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CURRENT LITERATURE

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

General and Historical

Chen, X. S., Li, W. Z., Jiang, X., Zhu, C. B. and Ye, G. Y. Studies of mode of detection of leprosy in China during the years 1981–1998. Lepr. Rev. **72** 302–310.

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

Chen, S., Zhang, L., Wang, Z., Zhou, J., Liu, Y. and Mao, C. Experiences from a collaborative project on the prevention of disability in leprosy patients in Shandong Province, the People's Republic of China. Lepr. Rev. **72** (2001) 330–336.

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

deterioration of existing disabilities in ex-patients through self-care and (b) prevent new neuritis in patients on MDT through early detection and the use of steroids.—Authors' Abstract

Dequeker, J., Fabry, G. and Vanopdenbosch, L. Hieronymus Bosch (1450–1516): paleopathology of the medieval disabled and its relation to the bone and joint decade 2000–2010. Isr. Med. Assoc. J. **3** (2001) 864–871.

Background: At the start of the Bone and Joint Decade 2000–2010, a paleopathologic study of the physically disabled may yield information and insight on the prevalence of crippling disorders and attitudes towards the afflicted in the past compared to today. Objective: To analyze "The procession of the Cripples," a representative drawing of 31 disabled individuals by Hieronymus Bosch in 1500. Methods: Three specialists—a rheumatologist, an orthopedic surgeon and a neurologist—analyzed each case by problem-solving means and clinical reasoning in order to formulate a consensus on the most likely diagnosis. Results: This iconographic study of cripples in the sixteenth century reveals that the most common crippling disorder was not a neural form of leprosy, but rather that other disorders were also prevalent, such as congenital malformation, dry gangrene due to ergotism, post-traumatic amputations, infectious diseases (Pott's, syphilis), and even simulators. The drawings show characteristic coping patterns and different kinds of crutches and aids. Conclusion: A correct clinical diagnosis can be reached through the collaboration of a rheumatologist, an orthopedist and a neurologist. The Bone and Joint Decade Project, calling for attention and education with respect to musculoskeletal disorders, should reduce the impact and burden of crippling diseases worldwide through early clinical diagnosis and appropriate treatment.—Authors' Abstract

Diggle, G. E. Thalidomide: 40 years on. Int. J. Clin. Pract. **55** (2001) 627–631.

Thalidomide was marketed in the late-1950s as a sedative and tranquillizer of exceptionally low general toxicity, but in 1961 it was implicated separately by Lenz and MacBride as the cause of the epidemic of congenital malformations that had been puzzling the world for some years. It is a very potent teratogen in humans, but in few other mammalian species; damage to the embryo is produced at specific stages of gestation, but the mechanism of embryopathic action is still not understood. Following the withdrawal of the drug worldwide, it was consigned to the history of medical tragedies. In 1965, however, Sheskin discovered that it was effective in treating erythema nodosum leprosum, a distressing complication of leprosy. As the drug is neither an antibiotic nor an analgesic, its action was assumed to be immunosuppressive. In Brazil the drug was used widely with few regulatory controls, since when more than 100 cases of congenital malformation have appeared. Sheskin's discovery led to the experimental use of thalidomide in many other indications thought to possess some immunological component. In some cases, e.g., Behcet's syndrome, graft-versus-host disease and aphthous ulceration in HIV-positive patients, the drug has been shown to possess some efficacy. And there is some evidence that it inhibits the replication of one of the immunodeficiency viruses. The AIDS community in the US has exerted much pressure on the FDA to allow the drug onto the market, although the use of a potent immunosuppressive drug of unknown mechanism in an immunodeficiency condition raises further questions. Thalidomide is not always beneficial; its use is associated with an increased mortality in epidermal necrolysis. In 1991, D'Amato confirmed it possessed antiangiogenic properties and this led to further trials in malignant conditions. Results were mixed, but those in multiple myeloma gave some grounds for optimism. In 1998, the FDA announced its extraordinary decision to grant marketing approval for thalidomide.—Author's Abstract

Lejbkowicz, F., Tsilman, B., Wexler, R. and Cohen, H. I. Leprosy in Israel: an imported disease—the support of histopathological examination for its de-

tection. Acta Histochem. **103** (2001) 433–436.

Leprosy is rare and non-endemic in Israel. Cases of leprosy are invariably imported by immigrants or foreign workers arriving from endemic areas. In view of the relative rarity of the disease, clinicians and pathologists are not always alert to the possibility of the disease or recognize potential symptoms. A case history is presented of a 31-year-old immigrant presenting symptoms of skin lesions and nodules on the hands and facial region, especially the ear lobe. Confirmation of the infection was provided by histopathology of suspected lesions stained for acid-fast bacilli (modified Fite-Faraco staining).—Authors' Abstract

Sasaki, S., Takeshita, F., Okuda, K. and Ishii, N. J. *Mycobacterium leprae* and leprosy. Microbiol. Immunol. **45** (2001) 729–736.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae,* which was discovered by G. H. A. Hansen in 1873. *M. leprae* is an exceptional bacterium because of its long generation time and no growth in artificial media. Entire sequencing of the bacterial genome revealed numerous pseudogenes (inactive reading frames with functional counterparts in *M. tuberculosis*) which might be responsible for the very limited metabolic activity of *M. leprae.* The clinical demonstration of the disease is determined by the quality of host immune response. Th1-type immune response helps to kill the bacteria, but hosts are encroached upon when Th2-type response is predominant. The bacteria have affinity to the peripheral nerves and are likely to cause neuropathy. *M. leprae*/laminin-alpha2 complexes bind to alpha/beta dystroglycan complexes expressed on the Schwann cell surface. WHO recommends a chemotherapy protocol [multidrug therapy (MDT)] which effectively controls the disease and contributes to the global elimination program. Leprosy has been stigmatized throughout history, and recent topics regarding the disease in Japan are also discussed.—Authors' Abstract

Chemotherapy

Chan, C. Y., Au-Yeang, C., Yew, W. W., Hui, M. and Cheng, A. F. Postantibiotic effects of antituberculosis agents alone and in combination. Antimicrob. Agents Chemother. **45** (2001) 3631–3634.

The postantibiotic effects (PAEs) of seven antimycobacterial agents, tested at their respective peak concentrations in serum alone and in different combinations, against *Mycobacterium tuberculosis* ATCC 27294 were studied with a radiometric culture system in parallel with the viable count method. Rifampin gave the longest PAE (67.8 hr) among the drugs used alone, and combinations of first-line drugs generally gave PAEs longer than 120 hr. The data obtained might help provide a better understanding of the scientific basis of intermittently administered antituberculosis chemotherapy—Authors' Abstract

Chen, B. J. Triptolide, a novel immunosuppressive and anti-inflammatory agent purified from a Chinese herb *Tripterygium wilfordii* hook F. Leuk. Lymphoma **42** (2001) 253–265.

Triptolide is a diterpenoid triepoxide purified from a Chinese herb *Tripterygium wilfordii* Hook F (TWHF). TWHF has been used in traditional Chinese medicine for more than two thousand years. However, its potential value was recognized by the western medicine only after investigators observed the effectiveness of TWHF in the treatment of leprosy and rheumatoid arthritis. Triptolide has been identified as the major component responsible for the immunosuppressive and anti-inflammatory effects of TWHF. Triptolide inhibits both Ca(2+)-dependent and Ca(2+)-independent pathways and affects T cell activation through

inhibition of interleukin-2 transcription at a site different from the target of cyclosporin A. Triptolide also has inhibitory effects on a variety of proinflammatory cytokines and mediators and on the expression of adhesion molecules by endothelial cells. Triptolide is effective for the treatment of a variety of autoimmune diseases and in prevention of allograft rejection and graft-versus-host disease in both animals and humans. Moreover, triptolide possesses antitumor and male anti-fertility effect. However, the toxicities of triptolide may be associated with renal, cardiac, hematopoietic and reproductive systems. Currently available data suggest that triptolide is a promising immunosuppressive and anti-inflammatory agent and should be explored further in autoimmune diseases and transplantation.—Author's Abstract

DeMaio, J., Schwartz, L., Cooley, P. and Tice, A. The application of telemedicine technology to a directly observed therapy program for tuberculosis: a pilot project. Clin. Infect. Dis. **33** (2001) 2082–2084.

We evaluated the use of videophone technology to provide directly observed therapy (DOT) to patients with active tuberculosis. During 304 treatment doses, adherence on videophone DOT was 95%, and patient acceptance of the technology was excellent. In selected cases, the use of videophone technology can maintain a high level of adherence to DOT in a cost-effective manner.—Authors' Abstract

Clinical Sciences

Cooke, G. S. and Hill, A. V. Genetics of susceptibility to human infectious disease. Nat. Rev. Genet. **2** (2001) 967–977.

Before Robert Koch's work in the late nineteenth century, diseases such as tuberculosis and leprosy were widely believed to be inherited disorders. Heritability of susceptibility to several infectious diseases has been confirmed by studies in the twentieth century. Infectious diseases, old and new, continue to be an important cause of mortality worldwide. A greater understanding of disease processes is needed if more effective therapies and more useful vaccines are to be produced. As part of this effort, developments in genetics have allowed a more systematic study of the impact that the human genome and infectious disease have on each other.—Authors' Abstract

Courtright, P., Kim, S. H., Tungpakorn, N., Cho, B. H., Lim, Y. K., Lee, H. J. and Lewallen, S. Lagophthalmos surgery in leprosy: findings from a population-based survey in Korea. Lepr. Rev. **72** (2001) 285–291.

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

education, (c) monitoring the uptake of surgery, and (d) monitoring the outcome of surgery to ensure the best possible outcome.—Authors' Abstract

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

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

Kulkarni, R. B., Rajeshwari, T. A. Patil and Praveena. S. Actinomycotic mycetoma due to *Nocardia brasiliensis* in a case of leprosy. Indian J. Lepr. **73** (2001) 263–272.

Various bacterial and fungal infections associated with non-healing ulcers in cases of leprosy have been reported (G Ebenzer, *et al.*, 2000, Rama Ramani *et al.*, 1990). There are no reports of mycetoma associated with leprosy patients in the literature. We report here a case of actinomycotic mycetoma due to *Nocardia brasiliensis* associated with the non-healing plantar ulcer of a leprosy patient.—Authors' Abstract

Petering, H., Kiehl, P., Vogelbruch, M., Sticht-Gron, V., Kapp, A. and Werfel, T. Chemotherapy-induced erythema nodosum leprosum: successful treatment with thalidomide. **52** (2001) 966–969 (in German).

The severity and outcome of a chronic granulomatous infection caused by *M. leprae* depend on the cell-mediated immunity towards the pathogen. The disease classification is based on the host's response to *M. leprae* ranging from high to low resistance (polar tuberculoid leprosy to polar lepromatous leprosy). The host's position in the spectrum is not stable; leprosy reactions reflecting changed immune status may occur spontaneously or during chemotherapy. The type II reaction or erythema nodosum leprosum can most often be seen in patients with lepromatous leprosy, a multiorgan disease characterized by an unrestricted bacillary replication. Clinically, this reaction is characterized by crops of painful bright pink, dermal and subcutaneous nodules arising in clinically normal skin, in association with fever, malaise, glomerulonephritis and arthralgias. Therefore, prompt institution of immunosuppressive therapy with corticosteroids or thalidomide is recommended. This case report describes the development of erythema nodosum leprosum during chemotherapy treated successfully with thalidomide. Furthermore, immunologic effects and potential side effects of this drug are discussed.—Authors' Abstract

Santos, A. R., Balassiano, V., Oliveira, M. L., Pereira, M. A., Santos, P. B., De-

grave, W. M. and Suffys, P. N. Detection of *Mycobacterium leprae* DNA by polymerase chain reaction in the blood of individuals, eight years after completion of anti-leprosy therapy. **96** (2001) 1123–1133.

Thirty-eight patients with indeterminate leprosy (HI), at least 4 to 6 years after discharge from multibacillary (MB) or paucibacillary (PB) schemes of anti-leprosy multidrug therapy (MDT), were submitted to traditional diagnostic procedures for leprosy and to polymerase chain reaction (PCR) analysis of different clinical samples for detection of *Mycobacterium leprae* DNA. No significant difference was observed for any of the parameters analyzed between PB or MB schemes of treatment and no indications were found for more efficient outcome of HI using the MB scheme. Remarkably, 18 (54.5%) of the individuals were PCR positive in at least one of the samples: positivity of PCR was highest in blood samples and four individuals were PCR positive in blood and some other sample. Upon comparison of PCR results with clinical and histopathological parameters, no correlation was found between PCR-positivity and eventual relapse. This is the first report on detection of *M. leprae* DNA in PB patients, more than half a decade after completion of MDT, suggesting that live bacilli are present and circulating much longer than expected, although reinfection of the individuals cannot be excluded. Overall, we feel that because of the high sensitivity of the assay, extreme care should be taken about association of PCR results, efficacy of treatment and disease status.—Authors' Abstract

Shanker Narayan, N. P., Ramu, G., Ilangumaran, S., Desikan, K. V. and Muthukaruppan, V. R. Poor correlation of systemic immunological parameters with clinical features in macular leprosy. Indian J. Lepr. **73** (2001) 239–246.

On the basis of clinical features and bacteriological status, macular skin lesions of nine cases of leprosy were classified as falling within a spectrum between the tuberculoid at one end and the lepromatous at the other. While histological correlation was seen in 60% of cases, humoral and cellular systemic immunologic features were found to be uncharacteristic. It is suggested that macular lesions form an early stage in the development of leprosy where the systemic immunological response is yet to set in fully.—Authors' Abstract

Shaw, I. N., Ebenezer, G., Babu, B. and Rao, G. S. Borderline tuberculoid leprosy of the scalp. Lepr. Rev. **72** (2001) 357–359.

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

Immuno-Pathology

Belkaid, Y., Hoffmann, K. F., Mendez, S., Kamhawi, S., Udey, M. C., Wynn, T. A. and Sacks, D. L. The role of Interleukin (IL)-10 in the persistence of *Leishmania major* in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. J. Exp. Med. **19** (2001) 1497–1506.

Some pathogens (e.g., *Mycobacterium tuberculosis, Toxoplasma gondii, Leishmania* spp.) have been shown to persist in their host after clinical cube, establishing the risk of disease reactivation. We analyzed the conditions necessary for the long term maintenance of *Leishmania major* in genetically resistant C57BL/6 mice after spontaneous healing of their dermal lesions. Interleukin (IL)-10 was found to play an essential role in parasite persistence as sterile cure was achieved in IL-10-deficient and IL-4/IL-10 double-deficient mice. The requirement for IL-10 in establishing latency associated with natural infection was con-

firmed in IL-10-deficient mice challenged by bite of infected sand flies. The host-parasite equilibrium was maintained by CD4(+) and CD8(+) T cells which were each able to release IL-10 or interferon (IFN)-gamma, and were found to accumulate in chronic sites of infection, including the skin and draining lymph node. A high frequency of the dermal CD4(+) T cells released both IL-10 and IFN-gamma. Wild-type mice treated transiently during the chronic phase with anti-IL-10 receptor antibodies achieved sterile cure, suggesting a novel therapeutic approach to eliminate latency, infection reservoirs, and the risk of reactivation disease.—Authors' Abstract

Brandt, L., Feino Cunha, J., Weinreich Olsen, A., Chilima, B., Hirsch, P., Appelberg, R. and Andersen, P. Failure of the *Mycobacterium bovis* BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to tuberculosis. **70** (2002) 672–678.

The efficacy of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) vaccine against pulmonary tuberculosis (TB) varies enormously in different populations. The prevailing hypothesis attributes this variation to interactions between the vaccine and mycobacteria common in the environment, but the precise mechanism has so far not been clarified. Our study demonstrates that prior exposure to live environmental mycobacteria can result in a broad immune response that is recalled rapidly after BCG vaccination and controls the multiplication of the vaccine. In these sensitized mice, BCG elicits only a transient immune response with a low frequency of mycobacterium-specific cells and no protective immunity against TB. In contrast, the efficacy of TB subunit vaccines was unaffected by prior exposure to environmental mycobacteria. Six different isolates from soil and sputum samples from Karonga district in Northern Malawi (a region in which BCG vaccination has no effect against pulmonary TB) were investigated in the mouse model, and two strains of the *Mycobacterium avium* complex were found to block BCG activity completely.—Authors' Abstract

Diniz, L. M., Zandonade, E., Dietze, R., Pereira, F. E. and Ribeiro-Rodrigues, R. Short report: do intestinal nematodes increase the risk for multibacillary leprosy? Am. J. Trop. Med. Hyg. **65** (2001) 852–854.

Intestinal helminths are known to subvert the host's immune response towards a Th2 response, which in turn may lead to both eosinophilia and high immunoglobulin E titers often associated with these parasites. *Mycobacterium leprae* infection may lead to different clinical and pathological forms. Multibacillary forms are associated with Th2 cytokines, whereas paucibacillary forms are associated with Th1 cytokines. We report a significantly higher frequency of intestinal helminthic infections in patients with the lepromatous form, a multibacillary form of leprosy (odds ratio, 2.99; 95% confidence interval, 1.82–4.95; $p = 0.006$) when compared with patients with paucibacillary leprosy or to a control group without leprosy. A direct correlation was also found between mycobacterial index and the frequency of intestinal helminths. Our results suggest that the presence of intestinal helminths may facilitate the establishment of *M. leprae* infection or the progression to more severe forms of leprosy.—Authors' Abstract

Garcia, V. E., Quiroga, M. F., Ochoa, M. T., Ochoa, L., Pasquinelli, V., Fainboim, L., Olivares, L. M., Valdez, R., Sordelli, D. O., Aversa, G., Modlin, R. L. and Sieling, P. A. Signaling lymphocytic activation molecule expression and regulation in human intracellular infection correlate with Th1 cytokine patterns. J. Immunol. **167** (2001) 5719–5724.

Induction of Th1 cytokines, those associated with cell-mediated immunity, is critical for host defense against infection by intracellular pathogens, including mycobacteria. Signaling lymphocytic activation molecule (SLAM, CD150) is a transmembrane protein expressed on lymphocytes that promotes T cell proliferation and IFN-gamma production. The expression and role of SLAM in human infectious disease were

investigated using leprosy as a model. We found that SLAM mRNA and protein were more strongly expressed in skin lesions of tuberculoid patients, those with measurable CMI to the pathogen, *Mycobacterium leprae*, compared with lepromatous patients, who have weak CMI against *M. leprae*. Peripheral blood T cells from tuberculoid patients showed a striking increase in the level of SLAM expression after stimulation with *M. leprae*, whereas the expression of SLAM on T cells from lepromatous patients show little change by *M. leprae* stimulation. Engagement of SLAM by an agonistic mAb up-regulated IFN-gamma production from tuberculoid patients and slightly increased the levels of IFN-gamma in lepromatous patients. In addition, IFN-gamma augmented SLAM expression on *M. leprae*-stimulated peripheral blood T cells from leprosy patients. Signaling through SLAM after IFN-gamma treatment of Ag-stimulated cells enhanced IFN-gamma production in lepromatous patients to the levels of tuberculoid patients. Our data suggest that the local release of IFN-gamma by *M. leprae*-activated T cells in tuberculoid leprosy lesions leads to up-regulation of SLAM expression. Ligation of SLAM augments IFN-gamma production in the local microenvironment, creating a positive feedback loop. Failure of T cells from lepromatous leprosy patients to produce IFN-gamma in response to *M. leprae* contributes to reduced expression of SLAM. Therefore, the activation of SLAM may promote the cell-mediated immune response to intracellular bacterial pathogens.—Authors' Abstract

Hill, A. V. The genomics and genetics of human infectious disease susceptibililty. Annu. Rev. Genomics Hum. Genetics **2** (2001) 373–400.

A genetic basis for interindividual variation in susceptibility to human infectious diseases has been indicated by twin, adoptee, pedigree, and candidate gene studies. This has led to the identification of a small number of strong genetic associations with common variants for malaria, HIV infection, and infectious prion diseases. Numerous other genes have shown less strong associations with these and some other infectious diseases, such as tuberculosis, leprosy, and persistent hepatitis viral infections. Many immunogenetic loci influence susceptibility to several infectious pathogens. Recent genetic linkage analyses of measures of infection as well as of infectious disease, including some genome-wide scans, have found convincing evidence of genetic linkage to chromosomal regions wherein susceptibility genes have yet to be identified. These studies indicate a highly polygenic basis for susceptibility to many common infectious diseases, with some emerging examples of interaction between variants of specific polymorphic host and pathogen genes.—Author's Abstract

Jayapal, V., Selvibai, G., Mahalakshmi, K., Pushkala, Ragunath, K. and Subramanian, S. Comparative study of anti-PGL-1, anti-35kDa and anti-lipoarabinomannan assays for serodiagnosis of leprosy. Indian J. Lepr. **73** (2001) 229–237.

Three antibody assays (anti-PGL-1, anti-35kDa and anti-LAM) were used to determine the levels of antibodies in the sera of untreated leprosy patients. All the three assays showed higher levels of antibodies in BL/LL patients as compared to I and TT/BT patients, as well as healthy controls. BL/LL patients showed positivity of 100%, 84.2% and 78.9% by anti-PGL-1, anti-35kDa and anti-LAM assays respectively. All the three assays were negative for leprosy in healthy controls. Anti-PGL-1 assay was positive in 20% of TT/BT patients and 17.9% of I patients. Anti-35kDa assay was negative in all the TT/BT patients and positive in 7.14% of I patients. Anti-LAM assay was positive in 13.3% of TT/BT patients and in 10.7% of I patients. Hence, while these assays are valuable in diagnosing BL/LL patients, their usefulness in diagnosing 1, BT or TT leprosy is limited.—Authors' Abstract

Meisner, S. J., Mucklow, S., Warner, G., Sow, S. O., Liendhardt, C. and Hill, A. V. Association of NRAMP1 polymorphism with leprosy type but not susceptibility to leprosy per se in west Africans.

Am. J. Trop. Med. Hyg. **65** (2001) 733–735.

Twin and family studies indicate that host genetic factors influence susceptibility to leprosy and, possibly, leprosy type. Murine studies have suggested a role for the natural resistance-associated macrophage protein 1 (Nramp1) gene, which can influence cellular immune responses to intracellular pathogens. We evaluated a variation in the human homolog, NRAMP1, recently associated with tuberculosis susceptibility in West Africa. A total of 273 patients with leprosy and 201 controls from Mali were genotyped for NRAMP1 polymorphisms previously associated with tuberculosis. No association was found with leprosy per se ($p = 0.83$), but the NRAMP1 3′-untranslated region 4-bp insertion/deletion polymorphism was associated with leprosy type ($p = 0.007$). Heterozygotes were more frequent among multibacillary than paucibacillary leprosy cases. Thus, variation in or near the NRAMP1 gene may exert an influence on the clinical presentation of leprosy, possibly by influencing cellular immune response type.—Authors' Abstract

Modlin, R. L. Learning from leprosy: insights into contemporary immunology from an ancient disease. Skin Pharmacol. Appl. Skin Physiol. **15** (2002) 1–6.

Leprosy provides an ideal model to study immune responses in humans and in skin. Learning from leprosy, we have gained insight into mechanisms of host resistance and susceptibility to infection. New paradigms include the role of Th1/Th2 cytokines, the ability of CD1 to present nonpeptide antigens to T cells, the ability of microbial lipoproteins to stimulate antimicrobial activity in monocytes and the demonstration that T cells can mediate a direct antimicrobial activity through release of granulysin. Together, these findings provide a rationale for developing new strategies to treat and prevent infectious disease.—Author's Abstract

Rook, G. A., Lightman, S. L. and Heijnen, C. J. Can nerve damage disrupt neuroendocrine immune homeostasis? Leprosy as a case in point. Trends Immunol. **23** (2002) 18–22.

The crucial clinical problem in leprosy is the occurrence of acute inflammatory episodes that lead to nerve damage, even after the infecting organisms have been killed by antibiotics. We suggest that the instability of these inflammatory sites is attributable to a disturbance of the role that nerves play in the regulation of inflammation. The destruction of sensory C fibers and sympathetic innervation will remove anti-inflammatory feedback circuits. Moreover, diminishing levels of neuropeptides and changes in the cytokine profile will affect the cortisol-sensitivity of infiltrating T cells, and modulate the cortisol-cortisone shuttle so that the inflammatory site becomes resistant to physiological levels of anti-inflammatory adrenocortical steroids.—Authors' Abstract

Schon, T., Hernandez-Pando, R. H., Negesse, Y., Leekassa, R., Sundqvist, T. and Britton, S. Expression of inducible nitric oxide synthase and nitrotyrosine in borderline leprosy lesions. Br. J. Dermatol. **145** (2001) 809–815.

Background: In the response to T-helper cell (Th1)-type cytokines and interactions with pathogens, high levels of nitric oxide (NO) are produced by activated macrophages expressing the inducible NO synthase (iNOS). The role and importance of reactive nitrogen intermediates (RNIs) such as NO and peroxynitrite in the host response to diseases caused by intracellular pathogens such as *Mycobacterium leprae* and *M. tuberculosis* is unclear. Objectives: The aim of this study was to investigate the presence of local production of NO and peroxynitrite in borderline leprosy by using antibodies product of peroxynitrite, nitrotyrosine (NT). Methods: We detected the presence of iNOS and NT in skin biopsies from borderline leprosy patients, with and without reversal reaction (RR), by immunohistochemistry ($N = 26$). Results: In general, the granulomas from borderline leprosy lesions with and without RR showed high and specific expression of iNOS and NT. Moreover, strong immunoreactivity to iNOS and NT was ob-

served in granulomas surrounding and infiltrating dermal nerves. The expression of iNOS and NT was also strong in keratinocytes, fibroblasts and endothelial cells in close relation to the granulomatous reaction. In contrast, normal human skin showed no expression of iNOS and NT in these cells. Conclusions: We conclude that iNOS and NT are expressed in granulomas from borderline leprosy patients with and without RR and propose that RNIs might be involved in the nerve damage following RR in leprosy.—Authors' Abstract

Seitzer, U., Kayser, K., Hohn, H., Entzian, P., Wacker, H. H., Ploetz, S., Flad, H. D., Gerdes, J. and Maeurer, M. J. Reduced T-cell receptor CD3zeta-chain protein and sustained CD3epsilon expression at the site of mycobacterial infection. Immunology **104** (2001) 269–277.

Control of mycobacterial infection by the cellular immune system relies both on antigen-presenting cells and on T lymphocytes. The quality of an effective cellular immune response is dependent on functional signal transduction residing in the cytoplasmic tails of the T-cell receptor CD3 components. In order to investigate potential effects of mycobacteria on T-cell receptor signalling, we examined the protein expression of T-cell signal transduction molecules (CD3zeta, ZAP-70, p59fyn, p561ck). In Western blots of peripheral blood mononuclear cells of *Mycobacterium tuberculosis* infected patients, only the CD3zeta-chain showed a marked reduction in protein expression. To investigate the situation *in situ,* immunoenzymatic and immunofluorescence stainings for CD3epsilon and CD3zeta expression were performed on sections of normal lymphoid tissue, *M. leprae* infected and sarcoid tissue. CD3epsilon and CD3zeta expression were similar with respect to intensity, localization and the number of cells stained in normal lymphoid tissue and in sarcoid granulomas. In contrast, the granulomas of *M. leprae* infected tissues showed a significantly reduced expression of CD3zeta compared to CD3epsilon. Using double immunofluorescence analysis, virtually no CD3zeta expression could be detected in comparison to the CD3epsilon expression in the lesions. Apparently, mycobacteria are capable of significantly reducing CD3zeta-chain expression, which may be restored by cytokines. IL-2-enhanced zeta-chain expression and T-cell effector functions, defined by interferon-gamma release, in *M. tuberculosis*-specific and human leucocyte antigen-DR restricted CD4+ T cells isolated from granuloma lesions from patients with pulmonary tuberculosis. Because CD3zeta is essential for CD3 signalling and for eliciting T-cell effector functions, reduced CD3-zeta protein expression could result in altered signal transduction and inefficient T-cell effector functions. Alternatively, reduced CD3zeta-chain expression may protect T cells from repetitive TCR stimulation associated with anergy or apoptosis.—Authors' Abstract

Shanker Narayan, N. P., Ramu, G., Desikan, K. V. and Vallishayee, R. S. Correlation of clinical histological and immunological features across the leprosy spectrum. Indian J. Lepr. **73** (2001) 329–342.

The Ridley-Jopling system of classification of the variegated clinical pattern of leprosy is based on the specific cell-mediated immunity observed in the histopathology of skin lesions conforming to a spectrum from TT at one end to LL at the other.

In this study a fairly large sample of 90 patients was classified on clinical grounds; the histopathology of the skin lesions was studied blind. There was an overall concordance of 90% between the clinical and histological classifications. In addition, the systemic cell-mediated and humoral immune responses were studied. The *in vivo* cell-mediated immune response, namely the Mitsuda skin response, mostly conformed to the clinical classification. While the *in vitro* lymphoproliferative responses to BCG and its sonicate were high, the lymphoproliferative responses to Dharmendra lepromin were surprisingly poor. Humoral responses to 35kDA protein of *M. leprae* and PGL-1 were good in most LL, BL patients and tapered off towards TT. IgG antibodies to recombinant ML 65kDa proteins denoted mycobacterial presence.—Authors' Abstract

Microbiology

Bentrup, K. H. and Russell, D. G. Mycobacterial persistence: adaptation to a changing environment. Trends Microbiol. **9** (2001) 597–605.

Mycobacterium tuberculosis is a bacterial pathogen that can persist within an infected individual for extended periods of time without causing overt, clinical disease, in a state normally referred to as latent or chronic tuberculosis. Although the replicative state of the bacterium during this period is a matter of some conjecture, recent developments have indicated that the bacterium requires the regulated expression of a set of genes and metabolic pathways to maintain a persistent infection in an immunocompetent host. The characterization of these gene products and their role in bacterial metabolism and physiology is starting to provide insights into the mechanisms that *M. tuberculosis* has evolved to adopt its highly successful mode of pathogenicity.—Authors' Abstract

Bhakta, S. and Basun, J. Overexpression, purification and biochemical characterization of a class A high-molecular-mass penicillin-binding protein (PBP), PBP1* and its soluble derivative from *Mycobacterium tuberculosis.* Biochem. J. **361** (2002) 635–639.

The product of the gene ponA present in cosmid MTCY21D4, one of the collection of clones representing the genome of *Mycobacterium tuberculosis,* has been named penicillin-binding protein 1* (PBP1*), by analogy to the previously characterized PBP1* of *M. leprae.* This gene has been overexpressed in *Escherichia coli.* His(6)-tagged PBP1* localizes to the membranes of induced *E. coli* cells. Its susceptibility to degradation upon proteinase K digestion of spheroplasts from *E. coli* expressing the protein supports the view that the majority of the protein translocates to the periplasmic side of the membrane. Recombinant PBP1* binds benzylpenicillin and several other beta-lactams, notably cefotaxime, with high affinity. Truncation of the N-terminal 64 amino acid residues results in an expressed protein present exclusively in inclusion bodies and unable to associate with the membrane. The C-terminal module encompassing amino acids 272–663 can be extracted from inclusion bodies under denaturing conditions using guanidine/HCl and refolded to give a protein fully competent in penicillin-binding. Deletion of Gly(95)-Gln(143) results in the expression of a protein, which is localized in the cytosol. The soluble derivative of PBP1* binds benzylpenicillin with the same efficiency as the full-length protein. This is the first report of a soluble derivative of a class A high-molecular-mass PBP.—Authors' Abstract

Braunstein, M., Brown, A. M., Kurtz, S. and Jacobs, W. R. Jr. Two nonredundant SecA homologues function in mycobacteria. J. Bacteriol. **183** (2001) 6979–6990.

The proper extracytoplasmic localization of proteins is an important aspect of mycobacterial physiology and the pathogenesis of *Mycobacterium tuberculosis.* The protein export systems of mycobacteria have remained unexplored. The Sec-dependent protein export pathway has been well characterized in *Escherichia coli* and is responsible for transport across the cytoplasmic membrane of proteins containing signal sequences at their amino termini. SecA is a central component of this pathway, and it is highly conserved throughout bacteria. Here we report on an unusual property of mycobacterial protein export—the presence of two homologs of SecA (SecA1 and SecA2). Using an allelic-exchange strategy in *Mycobacterium smegmatis,* we demonstrate that secA1 is an essential gene. In contrast, SecA2 cari be deleted and is the first example of a nonessential SecA homolog. The essential nature of secA1, which is consistent with the conserved Sec pathway, leads us to believe that secA1 represents the equivalent of *E. coli* SecA. The results of a phenotypic analysis of a DeltasecA2 mutant

of *M. smegmatis* are presented here and also indicate a role for SecA2 in protein export. Based on our study, it appears that SecA2 can assist SecA1 in the export of some proteins via the Sec pathway. However, SecA2 is not the functional equivalent of SecA1. This finding, in combination with the fact that SecA2 is highly conserved throughout mycobacteria, suggests a second role for SecA2. The possibility exists that another role for SecA2 is to export a specific subset of proteins.—Authors' Abstract

Brennan, M. J., Delogu, G., Chen, Y., Bardarov, S., Kraiakov, J., Alavi, M. and Jacobs, W. R. Jr. Evidence that mycobacterial PE_PGRS proteins are cell surface constituents that influence interactions with other cells. Infect. Immun. **69** (2001) 7326–7333.

The elucidation of the genomic sequence of *Mycobacterium tuberculosis* revealed the presence of a novel multigene family designated PE/PE_PGRS that encodes numerous, highly related proteins of unknown function. In this study, we demonstrate that a transposon insertion in a PE_PGRS gene (1818(PE_PGRS)) found in *Mycobacterium bovis* BCG Pasteur, which is the BCG homolog of the *M. tuberculosis* H37Rv gene Rv1818c, introduces new phenotypic properties to this BCG strain. These properties include dispersed growth in liquid medium and reduced infection of macrophages. Complementation of the 1818(PE_PGRS)::Tn5367 mutant with the wild-type gene restores both aggregative growth (clumping) in liquid medium and reestablishes infectivity of macrophages to levels equivalent to those for the parent BCG strain. Western blot analysis using antisera raised against the 1818(PE_PGRS) protein shows that PE_PGRS proteins are found in cell lysates of BCG and *M. tuberculosis* H37Ra and in the cell wall fraction of *M. tuberculosis* H37Rv. Moreover, immunofluorescent labeling of mycobacteria indicates that certain PE_PGRS proteins are localized at the cell surface of BCG and *M. tuberculosis*. Together these results suggest that certain PE_PGRS proteins may be found at the surface of mycobacteria and influence both cell surface interactions among mycobacteria as well as the interactions of mycobacteria with macrophages—Authors' Abstract

Brennan, P. J. and Vissa, V. D. Genomic evidence for the retention of the essential mycobacterial cell wall in the otherwise defective *Mycobacterium leprae*. Lepr. Rev. **72** (2001) 415–428.

The obligate intracellularism of *Mycobacterium leprae* may be attributable to the effects of mutations in major metabolic areas due to a genome capable of encoding only about 1600 proteins. Yet cell wall biosynthesis capability remains relatively intact and comparisons with the genome of *Mycobacterium tuberculosis* provide insights into the genetic basis of a minimal mycobacterial cell wall.—Authors' Abstract

Cambau, E., Bonnafous, P., Perani, E., Sougakoff, W., Ji, B. and Jarlier, V. Molecular detection of rifampin and ofloxacin resistance for patients who experience relapse of multibacillary leprosy. Clin. Infect. Dis. **34** (2002) 39–45.

Molecular detection of rifampin resistance (rpoB analysis) in *Mycobacterium leprae* was determined for 49 patients who experienced relapse of multibacillary leprosy and for 34 untreated patients. Molecular detection of ofloxacin resistance (gyrA analysis) was determined for the 12 patients who experienced relapse and who had received ofloxacin. Results of molecular tests were compared with the reference susceptibility test in the mouse footpad. Overall, the efficiency of molecular detection—that is, positive DNA amplification—was 95%, whereas that of the *in vivo* test was 55% ($p < 0.001$). Results of molecular detection and *in vivo* test were fully concordant when both were available—that is, for 35 rifampin-sensitive cases of leprosy (no rpoB mutation), 4 ofloxacin-sensitive cases (no gyrA mutation), 11 rifampin-resistant cases (rpoB missense mutations), and 1 ofloxacin-resistant case (gyrA mutation). rpoB and gyrA analysis appears to be an ef-

fective method for detection of rifampin and ofloxacin resistance in patients with leprosy.—Authors' Abstract

Chen, C. K., Barrow, E. W., Allan, P. W., Bansal, N., Maddry, J.A., Suling, W. J., Barrow, W. W, and Parker, W. B. The metabolism of 2-methyladenosine in *Mycobacterium smegmatis.* Microbiology **148** (2002) 289–295.

2-Methyladenosine (methyl-ado) has demonstrated selective activity against *Mycobacterium tuberculosis,* which indicates that differences in the substrate preferences between mycobacterial and human purine metabolic enzymes can be exploited to develop novel drugs for the treatment of mycobacterial diseases. Therefore, in an effort to better understand the reasons for the antimycobacterial activity of methyl-ado, its metabolism has been characterized in *Mycobacterium smegmatis.* In a wild-type strain, methyl-ado was phosphorylated by adenosine kinase to methyl-AMP, which was further converted to methyl-ATP and incorporated into RNA. In contrast, a mutant strain of *M. smegmatis* was isolated that was resistant to methyl-ado, deficient in adenosine kinase activity and was not able to generate methyl-ado metabolites in cells treated with methyl-ado. These results indicated that phosphorylated metabolites of methyl-ado were responsible for the cytotoxic activity of this compound. Methyl-ado was not a substrate for either adenosine deaminase or purine-nucleoside phosphorylase from *M. smegmatis.* Treatment of *M. smegmatis* with methyl-ado resulted in the inhibition of ATP synthesis, which indicated that a metabolite of methyl-ado inhibited one of the enzymes involved in *de novo* purine synthesis. These studies demonstrated the importance of adenosine kinase in the activation of methyl-ado to toxic metabolites in *M. smegmatis.*—Authors' Abstract

Cole, S. T., Supply, P. and Honoré, N. Repetitive sequences in *Mycobacterium leprae* and their impact on genome plasticity. Lepr. Rev. **72** (2001) 449–461.

About 2% of the genome of *Mycobacterium leprae* is composed of repetitive DNA. There are more than 26 extinct IS elements together with four families of dispersed repeats, present in five copies or more, RLEP (37 copies), REPLEP (15 copies), LEPREP (eight copies), and LEPRPT (five copies). Although there is no sequence similarity to known transposable elements, RLEP occurs predominantly at the 3′-end of genes and, in several cases, within pseudogenes, suggesting that it was capable of dissemination. Strikingly, on comparison of the genome sequences of *M. leprae* and the closely related tubercle bacillus, *Mycobacterium tuberculosis* H37Rv, many of these repetitive sequences were found at sites of discontinuity in gene order. Evidence is presented that loss of synteny, inversion and genome downsizing may have resulted from recombination between dispersed copies of these repetitive elements.—Authors' Summary

Dawes, S. S. and Mizrahi, V. DNA metabolism in *Mycobacterium leprae.* Lepr. Rev. **72** (2001) 408–414.

Understanding the molecular basis underlying the hallmark features of *Mycobacterium leprae,* such as its parasitism and extraordinarily slow growth rate, has stimulated research into the biology of this pathogen for decades, but it is through the completion of its genome sequence that a quantum leap of progress has been achieved. In this review, we analyze the genes in *M. leprae* that are involved in the synthesis and salvage of purines and pyrimidines and in DNA replication and repair in an attempt to uncover the relationship between the massive gene decay observed in the *M. leprae* genome and its DNA metabolic capacity. This analysis has provided insights into possible mechanisms for the genomic deterioration in the leprosy bacillus and supplements the sparse biochemical data hard won from this organism.—Authors' Abstract

Eiglmeier, K., Parkhill, J., Honoré, N., Garnier, T., Tekaia, F., Telenti, A., Klastser, P., James, K. D., Thomson,

N. R., Wheeler, P. R., Churcher, C., Harris, D., Mungall, K., Barrell, B. G. and Cole, S. T. The decaying genome of *Mycobacterium leprae.* Lepr. Rev. **72** (2001) 387–398.

Everything that we need to know about *Mycobacterium leprae,* a close relative of the tubercle bacillus, is encrypted in its genome. Inspection of the 3.27 Mb genome sequence of an armadillo-derived Indian isolate of the leprosy bacillus identified 1605 genes encoding proteins and 50 genes for stable RNA species. Comparison with the genome sequence of *Mycobacterium tuberculosis* revealed an extreme case of reductive evolution, since less than half of the genome contains functional genes while inactivated or pseudogenes are highly abundant. The level of gene duplication was ~34% and, on classification of the proteins into families, the largest functional groups were found to be involved in the metabolism and modification of fatty acids and polyketides, transport of metabolites, cell envelope synthesis and gene regulation. Reductive evolution, gene decay and genome downsizing have eliminated entire metabolic pathways, together with their regulatory circuits and accessory functions, particularly those involved in catabolism. This may explain the unusually long generation time and account for our inability to culture the leprosy bacillus.—Authors' Abstract

Eiglmeier, K., Simon, S., Garnier, T. and Cole, S. T. The integrated genome map of *Mycobacterium leprae.* Lepr. Rev. **72** (2001) 462–469.

The integrated map of the *Mycobacterium leprae* genome unveiled for the first time the genomic organization of this obligate intracellular parasite. Selected cosmid clones, isolated from a genomic library created in the cosmid vector Lorist6, were identified as representing nearly the complete genome and were subsequently used in the *M. leprae* genome sequencing project. Now a new version of the integrated map of *M. leprae* can be presented, combining the mapping results from the Lorist6 cosmids with data obtained from a second genomic library constructed in an *Escherichia coli*-mycobacterium shuttle cosmid, pYUB18. More than 98% of the *M. leprae* genome is now covered by overlapping large insert genomic clones representing a renewable source of well defined DNA segments and a powerful tool for functional genomics.—Authors' Summary

Grosset, J. H. and Cole, S. T. Genomics and the chemotherapy of leprosy. Lepr. Rev. **72** (2001) 429–440.

The information deduced from the genome sequence of *Mycobacterium leprae* is of immense value for the chemotherapy of leprosy. Knowing the complete set of genes, enzymes and proteins allows us to understand why some drugs are without effect whereas others are fully active. It may also enable better use to be made of existing drugs, such as β-lactams, and opens new avenues for the development of novel compounds. *M. leprae* is relatively susceptible to a wide range of drugs, unlike the highly related tubercle bacillus, and several new multidrug regimens are in clinical trials. Genomics provides a number of possible explanations for this broader susceptibility as some of the genes encoding enzymes involved in antibiotic inactivation have decayed whereas the number of transporters available to contribute to drug efflux is considerably lower than in *Mycobacterium tuberculosis.* Several leads for new drug targets have been uncovered.—Authors' Abstract

Haque, A. K. and Kashiwabara, Y. Detection of *Mycobacterium leprae* by polymerase chain reaction. Bangladesh Med. Res. Counc. Bull. **26** (2000) 87–91.

The improved procedure based on polymerase chain reaction (PCR) for detection of *M. leprae* has been developed. The sensitivity and specificity of this method were tested using different concentrations of genomic DNA of *M. leprae* Thai 53 and genomic DNAs from mycobacterial species and related microorganisms respectively. Application of this method to biopsy samples obtained from Bangladesh was conducted and detected *M. leprae* DNA in 7 of

the 10 clinical specimens. Acid fast bacilli were not detected in four of the seven positive cases under the microscopic observation. It was concluded that this method was sensitive and specific for detection of *M. leprae* in clinical specimens and also simple to detect in only one step of PCR.—Authors' Abstract

Honoré, N., Roche, P. W., Grosset, J. H. and Cole, S. T. A method for rapid detection of rifampicin-resistant isolates of *Mycobacterium leprae.* Lepr. Rev. **72** (2001) 441–448.

A genotypic method for predicting rifampicin resistance in *Mycobacterium leprae* has been developed and rigorously tested on mouse footpad-derived and clinical specimens. A series of immobilized oligonucleotide capture probes can discriminate between wild type and mutant *rpoB* alleles, and positive controls are available for the most frequent mutation affecting Ser425. Two different non-radioactive detection formats have been tested with comparable success in both an industrialized and a developing country. The standardized procedure could now be used in a prospective study of potential rifampicin resistance among multibacillary patients.—Authors' Abstract

Jones, L., Moszer, I. and Cole, S. T. *Leproma:* a *Mycobacterium leprae* genome browser. Lepr. Rev. **72** (2001) 470–477.

Leproma is a powerful Web-based tool for extracting information about annotations from a *Mycobacterium leprae* genome database. The URL for the Leproma web site is http://genolist.pasteur.fr/Leproma.

With *Leproma,* the user may search the *M. leprae* genome database using several search criteria. One can search by gene name or synonym, by region in the genome, by gene function or classification, by DNA or protein patterns, by a BLAST or FASTA search in the DNA sequence or the protein sequences, or by free text.

Search results can be in the form of a list where the columns in the list are set by the user or in the form of a drawing if the search results in a region in the genome. The user can also download or view the DNA sequence or the protein sequence from a single gene or from the list of a search result.—Authors' Summary

Maeda, S., Matsuoka, M., Nakata, N., Kai, M., Maeda, Y., Hashimoto, K., Kimura, H., Kobayashi, K. and Kashiwabara, Y. Multidrug resistance *Mycobacterium leprae* from patients with leprosy. Antimicrob. Agents Chemother. **45** (2001) 3635–3639.

Sequences of the folP1, rpoB, and gyrA genes were analyzed for 88 isolates of *Mycobacterium leprae* from leprosy patients in Japan, Haiti, Indonesia, Pakistan, and the Philippines. Thirteen isolates (14.8%) showed representative mutations in more than two genes, suggesting the emergence of multidrug-resistant. *M. leprae.*—Authors' Abstract

Martin, E., Triccas, J. A., Kamath, A. T., Winter, M. and Britton, W. J. Comparative protective effects of recombinant DNA and *Mycobacterium bovis* bacilli Calmette-Guerin vaccines against *M. avium* infection. Clin. Exp. Immunol. **126** (2001) 482–487.

A range of strategies are being explored to develop more effective vaccines against mycobacterial infection, including immunization with DNA plasmids encoding single mycobacterial bacterial genes and the use of recombinant live vectors based on the current vaccine, *Mycobacterium bovis* bacille Calmette-Guerin (BCG). We have compared these two approaches using a model of virulent *M. avium* infection, and the gene for the immunodominant 35 kDa protein which is shared by *M. avium* and *M. leprae,* but absent from BCG. Recombinant BCG over-expressing the *M. avium* 35 kDa protein (BCG-35) induced strong antigen-specific proliferative and interferon-gamma (IFN-gamma)-secreting T cell responses. These were comparable to those induced by a single immunization with a plasmid expressing the same antigen (DNA-35); however, repeat DNA-35 immunization evoked

the strongest IFN-gamma release. Immunization with BCG-35 significantly reduced the growth of virulent *M. avium*, although this effect was similar to that induced by wild-type BCG. Immunization with DNA-35 resulted in significantly greater ($2 \times \log_{10}$ reduction in the growth of *M. avium*. Prime-boost strategies combining DNA-35 and BCG-35 increased the protective effect above that achieved by BCG-35, but they were not more protective than DNA-35 alone. Therefore, recombinant BCG-35 and BCG induced similar levels of protection in this model, and maximal protection against *M. avium* infection was attained by immunization with DNA encoding the 35 kDa protein.—Authors' Abstract

Spencer, J. S., Marques, M. A., Lima, M. C., Jungueira-Kipnis, A. P., Gregory, B. C., Truman, R. W. and Brennan, P. J. Antigenic specificity of the *Mycobacterium leprae* homologue of ESAT-6. Infect. Immun. **70** (2002) 1010–1013.

The sequence of the *Mycobacterium leprae* homolog of ESAT-6 shows only 36% amino acid correspondence to that from *Mycobacterium tuberculosis*. Anti-*M. leprae* ESAT-6 polyclonal and monoclonal antibodies and T-cell hybridomas reacted only-with the homologous protein and allowed identification of the B- and T-cell epitopes. The protein is expressed in *M. leprae* and appears in the cell wall fraction. Thus, *M. leprae* ESAT-6 shows promise as a specific diagnostic agent for leprosy.—Authors' Abstract

Wheeler, P. R. The microbial physiologist's guide to the leprosy genome. Lepr. Rev. **72** (2001) 399–407.

Obtaining a genome sequence should be regarded as a springboard to research on the microbe in question. I hope in this short review I have shown that this is a time to push forward with research into *M. leprae*, a paradigm of obligate intracellular host-dependency. Fundamental questions for an intracellular pathogen about iron metabolism can be addressed. How the loss of redundancy throughout the genome in comparison with the tubercle bacilli has resulted in a specialized pathogen in contrast to the adaptable *M. tuberculosis* complex is another basic issue in mycobacteriology. Does the apparent limitation in virulence determinants and cell entry genes (Table 1) commit *M. leprae* to gaining access only to its narrow niche? While two of the three hemolysin genes found in *M. tuberculosis*, including *tlyA*, persist in *M. leprae*, all four *plc* genes are lost. Will we find out the role of PPEs and PEs in tubercle bacilli by making comparisons with *M. leprae*, a 'natural mutant' for most of them? What else can we learn about tubercle bacilli by comparative genomics now we have the leprosy genome? Why cannot *M. leprae* be grown axenically; do the lesions in energy metabolism only allow interrupted growth when conditions are just right in the host? Are media too toxic, at least in aerobic conditions? With a massive loss of regulatory functions have those that would allow *M. leprae* to adapt to axenic culture been lost? We are now in a position to generate better defined hypotheses. Soon the *Mycobacterium ulcerans* genome will be sequenced; will comparisons with this difficult to grow mycobacterium help us to formulate new hypotheses for *M. leprae?* Finally, we can design experiments that will provide a better understanding of the interaction between *M. leprae* and Schwann cells. With neurological reactions still a major clinical issue in the treatment of leprosy these are urgent experiments. My conclusion would be to recommend the leprosy research community develops post-genomic research and investigates the expression of *M. leprae* genes as a means of addressing the many biological questions that still remain.—Author's Abstract

Epidemiology and Prevention

Souza, W. V., Barcellos, C. C., Brito, A. M., Carvalho, M. S., Cruz, O. G., Alburquerque Md. Mde. F., Alves, K. R. and Lapa, T. M. Empirical Bayesian model applied to the spatial analysis of leprosy occurrence. (In Portuguese) Rev. Saude Publica. **35** (2001) 474–480.

Objective: To analyze the spatial distribution of leprosy, identify areas of potential case underreporting or high transmission risk, and to assess the ecological association of leprosy distribution with multibacillary cases. Methods: This study was carried out in 94 neighborhoods of Recife, Brazil. Data was obtained from the Ministry of Health's Disease Reporting System. An ecological approach with the empirical Bayesian method was applied for local rate flattening, using data from a neighborhood matrix. Results: The mean annual occurrence was 17.3% of new cases in individuals under the age of 15 (28.3% corresponded to multibacillary forms), revealing an intense disease transmission. The spatial distribution of leprosy indicated three areas where there was a concentration of high detection rates and low-income neighborhoods. Conclusions: The Bayesian method allowed to reassess epidemiological indicators based on data from neighboring spatial units. This enabled to identify areas that should be prioritized in municipal control programs, either because of underreporting of cases or the higher number of occurrences related to multibacillary forms in individuals under 15.—Authors' Abstract

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

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

Rehabilitation

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

This paper describes the development of self-care groups in Ethiopia by ALERT, and the successes and failures experienced in the process. The groups were started in 1995 in response to two main problems, the increasing number of people dependent on ALERT to heal their wounds despite years of health education, and the limited financial resources of ALERT for wound healing sup-

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

Bharath, S., Shamasundar, C., Raghuram, R. and Subbakrishna, D. K. Correlates of psychiatric morbidity in patients with leprosy. Indian J. Lepr. **73** (2001) 217–228.

The relationship between psychiatric morbidity in 30 leprosy patients under treatment as assessed by the General Health Questionnaire (GHQ-12) and certain variables of their illness and psychosocial factors are examined in this paper. Physical disability and duration of illness were the illness variables considered; knowledge and adjustment were the psychosocial variables included. Bell's Adjustment Inventory (BAI) measured the latter. Psychiatric morbidity was positively correlated with physical disability ($p < 0.05$), knowledge about the disease ($p < 0.01$) and social, emotional and health maladjustment ($p < 0.01$), but not with duration of illness ($p > 0.05$). The importance of appropriate knowledge, social stigma and physical disability in leprosy is discussed in addressing the psychiatric morbidity of leprosy patients.—Authors' Abstract

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

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

Other Mycobacterial Diseases and Related Entities

Amaral, L., Viveiros, M. and Kristiansen, J. E. Phenothiazines: potential alternatives for the management of antibiotic resistant infections of tuberculosis and malaria in developing countries. Trop. Med. Int. Health **6** (2001) 1061–1022.

The *in vitro* and *in vivo* activity of phenothiazines against antibiotic susceptible and antibiotic resistant *Mycobacterium tuberculosis* and malaria-causing Plasmodia is reviewed. Given the facts that pulmonary tuberculosis and malaria are the major causes of death in developing countries, that both of these infections continue to escalate in their resistance to antibiotics, that the cost for the management of these infections is beyond that afforded by most developing nations, and lastly, that new and ef-

fective agents are not forthcoming from the pharmaceutical industry, the scientific rationale for the potential use of select phenothiazines for the management of these infections is presented.—Authors' Abstract

Amato, R. J. Thalidomide: an antineoplastic agent. Cur. Oncol. Rep. **4** (2002) 56–62.

It has been more than three decades since the withdrawal of thalidomide from the marketplace. Thalidomide is attracting growing interest because of its reported immunomodulatory and anti-inflammatory properties. Current evidence indicates that thalidomide reduces the activity of the inflammatory cytokine tumor necrosis factor-a by accelerating the degradation of its messenger RNA. Thalidomide inhibits angiogenesis. Recently, thalidomide was approved for sale in the United States for the treatment of erythema nodosum leprosum, an inflammatory complication of Hansen's disease. Thalidomide has been used successfully in several other dermatologic disorders, including aphthous stomatitis, Behcet's syndrome, chronic cutaneous systemic lupus erythematosus, and graft-versus-host disease, the apparent shared characteristic of which is immune dysregulation. Many recent studies have evaluated thalidomide in patients with HIV infection, in which this drug is an efficacious agent against oral aphthous ulcers, HIV-associated wasting syndrome, HIV-related diarrhea, and Kaposi's sarcoma. Only in the last several years has thalidomide been aggressively investigated for its antiangiogenic potential and immunomodulatory properties in various tumor types. Current research on thalidomide in oncology covers investigation in a wide range of both solid tumors and hematologic malignancies.—Author's Abstract

Ances, B. M. New concerns about thalidomide. Obstet. Gynecol. **99** (2002) 125–128.

Recently, the Food and Drug Administration (FDA) approved thalidomide for the treatment of the painful symptoms of erythema nodosum leprosum. This most recent FDA decision is a marked reversal to its previous rejection of this drug in the 1960s. The initial rejection by the FDA in the 1960s spared countless American children as thalidomide was shown to cause birth defects and miscarriages worldwide. The FDA's reputation as one of the finest consumer safety authorities was strengthened because of this decision. The recent approval of thalidomide by the FDA, with accompanying strict guidelines and monitoring procedures, has not only brought forth potential benefits, but also created new potential problems—Author's Abstract

Bohlson, S. S., Strasser, J. A., Bower, J. J. and Schorey, J. S. Role of complement in *Mycobacterium avium* pathogenesis: *in vivo* and *in vitro* analyses of the host response to infection in the absence of complement component C3. Infect. Immun. **69** (2001) 597–605.

We investigated the importance of the host complement system in the pathogenesis of disease mediated by the intramacrophage pathogen *Mycobacterium avium.* Mycobacteria opsonized with complement are efficiently ingested by macrophages through various complement receptors. Furthermore, unlike other bacteria, mycobacteria can activate both the alternative and classical complement pathways in the absence of specific antibodies. Therefore, to examine the role of complement in the mycobacterial infection process *in vivo,* mice deficient in complement component C3 were infected with *M. avium.* Surprisingly, C3-deficient mice infected intravenously with *M. avium* displayed no difference in bacterial burden or granulomatous response compared to wild-type control mice. C3-sufficient mice and C3-deficient mice were equally susceptible to infection by *M. avium* regardless of the genotype at the bcg locus, a locus known to confer susceptibility to infection with intracellular pathogens. *In vitro* studies using mouse bone marrow-derived macrophages resulted in significant *M. avium* invasion of macrophages in the absence of C3; however, the kinetics of infection were delayed compared to complement-mediated invasion. The data indicate that complement does not play an essential role in mediating *M. avium*

infections in the mouse and suggest either that other invasion mechanisms can compensate for the absence of complement-mediated entry or that complement is not a major mycobacterial opsonin *in vivo*.—Authors' Abstract

Borgdorff, M. W., Nagelkerke, N. J., de Haas, P. E. and van Soolingen, D. Transmission of *Mycobacterium tuberculosis* depending on the age and sex of source cases. Am. J. Epidemiol. **154** (2001) 934–943.

This study estimated to what extent tuberculosis transmission in the Netherlands depends on the age and sex of source cases. DNA fingerprints of *Mycobacterium tuberculosis* isolates were matched to patient information in the Netherlands Tuberculosis Register for 1993–1998. Clusters were defined as groups of patients with pulmonary tuberculosis whose isolates had identical DNA fingerprints. Source cases were assigned by using two models. The first-case model assumed that the first diagnosed case was the source case. The incidence rate model estimated source case probabilities from the incidence rates of potential source cases and the time of diagnosis. DNA fingerprints of 6102 isolates were matched to patient information on 5080 (83%) cases, 3479 of whom had pulmonary disease. According to both models, the number of infectious cases generated per source case was lower for female than for male source cases and decreased with increasing age of the source case. The authors concluded that transmission of tuberculosis is associated with the age and sex of source cases as well as the age of secondary cases. Increased transmission among immigrant groups in the Netherlands is largely attributable to the relatively young age of immigrant source cases.—Authors' Abstract

Bryceson, A. A policy for leishmaniasis with respect to the prevention and control of drug resistance. Trop. Med. **6** (2001) 928–934.

At the moment no country has a policy designed to control or prevent drug resistance in leishmaniasis. The risk of resistance is high in areas of anthroponotic visceral leishmaniasis, for example North Bihar, India, where the rate in some areas is 60%. Post-epidemic Sudan is also at risk. Zoonotic areas in which HIV co-infection is common could also be at risk as sandflies can become infected from co-infected individuals. Many factors determine the choice of drug for the treatment of visceral leishmaniasis, and drug resistance may not be the over-riding priority. In anthroponotic areas reduction in transmission through public health measures will be important, but the use of two drugs in combination should be seriously considered. Pharmacokinetic and other features of the drugs available, relevant to their use in combination are discussed and tentative suggestions made concerning trials of possible combinations. These include miltefosine plus paromomycin and allopurinol plus an azole. Lessons may be learned from the experiences of similar problems in malaria, leprosy and tuberculosis. Guidelines are offered for the introduction of policies to use drugs in combination, which differ between anthroponotic and zoonotic areas of transmission.—Author's Abstract

Bryk, R., Lima, C. D., Erdjument-Bromage, H., Tempst, P. and Nathan, C. Metabolic enzymes of mycobacteria linked to antioxidant defense by a thioredoxin-like protein. Science **295** (2002) 1073–1077.

Mycobacterium tuberculosis (Mtb) mounts a stubborn defense against oxidative and nitrosative components of the immune response. Dihydrolipoamide dehydrogenase (Lpd) and dihydrolipoamide succinyltransferase (SucB) are components of alpha-ketoacid dehydrogenase complexes central to intermediary metabolism. We find that Lpd and SucB support Mtb's antioxidant defense. The peroxiredoxin alkyl hydroperoxide reductase (AhpC) is linked to Lpd and SucB by an adaptor protein, AhpD. The 2.0 A AhpD crystal structure reveals a thioredoxin-like active site that is responsive to lipoamide. We propose that Lpd, SucB (the only lipoyl protein detected in Mtb), AhpD, and AhpC together comprise an NADH-dependent peroxidase and peroxynitrite re-

ductase. AhpD represents a new class of thioredoxin-like molecules that enables a novel antioxidant defense.—Authors' Abstract

Cadapan, L. D., Arslanian, R. L., Carney, J. R., Zavala, S. M., Small, P. L. and Licari, P. Suspension cultivation of *Mycobacterium ulcerans* for the production of mycolactones. FEMS Microbiol. Lett. **205** (2001) 385–389.

Mycolactones are polyketide toxins produced by *Mycobacterium ulcerans,* the causative agent of the tropical skin disease known as Buruli ulcer. Development of novel therapeutic agents from mycolactones has been hindered by the difficulty of producing sufficient amounts of material. Here, we describe the successful adaptation of *M. ulcerans* to suspension cultivation and the development of a fed-batch fermentation process that was scaled up to 150 l. In addition to producing mycolactones A and B, a number of new mycolactone-related compounds were also observed.—Authors' Abstract

Cantrell, C. L., Franzblau, S. G. and Fischer, N. H. Antimycobacterial plant terpenoids. Planta med. **67** (2001) 685–694.

Tuberculosis (TB), mainly caused by *Mycobacterium tuberculosis,* is the leading killer among all infectious diseases worldwide and is responsible for more than two million deaths annually. For over thirty years no antitubercular agents with new mechanisms of action have been developed. The recent increase in the number of multidrug resistant clinical isolates of *M. tuberculosis* has created an urgent need for the discovery and development of new antituberculosis leads. This review covers recent reports on plant-derived terpenoids that have demonstrated moderate to high activity in *in vitro* bioassays against *M. tuberculosis.* In this review, mono-, sesqui-, di- and triterpenes, and sterols, their structural analogs and semisynthetic derivatives will be discussed, with particular emphasis on the structural features essential for antimycobacterial activity.—Authors' Abstract

Cappelli, G., Volpe, P., Sanduzzi, A., Sacchi, A., Colizzi, V. and Mariani, F. Human macrophage gamma interferon decreases gene expression but not replication of *Mycobacterium tuberculosis:* analysis of the shot-pathogen reciprocal influence on transcription in a comparison of strains H37Rv and CMT97. Infect. Immun. **69** (2001) 7262–7270.

Mycobacterium tuberculosis is an intracellular pathogen that readily survives and replicates in human macrophages (Mϕ). Host cells have developed different mycobactericidal mechanisms, including the production of inflammatory cytokines. The aim of this study was to compare the Mϕ response, in terms of cytokine gene expression, to infection with the *M. tuberculosis* laboratory strain H37Rv and the clinical *M. tuberculosis* isolate CMT97. Both strains induce the production of interleukin-12 (IL-12) and IL-16 at comparable levels. However, the clinical isolate induces a significantly higher and more prolonged Mϕ activation, as shown by reverse transcription-PCR analysis of IL-1beta, IL-6, IL-10, transforming growth factor beta, tumor necrosis factor alpha, and gamma interferon (IFN-gamma) transcripts. Interestingly, when IFN-gamma transcription is high, the number of *M. tuberculosis* genes expressed decreases and vice versa, whereas no mycobactericidal effect was observed in terms of bacterial growth. Expression of 11 genes was also studied in the two *M. tuberculosis* strains by infecting resting or activated Mϕ and compared to bacterial intracellular survival. In both cases, a peculiar inverse correlation between expression of these genes and multiplication was observed. The number and type of genes expressed by the two strains differed significantly.—Authors' Abstract

D'Amato, R. J., Lentzsch, S., Anderson, K. C. and Rogers, M. S. Mechanism of action of thalidomide and 3-aminothalidomide in multiple myeloma. Semin. Oncol. **28** (2001) 597–601.

We have explored the mechanism of the antiangiogenic effects of thalidomide by structure-activity studies. These investiga-

tions revealed that angiogenesis inhibition correlates with teratogenicity but not with tumor necrosis factor-alpha (TFA-alpha) inhibition. Additionally, one analog of thalidomide, 3-aminothalidomide, exhibited an unusual capacity to directly inhibit myeloma cell proliferation. This activity did not correlate with TNF-alpha inhibition. Thus 3-aminothalidomide was found to inhibit multiple myeloma through effects on both the tumor and vascular compartment. The effects of an inhibitor of both the tumor and vascular compartments of a tumor on tumor growth may be synergistic.—Authors' Abstract

Davies, P. and Grange, J. The genetics of host resistance and susceptibility to tuberculosis. Ann. N.Y. Acad. Sci. **953** (2001) 151–156.

The study of human genomics has the potential to aid our understanding of the interindividual and interpopulation differences in susceptibility to tuberculosis. Resistance to infection is affected by the ability of macrophages to phagocytose and destroy the bacilli. Several genes are involved in this process, and two have been the focus of recent interest: the natural resistance-associated protein (NRAMP1) gene and the genes coding for the vitamin D receptor. Susceptibility genes have also been discovered—for example, one on the X chromosome that may explain the increased susceptibility of males to tuberculosis. Studies have also focused on the variations in virulence of the bacillus in both its drug-susceptible and drug-resistant forms. These mechanisms must be understood in order to prevent, or combat, the emergence of a virulent, multidrug-resistant form of the bacillus that would be uncontrollable by means of today's treatment strategies—Authors' Abstract

Delogu, G., Li, A., Repique, C., Collins, F. and Morris, S. L. DNA vaccine combinations expressing either tissue plasminogen activator signal sequence fusion proteins or ubiquitin-conjugated antigens induce sustained protective immunity in a mouse model of pulmonary tuberculosis. Infect. Immun. **70** 292–302.

DNA vaccination has emerged as a powerful approach in the search for a more efficacious vaccine against tuberculosis. In this study, we evaluated the effectiveness of immunizing with combinations of 10 different tuberculosis DNA vaccines that expressed mycobacterial proteins fused at the N terminus to eukaryotic intracellular targeting sequences. In one vaccine combination, the genes were fused to the tissue plasminogen activator signal sequence (TPA), while in a second combination the same 10 genes were expressed as ubiquitin (Ub)-conjugated proteins. In *ex vivo* studies in which the secretion of gamma interferon was measured, cellular immune responses were detected in mice vaccinated with either the TPA DNA vaccine combination or the Ub DNA vaccine combination at 7 and 14 days following a low-dose *Mycobacterium tuberculosis* challenge. Moreover, mice vaccinated with the TPA combination, the Ub combination, and *Mycobacterium bovis* BCG were able to limit the growth of tubercle bacilli in the lung and spleen after a virulent tuberculous aerosol challenge. Histopathological analyses also showed that mice immunized with the DNA vaccine combinations had substantially improved postinfection lung pathology relative to the naive controls. Finally, in three different tong-term experiments, the survival periods following aerogenic challenge were extended as much as sevenfold for vaccinated mice compared to naive controls. Interestingly, in all three experiments, no significant differences were detected in the mean times to death for mice immunized with the TPA combination or the Ub combination relative to the BCG controls. In conclusion, these studies demonstrate the effectiveness of immunization with DNA vaccine combinations against tuberculosis and suggest that further testing of these plasmid cocktails is warranted.—Authors' Abstract

Dutta, T. K., Goci, A., Chotekar, L. H., Hamide, A., Badhe, B. A. and Basu, D. Dapsone in treatment of chronic idiopathic thrombocytopenic purpura in adults. J. Assoc. Physicians India **49** (2001) 421–425.

Background: When a patient is steroid-dependent, a currently available strategy in chronic idiopathic thrombocytopenic purpura (ITP) is to follow a trial and error approach with any of the known drugs which has been found effective in the condition. Objective: To evaluate the response of chronic ITP to dapsone, an inexpensive drug now reported to be effective in the disease. Design: A controlled trial of abstinence and rechallenge type. Subjects: Eight subjects with chronic ITP. Interventions: Phase I—Intake of 100 mg of dapsone daily until response (in form of rise of platelet count in blood), Phase II—Above followed by drug abstinence, minimum for four weeks, and then rechallenge with the drug. Main outcome measures: Platelet counts during various phases viz during drug intake, withdrawal and rechallenge. Results: Four (50%) patients responded to treatment. The mean pre-dapsone and post-dapsone platelet counts of blood were 29.6 $\times 10^9$/l and 142.5 $\times 10^9$/l respectively during the first phase of trial. The rechallenge was done in five patients following withdrawal of drug and the mean values of platelet count before and after rechallenge were 32.2 $\times 10^9$/l and 83 $\times 10^9$/l respectively. There was a remarkable response in two patients; one is now off the drug and the other on a maintenance dose of 50 mg of dapsone daily. Conclusion: Dapsone caused significant rise of platelet count in some patients of chronic ITP. It can be tried as an alternative to other second-line drugs in chronic ITP.—Authors' Abstract

Frances, C., El Khoury, S., Gompel, A., Becherel, P. A., Chosidow, O. and Piette, J. C. Transient secondary amenorrhea in women treated by thalidomide. Eur. J. Dermatol. **12** (2002) 63–65.

Compared with other side effects induced by thalidomide, amenorrhea has been relatively poorly investigated although it was first reported in 1989. The aim of this study was to determine the prevalence of amenorrhea in consecutive women treated with thalidomide in our institution from 1995 to 1999. During that period, 21 women received thalidomide associated with progestin contraception. Their clinical and biological data were retrospectively reviewed. Data concerning their genital life were systematically checked by phone with a simple questionnaire. Transient secondary amenorrhea occurred in 5 women (24%) treated for refractory cutaneous lupus erythematosus (4 cases) or complex aphthosis (1 case). The link between thalidomide and amenorrhea was suggested by the resumption of menses 2 to 3 months after drug withdrawal, recurrence of amenorrhea after reintroduction of thalidomide (1 case) and high serum levels of pituitary gonadotrophins. Amenorrhea secondary to ovarian failure is frequently observed in women treated with thalidomide. Its precise mechanism remains to be elucidated—Authors' Abstract

Fujita, J., Mestre, J. R., Zeldis, J. B., Subbaramaiah, K. and Dannenberg, A. J. Thalidomide and its analogues inhibit lipopolysaccharide-mediated induction of cyclooxygenase-2. Clin. Cancer Res. **7** (2001) 3349–3355.

We investigated the effect of thalidomide, a compound with immunomodulatory and antiangiogenic properties, on lipopolysaccharide (LPS)-mediated induction of cyclooxygenase-2 (Cox-2) and prostaglandin (PG) biosynthesis in murine macrophages. Thalidomide caused a dose-dependent inhibition of LPS-mediated induction of PGE(2) synthesis in RAW 264.7 cells. The induction of Cox-2 protein and mRNA by LPS was also suppressed by thalidomide. Based on the results of nuclear run-off assays and transient transfections, treatment with LPS stimulated Cox-2 transcription, an effect that was unaffected by thalidomide. Thalidomide decreased the stability of Cox-2 mRNA. A series of structural analogs of thalidomide also inhibited LPS-mediated induction of Cox-2 and PGE(2) synthesis. Taken together, these data provide new insights into the antineoplastic and anti-inflammatory properties of thalidomide.—Authors' Abstract

Juffermans, N. P., Leemans, J. C., Florquin, S., Verbon, A., Kolk, A. H., Speelman, P., van Deventer, S. J. and van der Poll, T. CpG oligodeoxynu-

cleotides enhance host defense during murine tuberculosis. Infect. Immun. **70** (2002) 147–152.

Oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs activate immune cells to produce cytokines. CpG ODNs protect mice against infections with intracellular bacteria by the induction of a T helper 1 (Th1) response. To determine the effect of CpG ODNs in pulmonary tuberculosis, mice were treated with CpG ODNs or control ODNs at the time of intranasal infection. CpG ODNs reduced mycobacterial outgrowth for up to 5 weeks after *Mycobacterium tuberculosis* infection and were associated with a decrease in inflammation in lung tissue. CpG treatment was also associated with elevated levels of gamma interferon (IFN-gamma) and decreased levels of interleukin 4 in the lungs and an increased capacity of splenocytes to secrete Th1-type cytokines. CpG ODNs given 2 weeks after infection were still able to reduce mycobacterial outgrowth and to enhance a Th1 response 5 weeks postinfection. Administration of CpG ODNs to IFN-gamma-gene-deficient mice failed to reduce mycobacterial outgrowth. These data suggest that CpG ODNs improve host defense during pulmonary tuberculosis by an IFN-gamma-dependent mechanism.—Authors' Abstract

Klein, M. R. and Fox, A. Mycobacterium-specific human CD8 T cell responses. Arch. Immunol. Ther. Exp. **49** (2001) 379–389.

Tuberculosis (TB) remains a global health problem. There is an intense effort to identify correlates of protective immunity and to design new TB vaccines. CD8 T cells are thought to play a significant role in controlling *Mycobacterium tuberculosis* infection. Relatively little has been published about the antigens and epitopes targeted by mycobacteria-specific CD8 T cells. Here we present an update of our 1999 overview of human CD8 T cell epitopes in mycobacterial antigens and discuss related issues relevant to TB diagnosis and vaccine development.—Authors' Abstract

Lounis, N., Bentoucha, A., Truffot-Pernot, C., Ji, B., O'Brien, R. J., Vernon, A., Roscigno, G. and Grosset, J. Effectiveness of once-weekly rifapentine and moxifloxacin regimens against *Mycobacterium tuberculosis* in mice. Antimicrob. Agents Chemother. **45** (2001) 3482–3486.

Mice infected with 1.6×10^7 CFU of *Mycobacterium tuberculosis* were treated 14 days later for 6 months with a regimen of once-weekly 10 mg of rifapentine and 75 mg of isoniazid per kg of body weight supplemented with either 150 mg of streptomycin per kg or 100 mg of moxifloxacin per kg during either both the 2-week daily initial and once-weekly continuation phases or only in the daily 2-week initial phase. On completion of treatment, all lung cultures were negative, except for three mice, each with a single colony: two whose rifapentine-isoniazid regimen was supplemented with streptomycin during the whole course of therapy and one whose rifapentine-isoniazid regimen had no initial daily phase, but was supplemented with streptomycin and moxifloxacin during the whole course of therapy. After 3 months of follow-up, positive lung cultures were obtained from 61 and 56% of mice supplemented with streptomycin during either the full course of therapy or only the daily 2-week initial phase, respectively, and 15 and 50% of mice supplemented with moxifloxacin during either the full course of therapy or only the daily 2-week initial phase, respectively. These results suggest that moxifloxacin has sterilizing activity against *M. tuberculosis.* —Authors' Abstract

Liu, S., Zheng, M., Li, Z. *et al.* The protective effects of thalidomide on acute liver failure. Zhonghua Nei Ke Za Zhi **38** (1999) 688–690 (in Chinese).

Objective: To understand whether thalidomide (Thal) has the same protective effects as it does in leprosy reaction type II, immunologic and inflammatory diseases. Methods: D-Galactosamine (D-GalN, 600 mg/kg body weight, intraperitoneally) and lipopolysaccharide (LPS, 5 μg/rat, hypodermically) were used to produce a model of

acute liver failure in Wistar rats. The mortality of the models was counted. The plasma peak levels of various cytokines and transaminases were measured. The pathological alterations of the livers were examined with hematoxylin and eosin stain under light microscopy. The expression of tumor necrosis factor (TNF)alpha mRNA was determined by dot blot hybridization combined with semiquantitative analysis. Results: It was found that Thal could lower the mortality of the models ($p < 0.05$); reduce the peak levels of plasma cytokines, especially TNFalpha, and transminases $p < 0.05$), alleviate the degree of liver injury and suppress the expression of TNFalpha mRNA ($p < 0.05$). Conclusion: It is fully demonstrated that Thal has definite protective effect on acute liver failure, as it does in other diseases. Even though Thal possesses teratogenic effect in patients with pregnancy, it is still believed that Thal has an important curative significance in the treatment of patients who are not pregnant.—Authors' Abstract

Marriott, J. B., Muller, G., Stirling, D., and Dalgleish, A. G. Immunotherapeutic and antitumour potential of thalidomide analogues. Expert. Opin. Biol. Ther. **1** (2001) 675–682.

The immunomodulatory drug thalidomide has been shown to be clinically useful in a number of conditions including various immunological disorders and cancers. Clinical activity *in vivo* is attributed to the wide ranging immunological and non-immunological properties possessed by this drug; these include anti-TNF-alpha, T-cell co-stimulatory, anti-angiogenic activities and also direct antitumor activity. Recently, the design of compounds based on the thalidomide structure has led to the synthesis of analogs with greatly enhanced immunological activity and with similarly decreased toxicity. These derivatives faLl into at least two categories; selective cytokine inhibitory drugs (SelCID), which are phosphodiesterase Type 4 (PDE4) inhibitors and immunomodulatory drugs (IMiD), similar to thalidomide which act via unknown mechanism(s). These compounds are in the process of being characterized in laboratory studies and are also now being assessed in Phase I and Phase I/II clinical studies. In this review we will highlight the properties of these two novel classes of compound in terms of their effects on both immunological and non-immunological systems *in vitro*. We will also describe how these studies are enabling the characterization and development of these compounds into clinically relevant drugs in widely varying diseases. To this end we will describe the various clinical studies of lead compounds that are in progress and speculate as to the potential and future development of these exciting compounds.—Authors' Abstract

Miller, B. H. and Shinnick, T. M. Identification of two *Mycobacterium tuberculosis* H37Rv ORFs involved in resistance to killing by human macrophages. BMC Microbiol. **1** (2001) 26.

Background: The ability of *Mycobacterium tuberculosis* to survive and replicate in macrophages is crucial for the mycobacterium's ability to infect the host and cause tuberculosis. To identify *Mycobacterium tuberculosis* genes involved in survival in macrophages, a library of non-pathogenic *Mycobacterium smegmatis* bacteria, each carrying an individual integrated cosmid containing *M. tuberculosis* H37Rv genomic DNA, was passed through THP-1 human macrophages three times. Results: Two of the clones recovered from this enrichment process, sur2 and sur3, exhibited significantly increased survival relative to wild-type bacteria. In coinfection experiments, the ratio of sur2 colonies to wild-type colonies was 1:1 at 0 hours but increased to 20:1 at 24 hours post phagocytosis. The ratio of sur3 colonies to wild-type colonies was 1:1 at 0 hours and 5:1 at 24 hours. The *M. tuberculosis* ORFs responsible for increased survival were shown to be Rv0365c for the sur2 clone and Rv2235 for the sur3 clone. These ORFs encode proteins with as-of-yet unknown functions. Conclusions: We identified two *M. tuberculosis* ORFs which may be involved in the ability of tubercle bacilli to survive in macrophages.—Authors' Abstract

Mueller-Ortiz, S. L., Wanger, A. R and Norris, S. J. Mycobacterial protein HbhA binds human complement component C3. Infect. Immun. **69** (2001) 7501–7511.

Mycobacterium tuberculosis and *Mycobacterium avium* are facultative intracellular pathogens that are able to survive and replicate in mononuclear phagocytes. Human complement component C3 has previously been shown to mediate attachment and phagocytosis of these bacteria by mononuclear phagocytes. In this study, a C3 ligand affinity blot protocol was used to identify a 30-kDa C3-binding protein in *M. tuberculosis* and *Mycobacterium smegmatis* and a 31-kDa C3-binding protein in *M. avium.* The C3-binding proteins in *M. tuberculosis* and *M. avium* localized to the cell membrane fraction and partitioned to the detergent fraction during Triton X-114 phase partitioning. The C3-binding protein from *M. tuberculosis* was partially purified using a cation exchange column and was shown to bind concanavalin A. The N terminus and an internal fragment of the partially purified C3-binding protein were subjected to amino acid sequence analysis. The resulting amino acid sequences matched the *M. tuberculosis* heparin-binding hemagglutinin (HbhA) protein. Recombinant full-length HbhA and the C terminus of HbhA fused to maltose-binding protein, but not recombinant HbhA lacking the C-terminal region, bound human C3. Recombinant full-length HbhA coated on polystyrene beads, was found to enhance the adherence and/or phagocytosis of the coated beads to J774.A1 cells in both the presence and absence of human serum. The presence of complement-sufficient serum increased the adherence of the HbhA-coated beads to the J774.A1 cells in a C3-dependent manner. If HbhA within the bacterial cell membrane functions similarly to isolated HbhA, this protein may enhance the adherence and phagocytosis of *M. tuberculosis* and *M. avium* to mononuclear phagocytes through the binding of C3 and interaction with C3 receptors on mononuclear phagocytes.—Authors' Abstract

Price, D. K., Ando, Y., Druger, E. A., Weiss, M. and Figg, W. D. 5:-OH-Thalidomide, a metabolite of thalidomide, inhibits angiogenesis. Ther. Drug Monit. **24** (2002) 104–110.

Despite its known teratogenic effects, thalidomide has been used to treat a variety of diseases ranging from alleviation of autoimmune disorders to prevention of metastasis of cancers. The exact method of action of thalidomide and its derivatives is still under investigation. Thalidomide undergoes very little metabolism by the cytochrome P 450 system *in vitro,* but at least two hydroxylated metabolites have been found in humans. The two metabolites are 5-hydroxythalidomide, formed by hydroxylation of the phthalimide ring, possibly via arene oxides, and 5;-hydroxythalidomide, formed by hydroxylation of the glutarimide ring, leading to diastereomeric products. These two metabolites, along with another minor metabolite of thalidomide, were tested in a rat aortic ring assay, a human saphenous vein model, and a tube formation assay to assess the metabolite's ability to inhibit angiogenesis. Of the metabolites tested, only 5;-OH-thalidomide showed biologic activity in the rat aortic ring assay, and none of the metabolites showed activity in the human model. The studies with thalidomide and thalidomide metabolites underline the difficulty and complexity of trying to isolate and evaluate a single biologically active agent. These studies, however, do suggest that at least one metabolite, 5;-OH-thalidomide, has moderate antiangiogenic activity at high concentrations. Unfortunately, because of the lack of observed activity of 5;-OH-thalidomide in the human saphenous vein assay, it remains unclear whether there is species specificity for the activity of this metabolite.—Authors' Abstract

Ramachandra, L., Noss, E., Boom, W. H. and Harding, C. V. Processing of *Mycobacterium tuberculosis* antigen 85B involves intraphagosomal formation of peptide-major histocompatibility complex II complexes and is inhibited by live bacilli that decrease phagosome maturation. J. Exp. Med. **194** (2001) 1421–1432.

Mycobacterium tuberculosis (MTB) inhibits phagosomal maturation to promote

its survival inside macrophages. Control of MTB infection requires CD4 T cell responses and major histocompatibility complex (MHC) class II (MHC-II) processing of MTB antigens (Ags). To investigate phagosomal processing of MTB Ags, phagosomes containing heat-killed (HK) or live MTB were purified from interferon-gamma (IFN-gamma)-activated macrophages by differential centrifugation and Percoll density gradient subcellular fractionation. Flow organellometry and Western blot analysis showed that MTB phagosomes acquired lysosome-associated membrane protein-1 (LAMP-1), MHC-II, and H2-DM. T hybridoma cells were used to detect MTB Ag 85B(241–256)-I-A(b) complexes in isolated phagosomes and other subcellular fractions. These complexes appeared initially (within 20 min) in phagosomes and subsequently (>20 min) on the plasma membrane, but never within late endocytic compartments. Macrophages processed HK MTB more rapidly and efficiently than live MTB; phagosomes containing live MTB expressed fewer Ag 85B(241–256)-I-A(b) complexes than phagosomes containing HK MTB. This is the first study of bacterial Ag processing to directly show that peptide-MHC-II complexes are formed within phagosomes and not after export of bacterial Ags from phagosomes to endocytic Ag processing compartments. Live MTB can alter phagosome maturation and decrease MHC-II Ag processing, providing a mechanism for MTB to evade immune surveillance and enhance its survival within the host.—Authors' Abstract

Sonnenberg, P., Murray, J., Glynn, J. R., Shearer, S., Kambashi, B. and Godfrey-Faussett, P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet **358** (2001) 1687–1693.

Background: The proportion of recurrent tuberculosis cases attributable to relapse or reinfection and the risk factors associated with these different mechanisms are poorly understood. We followed up a cohort of 326 South African mineworkers, who had successfully completed treatment for pulmonary tuberculosis in 1995, to determine the rate and mechanisms of recurrence. Methods: Patients were examined 3 and 6 months after cure, and then were monitored by the routine tuberculosis surveillance system until December, 1998. IS6110 DNA fingerprints from initial and subsequent episodes of tuberculosis were compared to determine whether recurrence was due to relapse or reinfection. All patients gave consent for HIV-1 testing. Findings: During follow-up (median 25.1 months, IQR 13.2–33.4), 65 patients (20%) had a recurrent episode of tuberculosis, a recurrence rate of 10.3 episodes per 100 person-years at risk (PYAR)-16.0 per 100 pyar in HIV-1-positive patients and 6.4 per 100 pyar in HIV-1-negative patients. Paired DNA fingerprints were available in 39 of 65 recurrences: 25 pairs were identical (relapse) and 14 were different (reinfection). 93% (13/14) of recurrences within the first 6 months were attributable to relapse compared with 48% (12/25) of later recurrences. HIV-1 infection was a risk factor for recurrence (hazard ratio 2.4, 95% CI 1.5–4.0), due to its strong association with disease caused by reinfection (18.7 2.4–143), but not relapse (0.58; 0.24–1.4). Residual cavitation and increasing years of employment at the mine were risk factors for relapse. Interpretation: In a setting with a high risk of tuberculous infection, HIV-1 increases the risk of recurrent tuberculosis because of an increased risk of reinfection. Interventions to prevent recurrent disease, such as lifelong chemoprophylaxis in HIV-1-positive tuberculosis patients, should be further assessed—Authors' Abstract

Tansuphasiri, U. Detection of *Mycobacterium tuberculosis* from sputum collected on filter paper and stored at room temperature for 5 days by PCR assay and culture. J. Med. Assoc. Thai **84** (2001) 1183–1189.

The efficacy of PCR assay and culture for direct detection of *M. tuberculosis* (MTB) from sputum specimens collected on filter paper and stored at room temperature for 5

days was evaluated in comparison with conventional culture of fresh sputum specimen. A total of 231 sputum specimens were examined. MTB was recovered from 124 samples by culture from fresh sputum samples before storage. The culture positivity rate was significantly decreased to 70 per cent after 5 day's storage on filter paper. For PCR assay, a fragment of 377-bp of the IS6110 sequence was amplified and detected using nested PCR. Compared with culture results performed on fresh sputum samples, the sensitivity, specificity, and efficiency for the nested PCR were 96.0, 97.2 and 96.5 per cent, respectively. The nested PCR showed sensitivity and specificity with no significant difference ($p > 0.05$) from culture of fresh sputum specimens. Conclusion: The collection and storage of sputum on filter paper at room temperature for 5 days had no apparent effect on the performance of nested PCR. Sputum samples collected by this method could be sent by post in a minimum of space and inexpensive way and will enable a large number of samples collected in the field or from peripheral health centers to be sent to central laboratories for analysis by trained technicians and under a well-equipped and well-established quality control system. The rapid and reliable detection by PCR-based assay will be helpful for optimal patient management of therapy and effective control of tuberculosis.—Author's Abstract

Terry, S., Timothy, N. H., Zurlo, J. J. and Manders, E. K. *Mycobacterium chelonae:* nonhealing leg ulcers treated successfully with an oral antibiotic. J. Am. Board Fam. Pract. **14** (2001) 457–461.

Background: *Mycobacterium chelonae* is an important human pathogen and should be considered when a physician is faced with nonhealing cutaneous wounds, including ulcers of the lower leg. Methods: The medical literature was searched from 1965 to the present using the key words "*Mycobacterium chelonae*" and "leg ulcers." A case of *Mycobacterium chelonae* infection is reported. Results and Conclusion: Clarithromycin as single-agent oral therapy has been effective in treating these infections once the proper diagnosis is established. Diagnosis of *M. chelonae* infection requires being alert to this infectious agent and obtaining cultures for mycobacteria. Aggressive surgical debridement with direct excision of the wound might now be unnecessary because of the effectiveness of oral clarithromycin administered as a single oral agent.—Authors' Abstract

van Crevel, R., Nelwan, R. H., de Lenne, W., Veeraragu, Y., van Der Zanden, A. G., Amin, Z., van Der Meer, J. W. and van Soolingen, D. *Mycobacterium tuberculosis* Beijing genotype strains associated with febrile response to treatment. Emerg. Infect. Dis. **7** (2001) 880–883.

DNA fingerprinting has demonstrated predominance of the Beijing genotype among *Mycobacterium tuberculosis* strains isolated in Southeast Asia. We prospectively examined the occurrence of Beijing genotype strains in tuberculosis patients in Indonesia. Early in treatment, patients infected with Beijing genotype strains more often had fever unrelated to disease severity, toxicity, or drug resistance, indicating that Beijing genotype strains may have specific pathogenic properties.—Authors' Abstract

Velmulapalli, R. K., Cantey, J. R., Steed, L. L., Knapp, T. L. and Thielman, N. M. Emergence of resistance to clarithromycin during treatment of disseminated cutaneous *Mycobacterium chelonae* infection: case report and literature review. J. Infect. **43** (2001) 163–168.

Results of *in vitro* susceptibility studies and one clinical trial have led to recommendations of clarithromycin monotherapy for the treatment of disseminated cutaneous *Mycobacterium chelonae* infections. We describe the case of a 65-year-old woman, immunocompromised by the use of chronic steroid therapy, who developed disseminated cutaneous infection with *M. chelonae* and failed clarithromycin monotherapy due to the development of drug resistance. In the relapse isolate we document the presence of a single point mutation at position 2058 in the gene coding for 23S rRNA pep-

tidyltransferase regions, a mutation previously implicated in the development of resistance to clarithromycin. Two susceptible control isolates lacked the mutation. Three additional reports in the literature of patients developing recurrent skin lesions with clarithromycin-resistant *M. chelonae* following initial response to monotherapy are summarized. We demonstrate that clarithromycin monotherapy in patients with disseminated cutaneous infections can lead to clarithromycin resistance and therapeutic failure associated with a single point mutation at position 2058 of 23S rRNA.—Authors' Abstract

Wang, C. H., Lin, H. C., Liu, C. Y., Huang, K. H., Huang, T. T., Yu, C. T. and Kuo, H. P. Upregulation of inducible nitric oxide synthase and cytokine secretion in peripheral blood monocytes from pulmonary tuberculosis patients. J. Tubercul. Lung Dis. **5** (2001) 283–291.

A study was conducted to identify whether inducible nitric oxide synthase (iNOS) expression and cytokine release of peripheral blood monocytes (PBM) are upregulated and have a connection in tuberculosis (TB) infection [date not given]. The expression of iNOS immunoreactivity on PBM from TB patients (N = 18; 12 males, 6 females; aged 57.0 ± 4.5 years) and normal subjects (N = 14; 8 males, 6 females; mean age, 46.5 ± 4.3 years) from Taiwan, was measured by loading with anti-macrophage iNOS polycolonal primary antibody analyzed by flow cytometry. Expression of iNOS mRNA in PBM was detected by RT-PCR. The spontaneous generation of nitrite and cytokines (IL-β and TNF-α) by cultured monocytes was also determined. Results revealed that iNOS immunoreactivity, the capacity for spontaneous nitrite generation and the level of TNF-α or IL-1β secretion of PBM were significantly higher in TB patients than in normal subjects. The amount of nitrite, TNF-α and IL-1β released from PBM of TB patients was inhibited by N^G-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NOS. The level of iNOS immunoreactivity on PBM was highly correlated with nitrite generation both in all the subjects studied and in TB patients alone. Spontaneous TNF-α production showed a stronger correlation with nitrite production than with IL-1β. It is concluded that the NO and cytokine synthase activities of monocytes appear to be concomitantly upregulated in response to mycobacterial infection. The enhanced NO generation by monocytes in TB patients may play an autoregulatory role in amplifying the synthesis of pro-inflammatory cytokines.—Trop. Dis. Bull.

Yerokhin, V. V., Punga, V. V. and Rybka, L. N. Tuberculosis in Russia and the problem of multiple drug resistance. Ann. N.Y. Acad. Sci. **953** (2001) 133–137.

Tuberculosis cases in Russia doubled from 1991 to 2000, when the incidence reached 90 per 100,000; and Russian TB mortality rates are the highest in Europe. Implementation of WHO-recommended DOTS strategy is in the preliminary stages. The epidemiological data are presented as is the implementation strategy for stabilizing the epidemic.—Authors' Abstract