date. In addition, the putative aac(2′)-le gene has been located in a recent release of the *M. leprae* genome. The expression of the aac(2′)-lc and aac(2′)-ld genes has been studied in *M. smegmatis* and only aac(2′)-ld is correlated with aminoglycoside resistance. In order to elucidate the role of the aminoglycoside 2′-N-acetyltransferase genes in mycobacteria and to determine whether they are silent resistance genes or whether they have a secondary role in mycobacterial metabolism, the aac(2′)-ld gene from *M. smegmatis* has been disrupted in the chromosome of *M. smegmatis* mc(2)155. The disruptant shows an increase in aminoglycoside susceptibility along with a slight increase in the susceptibility to lysozyme.—Authors' Abstract

Azad, A.K., Sirakova, T. D., Fernandes, N. D. and Kolattukudy, P. E. Gene knockout reveals a novel gene cluster for the synthesis of a class of cell wall lipids unique to pathogenic mycobacteria. J. Biol. Chem. **272** (1997) 16741–16745.

Surface-exposed unusual lipids containing phthiocerol and phenolphthiocerol are found only in the cell wall of slow-growing pathogenic mycobacteria and are thought to play important roles in host-pathogen interaction. The enzymology and molecular genetics of biosynthesis of phthiocerol and phenolphthiocerol are unknown. We postulate the domain organization of a set of multifunctional enzymes and a cluster of genes (pps) that would encode these enzymes for the biosynthesis of phthiocerol and phenolphthiocerol. A cosmid containing the postulated pps gene cluster was identified by screening a genomic library of *Mycobacterium bovis* BCG with the postulated homologous domains from mycocerosic acid synthase and fatty acid synthase genes as probes. Homologous cosmids were also identified in the genomic libraries of *M. tuberculosis* and *M. leprae. M. bovis* BCG was transformed with a pps dis-

ruption construct, made from the BCG cosmid by introducing the hygromycin resistance gene as the positive-selectable marker and the sacB gene as the counter-selectable marker. Gene disruption by homologous recombination with double crossover was confirmed by polymerase chain reaction and Southern hybridization. Chromatographic analysis showed that the phenolphthiocerol derivative, mycoside B, and phthiocerol dimycocerosates were not produced by the gene knockout mutants. This result confirms the identity of the pps genes. With the identification of the pps gene clusters in both *M. tuberculosis* and *M. leprae,* it should be possible to test the postulated roles of these unique lipids in tuberculosis and leprosy.—Authors' Abstract

Belisle, J. T., Vissa, V. D., Sievert, T., Takayama, K., Brennan, P. J. and Besra, G. S. Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. Science **275** (1997) 1420–1422.

The dominant exported proteins and protective antigens of *Mycobacterium tuberculosis* are a triad of related gene products called the antigen 85 (Ag85) complex. Each has also been implicated in disease pathogenesis through its fibronectin-binding capacities. A carboxylesterase domain was found within the amino acid sequences of Ag85A, B, and C, and each protein acted as a mycolyltransferase involved in the final stages of mycobacterial cell wall assembly, as shown by direct enzyme assay and site-directed mutagenesis. Furthermore, the use of an antagonist (6-azido-6-deoxy-alpha,alpha′-trehalose) of this activity demonstrates that these proteins are essential and potential targets for new antimycobacterial drugs.—Authors' Abstract

Harth, G., Lee, B. Y. and Horwitz, M. A. High-level heterologous expression and secretion in rapidly growing nonpathogenic mycobacteria of four major *Mycobacterium tuberculosis* extracellular proteins considered to be leading vaccine candidates and drug targets. Infect. Immun. **65** (1997) 2321–2328.

Mycobacterium tuberculosis, the primary etiologic agent of tuberculosis, is the world's leading cause of death from a single infectious agent, and new vaccines and drugs to combat it are urgently needed. The major extracellular proteins of *M. tuberculosis,* which are released into its phagosome in macrophages, its host cells in humans, are leading candidates for a vaccine and prime targets for new drugs. However, the development of these biologicals has been hampered by the unavailability of large quantities of recombinant extracellular proteins identical to their native counterparts. In this report, we describe the heterologous expression and secretion of four major *M. tuberculosis* extracellular proteins (the 30-, 32, 16-, and 23.5-kDa proteins—the first, second, third, and eighth most abundant, respectively) in rapidly growing, nonpathogenic mycobacterial species. Multiple attempts to obtain secretion of the proteins by using *Escherichia coli*- and *Bacillus subtilis*-based expression systems were unsuccessful, suggesting that high-level expression and secretion of these *Mycobacterium*-specific proteins require a mycobacterial host. All four recombinant proteins were stably expressed from the cloned genes' own promoters at yields that were 5- to 10-fold higher than those observed for the native proteins. The four proteins were purified to apparent homogeneity from culture filtrates by ammonium sulfate precipitation and ion-exchange and molecular sieve chromatography. The recombinant proteins were indistinguishable from their native counterparts by multiple criteria. First, N-terminal amino acid sequence determination demonstrated that processing of the leader peptides was highly accurate. Second, sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis revealed identical migration patterns. Third, mass spectrometry analysis confirmed that differences in mass were less than or equal to 5 Ha. A homolog of the *M. tuberculosis* 30-kDa protein was identified in *M. smegmatis* by means of DNA analyses and immunoscreening. This is the first time that secretion of recombinant *M. tuberculosis* extracellular proteins in their native form has been achieved. This study opens the door to mass production of correctly processed and secreted extracellular proteins of *M. tuber-*

culosis in a heterologous host and allows ready evaluation of their biologic and immunologic function.—Authors' Abstract

Knipfer, N., Seth, A. and Shrader, T. E. Unmarked gene integration into the chromosome of *Mycobacterium smegmatis* via precise replacement of the pyrF gene. Plasmid **37** (1997) 129–140.

After integration into the bacterial chromosome an exogenuous gene may be stably expressed without continued selection for the recombinant locus. However, chromosomal integration events occur infrequently, requiring the concomitant integration of a drug-resistance marker in order to identify colonies of recombinant cells. The generation of a drug-resistant recombinant strain can both reduce the *in vivo* applicability of the strain and preclude the use of recombinant vectors which use the same drug-resistance marker. We have constructed a plasmid, pINT-delta, which allows recombination of exogenous genes onto the *Mycobacterium smegmatis* chromosome. The exogenous gene completely replaces the pyrF gene and the resultant strain lacks any exogenous drug-resistance marker. The methodologies described herein are general and applicable even to those bacteria for which extrachromosomal plasmids are not available. Using pINT-delta we integrated the lacZ gene into the *M. smegmatis* chromosome via a precise exchange of lacZ and pyrF. The resultant strain was used to demonstrate that the expression of genes integrated at the pyrF locus is repressed twofold by inclusion of uracil in the growth medium. In addition, we used pINT-delta to construct an *M. smegmatis* strain with a precise deletion of its pyrF locus. This strain, TSm-627, grows normally in rich medium but does not grow in medium lacking uracil. TSm-627 cells allow the pyrF gene to be used as a selectable marker for growth on medium lacking uracil. In TSm-627 cells, the pyrF gene is also useful as a counterselectable marker on complete medium containing 5′-fluoroorotic acid and uracil. Two pyrF-containing plasmids, designed to exploit the new delta pyrF strain, have been constructed and their possible applications to problems in mycobacteriology are discussed.—Authors' Abstract

Nakata, N., Matsuoka, M., Kashiwabara, Y., Okada, N. and Sasakawa, C. Nucleotide sequence of the *Mycobacterium leprae* katG region. J. Bacteriol. **179** (1997) 3053–3057.

Synthetic oligonucleotide primers based on the DNA sequence data of the *Escherichia coli, Mycobacterium tuberculosis,* and *M. intracellulare* katG genes encoding the heme-containing enzyme catalase-peroxidase were used to amplify and analyze the *M. leprae* katG region by PCR. A 1.6-kb DNA fragment, which hybridized to a *M. tuberculosis* katG probe, was obtained from a *M. leprae* DNA template. Southern hybridization analysis with a probe derived from the PCR-amplified fragment showed that the *M. leprae* chromosome contains only one copy of the putative katG sequence in a 3.4-kb EcoRI-BamHI DNA segment. Although the nucleotide sequence of the katG region of *M. leprae* was approximately 70% identical to that of the *M. tuberculosis* katG gene, no open reading frame encoding a catalase-peroxidase was detectable in the whole sequence. Moreover, two DNA deletions of approximately 100 and 100 bp were found in the *M. leprae* katG region, and they seemed to be present in all seven *M. leprae* isolates tested. These results strongly suggest that *M. leprae* lacks a functional katG gene and catalase-peroxidase activity.—Authors' Abstract

Shankar, S., Hershberger, C. D. and Chakrabarty, A. M. The nucleoside diphosphate kinase of *Mycobacterium smegmatis*: identification of proteins that modulate specificity of nucleoside triphosphate synthesis by the enzyme. Mol. Microbiol. **24** (1997) 477–487.

We report the purification and characterization of the enzyme nucleoside diphosphate kinase (Ndk) from *Mycobacterium smegmatis.* The N-terminus of the enzyme was blocked but an internal sequence showed approximately a 70% homology

with the same enzymes from *Pseudomonas aeruginosa* and *Escherichia coli.* Immobilization of the mycobacterial nucleoside diphosphate kinase on a Sepharose 4B matrix and passing the total cell extract through it revealed four proteins (P_{70}, P_{65}, P_{60}, and P_{50}, respectively) of M_r 70 kDa, 65 kDa, 60 kDa and 50 kDa that were retained by the column. While the proteins of M_r 70 kDa and 50 kDa modulated the activity of Ndk directing it toward GTP synthesis, the 60-kDa protein channelled the specificity of Ndk entirely towards CTP synthesis. The 65-kDa protein modulated the specificity of Ndk directing it entirely toward UTP synthesis. The specificity for such mycobacterial proteins toward NTP synthesis is retained when they are complexed with *P. aeruginosa* Ndk. We further demonstrate that the P_{70} protein is pyruvate kinase and that each of the four proteins forms a complex with Ndk and alters its substrate specificity. Given the ubiquitous nature of Ndk in the living cell and its role in maintaining correct ratios of intracellular nucleoside triphosphates, the implications of the occurrence of these complexes have been discussed in relation to the precursor pool for cell wall biosynthesis as well as RNA/DNA synthesis.—Authors' Summary

Sinha, R. K., Verma, I. and Khuller, G. K. Immunobiological properties of a 30 kDa secretory protein of *Mycobacterium tuberculosis* H37Ra. Vaccine **15** (1997) 689–699.

Six different secretory proteins of molecular weights (15, 26, 30, 41, 55 and 70 kDa) were isolated from 8-day-old culture filtrate of *Mycobacterium tuberculosis* H37Ra using different column chromatography techniques. These proteins were further examined for their ability to induce cell-mediated (T-cell proliferation assay) and humoral immune response (ELISA) in mice immunized with total culture filtrate proteins. Out of six proteins, three proteins showed good reactivity. However, the activity was at a maximum with a 30-kDa antigen. The immune response induced by the 30-kDa antigen emulsified in Freund's incomplete adjuvant (FIA) was investigated and was found to be dose dependent. The T-cell response induced by this protein was skewed toward T-helper (Th1) cells as determined by the pronounced secretion of interleukin-2 (IL-2) and gamma-interferon (IFN-γ). The protective activity of the 30-kDa protein was also evaluated and compared with reference to bacillus Calmette Guerin (BCG) vaccine in the mice challenged with virulent *M. tuberculosis* H37Rv. The degree of protection afforded by the 30-kDa antigen on the basis of mortality and the significant decrease in cfu recovered from different organs (lung, liver, spleen) after 30 days of challenge with LD50 of *M. tuberculosis* H37Rv was significantly higher in comparison to BCG vaccinated animals. However, the degree of immunity induced by this antigen decreased with time (when challenged 8 and 12 weeks post-immunization) but it was still comparable with BCG. These findings suggest that the 30-kDa secretory protein of *M. tuberculosis* is the key immunoprotective antigen and may be a suitable candidate for the development of an alternative subunit vaccine against tuberculosis.—Authors' Abstract

Sougakoff, W., Lemaitre, N., Cambau, E., Szpytma, M., Revel, V. and Jarlier, V. Nonradioactive single-strand conformation polymorphism analysis for detection of fluoroquinolone resistance in mycobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **16** (1997) 395–398.

A simple, rapid, and nonradioactive method for routine detection of fluoroquinolone resistance in mycobacteria is described. A single-strand conformation polymorphism (SSCP) methodology, based on the use of mini-gels and silver staining of DNA, was optimized for the analysis of denatured DNA products obtained by polymerase chain reaction (PCR) from the gyrA gene involved in fluoroquinolone resistance in mycobacteria. The method was successfully applied to fluoroquinolone-susceptible and -resistant laboratory strains of *Mycobacterium smegmatis* and to clinical strains of *M. tuberculosis* isolated from patients who developed resistance during the

course of fluoroquinolone treatment.—Authors' Abstract

Wieles, B., Ottenhoff, T. H. M., Steenwijk, T. M., Franken, K. L. M. C., de Vries, R. R. P. and Langermans, J. A. M. Increased intracellular survival of *Mycobacterium smegmatis* containing the *Mycobacterium leprae* thioredoxin-thioredoxin reductase gene. Infect. Immun. **65** (1997) 2537–2541.

The thioredoxin (Trx) system of *Mycobacterium leprae* is expressed as a single hybrid protein containing thioredoxin reductase (TR) at its N terminus and Trx at its C terminus. This hybrid Trx system is unique to *M. leprae,* since in all other organisms studied to date, including other mycobacteria, both TR and Trx are expressed as two separate proteins. Because Trx has been shown to scavenge reactive oxygen species, we have investigated whether the TR-Trx gene product can inhibit oxygen-dependent killing of mycobacteria by human mononuclear phagocytes and as such could contribute to mycobacterial virulence. The gene encoding *M. leprae* TR-Trx was cloned into the apathogenic, fast-growing bacterium *M. smegmatis.* Recombinant *M. smegmatis* containing the gene encoding TR-Tm was killed to a significantly lesser extent than *M. smegmatis* containing the identical vector with either no insert or a control *M. leprae* construct unrelated to TR-Trx. Upon phagocytosis, *M. smegmatis* was shown to be killed predominantly by oxygen-dependent macrophage-killing mechanisms. Coinfection of *M. smegmatis* expressing the gene encoding TR-Trx together with *Staphylococcus aureus,* which in known to be killed via oxygen-dependent microbicidal mechanisms, revealed that the TR-Trx gene product interferes with the intracellular killing of this bacterium. A similar coinfection with *Streptococcus pyogenes,* known to be killed by oxygen-independent mechanisms, showed that the TR-Trx gene product did not influence the oxygen-independent killing pathway. The data obtained in this study suggest that the Trx system of *M. leprae* can inhibit oxygen-dependent killing of intracellular bacteria and thus may represent one of the mechanisms by which *M. leprae* can deal with oxidative stress within human mononuclear phagocytes.—Authors' Abstract

Wu, Q. L., Kong, D. Q., Lam, K. and Husson, R. N. A mycobacterial extracytoplasmic function sigma factor involved in survival following stress. J. Bacteriol. **179** (1997) 2922–2929.

The extracytoplasmic function (ECF) sigma factors constitute a diverse group of alternative sigma factors that have been demonstrated to regulate gene expression in response to environmental conditions in several bacterial species. Genes encoding an ECF sigma factor of *Mycobacterium tuberculosis, M. avium,* and *M. smegmatis,* designated sigE, were cloned and analyzed. Southern blot analysis demonstrated the presence of a single copy of this gene in these species and in *M. bovis* BCG, *M. leprae,* and *M. fortuitum.* Sequence analysis showed the sigE gene to be highly conserved among *M. tuberculosis, M. avium, M. smegmatis,* and *M. leprae.* Recombinant *M. tuberculosis* sigE, when combined with core RNA polymerase from *M. smegmatis,* reconstituted specific RNA polymerase activity on sigE *in vitro,* demonstrating that this gene encodes a functional sigma factor. Two *in vivo* transcription start sites for sigE were also identified in *M. smegmatis* and *M. bovis* BCG. Comparison of wild-type *M. smegmatis* with a sigE mutant strain demonstrated decreased survival of the mutant under conditions of high-temperature heat shock, acidic pH, exposure to detergent, and oxidative stress. An inducible protective response to oxidative stress present in the wild type was absent in the mutant. The mycobacterial sigE protein, although nonessential for viability *in vitro,* appears to play a role in the ability of these organisms to withstand a variety of stresses.—Authors' Abstract

Xin, Y., Lee, R. E., Scherman, M. S., Khoo, K. H., Besra, G. S., Brennan, P. J. and McNeil, M. Characterization of the *in vitro* synthesized arabinan of mycobacterial cell walls. Biochim. Biophys. Acta—Gen. Subj. **1335** (1997) 231–234.

Previous studies have shown that polymerized [C-14]arabinan can be synthesized from polyprenylphosphate-[C-14]arabinose by the particulate enzymes of *Mycobacterium smegmatis* [R. E. Lee, K. Mikusova, P. J. Brennan and G. S. Besra (1995) J. Am. Chem. Sec. 117, 11829–11832]. In the present investigation, the [C-14]arabinan product was biochemically characterized. Sizing chromatography revealed a molecular weight consistent with that expected from mature arabinan. Digestion of the [C-14]arabinan with a mixture of arabinases produced oligo[C-14]arabinoside fragments including hexa[C-14]arabinoside and tetra[C-14]arabinoside which originated from the non-reducing terminal regions of the polymer, and di[C-14]arabinoside from the internal regions of the polymer. These arabinoside fragments represent the major known structural motifs that comprise the arabinan segment of arabinogalactan and lipoarabinomannan. The presence of [C-14]arabinose in both the internal and external regions of the [C-14]arabinan suggests that polyprenylphosphate-arabinose is the major, and perhaps the only, donor of arabinosyl residues in mycobacteria.—Authors' Abstract

Experimental Infections

Doherty, T. M. and Sher, A. Defects in cell-mediated immunity after chronic, but not innate, resistance of mice to *Mycobacterium avium* infection. J. Immunol. **158** (1997) 4822–4831.

To investigate the role of cell-mediated immunity in the control of *Mycobacterium avium* infection, we studied the effects of targeted gene disruptions in components of the T lymphocyte-dependent, macrophage-mediated response on resistance of mice to this pathogen. Normal mice developed a chronic, asymptomatic infection, with rapid induction of mRNAs for IFN-gamma, IL-12, and TNF-alpha in spleen, liver, and lung. Bacterial loads in gene knockout, scid, and wild-type mice were indistinguishable for the first 4 wk of infection. However, by 8 wk postinfection, scid mice as well as animals with a targeted disruption of the IFN-gamma gene showed enhanced bacterial growth compared with wild-type controls. In contrast, knockout mice lacking the genes for the TNF-alpha p55/p75 receptors or inducible nitric oxide synthase not only developed comparable bacterial loads to wild-type animals they also failed to display the splenomegaly and profound suppression of mitogen-induced lymphocyte proliferative responses evident in infected wild-type controls. Thus, *M. avium* is clearly distinct from other intracellular pathogens (e.g., *Leishmania monocytogenes, Toxoplama gondii,* and *M. tuberculosis*) whose initial replication in the host is tightly controlled by Th1-dependent effector mechanisms. Instead, the major effect of host cell-mediated immunity is to limit bacterial growth during the chronic phase of infection. Surprisingly, inducible nitric oxide appears to be more important for the immunopathology than for the host resistance induced by this bacterial pathogen.—Authors' Abstract

Epidemiology and Prevention

Andrade, V., Militao de Albuquerque, M. D. F. and Chagastelles Sabroza, C. The importance of operational factors for the interpretation of indicators in the Hansen's disease endemic in Brazil. Acta Leprol. **10** (1997) 131–139.

In Brazil, an increase has been recorded in recent years in the magnitude of detection coefficients for new cases of Hansen's disease, which is frequently interpreted as evidence of the endemic's expansion. The objective of this work is to determine the

role of operational factors for interpreting the trend displayed by the morbidity coefficients for Hansen's disease from 1982 to 1995 in the country. We observed a strong correlation between the adjusted detection coefficients and the number of technicians trained (r = 0.80), a decrease in the proportion of new cases with disabilities at the time of diagnosis (r = –0.86), and a downward trend in tuberculoid forms (r = –0.70). Patient time on the active register is correlated negatively with MDT-WHO coverage (r = –0.95) and the percentage of patients discharged from treatment due to cure (r = –0.91). These results suggest that the increase in the potential for detection of new cases of Hansen's disease resulting from new strategies adopted by the program, i.e., mainly extensive training of health personnel, could be a coherent explanation for the increase in detection coefficients for new cases observed in Brazil in the last 10 years.—Authors' Summary

Andrade, V., Moreira Alves, T., Regazzi Avelleira, J. C. and Bayona, M. Prevalence of HIV1 in leprosy patients in Rio de Janeiro, Brazil. Acta Leprol. **10** (1997) 159–163.

The purpose of this study was to learn if HIV1 infection was associated with leprosy in Rio de Janeiro, Brazil, by comparing the prevalence rates of 1016 leprosy patients tested on a voluntary basis and 78,482 blood donors. A cross-sectional survey of anti-HIV1 antibodies was conducted in Rio de Janeiro, from 1990 to 1992, for this purpose. HIV1 prevalence found among leprosy patients was (3 cases) 2.9 per 1000, and among blood donors was (282 cases) 3.8 per 1000. Such a difference was not significant (OR = 0.79; p = 0.69). Since HIV1 cases were only found among male leprosy patients, further analysis excluded females. Male leprosy patients showed a slightly higher prevalence of HIV1 than blood donors before and after age adjustment. However, this result was not statistically significant (adjusted OR = 1.38, 95% CI 0.35–4.5; p = 0.83). These data do not provide evidence that leprosy and HIV1 infection are associated in the state of Rio de Janeiro. This is consistent with similar investigations conducted elsewhere.—Authors' Summary

Karonga Prevention Trial Group (Malawi). Randomised controlled trial of single BCG, repeated BCG, or combined BCG and killed *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. Lancet **348** (1996) 17–24.

Between 1986 and 1989, individuals lacking a BCG scar in Karonga District, Malawi, were randomly assigned BCG alone (n = 27,904) or BCG plus killed *Mycobacterium leprae* (n = 38,251). Individuals with a BCG scar were randomly allocated placebo (n = 23,307), a second BCG (n = 23,456), or BCG plus killed *M. leprae* (n = 8102). Incident cases of leprosy and tuberculosis were ascertained over the subsequent 5–9 years; 139 cases of leprosy were identified by May 1995, 93 of these were diagnostically certain, definitely post-vaccination cases. Among scar-positive individuals, a second BCG vaccination gave further protection against leprosy (about 50%) over a first BCG vaccination. The rate ratio for all diagnostically certain, definitely post-vaccination cases, all ages, was 0.51 (95% CI 0.25–1.03, p = 0.05) for BCG vs placebo. This benefit was apparent in all subgroups, although the greatest effect was among individuals vaccinated <15 years old (RR = 0.40 [95% CI 0.15–1.01] p = 0.05). The addition of killed *M. leprae* did not improve the protection afforded by a primary BCG vaccination. The rate ratio for BCG plus killed *M. leprae* versus BCG alone among scar-negative individuals was 1.06 (0.62–1.82, p = 0.82) for all ages, though 0.37 (0.11–1.24, p = 0.09) for individuals. vaccinated <15 years old. Three hundred seventy-six cases of post-vaccination pulmonary tuberculosis and 31 of glandular tuberculosis were ascertained by May 1995. The rate of diagnostically certain tuberculosis was higher among scar-positive individuals who had received a second BCG (1.43 [0.88–2.35], p = 0.15) than among those who had received placebo and there was no evidence that any of the trial vaccines contributed to protection against pulmonary tuberculosis. It was concluded that in a popu-

lation in which a single BCG vaccination affords 50% or more protection against leprosy, but none against tuberculosis, a second vaccination can add appreciably to the protection against leprosy, without providing any protection against tuberculosis.—Trop. Dis. Bull. **94** (1997) 358

Shen, J., *et al.* [The third year's follow up after a serological test in Wenshan, Yunnan.] China Lepr. J. **13** (1997) 8–12. (in Chinese)

Third year's follow up of leprosy household contacts and healthy residents in Wenshan and Guangnan Counties, Yunnan Province, including a total of 765 persons, of which 184 residents and 135 contacts were previously antibody-positive and 317 residents and 129 contacts were negative, was completed. Among both the residents and contacts with previous antibody positivity, 55 (29.5%) and 20 (14.8%) remained positive, respectively. Among both the residents and contacts with previous negativity, 47 (14.8%) and 11 (8.5%) became positive, respectively. During the 3 years, 11 persons out of them have developed leprosy clinically, including BT 3 and BL 8. All the BL cases were antibody-positive at attack of the disease and in six cases the titers of the antibody have been increasing. The average time from reaching the cut-off value of antibody to the attack of the disease was 26 months. All three BT cases were antibody-negative at attack of the disease. Authors consider that serological screening plus skin smear in villages with higher leprosy prevalence, together with the follow up 2 years later, may be helpful to early detection of MB leprosy but it might be costly to be used as a routine in the field.—Authors' English Abstract

Sun, X. [The relation between subclinical infection and clinical disease of leprosy.] China Lepr. J. **13** (1997) 13–15. (in Chinese)

In 12 counties of Guizhou province, 1715 contacts with leprosy patients being before, during or after MDT have been examined with NT-O-BAS ELISA and lepromin test, and then followed up for 5 and 9 years. Among them, 306 persons showed positive in ELISA, of which 61 with Mitsuda negativity are considered as persons at higher risk. The result showed that 34.62% (9/26) of the persons at higher risk have been diagnosed as leprosy for 9 years of follow up and 14.29% (5/35) for 5 years ($p < 0.05$). It was proved that the contact with MB case, in household and before the treatment is in more danger.—Author's English Abstract

Yuan, L., *et al.* [Characteristics of new leprosy patients detected before and after MDT in Weifang City and Wenshan Prefecture.] China Lepr. J. **13** (1997) 3–7. (in Chinese)

Weifang City, Shandong Province, and Wenshan Prefecture, Yunnan Province, were highly endemic areas for leprosy in the past. But since 1950s the detection rate decreased by 99.0%, i.e., being from 35.23 to 0.05 per 100,000 residents in Weifang and by 93.2%, i.e., from 69.93 to 4.74 per 100,000 in Wenshan. The decrease was more apparent after the implementation of MDT in 1986. The data such as age-specific detection rate, ratio of MB/PB, and disability rate in the patients detected since 1980 were analyzed.—Authors' English Abstract

Zhao, Z., *et al.* [Analysis of onset and detection of leprosy at the seasons with cosine model.] China Lepr. J. **13** (1997) 34–36. (in Chinese)

Distribution of onset and detection of leprosy at all seasons was analyzed with the cosine model in 2614 leprosy patients in Dali Prefecture, Yunnan, and showed that peak point was in the end of April and peak period in March to June for the onset, but in the end of June and in May to August for the detection, respectively. The authors suggest that leprosy control actions should be done in the peak seasons.—Author's English Abstract

Rehabilitation

Bernardin, R. and Thomas, B. Surgery for neuritis in leprosy: indications for and results of different types of procedures. Lepr. Rev. **68** (1997) 147–154.

From December 1988 to December 1992, 129 surgical procedures were performed on the peripheral nerves of 64 leprosy patients at the Hospital Cardinal Léger de l'Institut Fame Pareo for leprosy control in Haiti. Sixty-four patients totalizing 129 nerves with sufficient clinical data form the basis of this study. Based on the retrospective analysis of the operated cases, a new classification built on macroscopic findings of the involved nerves is presented. Five grades, according to the presenting aspects of these nerves, are set up as guides for different surgical procedures to be performed on the nerves: external decompression for the lesser grades I and II, intraneural neurolysis, interfascicular neurolysis for the higher grades III and IV, cleaning, and debridement for grade V. The final results are discussed. This new macroscopic grading done at surgery helps to minimize the aggressive procedures performed on nerve trunks, decrease the morbidity of surgical action on the nerve vascular structures and consequently, preserves all possible sensory and motor functions of a nerve.—Authors' Summary

Dong, L., *et al.* [Anatomy and clinical use of the first toe web flap in leprosy.] China Lepr. J. **13** (1997) 19–20. (in Chinese)

A flap consisting of the lateral side of the great toe and medial side of the second toe, with the first arteriae metatarseae dorsales or arteriae digitales plantares communes as a pedicle, can be used free or in an island-like transplantation to repair defects of the skin. The authors have repaired 16 planter ulcers at the position of heads of metatarsus I and II with the flap, and found no relapse during follow up of 48 to 124 months, except for one case whose ulcer relapsed because of fissures 12 months after the operation.—Authors' English Abstract

Grauwin, M. Y., Hirzel, C., Mane, I., Cartel, J. L. and Lepers, J. P. [Simplification and codification of the treatment of plantar ulcers.] Acta Leprol. **10** (1997) 165–168. (in French)

Practically leprous plantar ulcers (PU) are difficult to treat and heal under field conditions. Considering the important number of patients showing a PU, the directors of the national leprosy control programs are determined, within the programs on prevention of disabilities (POD), to treat the PU in the field. Therefore, it appears to be essential to codify and simplify their treatment, thus enabling it to be effective; the healing of PU being the only criteria of effectiveness of the technique. Four clinical stages were defined, each corresponding to a precise way of treatment using only essential and basic products at low cost. During the training about the treatment techniques and attitudes much emphasis is given on the discharge of the PU, on the trimming of the wound and on the products to use according to PU's evolutionary stage.—Authors' English Summary

Jiang, Z., *et al.* [Four disableds of leprosy after becoming rich.] China Lepr. J. **13** (1997) 29–30. (in Chinese)

Four persons who were cured of leprosy with severe disability and inferiority complex have become very rich after fitting artificial limbs, doing orthopedic operation or only depending on their own effort to engage in productive activity. The authors pointed out that this should be the way to socioeconomic rehabilitation for those who had the same experience.—Authors' English Abstract

Tiendrebeogo, A., Djakeaux, D. S., Asse, H., Eba, M. E. and Sica, A. [A survey of leprosy disabilities among patients treated with MDT in Ivory Coast.] Acta Leprol. **10** (1997) 151–158. (in French)

Between 1990 and 1995, 20,000 cases of leprosy were treated with WHO-recommended multiple drug therapy (MDT) in

Ivory Coast. A disability survey was conducted in April 1996 with a half-randomized sample of 500 patients. This survey showed that 28.73% of the patients had got grade two disabilities in WHO scale; 12.9% of the nondisabled patients at detection had developed leprosy impairments during or after treatment. Plantar ulcers (12.2% of the patients) appeared very frequent comparatively to the findings of a similar survey in Burkina Faso in 1995 (0.9% of plantar ulcers). With these results, the authors estimated the needs for disabilities care to enable the reinforcement of the prevention of disabilities and physical rehabilitation (POD and PR) in Ivory Coast.—Authors' English Summary

Xu, Y., *et al.* [A survey of the use of artificial limbs in 232 persons cured of leprosy.] China Lepr. J. **13** (1997) 21–23. (in Chinese)

In several districts of Guangdong Province, the use of 254 artificial limbs has been investigated among 232 people who had or have leprosy, including 22 bilateral amputees (9.48%), four with amputated thigh (1.72%) and the rest being amputees in their legs below the knee. In them the ratio of men/women was 1:0.4 with a mean age of 63.9 (35 to 87 years). The time of using artificial limbs was 6 months to 35 years. The artificial limbs were made of polyester in 122 persons and of iron-wood in 132. The results showed that 115 (99.1%) can stand steady in those with polyester type and 78 (67.2%) in those with iron-wood one, with good pace in 75 (64.7%) and in 27 (23.3%), respectively. There were 174 ulcers at the end of the residual limb, with 0.34/person in polyester ones and 1.01/person in iron-wood, and 0.29/person in those load-bearing with patellar ligament and 0.52/person in those with the thigh. The authors consider that the artificial limb made of polyester is better but too costly and iron-wood ones are more suitable to the users in villages.—Authors' English Abstract

Zu, Y., *et al.* [Knowledge of and attitude to leprosy among residents in Nanjing City.] China Lepr. J. **13** (1997) 26–28. (in Chinese)

Knowledge of leprosy and attitude to leprosy patients among the residents were investigated in Nanjing City. The results showed that the prejudice against leprosy is still serious and the knowledge is lacking. It is surprising that the higher the education level the more serious that situation is. So, health education on leprosy control must be generally strengthened.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Bahl, D., Miller, D. A., Leviton, I., Gialanella, P., Wolin, M. J., Liu, W., Perkins, R. and Miller, M. H. *In vitro* activities of ciprofloxacin and rifampin alone and in combination against growing and nongrowing strains of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **41** (1997) 1293–1297.

We characterized the effects of ciprofloxacin and rifampin alone and in combination on *Staphylococcus aureus in vitro.* The effects of drug combinations (e.g., indifferent, antagonistic, or additive interactions) on growth inhibition were compared by disk approximation studies and by determining the fractional inhibitory concentrations. Bactericidal effects in log-phase bacteria and in nongrowing isolates were characterized by time-kill methods. The effect of drug combinations was dependent upon whether or not cells were growing and whether killing or growth inhibition was the endpoint used to measure drug interaction. Despite bactericidal antagonism in time-kill experiments, our *in vitro* studies suggest several possible explanations for the observed benefits in patients treated with a

combination of ciprofloxacin and rifampin for deep-seated staphylococcal infections. Notably, when growth inhibition rather than killing was used to characterize drug interaction, indifference rather than antagonism was observed. An additive bactericidal effect was observed in nongrowing bacteria suspended in phosphate-buffered saline. While rifampin antagonized the bactericidal effects of ciprofloxacin, ciprofloxacin did not antagonize the bactericidal effects of rifampin. Each antimicrobial prevented the emergence of subpopulations that were resistant to the other.—Authors' Abstract

Chakrabarti, A., Bhattacharya, C. P., Acharya, D. P., Chakrabarty, A. N., Ghosh, K. and Dastidar, S. G. *In vitro* and *in vivo* experimental susceptibility of *Mycobacterium marinum* to augmentin. Indian J. Med. Res. **104** (1996) 281–283.

The effect of augmentin alone and in combination with various β-lactam antibiotics was studied against a pathogenic *Mycobacterium, M. marinum.* The *in vitro* studies did not reveal any additional advantage over that found with augmentin alone and this antibiotic seemed considerably inhibitory to *M. marinum* at <1 μg/ml concentration. *In vivo,* the effects of augmentin on experimentally produced lesions in mouse foot pads (MFP) showed a significant regression of the lesions, which was compatible with an early disappearance of *M. marinum* from the MFP, in contrast with those of the untreated control animals.—Authors' Abstract

Collins, L. A. and Franzblau, S. G. Microplate Alamar blue assay versus BACTEC 460 system for high throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium.* Antimicrob. Agents Chemother. **41** (1997) 1004–1009.

In response to the need for rapid, inexpensive, high through-put assays for antimycobacterial drug screening, a microplate-based assay which uses Alamar blue reagent for determination of growth was evaluated. MICs of 30 antimicrobial agents against *Mycobacterium tuberculosis* H37Rv, *M. tuberculosis* H37Ra, and *M. avium* were determined in the microplate Alamar blue assay (MABA) with both visual and fluorometric readings and compared to MICs determined in the BACTEC 460 system. For all three mycobacterial strains, there was less than or equal to 1 dilution difference between MABA and BACTEC median MICs in four replicate experiments for 25 of the 30 antimicrobics. Significant differences between MABA and BACTEC MICs were observed with 0, 3, and 5 of 30 antimicrobial agents against H37Rv, H37Ra, and *M. avium,* respectively. Overall, MICs determined either visually or fluorometrically in MABA were highly correlated with those determined in the BACTEC 460 system, and visual MABA and fluorometric MABA MICs were highly correlated. MICs of rifampin, rifabutin, minocycline, and clarithromycin were consistently lower for H37Ra compared to H37Rv in all assays but were similar for most other drugs. *M. tuberculosis* H37Ra may be a suitable surrogate for the more virulent H37Rv strain in primary screening of compounds for antituberculosis activity. MABA is sensitive, rapid, inexpensive, and nonradiometric and offers the potential for screening, with or without analytical instrumentation, large numbers of antimicrobial compounds against slow-growing mycobacteria.—Authors' Abstract

Converse, P. J., Jones, S. L., Astemborski, J., Vlahov, D. and Graham, N. M. H. Comparison of a tuberculin interferon-gamma assay with the tuberculin skin test in high-risk adults; effect of human immunodeficiency virus infection. J. Infect. Dis. **176** (1997) 144–150.

A novel, whole blood interferon-gamma (IFN-γ) assay was evaluated to determine its suitability for detecting *Mycobacterium tuberculosis* exposure in intravenous drug users with or without human immunodeficiency virus (HN) infection. Whole heparinized blood was incubated overnight in separate wells with tuberculin purified protein derivative (PPD), saline, and mitogen controls. Levels of IFN-γ in plasma supernatants were determined by rapid ELISA.

Participants were then administered the tuberculin skin test (TST) and tested for cutaneous anergy. The whole blood IFN-γ test agreed (89%–100%) with a positive TST in both HIV-seropositive and -seronegative subjects, but reactivity to PPD was more detectable by the whole blood assay among those with negative TSTs or anergy. TST induration diameter and IFN-γ responses were correlated (Spearman's p = 0.45, p = 0.0001), but both responses were blunted by HIV infection. In summary, tuberculin reactivity appears to be more detectable by the whole blood IFN-γ assay than by TST, and the assay requires no return visit for test reading.—Authors' Abstract

Cooper, A. M., Magram, J., Ferrante, J. and Orme, I. M. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis.* J. Exp. Med. **186** (1997) 39–45.

Immunity to *Mycobacterium tuberculosis* infection is associated with the emergence of protective CD4 T cells that secrete cytokines, resulting in activation of macrophages and the recruitment of monocytes to initiate granuloma formation. The cytokine-mediating macrophage activation is interferon-gamma (IFN-γ), which is largely dependent on interleukin-12 (IL-12) for its induction. To address the role of IL-12 in immunity to tuberculosis, IL-12 p40 (–/–) mice were infected with *M. tuberculosis* and their capacity to control bacterial growth and other characteristics of their immune response were determined. The IL-12 p40 (–/–) mice were unable to control bacterial growth, and this appeared to be linked to the absence of both innate and acquired sources of IFN-γ. T-cell activation as measured by delayed-type hypersensitivity and lymphocyte accumulation at the site of infection were both markedly reduced in the IL-12 p40 (–/–) mice. Therefore, IL-12 is essential to the generation of a protective immune response to *M. tuberculosis,* with its main functions being the induction of the expression of IFN-γ and the activation of antigen-specific lymphocytes capable of creating a protective granuloma.— Authors' Abstract

Cruciani, M., Gatti, G., Mengoli, C., Cazzadori, A., Lazzarini, L., Miletich, F., Graziani, M. S., Malena, M. and Bassetti, D. Penetration of dapsone into pulmonary lining fluid of human immunodeficiency virus type 1-infected patients. Antimicrob. Agents Chemother. **41** (1997) 1077–1081.

We studied the penetration of dapsone into the epithelial lining fluid (ELF) of 16 human immunodeficiency virus type 1-infected patients who had received the drug at a dose of 100 mg twice weekly as primary prophylaxis for *Pneumocystis carinii* pneumonia. Bronchoscopy, bronchoalveolar lavage (BAL), and venipuncture were performed for each patient at a specific time after administration of the last dose of dapsone. Dapsone concentrations in plasma and BAL were determined by high-performance liquid chromatography. The apparent volume of ELF recovered by BAL was determined by using urea as an endogenous marker. The mean concentrations of dapsone in ELF at 2 hr (five patients), 4 hr (three patients), 12 hr (two patients), 24 hr (three patients), and 48 hr (three patients) were 0.95, 0.70, 1.55, 0.23, and 0.45 mg/liter, respectively, while concentrations in plasma were 1.23, 0.79, 1.31, 0.83, and 0.18 mg/liter, respectively. Dapsone concentrations in ELF were 76, 79, 115, 65, and 291% of those observed in plasma at the same times, respectively.

These data show that dapsone is well distributed into ELF and that a twice-weekly 100-mg prophylactic regimen results in sustained concentrations in this compartment.—Authors' Abstract

Dhople A. M., Dhople, A. A. and Ibanez, M. A. Comparative *in vitro* activities of rifamycin analogues against rifampin-sensitive and rifampin-resistant *Mycobacterium tuberculosis.* Int. J. Antimicrob. Agents **8** (1997) 209–214.

Because of widespread emergence of multidrug resistant *Mycobacterium tuberculosis* worldwide, there is an urgent need for new bactericidal drugs against this organism. Several new analogs of rifamycin are being developed. Susceptibilities of five

of the most potent analogs were determined simultaneously on ten isolates each of rifampin-sensitive and rifampin-resistant *M. tuberculosis* using the radiometric method (BACTEC) with [C-14]palmltic acid. Against rifampin-sensitive isolates, all five analogs exhibited inhibitory activity, the most potent being KRM-1648, with MICs varying between 0.003 and 0.025 μg/ml (MICs of rifampin were between 0.05 and 0.4 μg/ml). Similar observations were also obtained for the MBCs of these five analogs—KRM-1648 was most potent, with nine out of ten isolates exhibiting a MBC/MIC ratio of 1.0. Among the rifampin-resistant isolates of *M. tuberculosis,* the most potent rifampin analog was, again, KRM-1648, with seven out of ten isolates exhibiting a MBC/MIC ratio of 1.0 and the remaining three exhibiting a ratio of 2.0. These results suggests that KRM-1648 should further be explored in the treatment of tuberculosis patients.—Authors' Abstract

El Zaatari, F. A. K., Graham, D. Y., Samuelsson, K. and Engstrand, L. Detection of *Mycobacterium avium* complex in cerebrospinal fluid of a sarcoid patient by specific polymerase chain reaction assays. Scand. J. Infect. Dis. **29** (1997) 202–204.

The etiology of sarcoidosis is unknown, but it has long been suspected to be mycobacterial. In the present study, we used 4 mycobacterial species-specific polymerase chain reaction assays on cerebrospinal fluid obtained from a patient with neurosarcoidosis. Positive hybridization was observed with both the *Mycobacterium avium* complex probe and the insertion element IS900-specific probe that has been found in *M. paratuberculosis* species. There was no hybridization with *M. tuberculosis* or *M. avium* woodpigeon strain-specific probes. This case report demonstrates that *M. paratuberculosis* or some closely related *M. avium* spp. which perhaps also carry IS900, or contain closely related DNA sequences, are associated with at least some cases of sarcoidosis disease.—Authors' Abstract

Frehel, C., Offredo, C., and de Chastellier, C. The phagosomal environment protects virulent *Mycobacterium avium* from killing and destruction by clarithromycin. Infect. Immun. **65** (1997) 2792–2802.

Murine bone marrow-derived macrophages infected with virulent strains of *Mycobacterium avium* (TMC 724 and 8 human clinical isolate) or with an avirulent opaque variant that spontaneously dissociates from the virulent human clinical isolate were subjected to a prolonged and continuous treatment with clarithromycin added at the MIC. The efficiency of this antibiotic in terms of inhibition of bacterial growth and bacterial degradation was evaluated during a 21-day treatment period. Growth was assessed by determination of CFU of intracellular bacteria and by a quantitative ultrastructural analysis which allowed us also to determine the extent of bacterial degradation. A similar treatment was applied to the same strains growing in liquid medium. Our data show that in liquid medium, clarithromycin caused a 90% decrease in CFU within 7 days of treatment. When applied to macrophages infected with virulent *M. avium,* clarithromycin immediately arrested bacterial growth but was unable to fully kill and degrade intracellularly growing virulent bacteria. After 21 days of treatment, 25% of intracellular bacteria were still morphologically intact. These bacteria resumed growth upon removal of the antibiotic, with a normal replication rate. These bacteria had not become more resistant to the drug, since the MIC was unchanged as compared to the one determined for the initial stock used to infect macrophages. Our data therefore suggest that the intraphagosomal environment protects bacteria from degradation. We propose that the inability of the drug to completely destroy bacteria is the result of a limited accessibility of the drug due to prevention of fusions between the immature phagosomes in which virulent bacteria reside and lysosomes in which clarithromycin accumulates. In accord with our proposal, we show that the avirulent opaque variant, which does not prevent phagosome-lysosome fusions (unpublished data), is finally destroyed by clarithromycin even within the phagosomal environment.—Authors' Abstract

Gilks, C. F., Godfrey Fausset, P., Batchelor, B. I. F., Ojoo, J. C., Ojoo, S. J., Brindle, R. J., Paul, J., Kimari, J.,

Bruce, M. C., Bwayo, J., Plummer, F. A. and Warrell, D. A. Recent transmission of tuberculosis in a cohort of HIV-1-infected female sex workers in Nairobi, Kenya. AIDS **7** (1997) 911–918.

Objectives: To describe the epidemiological and clinical characteristics of HIV-related tuberculosis in a female cohort, and to investigate the relative importance of recently transmitted infection and reactivation in the pathogenesis of adult HIV-related tuberculosis.

Design: Members of an established cohort of female sex workers in Nairobi were enrolled in a prospective study. Women were followed up regularly and seen on demand when sick.

Methods: Between October 1989 and September 1992 we followed 587 HIV-infected and 132 HIV-seronegative women. Standard protocols were used to investigate common presentations. Cases of tuberculosis were identified clinically or by culture. All available *Mycobacterium tuberculosis* strains underwent DNA fingerprint analysis.

Results: Forty-nine incident and four recurrent episodes of tuberculosis were seen in HIV-infected women; no disease was seen in seronegative sex workers ($p = 0.0003$). The overall incidence rate of tuberculosis was 34.5 per 1000 person-years among HIV-infected participants. In purified protein derivative (PPD) skin test-positive women the rate was 66.7 per 1000 person-years versus 18.1 per 1000 person-years in PPD-negative women. Twenty incident cases (41%) were clinically compatible with primary disease. DNA fingerprint analysis of strains from 32 incident cases identified two clusters comprising two and nine patients; allowing for index cases, 10 patients (28%) may have had recently transmitted disease. Three out of 10 (30%) patients who were initially PPD skin test-negative became PPD-positive. Taken together, 26 incident cases (53%) may have been recently infected. DNA fingerprint analysis also identified two (50%) of the four recurrent tuberculosis episodes as reinfection.

Conclusions: Substantial recent transmission of tuberculosis appears to be occurring in Nairobi among HIV-infected sex workers. It may be incorrect to assume in other regions of high tuberculosis transmission that active HIV-related tuberculosis usually represents reactivation of latent infection.—Authors' Abstract

Gomez Flores, R., Tamaz Guerra, R., Tucker, S. D. and Mehta, R. T. Bidirectional effects of IFN-gamma on growth of *Mycobacterium avium* complex in murine peritoneal macrophages. J. Interferon Res. **17** (1997) 331–336.

The effects of macrophage stimulation with interferon-gamma (IFN-γ) before or after infection on the intracellular growth of *Mycobacterium avium* complex (MAC) were investigated. Treatment of murine peritoneal macrophages before infection with IFN-γ (50 U/ml) for 24 hr and 48 hr, but not for 72 hr, was associated with a 41% and 52% significant MAC growth inhibition, respectively. N-G-monomethyl-L-arginine (NMA) did not affect the preinfection antimycobacterial activity of IFN-γ, thus indicating that nitric oxide was not involved in this phenomenon. In contrast, treatment of macrophages with IFN-γ (50 U/ml) for 24 hr and 48 hr after infection was ineffective; whereas treatment for 72 hr caused some MAC growth promotion. The use of NMA suppressed the IFN-γ-mediated MAC growth, suggesting that nitric oxide may affect postinfection microbicidal function of microphage. These results suggest that activation of macrophages with IFN-γ before or after infection may direct the course of the infection and that nitric oxide may be detrimental more than beneficial for MAC-infected macrophages.—Authors' Abstract

Hackbarth, C. J., Unsal, I. and Chambers, H. F. Cloning and sequence analysis of a class A beta-lactamase from *Mycobacterium tuberculosis* H37Ra. Antimicrob. Agents Chemother. **41** (1997) 1182–1185.

A cosmid library from *Mycobacterium tuberculosis* H37Ra was introduced into *M. smegmatis,* and eight recombinant clones with increased resistance to cefoxitin were identified. Isoelectric focusing detected an *M. tuberculosis*-derived beta-lactamase in one of these recombinant clones. A se-

quence analysis identified it as a class A beta-lactamase whose expression correlated with the increased resistance phenotype.—Authors' Abstract

Haslett, P., Tramontana, J., Burroughs, M., Hempstead, M. and Kaplan, G. Adverse reactions to thalidomide in patients infected with human immunodeficiency virus. Clin. Infect. Dis. **24** (1997) 1223–1227.

Thalidomide is emerging as a useful agent in the management of several complications of disease due to human immunodeficiency virus (HIV). we conducted three prospective studies of 56 HIV-infected patients who were treated with thalidomide for 14–21 days; 24 (43%) of these patients discontinued therapy owing to adverse reactions. Cutaneous and/or febrile reactions were the most frequent toxicities, arising in 20 (36%) of the patients. These reactions occurred after a mean interval (±SD) of 10 ± 3 days and were associated with significantly lower CD4 T lymphocyte counts in reactors than in nonreactors (median count, 52.5/mm^3 vs 242 cells/mm^3, respectively; p = 0.009). Four of four rechallenged patients experienced accelerated hypersensitivity; hypotension occurred in one case. Although sedation was an almost universal side effect among the patients, it was moderate or severe in only seven (13%); constipation was moderate or severe in five (9%) of the patients. Severe neuropathic symptoms and mood changes were each noted in two (4%) of the 56 patients. We conclude that the increasing use of thalidomide to treat HIV-infected patients must be accompanied by recognition of the drug's increased potential for toxicity in this population.—Authors' Abstract

Hauschild, A., Kroeger, H., Mitchison, N.A., Ugrinovic, S. and Zwingenberer, K. Thalidomide therapy of established collagen-induced arthritis (CIA) not accompanied by an evident Th2 shift. Clin. Exp. Immunol. **108** (1997) 428–431.

Thalidomide, a drug likely to affect the cytokine pattern, was administered orally to mice at various stages of CIA. Treatment (150 mg/kg per day by gavage, 5 days/week), started 6 weeks post-immunization, i.e., at the height of the disease, significantly reduced arthritis, and appeared also to reduce the level of inflammation as judged by neutrophil chemiluminescence. With treatment started 9 weeks post-immunization the effect on arthritis was no longer statistically significant, and when started at 14 weeks was lost. Over a dose range of up to 150 mg/kg per day the treatment had no effect on either interferon-gamma or IL-4 mRNA levels. The treatment is therefore not likely to have operated via a shift in the Th1/Th2 balance.—Authors' Abstract

Hawken, M. P., Meme, H. K., Elliott, L. C., Chakaya, J. M., Morris, J. S., Githui, W. A., Juma, E. S., Odhiambo, J. A., Thiongo, L. N., Kimari, J. N., Ngugi, E. N., Bwayo, J. J., Gilks, C. F., Plummer, F. A., Porter, J. D. H., Nunn, P. P. and McAdam, P. W. J. Isoniazid preventive therapy for tuberculosis in HIV-1-infected adults: results of a randomized controlled trial. AIDS **11** (1997) 875–882.

Objectives: To determine the efficacy of isoniazid 300 mg daily for 6 months in the prevention of tuberculosis in HIV-1-infected adults and to determine whether tuberculosis preventive therapy prolongs survival in HIV-1-infected adults.

Design and setting: Randomized, double-blind, placebo-controlled trial in Nairobi, Kenya.

Subjects: Six hundred eighty-four HIV-1-infected adults.

Main outcome measures: Development of tuberculosis and death.

Results: Three hundred forty-two subjects received isoniazid and 342 received placebo. The median CD4 lymphocyte counts at enrollment were 322 and 346 × 10^6/l in the isoniazid and placebo groups, respectively. The overall median follow up from enrollment was 1.83 years (range, 0–3.4 years). The incidence of tuberculosis in the isoniazid group was 4.29 per 100 person-years (PY) of observation [95% confidence interval (CI) 2.78–6.33] and 3.86 per 100 PY of observation (95% CI 2.45–5.79)

in the placebo group, giving an adjusted rate ratio for isoniazid versus placebo of 0.92 (95% CI 0.49–1.71). The adjusted rate ratio for tuberculosis for isoniazid versus placebo for tuberculin skin test (TST)-positive subjects was 0.60 (95% CI 0.23–1.60) and for the TST-negative subjects, 1.23 (95% CI 0.55–2.76). The overall adjusted mortality rate ratio for isoniazid versus placebo was 1.18 (95% CI 0.79–1.75). Stratifying by TST reactivity gave an adjusted mortality rate ratio in those who were TST-positive of 0.33 (95% CI 0.09–1.23) and for TST-negative subjects, 1.39 (95% CI 0.90– 2.12).

Conclusions: Overall, there was no statistically significant protective effect of daily isoniazid for 6 months in the prevention of tuberculosis. In the TST-positive subjects, where reactivation is likely to be the more important pathogenetic mechanism, there was some protection and some reduction in mortality, although this was not statistically significant. The small number of individuals in this subgroup made the power to detect a statistically significant difference in this subgroup low. Other influences that may have diluted the efficacy of isoniazid include a high rate of transmission of new infection and rapid progression to disease or insufficient duration of isoniazid in subjects with relatively advanced immunosuppression. The rate of drug resistance observed in subjects who received isoniazid and subsequently developed tuberculosis was low.—Authors' Abstract

Horsburgh, C. R., Schoenfelder, J. R., Gordin, F. M., Cohn D. L., Sullam, P. M. and Wynne, B. A. Geographic and seasonal variation in *Mycobacterium avium* bacteremia among North American patients with AIDS. Am. J. Med. Sci. **313** (1997) 341–345.

Analysis of geographic risk was performed for *Mycobacterium avium* complex (MAC) bacteremia among North American patients with AIDS. Monthly mycobacterial blood cultures were taken from patients who were placebo recipients in a prospective evaluation of MAC prophylaxis. Of 571 patients, 102 (17.9%) acquired MAC bacteremia during an average follow up of 256 days. The area with the highest risk for MAC was the South Central region (27.9%; $p < 0.02$); whereas the area with the lowest risk was Canada (11.3%; $p = 0.12$). When the Southern states were combined and compared with the Northern states and Canada, the incidence of MAC bacteremia was higher in the Southern states (21.6% versus 14.0%, $p < 0.03$). Proportional hazards analysis was performed for the difference between the North and South and controlled for baseline CD4 cell count. In this analysis, time to MAC was significantly longer in the North (hazard ratio = 0.587, 95% confidence interval 0.390 to 0.883, $p = 0.01$). Although overall variation in seasonality was not marked, there was a significant decrease in cases in the North during the summer months ($p < 0.01$). We conclude that geographic location is a risk factor for MAC bacteremia in patients with advanced AIDS, with decreased risk in northern North America.—Authors' Abstract

Jacobson, J. M., Greenspan, J. S., Spritzler, J., Ketter, N., Fahey, J. L., Jackson, J. B., Fox, L., Chernoff, M., Wu, A. W., MacPhail, L. A., Vasquez, G. J. and Wohl, D. A. Thalidomide for the treatment of oral aphthous ulcers in patients with human immunodeficiency virus infection. N. Engl. J. Med. **336** (1997) 1487–1493.

Background: In patients with advanced human immunodeficiency virus (HIV) infection, aphthous ulceration of the mouth and oropharynx can become extensive and debilitating. Preliminary reports suggest that thalidomide may promote the healing of oral aphthous ulcers.

Method: We performed a double-blind, randomized, placebo-controlled study of thalidomide as therapy for oral aphthous ulcers in HIV-infected patients. The patients received a 4-week course of either 200 mg of thalidomide or placebo orally once per day. They were evaluated weekly for the condition of the ulcers, their quality of life, and evidence of toxicity. Assays were performed for plasma tumor necrosis factor-alpha (TNF-α), soluble TNF-α receptors, and HIV RNA.

Results: Sixteen of 29 patients in the thalidomide group (55%) had complete

healing of their aphthous ulcers after 4 weeks, as compared with only 2 of 28 patients in the placebo group (7%; odds ratio, 15; 95% confidence interval after adjustment for group sequential testing, 1.8 to 499; unadjusted $p < 0.001$). Pain diminished and the ability to eat improved with thalidomide treatment. The adverse effects noted with thalidomide included somnolence and rash (7 patients each), and 6 of the 29 patients discontinued treatment because of toxicity. Thalidomide treatment increased HIV RNA levels (median increase, 0.42 $\log^{10}$ copies per milliliter; increase with placebo, 0.05; $p = 0.04$). With thalidomide treatment there were unexpected increases in the plasma concentrations of TNF-α and soluble TNF-α receptors.

Conclusions: Thalidomide is an effective treatment for aphthous ulceration of the mouth and oropharynx in patients with HIV infection.—Authors' Abstract

Kenyon, B. M., Browne, F. and D'Amato, R. J. Effects of thalidomide and related metabolites in a mouse corneal model of neovascularization. Exp. Eye Res. **64** (1997) 971–978.

Thalidomide, when administered orally, is an inhibitor of angiogenesis in the basic fibroblant growth factor (bFGF)-induced rabbit cornea micropocket assay. We now show in the mouse that thalidomide given intraperitoneally but not orally significantly inhibits bFGF-induced and vascular endothelial growth factor (VEGF)-induced corneal neovascularization. We further demonstrate that this inhibition is independent from thalidomide's ability to suppress tumor necrosis factor-alpha (TNF-α) production. Experiments examining thalidomide's enantiomers reveal that the S^--enantiomer has the strongest antiangiogenic activity in VEGF-induced and bFGF-induced corneal neovascularization. Structure activity studies suggest that thalidomide's antiangiogenic activity is related to the open ring metabolites resulting from hydrolysis. Together these data support a correlation between thalidomide's antiangiogenic and teratogenic activities.—Authors' Abstract

Lefevre, P., Braibant, M., DeWit, L., Kalai, M., Roeper, D., Grotzinger, J., Delville, J. P., Peirs, P., Ooms, J., Huygen, K. and Content, J. Three different putative phosphate transport receptors are encoded by the *Mycobacterium tuberculosis* genome and are present at the surface of *Mycobacterium bovis* BCG. J. Bacteriol. **179** (1997) 2900–2906.

A gene encoding a protein homologous to the periplasmic ARC phosphate binding receptor PstS from *Escherichia coli* was cloned and sequenced from a lambda-gt11 library of *Mycobacterium tuberculosis* by screening with monoclonal antibody 2A1–2. Its degree of similarity to the *E. coli* PstS is comparable to those of the previously described tuberculosis phosphate binding protein pab (Ag78, Ag5, or 38-kDa protein) and another *M. tuberculosis* protein which we identified recently. We suggest that the three *M. tuberculosis* proteins share a similar function and could be named PstS-1, PstS-2, and PstS-3, respectively. Molecular modeling of their three-dimensional structures using the structure of the *E. coli* PstS as a template and their inducibility by phosphate starvation support this view. Recombinant PstS-2 and PstS-3 were produced and purified by affinity chromatography. With PstS-1, these proteins were used to demonstrate the specificity of three groups of monoclonal antibodies. Using these antibodies in flow cytometry and immunoblotting analyses, we demonstrate that the three genes are expressed and their protein products are present and accessible at the mycobacterial surface as well as in its culture filtrate.

Together with the *M. tuberculosis* genes encoding homologs of the PstA, PstB, and PstC components we cloned before, the present data suggest that at least one, and possibly several, related and functional ABC phosphate transporters exist in mycobacteria. It is hypothesized that the mycobacterial gene duplications presented here may be a subtle adaptation of intracellular pathogens to phosphate starvation in their alternating growth environments.—Authors' Abstract

MacMicking, J. D., North, R. J., LaCourse, R., Mudgett, J. S., Shah, S. K. and Nathan, C. F. Identification of nitric

oxide synthase as a protective locus against tuberculosis. Proc. Natl. Acad. Sci. U.S.A. **94** (1997) 5243–5248.

Mutagenesis of the host immune system has helped identify response pathways necessary to combat tuberculosis. Several such pathways may function as activators of a common protective gene: inducible nitric oxide synthase (NOS2). Here we provide direct evidence for this gene controlling primary *Mycobacterium tuberculosis* infection using mice homozygous for a disrupted NOS2 allele. NOS2 (–/–) mice proved highly susceptible, resembling wild-type littermates immunosuppressed by high-dose glucocorticoids, and allowed *M. tuberculosis* to replicate faster in the lungs than reported for other gene-deficient hosts. Susceptibility appeared to be independent of the only known naturally inherited antimicrobial locus, NRAMP1. Progression of chronic tuberculosis in wild-type mice was accelerated by specifically inhibiting NOS2 via administration of N-6-(1-iminoethyl)-L-lysine. Together these findings identify NOS2 as a critical host gene for tuberculostasis.—Authors' Abstract

Moreira, A. L., Corral, L. G., Ye, W. G., Johnson, B., Stirling, D., Muller, G. W., Freedman, V. H. and Kaplan, G. Thalidomide and thalidomide analogs reduce HIV type 1 replication in human macrophages *in vitro.* AIDS Res. Hum. Retroviruses **13** (1997) 857–863.

Thalidomide is currently being evaluated for efficacy in alleviating some manifestations of HIV-1 infection. To determine whether thalidomide has any direct effects on HIV-1 infection, we investigated the effect of thalidomide and also of three structural analogs of thalidomide on HIV-1 replication *in vitro* in human monocyte-derived macrophages. The thalidomide analogs were previously shown to inhibit TNF-alpha production *in vitro* at much lower concentrations than thalidomide. In HIV-1-infected macrophages treated with thalidomide or thalidomide analogs, viral replication was reduced by 60% to 80% as determined by measuring viral RT activity in the culture supernatants. In all experiments the analogs inhibited HIV-1 replication more efficiently than did thalidomide The drugs also reduced HIV-1 gag mRNA expression. Furthermore, the drugs caused a decrease in NF-kappa B-binding activity in nuclear extracts of HIV-1-infected macrophages. The role of NF-kappa B in the drug-induced inhibition of HIV-1 replication was confirmed using an NF-kappa B-defective mutant virus to infect macrophages.—Authors' Abstract

Munk, M. E., Kern, P. and Kaufmann, S. H. E. Human CD30+ cells are induced by *Mycobacterium tuberculosis* and present in tuberculosis lesions. Int. Immunol. **9** (1997) 713–720.

CD30 is a member of the tumor necrosis factor/nerve growth factor receptor family and evidence has been presented that activated CD4+ CD45Ro+ T cells of Th2 type selectively express CD30. *Mycobacterium tuberculosis,* a facultative intracellular bacterium capable of replicating in resting macrophages, is a potent inducer of IFN-gamma secretion by Th1 cells. We find increased CD30 expression by *M. tuberculosis*-stimulated alpha beta and gamma delta T cells, and elevated numbers of CD30+ alpha beta T cells in tuberculosis pleuritis and affected lung tissue. Furthermore, surface CD30 was associated with intracytoplasmic IFN-gamma expression and IFN-gamma production by *M. tuberculosis*-stimulated alpha beta and gamma delta T cells. Thus, our results indicate that *M. tuberculosis* is a potent inducer of CD30 expression in Th1 cells and argue against exclusive correlation of CD30 expression with Th2 cell responses.—Authors' Abstract

O'Nuallain, E. M., Davis, W. C., Costello, E., Pollock, J. M. and Monaghan, M. L. Detection of *Mycobacterium bovis* infection in cattle using an immunoassay for bovine soluble interleukin-2 receptor-alpha (sIL-2R-alpha) produced by peripheral blood T lymphocytes following incubation with tuberculin PPD. Vet. Immunol. Immunopathol. **56** (1997) 65–76.

After activation of T lymphocytes with antigen there is an increase in the expres-

sion of interleukin-2 receptor-alpha (IL-2R-α) followed by the release of a soluble form of the molecule (sIL-2R-α) from the membrane of the stimulated cells. The present study investigates the novel use of the release of sIL-2R-α from activated T lymphocytes as a marker of cell-mediated immunity (CMI) in cattle infected with *Mycobacterium bovis.* An enzyme immunoassay was used to detect sIL-2R-α produced following incubation of bovine peripheral blood mononuclear cells with mycobacterial antigens. Using this assay, 63/67 cattle naturally infected with *M. bovis* were identified; whereas only 1/51 uninfected animals were considered to give a positive result. This assay is more convenient to use than lymphocyte proliferation assays which involve the use of radionucleosides. It should prove useful for monitoring the immunological activation of bovine T lymphocytes in a variety of situations including the development of CMI responses in cattle to novel mycobacterial antigens or potential vaccines.—Authors' Abstract

Pierre Audigier, C., Jouanguy, E., Lamhamedi, S., Altare, F., Rauzier J., Vincent, V., Canioni, D., Emile, J. F., Fischer, A., Blanche, S., Gaillard, J. L. and Casanova, J. L. Fatal disseminated *Mycobacterium smegmatis* infection in a child with inherited interferon gamma receptor deficiency. Clin. Infect. Dis. **24** (1997) 982–984.

Mycobacterium smegmatis is a common environmental mycobacterium that was first identified in 1884, yet is a rare pathogen in humans. The few *M. smegmatis* infections reported to date have been localized and have occurred in association with a primary lesion in otherwise immunocompetent individuals. To our knowledge, no case of disseminated *M. smegmatis* infection has ever been reported, even in patients with severe immune deficiencies. We report a case of disseminated mycobacterial infection that was diagnosed in a 3-year-old girl. The pathogen was not identified as *M. smegmatis* until the patient was 6 years old. Her condition gradually worsened, and she died when she was 8 years old despite appropriate antimycobacterial therapy. No other opportunistic infections were documented. Immunological investigations revealed an inherited interferon-gamma receptor deficiency. This report identifies *M. smegmatis* as a new opportunistic agent that may be responsible for disseminated disease in immunocompromised individuals.—Authors' Abstract

Pollock, J. M. and Andersen, P. Predominant recognition of the ESAT-6 protein in the first phase of infection with *Mycobacterium bovis* in cattle. Infect. Immun. **65** (1997) 2587–2592.

Tuberculosis continues to be a worldwide health problem for both humans and animals. The development of improved vaccines and diagnostic tests requires detailed understanding of the immune responses generated and the antigens recognized during the disease. This study examined the T-cell response which develops in cattle experimentally infected with *Mycobacterium bovis.* The first significant T-cell response was found 3 weeks after the onset of infection and was characterized by a pronounced gamma-interferon (IFN-γ) response from peripheral blood mononuclear cells directed to antigens in culture filtrates. Short-term culture filtrate (ST-CF) was separated into molecular mass fractions and screened for recognition by T cells from experimentally infected and field cases of bovine tuberculosis. Cattle in the early stages of experimental infection were characterized by strong IFN-γ responses directed predominantly toward the lowest-mass (<10-kDa) fraction of ST-CF, but cattle in later stages of experimental infection (16 weeks postinfection) exhibited a broader recognition of antigens of various molecular masses. Field cases of bovine tuberculosis, in comparison, preferentially recognized low-mass antigens, characteristic of animals in the early stages of infection. The major T-cell target for this dominant IFN-γ response was found to be the secreted antigen ESAT-6. This antigen was recognized strongly by the majority of field cases of bovine tuberculosis tested. As ESAT-6 is unique to pathogenic mycobacterial species, our study suggests that ESAT-6 is an antigen with major potential for vaccination against and spe-

cific diagnosis of bovine tuberculosis.—Authors' Abstract

Ross, B. C., Marino, L., Oppedisano, F., Edwards, R., Robins Browne, R. M. and Johnson, P. D. R. Development of a PCR assay for rapid diagnosis of *Mycobacterium ulcerans* infection. J. Clin. Microbiol. **35** (1997) 1696–1700.

The diagnosis of *Mycobacterium ulcerans* infection is hampered by the slow growth of the bacterium in culture, resulting in a delay of several months before a specific diagnosis can be obtained. In addition, *M. ulcerans* cannot be isolated from water even when there is convincing epidemiological evidence implicating this as the source of infection. The aim of the present study was to develop a PCR assay to circumvent the problems of delayed diagnosis and insensitivity of standard bacterial culture for *M. ulcerans*. For the PCR, we isolated an *M. ulcerans*-specific DNA fragment, 1109 bp long, which is repeated at least 50 times throughout the genome. Use of this sequence as a target for PCR allowed us to detect as few as 2 molecules of genomic DNA *in vitro*. The PCR was used to detect *M. ulcerans* in fresh tissue and paraffin-embedded sections from all seven patients with culture-confirmed cases of infection.—Authors' Abstract

Sharpstone, D., Rowbottom, A., Francis, N., Tovey, G., Ellis, D., Barrett, M. and Gazzard, B. Thalidomide: a novel therapy for microsporidiosis. Gastroenterology **112** (1997) 1823–1829.

Background & Aims: Microsporidiosis is a common cause of chronic diarrhea in human immunodeficiency virus (HIV)-seropositive individuals and often does not respond to treatment. Fecal tumor necrosis factor-alpha (TNF-α) is elevated in microsporidiosis; therefore, thalidomide, an anti-TNF-α agent, was used as therapy.

Methods: Eighteen subjects with chronic diarrhea caused by *Enterocytozoon bieneusi* that had not responded symptomatically to albendazole and 1 untreated subject with *Encephalitozoon intestinalis* received 1 month of thalidomide, 100 mg nightly. Clinical response was assessed by stool frequency and body weight, histological response by light microscopy with villus height/crypt depth ratios and electron microscopy, and immunologic response by fecal TNF-α level.

Results: Seven subjects with chronic diarrhea due to *E. bieneusi* had a complete clinical response, and 3 had a partial response to thalidomide. There was a significant decrease in stool frequency from 5.3 to 3.1 per day ($p = 0.001$), and weight increased significantly by 1.2 kg ($p < 0.02$). Thalidomide significantly increased the villus height/crypt depth ratio (1.95 to 2.07; $p = 0.045$) and number of abnormal forms of microsporidia ($p < 0.01$). Fecal TNF-α level nonsignificantly decreased from 17.9 to 8.9 U/mL. There was apparent disruption of all stages of the life cycle of *E. intestinalis*.

Conclusions: Thalidomide may be an effective therapy for diarrhea and weight loss from *E. bieneusi*.—Authors' Abstract

Sreevatsan, S., Stockbauer, K. E., Pan, X., Kreiswirth, B. N., Moghazeh, S. L., Jacobs, W. R., Jr., Telenti, A. and Musser, J. M. Ethambutol resistance in *Mycobacterium tuberculosis*: critical role of embB mutations. Antimicrob. Agents Chemother. **41** (1997) 1677–1681.

Ethambutol [(*S,S'*)-2,2'-(ethylenediimino)di-1-butanol; EMB], is a first-line drug used to treat tuberculosis. To gain insight into the molecular basis of EMB resistance, we characterized the 10-kb *embCAB* locus in 16 EMB-resistant and 3 EMB-susceptible genetically distinct *Mycobacterium tuberculosis* strains from diverse localities by automated DNA sequencing and single-stranded conformation polymorphism analysis. All 19 organisms had virtually identical sequences for the entire 10-kb region. Eight EMB-resistant organisms had mutations located in codon 306 of *embB* that resulted in the replacement of the wild-type Met residue with Ile or Val. Automated sequence analysis of the 5' region (1892 bp) of *embB* in an additional 69 EMB-resistant and 30 EMB-susceptible *M. tuberculosis* isolates from diverse geographic localities and representing 70 distinct IS*6110* finger-

prints confirmed the unique association of substitutions in amino acid residue 306 of EmbB with EMB resistance. Six other *embB* nucleotide substitutions resulting in four amino acid replacements were uniquely found in resistant strains. Sixty-nine percent of epidemiologically unassociated EMB-resistant organisms had an amino acid substitution not found in susceptible strains, and most (89%) replacements occurred at amino acid residue 306 of EmbB. For strains with the Met306Leu or Met306Val replacements EMB MICs were generally higher (40 μg/ml) than those for organisms with Met306Ile substitutions (20 μg/ml). The data are consistent with the idea that amino acid substitutions in EmbB alter the drug-protein interaction and thereby cause EMB resistance.—Authors' Abstract

Steitz, A., Feddersen, A., Freytag, C., Daniello, S., Schopf, R. E., Bocher, W. O., Bhakdi, S. and Husmann, M. Rapid identification of *Mycobacterium marinum* by comparative 16S-rRNA-gene analysis in five cases of progredient cutaneous infections. Eur. J. Dermatol. **7** (1997) 295–299.

Five cases of *Mycobacterium marinum* infection were diagnosed by automated sequencing of 16S-rDNA at our Department of Medical Microbiology over a period of 10 months. This compares to a single case of *M. marinum* infection recorded during the 5 years prior to the availability of this method at our clinic. While all of the patients shared typical features of fishtank granuloma, such as cutaneous lesions on an upper extremity, contact with aquatic organisms and wounds or scratches as potential portals of entry, none of the cases was diagnosed by the physicians who were initially consulted. This could partially be attributed to the different characteristics exhibited in some of the cases. All of the patients showed a marked improvement of their symptoms within several weeks under specific antibiotic treatment with combinations of rifampin, ethambutol and clarithromycin. The availability of 16S-rDNA-sequencing as a rapid and precise procedure for the identification of atypical mycobacteria is considered to be an incentive for the clinician to pursue more rigorously, an accurate diagnosis of such cutaneous lesions.—Authors' Abstract

Telenti, A., Philipp, W. J., Sreevatsan, S., Bernasconi, C., Stockbauer, K. E., Wieles, B., Musser, J. M. and Jacobs, W. R. The emb operon, a gene cluster of *Mycobacterium tuberculosis* involved in resistance to ethambutol. Nature Med. **3** (1997) 567–570.

Ethambutol (EMB), a front-line antituberculous drug, targets the mycobacterial cell wall, a unique structure among prokaryotes which consists of an outer layer of mycolic acids covalently bound to peptidoglycan via the arabinogalactan. EMB inhibits the polymerization of cell wall arabinan, and results in the accumulation of the lipid carrier decaprenol phosphoarabinose, which suggests that the drug interferes with the transfer of arabinose to the cell wall acceptor. Unfortunately, resistance to EMB has been described in up to 4% of clinical isolates of *Mycobacterium tuberculosis* and is prevalent among isolates from patients with multidrug-resistant tuberculosis. We used resistance to EMB as a tool to identify genes participating in the biosynthesis of the mycobacterial cell wall. This approach led to the identification of the embCAB gene cluster, recently proposed to encode for mycobacterial arabinosyl transferases. Resistance to EMB results from an accumulation of genetic events determining overexpression of the Emb protein(s), structural mutation in EmbB, or both. Further characterization of these proteins might provide information on targets for new chemotherapeutic agents and might help development of diagnostic strategies for the detection of resistant *M. tuberculosis.*—Authors' Abstract

Tortoli, E., Kirschner, P., Springer, B., Bartoloni, A., Burrini, C., Mantella, A., Scagnelli, N., Scarparo, C., Simonetti, M. T. and Bottger, E. C. Cervical lymphadenitis due to an unusual *Mycobacterium.* Eur. J. Clin. Microbiol. Infect. Dis. **16** (1997) 308–311.

A scotochromogenic acid-fast bacillus was isolated from a lymph node of a 2-year-old female. On the basis of conventional

testing, the mycobacterium appeared to be *Mycobacterium scrofulaceum.* Its mycolic acid profile, however, was not identical to that of *M. scrofulaceum* but was similar to that of *M. interjectum.* Direct sequencing of the 16s-rRNA gene revealed a unique nucleic acid sequence, suggesting that the isolate represents a previously undescribed pathogenic species.—Authors' Abstract