INTERNATIONAL JOURNAL OF LEPROSY

Volume 69, Number 2
Printed in the U.S.A.
(ISSN 0148-916X)

CURRENT LITERATURE

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

General and Historical

Begum, D. and Nath, S. C. Ethnobotanical review of medicinal plants used for skin diseases and related problems in northeastern India. J. Herbs Spices Med. Plants **7** (2000) 55–93.

The medicinal plants used for the treatments of skin diseases and related problems in northeastern India are reviewed based on the ethnobotanic reports. Of the 275 plant species examined, 224 species have been used for treatment of specific human ailments such as allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies, smallpox and sexually transmitted diseases. Some of the plant species, including *Artemisia nilagirica, Calotropis gigantea, Cannabis sativa, Cassia alata, C. fistula, Centella asiatica, Cyclea peltata, Datura metel, Drymaria cordata, Jatropha curcas, Litsea cordata, Mimosa pudica, Plantago major* and *Plumeria acutifolia* [*P. rubra*], are used among a range of ethnic groups for disease treatment.—Trop. Dis. Bull.

Boldsen, J. L. Epidemiological approach to the paleopathological diagnosis of leprosy. Am. J. Phys. Anthropol. **115** (2001) 380–387.

In paleopathology it is usually assumed that modern diagnostic criteria can be applied to infectious diseases in the past. However, as both the human species and populations of pathogenic microorganisms undergo evolutionary changes, this assumption is not always well-founded. To get valid estimates of the frequency (the point prevalence at death) of leprosy in skeletal samples, sensitivity, specificity, and sample frequency must be estimated simultaneously. It is shown that more than three symptoms must be evaluated in at least three samples in order to reach estimates with well-described properties. The method is applied to three skeletal samples from medieval Denmark; the samples were scored for the presence of seven osteological conditions indicating leprosy. For the osteological conditions, sensitivity varied from 0.36–0.80, and specificity from 0.58–0.98. The frequency of leprosy in the three samples was: Odense (a lepers' institution), 0.98, 95%CI 0.64–1.00; Malmo (urban cemetery), 0.02, 95%CI 0.00–0.07; and Tirup (rural cemetery), 0.36, 95%CI 0.23–0.46. It is concluded that it is indeed possible to estimate disease frequencies without reference to modern standards, and that leprosy occurred with widely differing frequencies in different segments of the medieval population in southern Scandinavia. —Author's Abstract

Chemotherapy

Griffiths, S. and Ready, N. Defaulting patterns in a provincial leprosy control programme in northern Mozambique. Lepr. Rev. **72** (2001) 199–205.

Cohort-based multidrug therapy (MDT) completion rates are used to assess adherence to MDT. However this measure gives no information about when during the treatment period defaulting occurs. Two districts in Cabo Delgado province in Northern Mozambique were selected for evaluation of multibacillary patient defaulter data between 1993 and 1997 to examine when patients default during the treatment period. In all, 548 (59.2%) of 926 MB patients completed treatment and 378 (40.8%) defaulted between 1993 to 1997. The percentage of defaulters fell steadily from 59.8% in 1993 to 23.2% in 1997. Of the 378 defaulters 57.7% defaulted treatment within 6 months and 83.1% within 1 year of starting treatment. It was observed that patients tend to default early rather than late in the treatment period and that this pattern is maintained over time despite a fall in defaulter rates. Patients established early into a treatment routine were more likely to complete treatment. A comprehensive effort to improve and maintain leprosy control services will probably influence adherence more than any single, specific strategy. Shortening MDT treatment from 2 years to 1 year is unlikely to affect the defaulter rate.—Authors' Summary

Gupte, M. D. Field trials of a single dose of the combination rifampicin-ofloxacin-minocycline (ROM) for the treatment of paucibacillary leprosy. Lepr. Rev. **71** Suppl. (2000) S77–S80.

This paper presents 2 trials evaluating the efficacy of single dose rifampin-ofloxacin-minocycline (ROM) for the treatment of paucibacillary leprosy (PB) in patients with different numbers of lesions in India. In trial 1, 697 patients with single-lesion leprosy (aged >5 years) were given a single dose of ROM (600 mg rifampin + 400 mg ofloxacin + 100 mg minocycline). Another 684 patients were given standard WHO regimen for PB leprosy consisting of a 6-month course of 600 mg rifampin monthly with 100 mg dapsone daily. Of 697 patients, 51.8% demonstrated marked improvement, while of those taking WHO regimen, 57.3% showed improvement. 46.9% of those administered ROM and 54.7% of those given WHO regimen demonstrated complete cure. In trial 2, 236 patients with 2 or 3 lesions were recruited between October 1995 and April 1996, and were given ROM (N = 118) and standard WHO (N = 118) regimens. Ninety-two percent of the patients completed 6 months of treatment, and ≈90% of the patients were followed for 18 months. Approximately 50% and ≈40% of patients in both groups had marked clinical improvement and complete clearance of lesions, respectively. Four patients in each group had treatment failure. Of the 26 patients treated with WHO regimen, 13 had complete clearance, compared with only 6 of 30 patients given ROM. Thus, in patients with 2 or 3 lesions, standard WHO regimen may be preferred.—Trop. Dis. Bull.

Mirochnick, M., Cooper, E., Caparelli, E., McIntosh, K., Lindsey, J., Xu, J., Jacobus, D., Mofenson, L., Bonagura, V. R., Nachman, S., Yogev, R., Sullivan, J. L. and Spector, S. A. Population pharmacokinetics of dapsone in children with human immunodeficiency virus infection. Clin. Pharmacol. Ther. **70** (2001) 24–32.

Background: Previous studies of dapsone pharmacokinetics in children have been too small to allow assessment of the relationships between dapsone pharmacokinetic parameters and patient characteristics or markers of efficacy and toxicity.

Methods: We used population analysis to estimate dapsone pharmacokinetic parameters in children participating in a phase I/II study of daily and weekly dapsone in children with human immunodeficiency virus (HIV) infection. With use of the program NONMEM and a 1-compartment open model, the influence of demographic and

clinical characteristics on oral clearance (CL/F) and oral volume of distribution (V/F) were examined. Measures of drug exposure (area under the concentration-time curve [AUC] and predicted concentrations just before and 2 hours after administration) were estimated for each patient and correlated with markers of efficacy and toxicity.

Results: Sixty children (median age, 3 years; age range, 2 months to 12 years) contributed 412 dapsone concentrations collected after 175 study doses. Final parameter estimates were 1.40 L/kg for V/F, 0.0283 L/kg/h for CL/F, and 2.66 for the absorption rate constant. Of the clinical characteristics evaluated, dapsone CL/F was significantly increased by 50% in children taking rifabutin, by 39% in black children, and by 38% in children younger than 2 years old. Although no significant correlations were found between any dapsone exposure parameter and markers of toxicity, increased AUC was associated with a decreased risk of *Pneumocystis carinii* pneumonia (PCP).

Conclusion: Ethnicity, age, and concomitant rifabutin use were associated with dapsone CL/F, with more rapid clearance observed in black children, children younger than 2 years old, and children receiving rifabutin. Dapsone pharmacokinetic parameters were not associated with toxicity, but higher dapsone AUC was associated with decreased risk of PCP. Monitoring of serum dapsone levels may be needed for optimal management of dapsone for PCP prophylaxis in children.—Authors' Abstract

Rao, P. N. and Lakshmi, T. S. Increase in the incidence of dapsone hypersensitivity syndrome—an appraisal. Lepr. Rev. **72** (2001) 57–62.

There has been an increase in the reports of dapsone hypersensitivity syndrome (DHS) in the past few years, coinciding with the introduction of multidrug therapy (MDT) for leprosy worldwide. The exact cause of this phenomenon is not clear. We report four cases of DHS observed among 252 leprosy patients on MDT and one case of DHS in a patient taking dapsone for nodulocystic acne in the Dermatology Department of the Osmania General Hospital, Hyderabad, India, between June 1997 and January 1999 with few unusual features. In two of these five patients maculopapular rash was severe and progressed to erythroderma. Introduction of MDT in 1982 has not only decreased the prevalence of leprosy but also brought about a positive change in the attitude of people which increased the voluntary reporting of leprosy patients. These coupled with improvements in the organization of leprosy control and awareness among medical personnel of DHS, are probably the most important reasons for the increased reporting of DHS in recent years.—Authors' Summary

CLINICAL SCIENCES

Bhatia, M. S., Jagawat, T. and Choudhary, S. Delusional parasitosis a clinical profile. Int. J. Psychiatry Med. **30** (2000) 83–91.

In the present study, a series of 52 consecutive cases of delusional parasitosis is reported from India. A majority of cases (92%) had insidious onset. The duration of symptoms in all but two cases was six months or more. Twenty-six cases presented with a delusion of infestation by insects over the body and twenty-three cases with a delusion of insects crawling over the scalp. Three cases had associated diabetes mellitus, five cases had leprosy, five cases dementia, four cases had depression, and three cases presented with trichotillomania. Pimozide was used in 46 cases, amitriptyline and fluoxetine in three cases each. Twenty-eight cases (54%) showed complete remission while receiving pharmacotherapy, 18 cases showed partial improvement, and six cases did not respond to treatment.—Trop. Dis. Bull.

Buhrer-Sekula, S., Cunha, M. G., Foss, N. T., Oskam, L., Faber, W. R. and Klatser, P. R. Dipstick assay to identify leprosy patients who have an increased risk of relapse. Trop. Med. Int. Health **6** (2001) 317–323.

Classification of leprosy patients into paucibacillary (PB) and multibacillary (MB) determines the duration of treatment; misclassification increases the risk of relapse because of insufficient treatment if an MB patient is classified as PB. We explored the possibility of using a simple dipstick assay based on the detection of antibodies to the *Mycobacterium leprae*-specific phenolic glycolipid-I (PGL-I) as a tool for classification of patients into PB and MB for treatment purposes. The sensitivity of the dipstick test for detection of MB patients was 85.1%, the specificity 77.7%. We found that of the 71 dipstick-negative PB patients, 25 (35.2%) were clinically cured at the end of treatment, compared with only two (9.5%) of the 21 dipstick-positive PB patients. Of 170 patients in the study population, nine (5.3%) relapsed within the 5-year follow-up period. Seven were MB patients, all dipstick positive. Two PB patients relapsed, one was dipstick negative and one was dipstick positive. Dipstick positivity is a risk factor for the future development of relapses, especially in those groups of patients who had received a shorter-than-usual course of treatment, and the dipstick can be used as an additional, simple tool for classification of patients and for identification of those patients who have an increased risk of relapse.—Authors' Abstract

Courtright, P., Lewallen, S., Tungpakorn, N., Cho, B. H., Lim, Y. K., Lee, H. J. and Kim, S. H. Cataract in leprosy patients: cataract surgical coverage, barriers to acceptance of surgery, and outcome of surgery in a population-based survey in Korea. Br. J. Ophthalmol. **85** (2001) 643–647.

Background/Aims: Cataract is the leading cause of blindness in leprosy patients. There is no population-based information on the cataract surgical coverage, barriers to use of surgical services, and outcome of surgery in these patients. We sought to determine these measures of cataract program effectiveness in a cured leprosy population in South Korea.

Methods: The population consisted of residents of six leprosy resettlement villages in central South Korea. All residents were invited to participate in a study of eye disease and interviewed regarding use of surgical services and reasons for not using these services.

Results: The cataract surgical coverage in this population was 55.4% when <6/18 was used as the cut off and increased to 78.3% when the cut off was <6/60. Barriers reported by patients included being told by the doctor that the cataract was not mature and a perception by the patient that there was no need for surgery. Among patients who had aphakic surgery, 71% were still blind in the operative eye while among patients who had pseudophakic surgery, 14% were still blind (presenting vision). Blindness in pseudophakic patients could be reduced to 3% with spectacle correction.

Conclusion: Cataract prevalence in leprosy patients will increase as life expectancy continues to increase. Leprosy control programs will need to develop activities aimed at reducing the burden of cataract. Recommendations include establishing collaborative agreements with ophthalmological services to provide high quality IOL surgery to these patients, training of health staff to identify and refer patients in need of surgery, monitoring the uptake of cataract surgery among patients needing services, and monitoring the outcome of surgery to improve refractive outcome.—Authors' Abstract

Illarramendi, X., Carregal, E., Nery, J. A. C. and Sarno, E. N. Progression of acral bone resorption in multibacillary leprosy. Acta Leprol. **12** (2000–2001) 29–37.

Although leprosy became a curable disease after implementation of the Global Strategy for the Elimination of Leprosy (WHO), mutilations and deformities are still commonplace in endemic countries. Hence, it remains important to evaluate the prevalence rate and the risk factors of acral bone resorption in the multidrug therapy (MDT) era. A cohort of 105 newly-diagnosed adult multibacillary leprosy patients admitted for treatment between 1990–1992 was surveyed until 1999. Progression of bone resorption (BR) in cured leprosy patients was observed up to 8 years after release from MDT. Twenty-three per-

cent of the patients were found to have acral resorption. BR was found to be associated with the male sex, grade of disability at diagnosis with other deformities and with the occurrence of four or more lepra reactions. Patient surveillance after release from MDT continues to be a necessary procedure in individuals with disabilities and recurrent or persistent reactions.—Authors' Summary

Ishibara, Y., Goto, S., Takigawa, M., Harigaya, Y. and Kosakai, M. [Evaluation of the conjunctiva of leprosy patients using impression cytology.] Nippon Ganka Gakkai Zasshi **105** (2001) 406–410. (in Japanese)

Objective: To evaluate ocular surface disorders (OSD) similar to cicatricial pemphigoid in leprosy patients.

Subject and Method: Sixteen leprosy patients were examined. They were under treatment as inpatients at the Tama Zenshoen National Sanatorium, an institution for the treatment of leprosy. In addition to routine ophthalmological examinations, the patients' conjunctival goblet cells were examined using impression cytology. OSD similar to ocular cicatricial pemphigoid were defined as the presence of at least two items of the following: symblepharon, cicatricial contraction of the conjunctival sac, corneal neovascularization, and palpebral entropion.

Results: OSD was present in 8 of the 16 patients (50%). Goblet cells were either decreased in number or absent in 7 cases (44%), and included 4 cases with OSD. Six of the 7 cases (86%) with reduced or absent goblet cells had been diagnosed as leprosy prior to 1944.

Conclusion: reduction or absence of goblet cells is a frequent feature in leprosy patients, particularly in longstanding ones of 56 years or more. Insufficient initial treatment appears to be a major cause of this finding.—Authors' English Summary

Kaplan, G. Potential of thalidomide and thalidomide analogues as immunomodulatory drugs in leprosy and leprosy reactions. Lepr. Rev. **71** Suppl. (2000) S117–S120.

The immune response of patients with erythema nodosum leprosum, tuberculosis, human immunodeficiency virus and scleroderma after thalidomide treatment is described. The effects of two groups of thalidomide analogs on T cells are also discussed. It is concluded that thalidomide is not successful in the treatment of reversal reactions, perhaps because T cell activation appears to be a significant component of reversal reactions and thalidomide was shown to stimulate T cell activation. Analogs of thalidomide may be candidate drugs for the treatment of reversal reactions.—Trop. Dis. Bull.

Klatser, P. R. Use of a *Mycobacterium leprae* dipstick to classify patients with leprosy. Lepr. Rev. **71** Suppl. (2000) S67–S72.

The use of *M. leprae* dipstick (ML dipstick), a newly developed simple and rapid method for the detection of IgM antiphenolic glycolipid-I antibodies, among 264 leprosy patients in Brazil [date not given] is discussed. The combined method of ML dipstick and number of skin lesions demonstrated 94% sensitivity and 77% specificity. It is concluded that this combined method can improve classification of leprosy patients for the purpose of treatment.—Trop. Dis. Bull.

Krupnick, A. I., Shim, H., Phelps, R. G., Cunningham-Rundles, C. and Sapadin, A. N. Cutaneous granulomas masquerading as tuberculoid leprosy in a patient with congenital combined immunodeficiency. Mt. Sinai J. Med. **68** (2001) 326–330.

Combined immunodeficiency disorders are characterized by abnormalities in cellular and humoral immunity. This classification includes common variable immunodeficiency (CVI), a primary immunodeficiency disorder characterized by hypogammaglobulinemia, recurrent bacterial infections, and significant T-cell abnormalities. Associated autoimmune diseases include rheumatoid arthritis, pernicious anemia, idiopathic thrombocytopenic purpura, and systemic lupus erythematous. Granulomatous lesions

in lymphoid tissues, solid organs, and skin have been reported. We describe a patient with CVI who developed cutaneous granulomas with perineural invasion; to our knowledge, this is a previously undescribed feature.—Authors' Abstract

Moses, A. E., Adelowo, K. A. and Nwankwo, E. A. Effect of HIV infection on the clinical spectrum of leprosy in Maiduguri. Niger Postgrad. Med. J. **8** (2001) 74–77.

The clinical features associated with different classes of leprosy patients co-infected with HIV in Maiduguri was studied and the classification of leprosy was done clinically and bacteriologically using Ridley-Jopling classification and bacteriological index respectively. The cases were classified as paucibacillary (PB) (Tuberculoid-TT and Borderline Tuberculoid-BT) and multibacillary (MB) (Borderline Borderline-BB, Borderline Lepromatous-BL and Lepromatous Leprosy-LL) leprosy. Eleven (10.5%) of 105 leprosy cases were HIV-seropositive comprising of 7 males and 4 females. Age range was 15 and 62 years. Among the HIV seropositive patients, those with PB leprosy were 6 (TT-1, BT-5) while those with MB leprosy were 5 (BB-1, BL-2, LL-2). The predominant clinical features were clawing of fingers (64%), ulcerations (64%), hand muscle atrophy (55%) and clawing of toes (45%). Some clinical features of PB leprosy such as sensory and hair losses (as is also seen in HIV negative patients) occurred in increased frequency in HIV positive patients belonging to the MB class. The HIV-infected leprosy patients are more likely to manifest advanced stages of the disease than the HIV-seronegative patients.—Authors' Abstract

Vijaikumar, M., D'Souza, M., Kumar, S. and Badhe, B. Fine needle aspiration cytology (FNAC) of nerves in leprosy. Lepr. Rev. **72** (2001) 171–178.

Leprosy is primarily a disease of the peripheral nerves and a technique that is simpler than nerve biopsy is required to evaluate nerve involvement, especially in pure neuritic (PN) leprosy. This study was designed to evaluate the role of fine needle aspiration cytology (FNAC) of the nerve in the diagnosis and classification of leprosy. A prospective study was carried out on 25 patients with clinically active leprosy and at least one thickened peripheral sensory nerve. Nerve aspirates were evaluated by May-Grunwald-Giemsa and Fite's staining. Lepromin test, slit skin smears (SSS), skin biopsies (except PN cases) and nerve biopsies were performed and compared with FNAC. FNAC of nerve from 23 cases (92%) yielded diagnostic aspirates. Acid-fast bacilli were observed in six cases by FNAC. FNAC and nerve pathology were equally comparable with the other parameters evaluated. Based on the results, cytological criteria were developed for interpreting nerve aspirates and the cases were classified as paucibacillary (18), BB (2), BL (2), LL (1) and non-diagnostic (2). All PN cases showed diagnostic paucibacillary-type cytology. FNAC of the nerve yields diagnostic aspirates in leprosy comparable with nerve pathology and the proposed cytological criteria may be useful in classification of nerve aspirates.—Authors' Summary

Immuno-Pathology

Dupuis, S., Dargemont, C., Fieschi, C., Thomassin, N., Rosenzweig, S., Harris, J., Holland, S. M., Schreiber, R. D. and Casanova, J. L. Impairment of mycobacterial but not viral immunity by a germ line human STAT1 mutation. Science **293** (2001) 300–303.

Interferons (IFNs) alpha/beta and gamma induce the formation of two transcriptional activators: gamma-activating factor (GAF) and interferon-stimulated gamma factor 3 (ISGF3). We report a natural heterozygous germline STAT1 mutation associated with susceptibility to mycobacterial but not viral

disease. This mutation causes a loss of GAF and ISGF3 activation but is dominant for one cellular phenotype and recessive for the other. It impairs the nuclear accumulation of GAF but not of ISGF3 in heterozygous cells stimulated by IFNs. Thus, the antimycobacterial but not the antiviral, effects of human IFNs are principally mediated by GAF.—Authors' Abstract

Kang, T. and Chae, G. Detection of toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. FEMS Immunol. Med. Microbiol. **31** (2001) 53–58.

Toll-like receptor 2 (TLR2) is critical in the immune response to mycobacterial infections and the mutations in the TLR2 have been shown to confer the susceptibility to severe infection with mycobacteria. To define this, we screened the intracellular domain of TLR2 in 131 subjects. Groups of 45 lepromatous and 41 tuberculoid leprosy (TT) patients and 45 controls were investigated. Ten subjects among the lepromatous leprosy (LL) patients had a band variant detected by single-stranded conformational polymorphism. DNA sequencing detected a C to T substitution at nucleotide 2029 from the start codon of the TLR2. The mutation would substitute Arg to Trp at amino acid residue 677, one of the conserved regions of TLR2. In our results, the mutation was involved in only LL, not TT and control. Thus, we suggest that the mutation in the intracellular domain of TLR2 has a role in susceptibility to LL.—Authors' Abstract

Kim, J., Uyemura, K., Van Dyke, M. K., Legaspi, A. J., Rea, T. H., Shuai, K. and Modlin, R. L. A role for IL-12 receptor expression and signal transduction in host defence in leprosy. J. Immunol. **167** (2001) 779–786.

The generation of cell-mediated immunity against intracellular infection involves the production of IL-12, a critical cytokine required for the development of Th1 responses. The biologic activities of IL-12 are mediated through a specific, high affinity IL-12R composed of an IL-12Rβ1/IL-12Rβ2 heterodimer, with the IL-12Rβ2 chain involved in signaling via Stat4. We investigated IL-12R expression and function in human infectious disease, using the clinical-immunologic spectrum of leprosy as a model. T cells from tuberculoid patients, the resistant form of leprosy, are responsive to IL-12; however, T cells from lepromatous patients, the susceptible form of leprosy, do not respond to IL-12. We found that the IL-12Rβ2 was more highly expressed in tuberculoid lesions compared with the lepromatous lesions. In contrast, IL-12Rβ1 expression was similar in both tuberculoid and lepromatous lesions. The expression of IL-12Rβ2 on T cells was up-regulated by *Mycobacterium leprae* in tuberculoid by not in lepromatous patients. Furthermore, IL-12 induced Stat4 phosphorylation and DNA binding in *M. leprae*-activated T cells from tuberculoid but not from lepromatous patients. Interestingly, IL-12Rβ2 in lepromatous patients could be up-regulated by stimulation with *M. tuberculosis*. These data suggest that Th response to *M. leprae* determines IL-12Rβ2 expression and function in host defense in leprosy.—Authors' Abstract

Lima, C. S., Ribeiro, M. L., Souza, L. A., Sardella, A. B., Wolf, V. M. and Pessolani, M. C. Intracellular signals triggered during association of *Mycobacterium leprae* and *Mycobacterium bovis* BCG with human monocytes. Microb. Pathog. **31** (2001) 37–45.

To gain a better understanding of mycobacteria-host cell interaction, the present study compared the signal transduction events triggered during the interaction of *Mycobacterium leprae* (the causative agent of leprosy) and of *Mycobacterium bovis* BCG (an attenuated strain used as a vaccine against leprosy and tuberculosis) with human monocytes. The assays consisted of pre-treating or not THP-1 cells (a human monocytic cell line) with different kinase inhibitors, followed by incubation with fluorescein-labelled bacteria and analysis of bacterial association via fluorescence microscopy. The specific tyrosine kinase (TK) inhibitor tyrphostin AG126 provided the highest rates of association inhibition

(>90% for BCG and >65% for *M. leprae*). The early activation of TKs during mycobacteria-host cell interaction was confirmed by immunoblot analysis, demonstrating that in several host cell proteins mycobacteria stimulated tyrosine phosphorylation. The use of the drugs wortmannin and bisindolylmaleimide I which, respectively, inhibit phosphatidylinositide 3-kinase (PI 3-kinase) and protein kinase C (PKC), produced lower but consistent results within a 35%–60% association inhibition range for both bacteria. Dose response curves with these inhibitors were obtained. Similar results were obtained when primary human monocytes were used as host cells, strongly suggesting that TK, PKC and PI 3-kinase signals are activated during the interaction of human monocytes with both pathogenic and attenuated species of mycobacteria.—Authors' Abstract

Nakayama, E. E., Ura, S., Fleury, R. N. and Soares, V. Renal lesions in leprosy: a retrospective study of 199 autopsies. Am. J. Kidney Dis. **38** (2001) 26–30.

In the present work, 199 patients with leprosy who underwent autopsy between 1970 and 1986 were retrospectively studied to determine the prevalence, types, clinical characteristics, and etiologic factors of renal lesions (RLs) in leprosy. Patients were divided into two groups: 144 patients with RLs (RL+) and 55 patients without RLs (RL–). RLs observed in 72% of the autopsied patients were amyloidosis (AMY) in 61 patients (31%), glomerulonephritis (GN) in 29 patients (14%), nephrosclerosis (NPS) in 22 patients (11%), tubulointerstitial nephritis (TIN) in 18 patients (9%), granuloma in 2 patients (1%), and other lesions in 12 patients (6%). AMY occurred most frequently in patients with lepromatous leprosy (36%; nonlepromatous leprosy, 5%; $p < 0.01$), recurrent erythema nodosum leprosum (33%; $p < 0.02$), and trophic ulcers (27%; $0.05 < p < 0.10$). Ninety-seven percent of AMY was found in patients with lepromatous leprosy, 88% showed recurrent trophic ulcers, and 76% presented with erythema nodosum leprosum. NPS was found in older patients with arterial hypertension, neoplastic diseases, infectious diseases, and vasculitis associated with GN. Most patients with AMY presented with proteinuria (95%) and renal failure (88%). The most frequent causes of death were renal failure in patients with AMY (57%), infectious diseases in patients with GN (41%) and TIN (45%), and cardiovascular diseases in patients with NPS (41%). No difference in survival rates was observed among RL– patients and those with AMY, GN, NPS, or TIN.—Authors' Abstract

Pieters, J. Entry and survival of pathogenic mycobacteria in macrophages. Microbes Infect. **3** (2001) 249–255.

Pathogenic mycobacteria, including *Mycobacterium tuberculosis*, are phagocytosed by macrophages but manage to survive within the mycobacterial phagosome. Recent work has shed some more light on the mechanisms of mycobacterial entry and survival inside macrophages. Two host cell components, the steroid cholesterol and a phagosomal coat protein termed TACO were found to play crucial roles in the establishment of an intracellular infection. This review describes how these findings may help to understand the circumvention of the normal trafficking routes inside host cells by mycobacteria.—Author's Abstract

Richard, B., Khatri, B., Knolle, E., Lucas, S. and Turkof, E. Leprosy affects the tibial nerves diffusely from the middle of the thigh to the sole of the foot, including skip lesions. Plast. Reconstr. Surg. **107** (2001) 1717–1724.

This study investigated where leprosy affects the posterior tibial nerve and whether neurolysis is beneficial. Nine patients with bilateral posterior tibial leprous neuropathy with no sensorimotor recovery were studied. Preoperative sensory-muscle and nerve conduction velocity testing revealed the tarsal tunnel to be the site of a severe lesion in all cases. During surgery, the most proximal site of the nerve lesion was detected by electrically stimulating the spinal roots from the second lumbar nerve to the fourth sacral nerve, evoking efferent mixed nerve compound action potentials that were

recorded from the exposed tibial nerve. In all patients, the nerve compound action potentials became normal only proximal to the sciatic nerve bifurcation. Epineuriotomy within these seemingly unaffected segments revealed fibrosis of the interfascicular epineurium. Interfascicular neurolysis was performed on all affected segments. A 2-year follow up showed an increase in girth of the proximal calf musculature in 6 of 8 patients (the ninth patient had no recordable nerve conduction velocity). It was concluded that 1) leprosy affects the tibial nerves in a scattered way from the sciatic nerve main trunk distally to the exit of the tarsal tunnel; and 2) interfascicular, microsurgical neurolysis is beneficial provided that it is performed on all affected nerve segments.—Authors' Abstract

Stenger, S. Cytolytic T cells in the immune response to *Mycobacterium tuberculosis.* Scand. J. Infect. Dis. **33** (2001) 483–487.

Cytolytic T cells (CTL) are of paramount importance in immune defense against tumors and viruses. Work over the past decade has revealed that lysis of infected cells is also involved in protective immunity to bacteria and parasites, including *Mycobacterium tuberculosis.* Experiments involving gene-deleted mice and the characterization of CTL lines derived from tuberculosis patients suggest an important role of CTL in immunity to tuberculosis. More recently, the identification of an effector pathway of human CTL provided evidence for direct antimicrobial activity of CTL. This pathway involves the combined action of the pore-forming perforin and the antibacterial granulysin, both expressed in the granules of CTL. Granulysin binds to the bacterial cell surface, thereby disrupting the membrane and causing osmotic lysis. The relevance of this pathway for protection against intracellular pathogens is suggested by the expression of high amounts of granulysin in tissue from patients with tuberculoid leprosy, which are able to contain the spread of the bacilli. These findings support the current concept of designing novel vaccination strategies which elicit not only CD4 + T-helper cells, but also CD8 + CTL with direct antibacterial activity.—Author's Abstract

Suneetha, S., Arunthathi, S., Kurian, N. and Chacko, C. J. G. Histological changes in the nerve, skin and nasal mucosa of patients with primary neuritic leprosy. Acta Leprol. **12** (2000–2001) 11–18.

Primary neuritic leprosy (PNL) presents as a peripheral neuropathy with no visible skin patches and skin smears negative for acid fast bacilli. The pathogenesis of PNL is poorly understood. The aim of the study was to document the histological changes in the nerve, apparently normal skin and nasal mucosa in PNL and to study its significance to the pathogenesis of leprosy lesions. The study is based on a cohort of 208 PNL patients registered at the Schieffelin Leprosy Research and Training Center, Karigiri. All patients had a nerve biopsy, 196 had a skin biopsy and 39 had a nasal mucosal biopsy. The findings reveal that PNL patients exhibit a spectrum of disease histologically in the nerve ranging from lepromatous to tuberculoid leprosy with a significant proportion (46%) manifesting a multibacillary leprosy histology. Findings in the apparently normal skin and nasal mucosa reveal that there are widespread changes due to leprosy in tissues such as the skin and nasal mucosa even when the disease appears clinically confined to a few nerves. PNL may be an early stage in the pathogenesis of the disease before the appearance of skin lesions. The number of nerves enlarged and lepromin status did not give any clue to the nature of underlying disease.—Authors' Summary

Wakhlu, A., Gaur, S. P. S., Kaushal, G. P., Misra, A., Asthana, O. P. and Sircar, A. R. Response of *Mycobacterium habana* vaccine in patients with lepromatous leprosy and their household contacts; a pilot clinical study. Lepr. Rev. **72** (2001) 179–191.

Single dose vaccination was carried out with *Mycobacterium habana* vaccine, 31 lepromatous leprosy cases receiving 1.5 mg (1.5 mg = 6.27×10^8 bacilli) and 36 house-

hold contacts randomly receiving 1.5, 2.0, 2.5 mg vaccine intradermally. Duration of the study was 18 weeks. Vaccination induced lepromin conversion in 100% of lepromatous leprosy cases and lepromin negative household contacts and augmentation of lepromin reactivity in 100% of lepromin positive household contacts, which was stable for the 15 weeks duration of follow-up. The maximum augmentation in lepromin reactivity was obtained with 1.5 mg of vaccine, which is probably the supramaximal dose. Overall, post-vaccination, those without prior BCG vaccination scars showed higher mean values of lepromin augmentation. Local vaccination site changes included induration, ulceration, itching, pain and uncomplicated regional lymphadenopathy, all of which remitted spontaneously by 15 weeks. Systemic side-effects noted were pyrexia, ENL and jaundice, and were seen with no greater frequency than that reported in other vaccine trials. Overall, systemic side effects were easily controlled and were not accompanied by clinically detectable nerve or ocular damage. The safety profile investigations revealed an increase in the mean values of Hb%, RBC count and PCV in household contacts and of PCV in lepromatous patients, post-vaccination. Alterations in the liver function tests were also observed in patients of lepromatous leprosy. Thus, *M. habana* vaccine appears to be useful in stimulating specific CMI against *M. leprae* as evidenced by increased lepromin reactivity.—Authors' Summary

Microbiology

Cole, S. T., Honore, N. and Eiglmeier, K. Preliminary analysis of the genome sequence of *Mycobacterium leprae*. Lepr. Rev. **71** Suppl. (2000) S162–S167.

The genome sequence of a strain of *Mycobacterium leprae* originally isolated in Tamil Nadu, India, and designated TN has been completed recently in accord with one of the priorities defined for leprosy research programs at the joint WHO/Sasakawa Memorial Health Foundation workshop held in Bangkok in 1995. The sequence was obtained using automated DNA sequence analysis of selected cosmids and whole genome shotgun clones. The genome sequence contained 3,268,203 base pairs and an average G + C content of 57.8%. A battery of identification programs was used to analyze the genome sequence and detailed comparisons with the genome and proteome sequences of *M. tuberculosis* were carried out. There were ≈1500 genes common to both *M. leprae* and *M. tuberculosis*, likely to be functional in both organisms. The genome of *M. leprae* contained at least 1000 pseudogenes with 2 or more mutations which should prevent their expression and that 1686 genes have been deleted from the genome. The chromosome of *M. leprae* contained approximately 65 segments varying in length from 5 to more than 200 genes that show synteny to the genome of *M. tuberculosis* but differ in their relative order and distribution. The implications of these results in the study of other mycobacteria and development of immunological and skin test for diagnosis of leprosy are also discussed.—Trop. Dis. Bull.

de Mendonca-Lima, L., Picardeau, M., Raynaud, C., Rauzier, J., Goguet De La Salmoniere, Y. O., Barker, L., Bigi, F., Cataldi, A., Gicquel, B. and Reyrat, J. M. Erp, an extracellular protein family specific to mycobacteria. Microbiology **147** (2001) 2315–2320.

Erp (exported repeated protein) was originally characterized as a virulence factor in *Mycobacterium tuberculosis* and was thought to be present only in *Mycobacterium leprae* and members of the TB complex. Here it is shown that Erp is a ubiquitous extracellular protein found in all of the mycobacterial species tested. Erp proteins have a modular organization and contain three domains: a highly conserved amino-terminal domain which includes a signal se-

quence, a central variable region containing repeats based on the motif PGLTS, and a conserved carboxy-terminal domain rich in proline and alanine. The number and fidelity of PGLTS repeats of the central region differ considerably between mycobacterial species. This region is, however, identical in all of the clinical *M. tuberculosis* strains tested. In addition, it is shown here that a *Mycobacterium smegmatis* erp::aph mutant displays altered colony morphology which is complemented by all the Erp orthologues tested. The genome sequence flanking the erp gene includes cell-wall-related ORFs and displays extensive conservation between saprophytic and pathogenic mycobacteria.—Authors' Abstract

Fischer, K., Chatterjee, D., Torrelles, J., Brennan, P. J., Kaufmann, S. H. and Schaible, U. E. Mycobacterial lysocardiolipin is exported from phagosomes upon cleavage of cardiolipin by a macrophage-derived lysosomal phospholipase a^2. J. Immunol. **167** (2001) 2187–2192.

Pathogenic mycobacteria are able to survive and proliferate in phagosomes within host macrophages (Mϕ). This capability has been attributed in part to their cell wall, which consists of various unique lipids. Some of these are important in the host-pathogen interaction, such as resistance against microbicidal effector mechanisms and modulation of host cell functions, and/or are presented as antigens (Ags) to T cells. Here we show that two lipids are released from the mycobacterial cell wall within the phagosome of infected Mϕ and transported out of this compartment into intracellular vesicles. One of these lipids was identified as lysocardiolipin. Lysocardiolipin was generated through cleavage of mycobacterial cardiolipin by a Ca^{2+}-independent phospholipase A_2 present in Mϕ lysosomes. This result indicates that lysosomal host-cell enzymes can interact with released mycobacterial lipids to generate new products with a different intracellular distribution. This represents a novel pathway for the modification of bacterial lipid Ags.—Authors' Abstract

Kang, T. J., You, J. C. and Chae, G. T. Identification of catalase-like activity from *Mycobacterium leprae* and the relationship between catalase and isonicotinic acid hydrazide (INH). J. Med. Microbiol. **50** (2001) 675–681.

As *Mycobacterium leprae* proliferate inside macrophages, it has been speculated that catalase encoded by katG may protect the bacilli from deleterious effects of peroxide generated from the macrophage and may also play a crucial role in the survival of *M. leprae in vivo*. However, unlike that of *M. tuberculosis*, the katG of *M. leprae* has been reported to be a pseudogene, implicating that isoniazid, which is activated to a potent tuberculocidal agent by catalase, is unlikely to be of therapeutic benefit to leprosy patients. These results raise a question as to how *M. leprae* avoids H_2O_2-mediated killing inside macrophages. To understand the survival of *M. leprae* in macrophages, the present study attempted to detect catalase-like activity in *M. leprae*. Catalase-like activity was found in *M. leprae* cell lysate by the diaminobenzidine (DAB) staining method with non-denaturing polyacrylamide gel electrophoresis. An ammonium sulfate precipitation study revealed that the catalase-like activity was precipitable with 80% ammonium sulfate. The effect of isoniazid (INH) on *M. leprae* growth was also tested by RT-PCR and radiorespirometric assay to examine catalase-like activity in *M. leprae*, because INH was activated by catalase. It was found that the viability of *M. leprae* was decreased at a concentration of 20 μg/ml by radiorespirometric assay and it was inhibited at higher concentrations as determined by RT-PCR. These data suggest that a catalase-like activity other than that encoded by katG is present in *M. leprae*.—Authors' Abstract

Williams, D. L., Pittman, T. L., Gillis, T. P., Matsuoka, M. and Kashiwabara, Y. Simultaneous detection of *Mycobacterium leprae* and its susceptibility to dapsone using DNA heteroduplex analysis. J. Clin. Microbiol. **39** (2001) 2083–2088.

Currently recommended control measures for treating leprosy with multidrug

therapy should control the spread of drug-resistant strains; however, dapsone (DDS) resistance continues to be reported. Comprehensive estimates of drug-resistant leprosy are difficult to obtain due to the cumbersome nature of the conventional drug susceptibility testing method using mouse foot pad inoculation, which requires at least 6 months to obtain results. Recently, it has been determined that DDS-resistant strains contain missense mutations in codon 53 or 55 of the folP1 gene of *Mycobacterium leprae,* and definitive evidence linking these mutations with DDS resistance in *M. leprae* has been obtained. Based on these mutations, a heteroduplex DDS *M. leprae* (HD-DDS-ML) assay was developed for the simultaneous detection of *M. leprae* and of its susceptibility to DDS. The assay relies on the PCR amplification of an *M. leprae*-specific 231-bp fragment of folP1 containing codons 53 and 55. The PCR products are allowed to anneal to a universal heteroduplex generator, and the separation of the resultant DNA duplexes is accomplished by polyacrylamide gel electrophoresis. *M. leprae* was detected in crude cell lysates of skin biopsy specimen homogenates from 8 leprosy patients and from *M. leprae*-infected mouse or armadillo tissues infected with 14 separate strains using the HD-DDS-ML assay. The assay was specific for *M. leprae* in a comparison with results obtained from 14 species of mycobacteria other than *M. leprae* and four bacterial species known to colonize human skin. The HD-DDS-ML assay detected as few as 100 *M. leprae* organisms present in homogenates of human skin and demonstrated a 93% correlation with DDS susceptibility as determined by both DNA sequencing of folP1 and mouse foot pad susceptibility testing. The HD-DDS-ML assay provides a new tool for the simultaneous detection of *M. leprae* and of its susceptibility to DDS from a single specimen. The assay should prove useful for drug resistance surveillance in leprosy control programs when combined with similar molecular tests developed for other drug resistance markers.—Authors' Abstract

Young, D. and Robertson, B. Genomics: leprosy—a degenerative disease of the genome. Curr. Biol. **11** (2001) R381–R383.

Analysis of the genome of the leprosy bacillus uncovers evidence of extensive deletion and inactivation of genes. Secluded in a specialized niche, it has discarded much of its genetic heritage, though retaining just enough to be a major human pathogen.—Authors' Abstract

Epidemiology and Prevention

Ebenso, B. E., Tureta, S. M. and Udo, S. O. Treatment outcome and impact of leprosy elimination campaign in Sokoto and Zamfara states, Nigeria. Lepr. Rev. **72** (2001) 192–198.

A Leprosy Elimination Campaign (LEC) was implemented in 37 districts of Sokoto and Zamfara states, Nigeria, from 13 August to 30 November 1998. The campaign utilized intensive community mobilization and training of local health personnel to detect hidden leprosy cases. During 8 weeks of case finding, 160,127 persons were screened; 353 new cases of leprosy were detected and placed on MDT; 236 (67%) of new cases detected were classified as MB, 64 cases (18%) suffered visible deformities and 24 patients (6.8%) were children. Follow up in December 1999 of patients placed on MDT revealed 97% PB and 96% MB cure rates, respectively. Detection of cases in communities led some community leaders to ask for repeat surveys in their communities. Repeat surveys continue to yield new cases. The authors recommend that LECs be maintained for 3 years to accelerate leprosy elimination in the region. The cost-effectiveness and impact of LEC in Sokoto-Zamfara are discussed.—Authors' Summary

Fine, P. E., Floyd, S., Stanford, J. L., Nkhosa, P., Kasunga, A., Chaguluka, S., Warndorff, D. R., Jenkins, P. A., Yates, M. and Ponnighaus, J. M. Environmental mycobacteria in northern Malawi: implications for the epidemiology of tuberculosis and leprosy. Epidemiol. Infect. **126** (2001) 379–387.

More than 36,000 individuals living in rural Malawi were skin tested with antigens derived from 12 different species of environmental mycobacteria. Most were simultaneously tested with RT23 tuberculin, and all were followed up for both tuberculosis and leprosy incidence. Skin-test results indicated widespread sensitivity to the environmental antigens, in particular to *Mycobacterium scrofulaceum, M. intracellulare* and one strain of *M. fortuitum.* Individuals with evidence of exposure to "fast growers" (i.e., with induration to antigens from fast growers which exceeded their sensitivity to tuberculin), but not those exposed to "slow growers," were at reduced risk of contracting both tuberculosis and leprosy compared to individuals whose indurations to the environmental antigen were less than that to tuberculin. This evidence for cross protection from natural exposure to certain environmental mycobacteria may explain geographic distributions of mycobacterial disease, and has important implications for the mechanisms and measurement of protection by mycobacterial vaccines.—Authors' Abstract

Gupte, M. D. South India immunoprophylaxis trial against leprosy: relevance of the findings in the context of trends in leprosy. Lepr. Rev. **71** Suppl. (2000) S43–S47.

Results of an immunoprophylaxis trial conducted in southern India during 1991 are presented. Four vaccines were compared in the trial: a combination of BCG and heat-killed *Mycobacterium leprae* (HKML); the ICRC bacillus; *Mycobacterium w* (Mw); and BCG. A normal saline was used as placebo. The protective efficacy of the vaccines was: BCG + HKML, 64% (95% confidence interval (CI), 50.4–73.9); the ICRC vaccine, 65.5% protection (CI, 48.0–77.0); Mw, 25.7% (CI, 1.9–43.8); and BCG, 34.1% (CI, 13.5–49.8).—Trop. Dis. Bull.

Klatser, P. R. Strategies for pro-active case finding in leprosy control. Lepr. Rev. **71** Suppl. (2000) S30–S32.

A total of 3987 of 4770 (84%) individuals, in South Sulawesi Province, Indonesia, was screened for leprosy between June and July 2000. Among these, 91 new leprosy cases were diagnosed, representing a case-detection rate of 2.3%, with a range of 1.2%–5.0% per island. The prevalence rate was 191/10,000, with a range of 87–442 per 10,000 per island. Forty-six percent of the patients had multibacillary leprosy, 40% single lesion paucibacillary (PB) leprosy and 14% paucibacillary leprosy with 2–5 lesions. Multidrug therapy was administered in 2 different regimens (blanket regimen using rifampin and contact treatment). Three (Pelokan, Kembanglemari and Tampaan) of the 5 islands, combined to form one group, and Sapuka, were given the different prophylactic regimens, respectively while Sailus served as the control island, in which only the patients received MDT. Effects of the prophylactic regimens are still being studied.—Trop. Dis. Bull.

Razafimalala, F. C., Rakotomanga, S., Rakotondramarina, D. B. and Rakotomalala, J. N. [Leprosy status in a southeastern zone in Madagascar between 1996 and 1998.] Acta Leprol. **12** (2000–2001) 7–10. (in French)

Leprosy is endemic in Madagascar. The authors report the results of an epidemiologic study performed between 1996 and 1998 in Farafanguna, localized in the southeastern portion of the country. During this period, 217 new cases have been diagnosed. Of the 130 cases included in the study, 69.23% were children aged lower than 15 years and 76.91% suffered from a multibacillary form. More than 50% of the cases belonged to a large family (6 persons or more) and at least 1 family case was found in more than 60% of the cases. These results enhance the severity of the disease in the country and shown the presence of mul-

tiple risk factors (promiscuity, family cases and multibacillary forms).—Authors' English Summary

Shaw, M. A., Donaldson, I. J., Collins, A., Peacock, C. S., Lins-Lainson, Z., Shaw, J. J., Ramos, F., Silveira, F. and Blackwell, J. M. Association and linkage of leprosy phenotypes with HLA class II and tumor necrosis factor genes. Genes Immun. **2** (2001) 196–204.

Previous analyses indicate major gene control of susceptibility to leprosy per se and the HLA class II region has been implicated in determining susceptibility and control of clinical phenotype. Segregation analysis using data from 76 Brazilian leprosy multi-case pedigrees (1166 individuals) supported a two locus model as the best fit: a recessive major gene and a recessive modifier gene(s) (single locus vs two locus model, p = 0.0007). Combined segregation and linkage analysis to the major locus, showed strong linkage to HLA class II (HLA-DQB1 p = 0.000002, HLA-DQA1 p = 0.000002, HLA-DRB1 p = 0.0000003) and tumor necrosis factor genes (TNF p = 0.00002, LTA p = 0.003). Extended transmission disequilibrium testing, using multiple affected family members, demonstrated that the common allele TNF*1 of the –308 promoter region polymorphism showed linkage and/or association with disease per se, at a high level of significance (p <0.0001). Two locus transmission disequilibrium testing suggested susceptibility (TNF*1/LTA*2) and protective (TNF*2/LTA*2) haplotypes in the class iii region. Taken together the segregation and HLA analyses suggest the possibility of more than one susceptibility locus in the MHC.—Authors' Abstract

Smith, C., Smith, W. C., Cree, I., Klatser, P., Harboe, M., Bjune, G., Edward, V. K. (U.K. MILEP2 Study Group). Approaches to studying the transmission of *Mycobacterium leprae*. Lepr. Rev. **71** Suppl. (2000) S26–S29.

A collaborative study has been undertaken [date not given] to establish the relationship between infection by *Mycobacterium leprae* and the development of immunity in a community in which multidrug therapy (MDT) has been used for more than 10 years, to elucidate the pathogenesis of infection in leprosy, and to develop and test an intervention strategy based on chemotherapy for interruption of transmission of the organism in the community. The first phase of the study included the establishment of laboratory facilities and pilot work in India. In the course of the second phase, the entire populations of three villages in India and one in Ethiopia have been surveyed, nasal swabs were obtained for detection of *M. leprae* DNA by means of the polymerase chain reaction (PCR), specimens of saliva were obtained for measurement of levels of anti-*M. leprae* IgA antibodies, and follow-up surveys have been carried out. A double-blind trial of chemotherapy among subjects whose PCR was positive is proposed, to determine if the course of the infection can be influenced by treatment. The performance of large numbers of PCR tests in endemic countries has required the development of rigorous internal and external quality control procedures. These have shown that many batches (as many as 50%) fail to meet quality control criteria, and must be retested. Despite this, development of these methods and their application to field studies should provide tools for studying the transmission of *M. leprae* and direct methods of testing innovative interventions.—Trop. Dis. Bull.

Truoc, L. V., Ly, H. M., Thuy, N. K., Trach, D. D., Stanford, C. A. and Stanford, J. L. Vaccination against leprosy at Ben San Leprosy Centre, Ho Chi Minh City, Vietnam. Vaccine **19** (2001) 3451–3458.

Three vaccines, BCG alone, BCG + 10^7 killed *Mycobacterium vaccae* and 10^8 killed *M. vaccae* alone, were studied in children living in close contact with leprosy. In the year before vaccination, 14/446 (3.1%) children had developed leprosy. Among those who were not vaccinated, 9/74 (12.2%) developed the disease in the first 4 years of the study and 5/65 (7.7%) developed the disease in the second 4 years. In comparison with this, among those vacci-

nated, 20/343 (5.8%) developed leprosy in the first 4 years and 5/323 (1.5%) developed leprosy in the second 4 years. This represents 52.5% protection in the first 4 years and 80.5% in the second 4 years. There were no significant differences in protection afforded by each of the three vaccines but the success of the killed preparation of *M. vaccae* is an important finding.—Authors' Abstract

Rehabilitation

Abera, M. and Shanko, M. Small loan schemes: the experience of Ethiopia. Lepr. Rev. **71** (2000) 517–520.

A study was conducted in December 1997 to evaluate the performance and impacts of a community-based rehabilitation program and small loan pilot project among beneficiaries (36 patients with leprosy; 22 men and 14 women) from Gondar, Gojjam, Wollo, and Addis Ababa, Ethiopia. Seventy-eight percent of clients had grade II disability. The project created self-employment opportunity for some clients; others expanded their existing trades and others were engaged in new trades. Savings, income, food intake, clothing and housing had improved in most clients. Sixty-one percent of clients always made regular repayments and 17% showed some irregularities. The overall repayment rate was 78%. Over 86% of the study population reported that attitudes of their community and family members towards them had improved considerably.—Trop. Dis. Bull.

Feenstra, W., van de Vijver, S., Benbow, C., Amenu, A. and Saunderson, P. Can people affected by leprosy at risk of developing plantar ulcers be identified? A field study from central Ethiopia. Lepr. Rev. **73** (2001) 151–157.

In the ALERT leprosy control program, 75 people affected by leprosy, in 3 different geographical areas, were investigated. Each person was documented as having anesthesia to the 10 g monofilament. The study sought to determine why some people developed ulcers while others did not. According to the records, 43 had an ulcer during the last 5 years but 32 had never had an ulcer. In order to examine protective sensation on the sole of the foot, various sensory modalities were tested and the functional anatomy of the foot was examined. The results showed, as may be expected, that it is not possible to define a specific threshold for protective sensation that could be applied to all cases. Some people with only slightly diminished sensation developed ulcers, while many others with almost complete anesthesia remained ulcer-free. In these rural communities, being a farmer reduced the risk of developing an ulcer, but no other demographic features were significant. Graded monofilaments were found to be the most appropriate test, with loss of sensation at any of five points tested being a "positive" result. The 10-g filament was the most sensitive, but only 43% of feet identified by this test actually developed an ulcer. As people with partial loss of sensation were excluded from this study, this figure may be lower under program conditions. The 50-g and 100-g filaments decrease the number of feet identified as at risk, but increase the percentage which actually develop an ulcer to 46% and 49%, respectively. An appropriate test for selecting those for special programs which may have a limited capacity, for example, the provision of subsidized footwear or involvement in self-care groups would be a 100-g filament which would detect 86% of those feet likely to develop an ulcer, while reducing the number of those selected who are not at great risk. Vibrometry was found to be no better than graded filaments and an examination of functional anatomy did not help in identifying those at risk.—Authors' Summary

Hogeweg, M. Cataract: the main cause of blindness in leprosy. (Editorial) Lepr. Rev. **72** (2001) 139–142.

Severely disabled patients often cluster in leprosy settlements and are therefore easily accessible. Often little can be done, but cataract blindness can be cured. Programs

responsible for such patients can arrange for annual eye screening and surgery for those with visual loss due to cataract and other treatable conditions, such as severe lagophthalmos with exposure keratitis. Surgery in age-related cataract, in otherwise unaffected eyes, should preferably be with IOL implantation, provided that the expertise and necessary infrastructure are available. Surgery can be offered at the leprosy institution or settlements.

In conclusion, cataract has become the most common cause of blindness in leprosy. Leprosy patients may experience the additional barrier of stigma and difficult access to the eye care services. The prevalence of cataract blindness among leprosy patients may therefore be higher than in the general population. Specific groups, such as those living in settlements, should be targeted for annual screening with arrangement for surgery. Otherwise, cataract surgery for leprosy patients should be part of regular prevention of disability services and preferably performed through the general eye care services.—From the Editorial

Kumar, K. V. Evaluation of a housing programme. Lepr. Rev. **71** (2000) 521–523.

Starting in 1987, the German Federal Ministry of Cooperation housing program gave former leprosy patients from India interest-free loans for the purpose of building a house of their own. The program was evaluated after 10 years to assess continuity and benefits. Sixty-eight beneficiaries were interviewed. The housing scheme has improved the lifestyle and standard of living of former leprosy patients. Those who already had houses improved the condition of their houses. Electricity was available in 48 of the 68 houses and 16 houses had their own water source. Thirty-six of the beneficiaries were found to be regular in their payments while the others paid when they were able. The social stigma associated with leprosy has vanished in the beneficiaries' newfound lives.—Trop. Dis. Bull.

Meima, A., Saunderson, P. R., Gebre, S., Desta, K. and Habbema, J. D. F. Dynamics of impairment during and after treatment: the AMFES cohort. Lepr. Rev. **72** (2001) 158–170.

This study investigates the dynamics of impairment during and after multidrug therapy treatment for the patient cohort of the prospective ALERT MDT Field Evaluation Study (AMFES). The impairment status was compared at intake, at release from treatment (rft), and at the time of the latest survey between 24 and 48 months after release from treatment (follow-up). The eye-hand-foot impairment score (EHF score), which is the sum of the WHO impairment grades of the eyes, hands, and feet, was used as a tool for comparison. In all, 433 out of the 592 patients (224 paucibacillary [PB] and 209 multibacillary [MB]) completed treatment in time and were assessed at release from treatment. The rise of getting impaired was 4% for the 113 PB and 21% for the 91 MB patients who were initially free from impairment. Out of the 111 initially impaired PB patients, 41% recovered or improved and 13% worsened in EHF score. For the 118 initially impaired MB patients, these figures were: recovery or improvement 43% and worsening in 13%. Three hundred and twenty-three out of the 433 patients (158 PB and 165 MB) had a follow-up examination in between the next 24–48 months after rft. The risks of impairment at follow-up were 6% for the 79 PB and 18% for the 77 MB patients without impairment at rft. Out of the 79 PB patients with impairment at rft, 35% recovered or improved and 28% worsened. For the 88 impaired MB patients, these figures were: recovery or improvement 26% and worsening 27%. Patients showed a tendency to compensate EHF score improvement before rft by worsening after rft and vice versa. The first main conclusion is that the impairment status at intake was by far the most important determinant for future impairment. The second one is that the dynamics of impairment were less favorable after rft than before. Little is known about the long-term fate of leprosy patients with irreversible nerve damage and the associated risk of developing severe secondary impairment. Especially in this era of the leprosy elimination goal, we should give this accumulating patient group due attention in research and health policy agendas.—Authors' Summary

Mitchell, P. D. The threshold for protective sensation that prevents neuropathic ulceration on the plantar aspect of the foot: a study of leprosy patients in a rural community in India. Lepr. Rev. **72** (2001) 143–150.

The protective sensation threshold is an important concept in the prevention of plantar ulceration in leprosy patients. Previous studies have suggested that skin with sensory nerve damage on the plantar aspect of the foot which can still detect the 5.07 Semmes-Weinstein monofilament (~10 g) is highly unlikely to develop ulceration. While the threshold is thought to be less than the 6.10 filament (~75 g), no work just testing adjacent to current ulcers has been undertaken to assess this more accurately. This is important, as it has been shown that a significant proportion of healthy individuals who wear sandals or go barefoot in India may fail to detect this 5.07 filament in at least some areas of the sole, especially in older age groups, and in certain cases the 5.46 filament (~30 g) is the lightest detected. In an attempt to address this problem, a cross-sectional study on 26 current plantar ulcers in male adults with stable neuropathy due to leprosy was carried out in the rural town of Salur, India. It was confirmed that the ability to detect the 5.07 filament (~10 g) did prevent the development of ulceration while in contrast the ability to detect the 5.46 filament (~30 g) did not. This suggests that the threshold for protective sensation lies between these two filaments. An approach is suggested which may help to differentiate feet genuinely at risk of ulceration from those merely unable to detect the 5.07 filament on account of thickened skin callus or advancing age.—Author's Summary

Nicholls, P. G. Guidelines for social and economic rehabilitation. Lepr. Rev. **71** (2000) 422–465.

Guidelines aimed at providing information and advice to managers involved in helping people with leprosy regain the social and economic status that allows a dignified life, and discussing the organizational implications of socioeconomic rehabilitation are presented.—Trop. Dis. Bull.

Rao, V. P., Rao, I. R. and Palande, D. D. Socio-economic rehabilitation programmes of LEPRA India—methodology, results and application of needs-based socioeconomic evaluation. Lepr. Rev. **71** (2000) 466–471.

There is now a better understanding of the scope and process of rehabilitation. The approach recognizes the impact of leprosy on the individual, aims to understand the needs and concerns of those affected, their families and community in the rehabilitation process, and that aims to restore the person to normal social life. LEPRA India has undertaken socioeconomic rehabilitation (SER) activities in its projects in Andrah Pradesh and Orissa States in India with a holistic approach that has been evolutionary, developmental and participatory. An SER Officer (SERO) was posted to each project. A plan was formulated by the SERO with participation of all project staff. The main emphasis of the program was on active participation of the affected person in the rehabilitation process. A needs-assessment study was conducted in the target population using a semi-structured questionnaire. Information was elicited about social and economic status, before and after the disease, and the current rehabilitation needs of the persons affected. The next step was meeting the needs through interventions by the SER staff. The impact of the program on restoration of social and economic status of the affected persons was analyzed. The paper stresses the importance of assessing the needs of persons affected by leprosy, structuring a rehabilitation program with the active participation of the affected person and evaluating the impact of the interventions in restoring normal social and economic life.—Trop. Dis. Bull.

Razafimalala, F. C., Rakotondramarina, D. B., Razafitsalama, C. and Randimbiarisoa, A. [Surgery in leprosy: a retrospective study in Madagascar.] Acta Leprol. **12** (2000–2001) 25–28. (in French)

Leprosy is endemic in Madagascar and the diagnosis of leprosy is still delayed. Thus, many patients suffer from multiple

and/or severe disabilities. For a long time, many leprologists have argued that surgery is necessary and useful in the treatment of these disabilities. We report the results of a retrospective study done in 25 patients re-evaluated 2 to 7 years after surgery. Of these patients, 17 (68%) were successful, 7 (28%) had partial improvement and only 1 (4%) had no benefit. These results are encouraging and suggest that surgery must be proposed more often to treat leprosy disabilities.—Authors' English Summary

Sanlorenzo, M., Rakotondrajao, J., Caldera, D. and Bellato, C. [Leprosy surgery in a bush hospital in Africa: experience in Madagascar.] Acta Leprol. **12** (2000–2001) 19–24. (in French)

We report our experience of leprosy surgery in terms of feasibility and efficacy in a small bush hospital in Madagascar during the period of September 1989 to January 1993. Operations of neurolysis, corrections of claw hands by the techniques of Lasso-Zancolli or Van Droogenbroeck, arthrodesis, resections and amputations have been performed. Our results suggest that at least a part of these surgical procedures may be performed by a non-specialized medical team, taught on the premises. Thus, the cost of treatment will be low and accessible to more leprosy patients.—Authors' English Summary

Scott, J. The psychosocial needs of leprosy patients. Lepr. Rev. **71** (2000) 486–491.

The psychological needs of patients with leprosy in South Africa were studied. Thirty leprosy patients (15 males, 15 females; 41 to 60 years-old) underwent interviews [date not given]. Intense grief was the most common general reaction on hearing the diagnosis of leprosy. All respondents experienced anxiety while admitted at a hospital and the majority felt that lengthy institutionalization had more disadvantages than advantages. Eleven subjects first consulted a traditional healer before visiting a doctor because of the belief that a spell had been cast on them due to family influences and traditional beliefs. In general, the subjects were very satisfied with the treatment that they received from the Leprosy Mission and a Westfort Hospital. Of 23 married subjects, 9 men and 7 women had been deserted by their marriage partners because of leprosy. It is concluded that the psychosocial need of leprosy sufferers in South Africa are similar to those of leprosy sufferers in other parts of the world in the need for self-acceptance, social acceptance and acceptance by the community.—Trop. Dis. Bull.

Thomas, M. V. and Sundar Rao, P. S. S. Unmet needs of rehabilitation in leprosy services. Lepr. Rev. **71** (2000) 526–527.

Of 16,601 leprosy patients from Gudiyatham Taluk, Vellore District, Tamil Nadu, India, cured during 1955 to 1985, 9,245 were interviewed. More females had no source of independent living compared with males (79% vs 65%). Approximately 75% of the persons affected by leprosy had no land of their own and ≈10% did not have their own shelter. Twenty-two percent were identified as having rehabilitation needs, 7.13% needed socioeconomic assistance and 49.2% required some medical assistance. Fourteen percent had grade 2 or greater deformity.—Trop. Dis. Bull.

Vaz, M., Diffey, B., Jacob, A. J. W. and Vaz, M. Should nutritional status evaluation be included in the initial needs assessment of leprosy patients with disability prior to socioeconomic. Lepr. Rev. **72** (2001) 206–211.

Because of the large numbers of leprosy patients with disability and the limited resources available, it is important that socioeconomic rehabilitation (SER) is targeted towards those who are most in need. Toward this purpose, current assessments of leprosy patients prior to initiating SER include the evaluation of income, assets and household possessions. Conspicuously absent is the nutritional assessment of the patient. In the absence of weight loss associated with illness, population studies indicate that undernutrition reflects poor socioeconomic conditions. In this study of 151 cured leprosy patients with disability, 57% of the patients were found to be under-

nourished using body mass index (kg/m^2) derived from body weight and height, and 10% of the patients were severely undernourished (grade III). Undernutrition in the patients was poorly though significantly correlated with personal income ($r = 0.18$, $p < 0.05$). Total household income, reported amount of money spent on food and estimated cereal intakes were not correlated with the BMI of the patient, possibly due to reporting bias and other methodological issues. We propose the inclusion of nutritional status evaluation by anthropometry as part of the initial screening of leprosy patients prior to instituting SER. We believe that this simple and objective evaluation can add to the assessment of 'threat' of economic deprivation or actual economic 'dislocation,' and thus help in the prioritization of leprosy patients for SER.—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Balagon, M. V., Tan, P. L., Prestidge, R., Cellona, R. V., Abalos, R. M., Tan, E. V., Walsh, G. P., Watson, J. D. and Walsh, D. S. Improvement in psoriasis after intradermal administration of delipidated, deglycolipidated *Mycobacterium vaccae* (PVAC): results of an open-label trial. Clin. Exp. Dermatol. **26** (2001) 233–241.

The aim of new treatments for psoriasis is to induce extended remissions with fewer side-effects. Previous studies suggest that *Mycobacterium vaccae,* a harmless organism prepared as a heat-killed suspension, may induce periods of remission in some psoriasis patients after intradermal administration. To assess a more potent derivative of *M. vaccae,* we conducted an open-label study in which 20 patients with moderate to severe psoriasis (Psoriasis Area and Severity Index [PASI] of 12–35) received two intradermal inoculations of heat-killed, delipidated, deglycolipidated *M. vaccae* (DDMVAC or 'PVAC') in lesion-free deltoid skin, separated by a period of 3 weeks. Twelve weeks after the injections, 13 out of 20 patients (65%) showed marked improvement in the PASI score (>50% reduction), three were unchanged (<25% reduction), three had worsened (>5% increase), and one was withdrawn from the trial because of an exfoliative flare. At 24 weeks, 13 out of 19 patients continued to show >50% improvement that, in some, lasted for 6 months or longer. Patients classified as good responders at 12 or 24 weeks were then offered additional PVAC injections after 24 weeks if the PASI reached 8 or higher. Intradermal administration of PVAC was safe, well-tolerated, and induced clinically significant improvement in many psoriasis patients. A randomized, double-blind, controlled study is warranted.—Authors' Abstract

Besser, R. E., Pakiz, B., Schulte, J. M., Alvarado, S., Zell, E. R., Kenyon, T. A. and Onorato, I. M. Risk factors for positive Mantoux tuberculin skin tests in children in San Diego, California: evidence for boosting and possible foodborne transmission. Pediatrics **108** (2001) 305–310.

Objectives: Source case finding in San Diego, California, rarely detects the source for children with tuberculosis (TB) infection or disease. One third of all pediatric TB isolates in San Diego are *Mycobacterium bovis,* a strain associated with raw dairy products. This study was conducted to determine risk factors for TB infection in San Diego.

Design: Case-control study of children ≤5 years old screened for TB as part of routine health care visit. Asymptomatic children with a positive (≥10 mm) Mantoux skin test (TST) were matched by age to 1 to 2 children with negative TST from the same clinic. We assessed risk factors for TB infection through parental interview and chart review.

Results: A total of 62 cases and 97 controls were enrolled. Eleven cases and 25 controls were excluded from analysis because of previous positive skin tests. Compared with controls, cases were more likely to have received BCG vaccine (73% vs 7%,

odds ratio [OR] 44), to be foreign born (35% vs 11%, OR 4.3), and to have eaten raw milk or cheese (12% vs 8%, OR 3.76). The median time between the most recent previous TST and the current test was 12 months for cases and 25 months for controls. Other factors associated with a positive TST included foreign travel, staying in a home while out of the country, and having a relative with a positive TST. There was no association between contact with a known TB case. In a multivariable model, receipt of BCG, contact with a relative with a positive TST, and having a previous TST within the past year were independently associated with TB infection.

Conclusions: We identified several new or reemerging associations with positive TST including cross border travel, staying in a foreign home, and eating raw dairy products. The strong associations with BCG receipt and more recent previous TST may represent falsely positive reactions, booster phenomena, or may be markers for a population that is truly at greater risk for TB infection. Unlike studies conducted in nonborder areas, we found no association between positive TB skin tests and contact with a TB case or a foreign visitor. Efforts to control pediatric TB in San Diego need to address local risk factors including consumption of unpasteurized dairy products and cross-border travel. The interpretation of a positive TST in a young child in San Diego who has received BCG is problematic.—Authors' Abstract

Black, G. F., Dockrell, H. M., Crampin, A. C., Floyd, S., Weir, R. E., Bliss, L. Sichali, L., Mwaunqulu, L., Kanyongoloka, H., Ngwira, B., Warndorff, D. K. and Fine, P. E. Patterns and implications of naturally acquired immune responses to environmental and tuberculous mycobacterial antigens in northern Malawi. J. Infect. Dis. **184** (2001) 322–329.

Interferon (IFN)-gamma responsiveness to 12 purified protein derivative (PPD) and new tuberculin antigens from 9 species of mycobacteria was assessed, using a whole blood assay, in 616 young adults living in northern Malawi, where *Mycobacterium bovis* bacille Calmette-Guerin (BCG) vaccination provides no protection against pulmonary tuberculosis. The prevalence of IFN-gamma responsiveness was highest for PPDs of *M. avium, M. intracellulare,* and *M. scrofulaceum* (the MAIS complex). Correlations between responsiveness paralleled genetic relatedness of the mycobacterial species. A randomized, controlled trial was carried out, to assess the increase in IFN-gamma responsiveness to *M. tuberculosis* PPD that can be attributed to *M. bovis* BCG vaccination. The BCG-attributable increase in IFN-gamma response to *M. tuberculosis* PPD was greater for individuals with low initial responsiveness to MAIS antigens than for those with high initial responsiveness. Although not statistically significant, the trend is consistent with the hypothesis that prior exposure to environmental mycobacteria interferes with immune responses to BCG vaccination.—Authors' Abstract

Brooks, P. C., Movahedzadeh, F. and Davis, E. O. Identification of some DNA damage-inducible genes of *Mycobacterium tuberculosis:* apparent lack of correlation with LexA binding. J. Bacteriol. **183** (2001) 4459–4467.

The repair of DNA damage is expected to be particularly important to intracellular pathogens such as *Mycobacterium tuberculosis,* and so it is of interest to examine the response of *M. tuberculosis* to DNA damage. The expression of recA, a key component in DNA repair and recombination, is induced by DNA damage in *M. tuberculosis.* In this study, we have analyzed the expression following DNA damage in *M. tuberculosis* of a number of other genes which are DNA damage inducible in *Escherichia coli.* While many of these genes were also induced by DNA damage in *M. tuberculosis,* some were not. In addition, one gene (ruvC) which is not induced by DNA damage in *E. coli* was induced in *M. tuberculosis,* a result likely linked to its different transcriptional arrangement in *M. tuberculosis.* We also searched the sequences upstream of the genes being studied for the mycobacterial SOS box (the binding site for LexA) and assessed LexA binding to potential sites identified. LexA is the repressor

protein responsible for regulating expression of these SOS genes in *E. coli*. However, two of the genes which were DNA damage inducible in *M. tuberculosis* did not have identifiable sites to which LexA bound. The absence of binding sites for LexA upstream of these genes was confirmed by analysis of LexA binding to overlapping DNA fragments covering a region from 500 bp upstream of the coding sequence to 100 bp within it. Therefore, it appears most likely that an alternative mechanism of gene regulation in response to DNA damage exists in *M. tuberculosis*.—Authors' Abstract

Canaday, D. H., Wilkinson, R. J., Li, Q., Harding, C. V., Silver, R. F. and Boom, W. H. CD4+ and CD8+ T cells kill intracellular *Mycobacterium tuberculosis* by a perforin and Fas/Fas ligand-independent mechanism. J. Immunol. **167** (2001) 2734–2742.

Cytotoxic effector phenotype and function of MHC-restricted *Mycobacterium tuberculosis* (MTB)-reactive CD4+ and CD8+ T lymphocytes were analyzed from healthy tuberculin skin test-positive persons. After stimulation *in vitro* with MTB, both CD4+ and CD8+ T cells upregulated mRNA expression for granzyme A and B, granulysin, perforin, and CD95L (Fas ligand). mRNA levels for these molecules were greater for resting CD8+ than CD4+ T cells. After MTB stimulation, mRNA levels were similar for both T-cell subsets. Increased perforin and granulysin protein expression was confirmed in both in CD4+ and CD8+ T cells by flow cytometry. Both T-cell subsets lysed MTB-infected monocytes. Biochemical inhibition of the granule exocytosis pathway in CD4+ and CD8+ T cells decreased cytolytic function by >90% in both T-cell subsets. Ab blockade of the CD95–CD95L interaction decreased cytolytic function for both T-cell populations by 25%. CD4+ and CD8+ T cells inhibited growth of intracellular MTB in autologous monocytes by 74% and 84%, respectively. However, inhibition of perforin activity, the CD95–CD95L interaction, or both CTL mechanisms did not affect CD4+ and CD8+ T cell-mediated restriction of MTB growth. Thus, perforin and CD95–CD95L were not involved in CD4+ and CD8+ T cell-mediated restriction of MTB growth.—Authors' Abstract

Chemlal, K., De Ridder, K., Fonteyne, P. A., Meyers, W. M., Swings, J. and Portaels, F. The use of IS2404 restriction fragment length polymorphisms suggests the diversity of *Mycobacterium ulcerans* from different geographical areas. Am. J. Trop. Med. Hyg. **64** (2001) 270–273.

Buruli ulcer, caused by *Mycobacterium ulcerans*, has been reported in five continents: Africa, Asia, Australia, and North and South America. In the present study, restriction fragment length polymorphism with the recently described *M. ulcerans* specific insertion sequence IS2404 as a probe was applied to *Mycobacterium shinshuense, Mycobacterium marinum*, and 14 clinical *M. ulcerans* isolates originating from six geographic areas: Africa (N = 6), Australia (N = 2), Mexico (N = 1), South Asia (N = 2), Asia (N = 1), and South America (N = 2). Using this probe, six subtypes of *M. ulcerans* related to the six geographic origins of the isolates were distinguished, confirming that *M. ulcerans* can be divided into subgroups corresponding to different geographic variants of the same species.—Authors' Abstract

Desjardin, L. E., Hayes, L. G., Sohaskey, C. D., Wayne, L. G. and Eisenach, K. D. Microaerophilic induction of the alpha-crystallin chaperone protein homologue (hspX) mRNA of *Mycobacterium tuberculosis*. J. Bacteriol. **183** (2001) 5311–5316.

Among the products that are expressed then *Mycobacterium tuberculosis* undergoes hypoxic shiftdown to nonreplicating persistence (NRP) is the alpha-crystallin chaperone protein homolog (Acr). This expression coincides with the previously reported appearance of a respiratory type of nitrate reductase activity, the increase in glycine dehydrogenase activity, and the production of a unique antigen, URB-1. In a timed sampling study, using a slowly stirred oxygen depletion culture model, we have

demonstrated that the hspX mRNA that codes for Acr protein as well as the protein itself is induced just as the bacilli enter the microaerophilic NRP stage 1 (NRP-1). In contrast to the induction observed for hspX mRNA, levels of 16S rRNA, fbpB mRNA (encoding the 85B alpha antigen), and aroB mRNA (encoding dehydroquinate synthase) demonstrate relatively small-to-no change upon entering NRP-1. Acr protein was shown to be identical to URB-1 by Western analysis with anti-URB-1 antibody. The fact that antibody to Acr is found in a high percentage of tuberculosis patients suggest that the hypoxic shiftdown of tubercle bacilli to the NRP state that occurs *in vitro,* resulting in production of the alpha-crystallin protein, occurs *in vivo* as well. Simultaneous abrupt increases in hspX mRNA and Acr protein suggest that Acr protein expression is controlled at the level of transcription.—Authors' Abstract

Dhople, A. M. *In vivo* susceptibility of *Mycobacterium ulcerans* to KRM-1648, a new benzoxazinorifamycin, in comparison with rifampicin; anti-mycobacterial activity of KRM-1648. Arzeneimittelforschung **51** (2001) 501–505.

The antibacterial effects of a new benzoxazinorifamycin, KRM-1648 (3′-hydroxy-5′-(4-isobutyl-1-piperazinyl, CAS 129791-92-0), against *Mycobacterium ulcerans* were evaluated *in vivo* in mouse foot pads, and the results were compared against those obtained with rifampicin (rifampin, CAS 13292-46-1). When mice were fed with the drugs from the day of foot pad inoculations, KRM-1648, at concentrations of 0.001% and higher, mixed in mouse food, was effective in inhibiting the growth of *M. ulcerans* in the foot pads, and the effects were bactericidal. Effects of KRM-1648 at 0.0005% were bacteriostatic. Similar results were obtained with rifampin, but only at concentrations of 0.008% and above. In established infection, i.e., when *M. ulcerans* were growing actively in foot pads, bactericidal effects were observed with KRM-1648 at concentrations of 0.002% and above; to obtain similar results with rifampin, the minimum dose was 0.032%. Thus, the results suggest the superiority of KRM-1648 over rifampin in the treatment of *M. ulcerans* infection. The possibility of using KRM-1648 in combination with other antimycobacterial agents is discussed.—Author's Abstract

Dimopoulos, M. A., Zomas, A., Viniou, N. A., Grigoraki, V., Galani, E., Matsouka, C., Economou, O., Anagnostopoulos, N. and Panayiotidis, P. Treatment of Waldenstrom's macroglobulinemia with thalidomide. J. Clin. Oncol. **19** (2001) 3596–3601.

Purpose: We performed a prospective phase II study to assess the activity of thalidomide in patients with Waldenstrom's macroglobulinemia (WM).

Patients and Methods: Twenty patients with WM were treated with thalidomide at a starting dose of 200 mg daily with dose escalation in 200-mg increments every 14 days as tolerated to a maximum of 600 mg. All patients were symptomatic, their median age was 74 years, and 10 patients were previously untreated.

Results: On an intent-to-treat basis, 5 (25%) of 20 patients achieved a partial response after treatment. Responses occurred in 3 of 10 previously untreated and in 2 of 10 pretreated patients. None of the patients treated during refractory relapse or with disease duration exceeding 2 years responded to thalidomide. Time to response was short, ranging between 0.8 months to 2.8 months. Adverse effects were common but reversible and consisted primarily of constipation, somnolence, fatigue, and mood changes. The daily dose of thalidomide was escalated to 600 mg in only 5 patients (25%), and in 7 patients (35%), this agent was discontinued within 2 months because of intolerance.

Conclusion: Our data indicate that thalidomide has activity in WM but only low doses were tolerated in this elderly patient population. Confirmatory studies as well as studies that will combined thalidomide with chemotherapy or with rituximab may be relevant.—Authors' Abstract

Duan, L., Gan, H., Arm, J. and Remold, H. G. Cytosolic phospholipase A^2 partic-

ipates with TNF-alpha in the introduction of apoptosis of human macrophages infected with *Mycobacterium tuberculosis* H37Ra. J. Immunol. **166** (2001) 7469–7476.

Macrophage (Mϕ) apoptosis, an important innate microbial defense mechanism induced by *Mycobacterium tuberculosis* (Mtb) H37Ra, depends on the induction of tumor necrosis factor-alpha (TNF-α) synthesis. When protein synthesis is blocked, both infection with Mtb and addition of TNF-α are required to induce caspase 9 activation, caspase 3 activation and apoptosis. In this study, we show that the second protein synthesis-independent signal involves activation of group IV cytosolic phospholipase A^2 ($cPLA^2$). Apoptosis of Mtb-infected Mϕ and concomitant arachidonic acid release are abrogated by group IV $cPLA^2$ inhibitors (methyl arachidonyl fluorophosphate and methyl trifluoromethyl ketone), but not by inhibitors of group VI Ca^{2+}-independent ($iPLA^2$); bromoenol lactone) or of secretory low molecular mass PLA^2. In Mϕ homogenates, the predominant PLA^2 activity showed the same inhibitor sensitivity pattern and preferred arachidonic acid over palmitic acid in substrates, also indicating the presence of one or more group IV $cPLA^2$ enzymes. In concordance with these findings, Mϕ lysates contained transcripts and protein for group IV $cPLA^2$-alpha and $cPLA^2$-gamma. Importantly, group IV $cPLA^2$ inhibitors significantly reduced Mϕ antimycobacterial activity and addition of arachidonic acid, the major product of group IV $cPLA^2$, to infected Mϕ treated with $cPLA^2$ inhibitors completely restored the antimycobacterial activity. Importantly, addition of arachidonic acid alone to infected Mϕ significantly reduced the mycobacterial burden. These findings indicate that Mtb induces Mϕ apoptosis by independent signaling through at least two pathways, TNF-α and $cPLA^2$, which are both also critical for antimycobacterial defense of the Mϕ.—Authors' Abstract

Feng, C. G., Palendira, U., Demangel, C., Spratt, J. M., Malin, A. S. and Britton, W. J. Priming by DNA immunization augments protective efficacy of *Mycobacterium bovis* bacille Calmette-Guerin against tuberculosis. Infect. Immun. **69** (2001) 4174–4176.

Sequential immunization with mycobacterial antigen Ag85b-expressing DNA and *Mycobacterium bovis* bacille Calmette-Guerin (BCG) were more effective than BCG immunization in protecting against *Mycobacterium tuberculosis* infection. Depletion of the CD8+ T cells in the immunized mice impaired protection in their spleens, indicating that this improved efficacy was partially mediated by CD8+ T cells.—Authors' Abstract

Greinert, U., Ernst, M., Schlaak, M. and Entzian, P. Interleukin-12 as successful adjuvant in tuberculosis treatment. Eur. Respir. J. **17** (2001) 1049–1051.

Interleukin-12 (IL-12) proved to be an effective and successful adjuvant to a standard antituberculotic medication in a patient suffering from progressive clinical tuberculosis (TB). IL-12 is a potent enhancer of interferon-γ production which is necessary for killing intracellular bacteria like mycobacteria. This patient's TB was progressive, although sensitivity to first-line antituberculotics was proven and medication was given as directly observed therapy over more than 8 months. The 3-month adjuvant therapy with IL-12 significantly and convincingly improved results. It is believed that this case, the first in the literature to describe adjuvant interleukin-12 therapy in tuberculosis, strongly encourages the study of adjuvant interleukin-12 therapy on a more systematic basis.—Authors' Abstract

Giacomini, E., Iona, E., Ferroni, L., Miettinen, M., Fattorini, L., Orefici, G., Julkunen, I. and Coccia, E. M. Infection of human macrophages and dendritic cells with *Mycobacterium tuberculosis* induces a differential cytokine gene expression that modulates T cell response. J. Immunol. **166** (2001) 7033–7041.

Macrophages and dendritic cells (DC)

play an essential role in the initiation and maintenance of immune response to pathogens. To analyze early interactions between *Mycobacterium tuberculosis* (Mtb) and immune cells, human peripheral blood monocyte-derived macrophages (MDM) and monocyte-derived dendritic cells (MDDC) were infected with Mtb. Both cells were found to internalize the mycobacteria, resulting in the activation of MDM and maturation of MDDC as reflected by enhanced expression of several surface antigens (Ags). After Mtb infection, the proinflammatory cytokines TNF-alpha, IL-1, and IL-6 were secreted mainly by MDM. As regards the production of IFN-gamma-inducing cytokines, IL-12 and IFN-alpha, was seen almost exclusively from infected MDDC, while IL-18 was secreted preferentially by macrophages. Moreover, Mtb-infected MDM also produce the immunosuppressive cytokine IL-10. Because IL-10 is a potent inhibitor of IL-12 synthesis from activated human mononuclear cells, we assessed the inhibitory potential of this cytokine using soluble IL-10R. Neutralization of IL-10 restored IL-12 secretion from Mtb-infected MDM. In line with these findings, supernatants from Mtb-infected MDDC induced IFN-gamma production by T cells and enhanced IL-18R expression; whereas supernatants from MDM failed to do that. Neutralization of IFN-alpha, IL-12, and IL-18 activity in Mtb-infected MDDC supernatants by specific antibodies suggested that IL-12 and, to a lesser extent, IFN-alpha and IL-18 play a significant role in enhancing IFN-gamma synthesis by T cells. During Mtb infection, macrophages and DC may have different roles: macrophages secrete proinflammatory cytokines and induce granulomatous inflammatory response; whereas DC are primarily involved in inducing antimycobacterial T-cell immune response.—Authors' Abstract

Gordon, S. V., Eiglmeier, K., Garnier, T., Brosch, R., Parkhill, J., Barrell, B., Cole, S. T. and Hewinson, R. C. Genomics of *Mycobacterium bovis*. Tuberculosis (Edinburgh) **81** (2001) 157–163.

The imminent completion of the genome sequence of *Mycobacterium bovis* will reveal the genetic blueprint for this most successful pathogen. Comparative analysis with the genome sequences of *M. tuberculosis* and *M. bovis* BCG promises to expose the genetic basis for the phenotypic differences between the tubercle bacilli, offering unparalleled insight into the virulence factors of the *M. tuberculosis* complex. Initial analysis of the sequence data has already revealed a novel deletion from *M. bovis*, as well as identifying variation in members of the PPE family of proteins. As the study of bacterial pathogenicity enters the postgenomic phase, the genome sequence of *M. bovis* promises to serve as a cornerstone of mycobacterial genetics.—Authors' Abstract

Hirota, M., Totsu, T., Adachi, F., Kamikawa, K., Watanabe, J., Kanegasaki, S. and Nakata, K. Comparison of antimycobacterial activity of grepafloxacin against *Mycobacterium avium* with that of levofloxacin: accumulation of grepafloxacin in human macrophages. J. Infect. Chemother. **7** (2001) 16–21.

The bactericidal activity of two new quinolones, grepafloxacin and levofloxacin, against five strains of *Mycobacterium avium* was investigated *in vitro*. The minimum inhibitory concentrations (MICs) of these two quinolones, determined by the broth microdilution method, were comparable for all strains tested. In contrast, grepafloxacin suppressed the intracellular growth of all the strains in monocyte-derived macrophages more strongly than levofloxacin, when the cells infected with these strains were incubated for 7 days in the presence of various concentrations of the two new quinolones. To find the reason for the strengthened intracellular killing activity of grepafloxacin, we determined the ratio of the concentration of the new quinolones in the cells to that in the medium (C/M concentration ratio). The C/M concentration ratio of grepafloxacin was increased to 34.7 by 7 days, whereas that of levofloxacin at 7 days was only 12.3. These data suggested that a higher level of intraphagocytic accumulation of grepafloxacin endows it with greater mycobactericidal activity.—Authors' Abstract

Jepson, A., Fowler, A., Banya, W., Singh, M., Bennett, S., Whittle, H. and Hill, A. V. Genetic regulation of acquired immune responses to antigens of *Mycobacterium tuberculosis:* a study of twins in West Africa. Infect. Immun. **69** (2001) 3989–3994.

The role of genetic factors in clinical tuberculosis is increasingly recognized; how such factors regulate the immune response to *Mycobacterium tuberculosis* in healthy individuals is unclear. In this study of 255 adult twin pairs residing in The Gambia, West Africa, it is apparent that memory T-cell responses to secreted mycobacterial antigens (85-kDa antigen complex, "short-term culture filtrate," and peptides from the ESAT-6 protein), as well as to the 65-kDa heat shock protein, are subject to effective genetic regulation. The delayed hypersensitivity response to intradermal tuberculin also demonstrates significant genetic variance, while quantitative T-cell and antibody responses to the 38-kDa cell membrane protein appear to be determined largely by environmental factors. Such findings have implications for vaccine development.—Authors' Abstract

Khoor, A., Leslie, K. O., Tazelaar, H. D., Helmers, R. A. and Colby, T. V. Diffuse pulmonary disease caused by nontuberculous mycobacteria in immunocompetent people (hot tub lung). Am. J. Clin. Pathol. **115** (2001) 755–762.

The clinicopathologic spectrum of infections due to nontuberculous mycobacteria (NTM) includes cavitary disease, opportunistic infection, and nodular disease associated with bronchiectasis. We report a less well-described manifestation of NTM infection: 10 immunocompetent patients without preexisting bronchiectasis had radiographic evidence of diffuse infiltrative lung disease. The most common symptoms were dyspnea, cough, hypoxia, and fever. All 10 patients had used a hot tub. Histologic examination revealed exuberant nonnecrotizing, frequently bronchiolocentric, granulomatous inflammation in all cases. In 1 case, necrotizing granulomas were also noted. The inflammation often was associated with patchy chronic interstitial pneumonia and organization. Cultures revealed NTM in all cases (*Mycobacterium avium* complex in all but 1 case), but staining for acid-fast bacilli was positive in only 1 case. Four patients received corticosteroids alone for presumed hypersensitivity pneumonia, 4 were treated with antimycobacterial therapy, and 2 received both. All patients demonstrated significant improvement at the time of follow-up. These findings suggest that disease due to NTM may manifest as diffuse infiltrates in immunocompetent adults and that hot tub use may be an important risk factor for this disease pattern.—Authors' Abstract

Lim, A. and Dick, T. Plate-based dormancy culture system for *Mycobacterium smegmatis* and isolation of metronidazole-resistant mutants. FEMS Microbiol. Lett. **200** (2001) 215–219.

Mycobacterium smegmatis is an obligate aerobe. However, growth analyses in oxygen-limited liquid cultures have shown that the bacillus is able to survive anoxia with a half-life of 4 days by shifting down to a drug-resistant, dormant state. Metronidazole is the first lead against dormant bacilli and shows selective toxicity for this physiological state. Here, we report a plate-based dormancy culture system employing anoxic jars for *M. smegmatis.* Its usefulness for the genetic analysis of dormancy was demonstrated by isolating the first metronidazole-resistant mutants. Highly resistant mutants formed slightly yellow (as opposed to creamy) colonies. Furthermore, high-level metronidazole resistance correlated with an increased half-life of 12 days under anoxic conditions. This suggests a link between metronidazole susceptibility and anaerobic survival.—Authors' Abstract

Mollenkopf, H., Groine-Triebkorn, D., Andersen, P., Hess, J. and Kaufmann, S. H. Protective efficacy against tuberculosis of ESAT-6 secreted by a live *Salmonella typhimurium* vaccine carrier strain and expressed by naked DNA. Vaccine **19** (2001) 4028–4035.

We have constructed a recombinant (r) attenuated *Salmonella typhimurium* strain which secretes ESAT-6 of *Mycobacterium tuberculosis* via the hemolysin secretion system of *E. coli*. Additionally, we have ligated ESAT-6 to different commercially available mammalian expression systems for use as naked DNA vaccines. We studied protection against *M. tuberculosis* induced by vaccination with each of these constructs alone or in combination in mice. Vaccination with a single dose of r *S. typhimurium* secreting ESAT-6 reduced numbers of tubercle bacilli in the lungs throughout the course of infection. The combined prime-boost vaccination did not considerably enhance protection.—Authors' Abstract

Nakamura, R. M., Einck, L., Velmonte, M. A., Kawajiri, K., Ang, C. F., Delasllagas, C. E. and Nacy, C. A. Detection of active tuberculosis by an MPB-64 transdermal patch: a field study. Scand. J. Infect. Dis. **33** (2001) 405–407.

The mycobacterial antigen MPB-64 was formulated for delivery in a transdermal patch and used as a diagnostic skin test reagent to detect active tuberculosis (TB) in patients attending a clinic in Manila, The Philippines. The MPB-64 Transdermal Patch was applied to 62 patients, 49 with sputum-positive active disease and 13 who had completed TB chemotherapy, and to 28 non-TB but tuberculin-positive controls. The results were read at 72 hr. The sensitivity of the Transdermal Patch was 87.8%, with an efficacy of 92.9% and a specificity of 100%. The 13 TB patients who had completed 6 months of TB chemotherapy showed different reactions to the MPB64 patch test: those who had completed chemotherapy <4 months before testing were positive; 50% of patients who completed chemotherapy 5 months previously were positive; and those who had completed chemotherapy 7 and 8 months before were negative. All the non-TB controls with positive tuberculin tests were negative to the MPB-64 Transdermal Patch, even at the highest protein dose tested. This test may be a useful method to distinguish active TB patients from TB-infected but asymptomatic individuals. Moreover, the MPB64 Transdermal Patch may be useful to monitor successful chemotherapy.—Authors' Abstract

Neufert, C., Pai, R. K., Noss, E. H., Berger, M., Boom, W. H. and Harding, C. V. *Mycobacterium tuberculosis* 19-kDa lipoprotein promotes neutrophil activation. J. Immunol. **167** (2001) 1542–1549.

Certain microbial substances, e.g., lipopolysaccharide (LPS), can activate neutrophils or prime them to enhance their response to other activating agents, e.g., N-formyl-methionyl-leucyl-phenylalanine (fMLP). We investigated the role of the *Mycobacterium tuberculosis* (MTB) 19-kDa lipoprotein in activation of human neutrophils. MTB 19-kDa lipoprotein initiated phenotypic changes characteristic of neutrophil activation, including down-regulation of CD62 ligand (L-selectin) and up-regulation of CD35 (CR1) and CD11b/CD18 (CR3, Mac-1). In addition, exposure of neutrophils to MTB 19-kDa lipoprotein enhanced the subsequent oxidative burst in response to fMLP as assessed by oxidation of dihydrorhodamine 123 (determined by flow cytometry). LPS also produced these effects with similar kinetics, but an oligodeoxynucleotide containing a CpG motif failed to induce any priming or activation response. Although the effects of LPS required the presence of serum, neutrophil activation by MTB 19-kDa lipoprotein occurred independently of serum factors, suggesting the involvement of different receptors and signalling mechanisms for LPS and MTB 19-kDa lipoprotein. Thus, MTB 19-kDa lipoprotein serves as a pathogen-associated molecular pattern that promotes neutrophil priming and activation.—Authors' Abstract

Ng, J. C., Foley, P. A., Crouch, R. B. and Baker, C. S. A case of severe actinic prurigo successfully treated with thalidomide. Australas J. Dermatol. **42** (2001) 192–195.

Actinic prurigo is an uncommon and usually persistent idiopathic photodermatosis with typical human leukocyte antigen (HLA)

associations (HLA-DR4, particularly subtypes DRB1*0407 and DRB1*0401). Although its mechanism of action is not clearly understood, thalidomide has been shown to be particularly efficacious in treating actinic prurigo, among other conditions. A 31 year-old Australian woman who had suffered actinic prurigo for most of her life was treated with two courses of thalidomide (50–100 mg nocte) over consecutive summers. Remission was observed after cessation of the second course of thalidomide and had continued 4 years later. Abnormalities in the cutaneous response to ultraviolet radiation at the time of diagnosis, detected by monochromator phototesting, reverted to normal following treatment.—Authors' Abstract

Orme, I. M. The search for new vaccines against tuberculosis. J. Leukoc. Biol. **70** (2001) 1–10.

The failure of the BCG vaccine for tuberculosis in large, controlled clinical trials, coupled with the gradual consensus that it is mostly ineffective in preventing adult pulmonary disease in endemic areas, has led to a concerted effort to develop a new generation of vaccines. This work is ongoing in a variety of areas, including DNA vaccines, subunit vaccines, recombinant vaccines, and auxotrophic vaccines. Several such candidates are giving promising results in mouse and guinea pig, aerosol-challenge infection models and should move to clinical trials in the near future.—Author's Abstract

Peters, W., Scott, H. M., Chambers, H. F., Flynn, J. L., Charo, I. F. and Ernst, J. D. Chemokine receptor 2 serves an early and essential role in resistance to *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. U.S.A. **98** (2001) 7958–7963.

Although the protective cellular immune response to *Mycobacterium tuberculosis* requires recruitment of macrophages and T lymphocytes to the site of infection, the signals that regulate this trafficking have not been defined. We investigated the role of C-C chemokine receptor 2 (CCR2)-dependent cell recruitment in the protective response to *M. tuberculosis*. CCR2–/– mice died early after infection and had 100-fold more bacteria in their lungs than did CCR2+/+ mice. CCR2–/– mice exhibited an early defect in macrophage recruitment to the lung and a later defect in recruitment of dendritic cells and T cells to the lung. CCR2–/– mice also had fewer macrophages and dendritic cells recruited to the mediastinal lymph node (MLN) after infection. T cell migration through the MLN was similar in CCR2–/– and CCR2+/+ mice. However, T cell priming was delayed in the MLNs of the CCR2–/– mice, and fewer CD4+ and CD8+ T cells primed to produce IFN-gamma accumulated in the lungs of the CCR2–/– mice. These data demonstrate that cellular responses mediated by activation of CCR2 are essential in the initial immune response and control of infection with *M. tuberculosis*.—Authors' Abstract

Pethe, K., Alonso, S., Biet, F., Delogu, G., Brennan, M. J., Locht, C. and Menozzi, F. D. The heparin-binding haemagglutinin of *M. tuberculosis* is required for extrapulmonary dissemination. Nature **412** (2001) 190–194.

Tuberculosis remains the world's leading cause of death due to a single infectious agent, *Mycobacterium tuberculosis,* with 3 million deaths and 10 million new cases per year. The infection initiates in the lungs and can then spread rapidly to other tissues. The availability of the entire *M. tuberculosis* genome sequence and advances in gene disruption technologies have led to the identification of several mycobacterial determinants involved in virulence. However, no virulence factor specifically involved in the extrapulmonary dissemination of *M. tuberculosis* or *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) hbhA gene encoding the heparin-binding hemagglutinin adhesin (HBHA) markedly affects mycobacterial interactions with epithelial cells, but not with macrophage-like cells. When nasally administered to mice, the mutant strains were severely impaired in spleen colonization, but not in lung colonization. Coating wild-type mycobacteria with anti-HBHA antibodies also impaired dissemina-

tion after intranasal infection. These results provide evidence that adhesins such as HBHA are required for extrapulmonary dissemination, and that interactions with nonphagocytic cells have an important role in the pathogenesis of tuberculosis. They also suggest that antibody responses to HBHA may add to immune protection against tuberculosis.—Authors' Abstract

Rajkumar, S. V. Current status of thalidomide in the treatment of cancer. Oncology **15** (2001) 867–874; 877–879.

Tumor angiogenesis is a critical factor in the growth and metastasis of most malignant neoplasms. Thalidomide (Thalomid), banned from clinical use in the 1960s because of severe teratogenicity, has been shown to possess antiangiogenic properties. A recent clinical trial of antiangiogenic therapy with thalidomide demonstrated significant activity in a group of patients with relapsed refractory myeloma. Although its mechanism of action remains unclear, several trials have since confirmed that thalidomide is active in 25% to 35% of patients with relapsed myeloma. As a result, thalidomide had reemerged in clinical practice and is now actively being studied in the treatment of several cancers. Major toxicities associated with the use of thalidomide include constipation, sedation, skin rash, fatigue, and peripheral neuropathy. This article summarizes the current status of thalidomide therapy in cancer.—Author's Abstract

Roach, D. R., Martin, E., Bean, A. G., Rennick, D. M., Briscoe, H. and Britton, W. J. Endogenous inhibition of antimycobacterial immunity by IL-10 varies between mycobacterial species. Scand. J. Immunol. **54** (2001) 163–170.

Interleukin-10 (IL-10) is an immunoregulatory cytokine that inhibits both Th1-like T-cell responses and macrophage activation. Deficiency of IL-10 has been associated with increased Th1-like CD4+ T-cell responses and increased clearance of some intracellular pathogens, however, its role in mycobacterial infections is controversial. In order to examine the effects of mycobacterial virulence on the outcome of infection we compared infection with *Mycobacterium avium* and virulent *Mycobacterium tuberculosis* in C57BL/6 IL-10 –/– mice. *M. avium* infection in IL-10 –/– mice resulted in sustained increases in interferon-gamma (IFN-γ)-secreting T-cell responses and was associated with the increased clearance of *M. avium* from the liver and lung. By contrast, *M. tuberculosis* infection in IL-10 –/– mice led to a transient increase in IFN-γ T-cell responses at 4 weeks postinfection, with reduced bacterial burden in the lungs. This was not sustained so that by 8 weeks there was no difference to wild-type (WT) mice. *In vitro* infection of IL-10 –/– macrophages with *M. avium,* but not *M. tuberculosis,* led to an increased IL-12 production. Therefore, endogenous IL-10 exerts a significant inhibition on specific IFN-γ T-cell responses to *M. avium* infection, however, this effect is short-lived during the *M. tuberculosis* infection, and fails to influence the long-term course of infection.—Authors' Abstract

Rowland, T. L., McHugh, S. M., Deighton, J., Ewan, P. W., Dearman, R. J. and Kimber, I. Differential effect of thalidomide and dexamethasone on the transcription factor NF-kappa B. Int. Immunopharmacol. **1** (2001) 49–61.

Thalidomide was initially used as a sedative during pregnancy but was withdrawn from the market due to its teratogenic effects. *In vitro* studies have shown that thalidomide inhibits tumor necrosis factor-alpha (TNF-α) mRNA expression and protein production by mitogen-stimulated macrophages and activated T cells. Even at the highest concentration (10^{-1} mM) tested, however, TNF-α levels are inhibited only partially and the mechanism of action is unknown. In the present investigations, we have examined the influence of thalidomide on nuclear levels of NF-kappa B in human peripheral blood mononuclear cells (PBMC) following activation with mitogen or phorbol myristate acetate (PMA)/ionophore. Dexamethasone was used as a positive control due to its well-characterized mechanism of action and NF-kappa B-mediated

effects on TNF-α expression. PBMC from healthy human volunteers were stimulated optimally with phytohemagglutinin (PHA) or PMA/ionophore in the presence of 10^{-1}–10^{-5} mM thalidomide or dexamethasone, concentrations that displayed a range of inhibitory effects on TNF-α production. Cells were harvested at varying time points and nuclear extracts prepared. Nuclear levels of NF-kappa B were measured using electrophoretic mobility shift assays (EMSA) with a radiolabelled DNA probe specific for NF-kappa B. Results were analyzed using optical densitometry. Nuclear levels of NF-kappa B were found to be unaffected by thalidomide at all concentrations tested, including concentrations (10^{-1}–10^{-3} mM) that exhibited significant inhibition of TNF-α protein and mRNA expression. In concurrent experiments, dexamethasone was found to reduce NF-kappa B expression in a dose-dependent manner with maximal inhibition at the highest dose tested (10^{-1} mM). TNF-α gene expression is controlled by at least three separate transcription factors that are involved in binding to the promoter region. These observations suggest that thalidomide does not act directly on NF-kappa B and therefore inhibits TNF-α production through another independent mechanism.—Authors' Abstract

Schaeffer, M. L., Agnihotri, G., Kallender, H., Brennan, P. J. and Lonsdale, J. T. Expression, purification, and characterization of the *Mycobacterium tuberculosis* acyl carrier protein, AcpM. Biochim. Biophys. Acta **1532** (2001) 67–78.

Mycolic acids are generated in *Mycobacterium tuberculosis* as a result of the interaction of two fatty acid biosynthetic systems: the multifunctional polypeptide, FASI, in which the acyl carrier protein (ACP) domain forms an integral part of the polypeptide, and the dissociated FASII system, which is composed of monofunctional enzymes and a discrete ACP (AcpM). In order to characterize enzymes of the FASII system, large amounts of AcpM are required to generate substrates such as holo-AcpM, malonyl-AcpM and acyl-AcpM. The *M. tuberculosis* acpM gene was over-expressed in *Escherichia coli* and AcpM purified, yielding approximately 15–20 mg/l of culture. Analysis of AcpM by mass spectrometry, N-terminal sequencing, amino acid analysis, and gas chromatography indicated the presence of 3 species, apo-, holo-, and acyl-AcpM, the former comprising up to 65% of the total pool. The apo-AcpM was purified away from the *in vivo* generated holo- and acyl-forms, which were inseparable and heterogeneous with respect to acyl chain lengths. Once purified, we were able to convert apo-AcpM into holo- and acyl-forms. These procedures provide the means for the preparation of the large quantities of AcpM and derivatives needed for characterization of the purified enzymes of the mycobacterial FASII system.—Authors' Abstract

Schoeman, J. F., Ravenscroft, A. and Hartzenberg, H. B. Possible role of adjunctive thalidomide therapy in the resolution of a massive intracranial tuberculous abscess. Childs. Nerv. Syst. **17** (2001) 370–372.

We present the case of a young child who developed a massive tuberculous abscess of the posterior fossa while being treated for pulmonary tuberculosis. Clinical improvement after surgical excision of the abscess was followed by recurrence of symptoms of acutely raised intracranial pressure on standard antituberculosis and corticosteroid therapy. Magnetic resonance (MR) imaging of the brain showed that a multiloculated abscess had developed anterior to the excision site of the original abscess. The recurring abscess was partly excised and drained but could not be removed completely because of its proximity to the brain stem.

Thalidomide, a potent inhibitor of tumor necrosis factor alpha (TNF-α), was added to the treatment regimen and resulted in marked clinical improvement with resolution of the abscess within 4 months. The remaining CT lesion had the appearance of a small granuloma. Both the clinical and the radiological response was maintained after 1 year of antituberculosis treatment.—Authors' Abstract

Serbina, N. V. and Flynn, J. L. CD8+ T

cells participate in the memory immune response to *Mycobacterium tuberculosis.* Infect. Immun. **69** (2001) 4320–4328.

The contribution of CD8+ T cells to the control of tuberculosis has been studied primarily during acute infection in mouse models. Memory or recall responses in tuberculosis are less well-characterized, particularly with respect to the CD8 T-cell subset. In fact, there are published reports that CD8+ T cells do not participate in the memory immune response to *Mycobacterium tuberculosis.* We examined the CD8+ T-cell memory and local recall response to *M. tuberculosis.* To establish a memory immunity model, C57BL/6 mice were infected with *M. tuberculosis,* followed by treatment with anti-mycobacterial drugs and prolonged rest. The lungs of memory immune mice contained CD4+ and CD8+ T cells with the cell surface phenotype characteristic of memory cells (CD69(low) CD25(low) CD44(high)). At 1 week postchallenge with *M. tuberculosis* via aerosol, ≥30% of both CD4+ and CD8+ T cells in the lungs of immune mice expressed the activation marker CD69 and could be restimulated to produce interferon-gamma (IFN-γ). In contrast, <6% of T cells in the lungs of naive challenged mice were CD69+ at 1 week postchallenge, and IFN-γ production was not observed at this time point. CD8+ T cells from the lungs of both naive and memory mice after challenge were cytotoxic toward *M. tuberculosis*-infected macrophages. Our data indicate that memory and recall immunity to *M. tuberculosis* is comprised of both CD4+ and CD8+ T lymphocytes and that there is a rapid response of both subsets in the lungs following challenge.—Authors' Abstract

Sherman, D. R., Voskuil, M., Schnappinger, D., Liao, R., Harrell, M. I. and Schoolnik, G. R. Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding alpha-crystallin. Proc. Natl. Acad. Sci. U.S.A. **98** (2001) 7534–7539.

Unlike many pathogens that are overtly toxic to their hosts, the primary virulence determinant of *Mycobacterium tuberculosis* appears to be its ability to persist for years or decades within humans in a clinically latent state. Since early in the 20th century latency has been linked to hypoxic conditions within the host, but the response of *M. tuberculosis* to a hypoxic signal remains poorly characterized. The *M. tuberculosis* alpha-crystallin (acr) gene is powerfully and rapidly induced at reduced oxygen tensions, providing us with a means to identify regulators of the hypoxic response. Using a whole genome microarray, we identified >100 genes whose expression is rapidly altered by defined hypoxic conditions. Numerous genes involved in biosynthesis and aerobic metabolism are repressed, whereas a high proportion of the induced genes have no known function. Among the induced genes is an apparent operon that includes the putative two-component response regulator pair Rv3133c/Rv3132c. When we interrupted expression of this operon by targeted disruption of the upstream gene Rv3134c, the hypoxic regulation of acr was eliminated. These results suggest a possible role for Rv3132c/3133c/3134c in mycobacterial latency.—Authors' Abstract

Short, S. C., Traish, D., Dowe, A., Hines, F., Gore, M. and Brada, M. Thalidomide as an anti-angiogenic agent in relapsed gliomas. N. Neurooncol. **51** (2001) 41–45.

Background: Thalidomide (alpha-phthalimidoglutarimide), a synthetic sedative drug, has anti-angiogenic properties due to inhibition of growth-factor mediated neovascularization and has been shown to inhibit tumor growth in experimental solid tumor models.

Aim: To assess response of recurrent malignant gliomas to thalidomide.

Methods: Eighteen patients with recurrent gliomas were enrolled to an open, nonrandomized phase II trial between October 1997 and December 1999. All patients had failed following treatment with radiotherapy and chemotherapy with PCV and/or temozolomide regimens. Eleven patients had high-grade gliomas *de novo* and 7 high-grade gliomas following transformation of low-grade gliomas. Thalidomide was prescribed at 100 mg/day p.o. continuously.

Response was assessed at 4-weekly intervals. Disease progression was defined as neurological deterioration and/or radiological evidence of increased tumor size. Treatment was discontinued at the time of disease progression, or if toxicity occurred, or at patients' request.

Results: Thalidomide was prescribed for a median of 42 days (range 7–244). Treatment was discontinued due to toxicity (peripheral sensory neuropathy) in 1 patient. Six patients died before response could be fully assessed and are classified as nonresponders. Of 12 who continued treatment for more than 4 weeks, 1 patient had clinical and radiological response (PR), 2 patients had stable disease for 2 and 4 months respectively and 9 patients had disease progression. The median survival from the start of thalidomide was 2.5 months.

Conclusion: The efficacy of thalidomide in terms of response in recurrent gliomas is low, with a partial response rate of only 6%. Future studies should investigate thalidomide in combination with other agents and at an earlier stage of disease. Methods to assess anti-angiogenic properties such as changes in tumor vasculature could be employed as initial surrogate end-points in the investigation of efficacy.—Authors' Abstract

Singhal, S. and Mehta, J. Thalidomide in cancer: potential uses and limitations. BioDrugs **15** (2001) 163–172.

In addition to immunomodulatory and cytokine-modulatory properties, thalidomide has antiangiogenic activity. It has been investigated in a number of cancers including multiple myeloma, myelodysplastic syndromes, gliomas, Kaposi's sarcoma, renal cell carcinoma, advanced breast cancer, and colon cancer. Its role has been best explored in myeloma, where, at daily doses of 100 mg to 800 mg, it is remarkably active, causing clinically meaningful responses in one-third of extensively pretreated patients and in over half of patients treated early in the course of the disease. It also acts synergistically with corticosteroids and chemotherapy in myeloma. Thalidomide produces improvement of cytopenias characteristic of myelodysplastic syndrome, resulting in the reduction or elimination of transfusion dependence in some patients. Responses have also been seen in one-third of patients with Kaposi's sarcoma, in a small proportion of patients with renal cell carcinoma and high grade glioma and, in combination with irinotecan, in some patients with colon cancer. Thalidomide is being investigated currently in a number of clinical trials for cancer. Drowsiness, constipation and fatigue are common adverse effects seen in 75% of patients. Symptoms of peripheral neuropathy and skin rash are seen in 30%. A minority of patients experience bradycardia and thrombotic phenomena. Despite the high frequency of adverse effects, those severe enough to necessitate cessation of therapy are seen in only 10% to 15% of patients. A therapeutic trial of thalidomide should be considered in all patients with myeloma who are unresponsive to or relapse after standard therapy. In other malignant diseases, the most appropriate way to use the drug is in the setting of well-designed clinical trials. In the absence of access to such studies, thalidomide could be considered singly or in combination with standard therapy in patients with no meaningful therapeutic options.—Authors' Abstract

Tanghe, A., Content, J., Van Vooren, J. P., Portaels, F. and Huygen, K. Protective efficacy of a DNA vaccine encoding antigen 85A from *Mycobacterium bovis* BCG against Buruli ulcer. Infect. Immun. **69** (2001) 5403–5411.

Buruli ulcer, caused by *Mycobacterium ulcerans,* is characterized by deep and necrotizing skin lesions, mostly on the arms and legs. Together with tuberculosis and leprosy, this mycobacterial disease has become a major health problem in tropical and subtropical regions, particularly in central and western Africa. No specific vaccine is available for Buruli ulcer. There is, however, evidence in the literature that suggests a cross-reactive protective role of the tuberculosis vaccine *M. bovis* BCG. To identify potential mechanisms for this cross-protection, we identified and characterized the *M. ulcerans* homologue of the important protective mycobacterial antigen 85 (Ag85A) from BCG. The homologue is

well conserved in *M. ulcerans*, showing 84.1% amino acid sequence identity and 91% conserved residues compared to the sequence from BCG. This antigen was sufficiently conserved to allow cross-reactive protection, as demonstrated by the ability of *M. ulcerans*-infected mice to exhibit strong cellular immune responses to both BCG and its purified Ag85 complex. To further address the mechanism of cross-reactive protection, we demonstrate here that prior vaccination with either BCG or plasmid DNA encoding BCG Ag85A is capable of significantly reducing the bacterial load in the foot pads of *M. ulcerans*-infected mice, as determined by Ziehl-Neelsen staining and by actual counting of CFU on 7H11 Middlebrook agar. Together, the results reported here support the potential of a cross-protective Ag85-based future vaccine against tuberculosis, Buruli ulcer, and leprosy.—Authors' Abstract

Thomas, G. A., Williams, D. L. and Soper, S. A. Capillary electrophoresis-based heteroduplex analysis with a universal heteroduplex generator for detection of point mutations associated with rifampin resistance in tuberculosis. Clin. Chem. **47** (2001) 1195–1203.

Background: Slab gel heteroduplex analysis (HDA), a popular scanning method for genetic mutations, uses DNA fragments typically generated by PCR to create homo- and heteroduplex molecules with conformational differences and sequence-dependent electrophoretic profiles. Use of a universal heteroduplex generator (UGH) enhances the subtle variations caused by single-base substitutions.

Methods: The HDA-UHG slab gel format was modified for an efficient capillary-based method. The effect of staining dyes TOPRO5 and YOPRO1 on the analysis of heteroduplexes was studied, as well as ultraviolet absorbance and laser-induced fluorescence (LIF) detection methods. In addition, the entangled polymers hydroxyethyl cellulose, methyl cellulose, and linear polyacrylamide were evaluated as separation matrices.

Results: This assay was able to detect the presence of *Mycobacterium tuberculosis* and its rifampin susceptibility directly from clinical specimens in dramatically reduced analysis time (30 min vs 2.5 hr). Optimized conditions included 0.3% methyl cellulose as the separation matrix, on-line staining using 1 micromol/L YOPRO1, and LIF detection for quantitative and reproducible analysis of single-base substitutions in the rifampin resistance-determining region of rpoB that give rise to the rifampin-resistant phenotype of *M. tuberculosis*. We generated 95% confidence limits using the wild-type sequence and used these limits to determine rifampin susceptibility in samples.

Conclusions: Capillary electrophoresis, combined with the HDA-UHG technique, may be of value for rapid and efficient clinical diagnosis of rifampin-resistant tuberculosis strains.—Authors' Abstract

Ulmer, J. B. Tuberculosis DNA vaccines. Scand. J. Infect. Dis. **33** (2001) 246–248.

DNA vaccines have been the subject of intense investigation for the past 10 years, during which time several tuberculosis (TB) DNA vaccines have been shown to confer protective immunity in animal models. So far, proof of principle for priming of immune responses by a naked DNA vaccine (malaria) has been demonstrated in humans, but potency remains a significant limitation. However, new DNA vaccine formulations and delivery systems are being developed with markedly improved potency in animal models. Therefore, there is a clear path to the human clinical testing of TB DNA vaccines.—Author's Abstract

Viader-Salvado, M., Garza-Gonzalez, E., Valdez-Leal, R., de Los Angeles Del Bosque-Moncayo, M., Tijerina-Menchaca, R. and Guerrero-Olazaran, M. Mycolic acid index susceptibility method for *Mycobacterium tuberculosis*. J. Clin. Microbiol. **38** (2001) 2642–2645.

A rapid drug susceptibility test to measure the susceptibility of *Mycobacterium tuberculosis* to isoniazid (INH) and rifampin (RIF) using clinical isolates and a newly defined mycolic acid index (MAI) was evaluated. A total of 200 clinical iso-

lates of *M. tuberculosis* were tested for susceptibility or resistance to INH and RIF by the MAI susceptibility and indirect-proportion methods. Overall, there was agreement between the 2 methods for 398 (99.5%) of the 400 total tests. Specifically, the sensitivity of the MAI susceptibility method for INH and RIF was 97.6% and 100%, respectively. The specificity and positive predictive value were 100% for both drugs, and the negative predictive value for INH and RIF was 98.3% and 100%, respectively. In conclusion, the MAI susceptibility method described here can be used for rapid drug susceptibility testing of *M. tuberculosis* clinical isolates within 5 days after clinical isolates are incubated in the presence or absence of an antituberculosis drug.—Authors' Abstract

Xing, Z. The hunt for new tuberculosis vaccines: anti-TB immunity and rational design of vaccines. Curr. Pharm. Des. **7** (2001) 1015–1037.

Tuberculosis (TB) remains a leading infectious cause of death worldwide. Apparently, the current BCG vaccine that has been used for 80 years, has failed to control the TB epidemic. Hunting for improved TB vaccine formulations represents a daunting task to the TB research community. Anti-TB host defense requires T cell-mediated immunity and we are in desperate need of understanding how to develop a new generation of TB vaccines that are able to provoke potent and long-lasting protective cell-mediated immunity, different from almost all the vaccines currently in use. It is of importance to successful TB vaccine development to identify the key cellular and molecular events governing the generation of anti-TB immunity, but unfortunately little has been understood as to why 90% of infected humans never develop active TB. However, waiting would not help us to win the battle and an ever-intensifying effort is being made to develop various new formulations according to the immunology that we have been learning, in large part, from experimental models. This review article attempts to unite the current understanding of anti-TB immunity with the rational design of anti-TB vaccines. It examines what may have confounded the immunogenicity of current BCG vaccine and the major obstacles to successful development of TB vaccines. It also discusses antigen presentation, activation of Th1 and Tc1 cells, anti-TB immune effectors and the generation of memory T cells. The vaccine section describes four types of major TB vaccines under development: mycobacterial-, subunit-, plasmid DNA- and viral-based vaccines. A special section is dedicated to the rationale and current design of cytokine-based adjuvant formulations for TB vaccines. We also take this opportunity to introduce our recent development in cytokine transgene adjuvanted BCG vaccination and recombinant adenoviral-based TB vaccines.—Author's Abstract

Zangari, M., Anaissie, E., Barlogie, B., Badros, A., Desikan, R., Gopal, A. V., Morris, C., Toor, A., Siegel, E., Fink, L. and Tricot, G. Increased risk of deep-vein thrombosis in patients with multiple myeloma receiving thalidomide and chemotherapy. Blood **98** (2001) 1614–1615.

The occurrence of deep-vein thrombosis (DVT) in patients with newly diagnosed multiple myeloma, who were randomly assigned to receive identical induction chemotherapy with or without thalidomide, are reported in this study. The two study arms were comparable with respect to key myeloma prognostic factors and known risk factors for DVT. One hundred patients received induction chemotherapy including 4 cycles of continuous infusion of combinations of dexamethasone, vincristine, doxorubicin, cyclophosphamide, etoposide, and cisplatin, and each patient completed at least one induction cycle. DVT developed in 14 of 50 patients (28%) randomly assigned to receive thalidomide but in only 2 of 50 patients (4%) not given the agent ($p = 0.002$). All episodes of DVT occurred during the first 3 cycles of induction. Administration of thalidomide was resumed safely in 75% of patients receiving anticoagulation therapy. Thus, thalidomide given in combination with multiagent chemotherapy and dexamethasone is associated with a significantly increased risk of DVT, which ap-

pears to be safely treated with anticoagulation and does not necessarily warrant discontinuation of thalidomide.—Authors' Abstract

Zhang, Y., Yang, Y., Woods, A., Cotter, R. J. and Sun, Z. Resuscitation of dormant *Mycobacterium tuberculosis* by phospholipids or specific peptides. Biochem. Biophys. Res. Commun. **284** (2001) 542–547.

The presence of dormant tubercle bacilli presents a major problem for tuberculosis treatment. The culture supernatant of *Mycobacterium tuberculosis* was previously shown to resuscitate dormant bacilli *in vitro*. Here we report identification of active components as phospholipids and a tuberculosis protein Rv1174c. Remarkably, dormant bacilli from a 1 year-old culture which failed to form any colonies could be resuscitated with peptides derived from Rv1174c and formed 10^{5-7} colonies/ml. This finding represents the first unambiguous demonstration of resuscitation of dormant tubercle bacilli *in vitro* and may have implication for the study of mycobacterial dormancy and the design of novel strategies for improved treatment of tuberculosis.—Authors' Abstract