Volume 72, Number 1
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

Coker, R. J. Public health impact of detention of individuals with tuberculosis: systematic literature review. Public Health **117(4)** (2003) 281–287.

As the world witnesses ever-increasing rates of tuberculosis, particularly of drug-resistant strains affecting some of society's most marginalized individuals, policy makers and legislators may again visit the statute books in order to strengthen their armamentarium of tools to protect public health. This paper assesses the evidence in support of the sanction to detain those with tuberculosis who are perceived as posing a public health threat, and shows that little research has been conducted to inform policy, probably because traditional epidemiological methods used to assess the impact of interventions are not feasible.—Author's Abstract

Lu, H. M. [A study on the measures against Hansen's disease during the Colonial Korea]. Nippon Ishigaku Zasshi **49(2)** (2003) 223–261. [Article in Japanese].

In order to evaluate the measures taken against Hansen's diseases during the colonial era in Korea, from 1910–1945, I analyzed both Korean and Japanese materials and carried out field research. The Korean government-general established a hospital in 1916 and executed measures against Hansen's disease. These efforts can be divided into three periods. At first they started as a part of colonial policy. Then, in the middle period, with the change of Japanese policy on Hansen's disease, a Korean association was established and the Hansen's Disease Prevention Act was issued in Korea, aiming at the compulsory isolation of lepers. In the later period, during the war, the inmates were forced into an extremely severe environment and deprived of their human rights. My study shows that their policies changed greatly with the passage of time. Though they started them to relieve the suffering of the lepers in the beginning, they turned to be compulsory isolation of the patients in the later period and to the violation of their human rights.—Author's Abstract

Muller, P., Frederic, M., Salzer, B., and Strobel, M. [Leprosy in Guadeloupe (French West Indies): declining disease,

increasing diagnosis delay]. Ann. Dermatol. Venereol. **130(6–7)** (2003) 619–621. [Article in French].

INTRODUCTION: Endemic for nearly three centuries, leprosy is declining in Guadeloupe: its prevalence has decreased by 75 p. 100 over the last decade. Because it has become rare, it may well be overlooked. PATIENTS AND METHODS: Retrospective study of all the new cases of leprosy diagnosed in Guadeloupe from May 1996 to May 2001. RESULTS: In 10 cases of the 41 reported in this study, diagnosis had been delayed by more than 6 months. Nine of these 10 cases presented with classical clinical signs. The mean delay before diagnosis in these 10 cases was of 22 months (range: 7 to 36 months); the mean number of consultations with a physician before the final diagnosis was of 3.2 (range: 2–8). The mean age at the time of diagnosis in patients in whom diagnosis was delayed was significantly greater than those in whom diagnosis was confirmed rapidly (55 *vs* 37 years). DISCUSSION: In Guadeloupe, one patient out of 4 presenting with leprosy is diagnosed with a delay of more than 6 months, despite a classical clinical presentation. This is deleterious to the patients and health economics. The patients in whom diagnosis was delayed were older. This epidemiological tendency appears inherent to this form of "residual leprosy". The present rareness of the disease is responsible for a lack of knowledge of the disease by the physicians through lack of experience. This phenomenon is also observed for syphilis and measles. There is a real risk of underestimation or erroneous diagnosis.—Authors' Abstract

Chemotherapy

Fenniche, S., Maalej, S., Fekih, L., Hassene, H., Belhabib, D., and Megdiche, M. L. [Manifestations of rifampicin-induced hypersensitivity] Presse Med. **32(25)** (2003) 1167–1169. [Article in French].

INTRODUCTION: The side effects of rifampicine due to an immunoallergic mechanism are rare and usually observed during discontinued treatment or administration of high doses. OBSERVATIONS: An immediate hypersensitivity reaction with anaphylactic manifestations and increase in IgE occurred in a 39-year-old man suffering from resistant tuberculosis. The reaction occurred within the first hour following a low dose of rifampicin administered in a desensitization attempt, the outcome of which was favorable after administration of corticosteroids and antihistamines. A type II hypersensitivity reaction occurred in a 76 year-old male patient in the form of thrombopenia on D76 of a twice weekly treatment, diagnosed because of hemoptysis with normalization of platelet level on withdrawal of rifampicin. An immune complex hypersensitivity reaction was responsible for hepato-renal failure on D68 of twice weekly treatment and required permanent withdrawal of rifampicin and dialysis, which led to subsequent improvement. COMMENTS: These clinical cases illustrate the variability of the hypersensitivity mechanisms observed with rifampicin, the difficulty in imputability tests and methods for immunological confirmation, the interest of continuous treatment which avoids a certain number of these accidents, and that of desensitization during immediate hypersensitive reactions which permits the continuation this major anti-tuberculosis drug. —Authors' Abstract

Field, S. K., and Cowie, R. L. Treatment of *Mycobacterium avium*-intracellulare complex lung disease with a macrolide, ethambutol, and clofazimine. Chest **124(4)** (2003) 1482–1486.

BACKGROUND: *Mycobacterium avium*-intracellulare (MAC) causes progressive lung disease. Recommended treatment regimens include a macrolide and a rifamycin, but drug intolerance and relapse

after treatment is completed often limit successful therapy. METHODS: Consecutive individuals referred for treatment of MAC lung disease were treated with a regimen that included either clarithromycin, 500 mg bid, or azithromycin, 250 mg/d, on weekdays; ethambutol, 15 mg/kg/d; and clofazimine, 100 mg/d. The intention was to treat patients for a minimum of 12 months. The diagnosis of MAC lung disease was confirmed by multiple positive sputum culture findings in patients with typical symptoms and radiologic findings. RESULTS: Thirty patients (27 women and 3 men; mean age, 70 ± 9.4 years [S.D.]) were treated. A total of 22 of the patients reported adverse effects from clarithromycin or azithromycin. Intolerance of clarithromycin resulted in the withdrawal of four patients before sputum conversion. The remaining patients continued treatment for an average of 10 months, and sputum findings converted to negative in all 26 patients (87%). One patient died of unrelated causes while still receiving therapy, and five patients (19%) relapsed an average of 17 months after treatment was completed. CONCLUSIONS: Treatment with a macrolide, ethambutol, and clofazimine was successful in 20 of 30 patients (67%) with MAC lung disease and is a reasonable alternative to rifamycin-containing regimens.—Authors' Abstract

Jaso, A., Zarranz, B., Aldana, I., and Monge, A. Synthesis of new 2-acetyl and 2-benzoyl quinoxaline 1,4-di-N-oxide derivatives as anti-*Mycobacterium tuberculosis* agents. Eur. J. Med. Chem. **38(9)** (2003) 791–800.

A series of 2-acetyl and 2-benzoyl-6(7)-substituted quinoxaline 1,4-di-N-oxide derivatives were synthesized and evaluated for *in vitro* antituberculosis activity. The results show that 2-acetyl-3-methylquinoxaline 1,4-di-N-oxide derivatives with chlorine, methyl or methoxy group in position 7 of the benzene moiety (compounds 2, 4 and 6, respectively) and unsubstituted (3) have good antitubercular activity, exhibiting EC(90)/MIC values between 0.80 and 4.29. In conclusion, the potency, selectivity and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity.—Authors' Abstract

Jha, S. H., Reddy, J. A., and Dave, J. K. Dapsone-induced acute pancreatitis. Ann. Pharmacother. **37(10)** (2003) 1438–1440.

OBJECTIVE: To report a case of acute pancreatitis associated with dapsone use. CASE SUMMARY: An 87-year-old white man was prescribed dapsone for dermatitis herpetiformis. Four weeks later, he developed acute abdominal pain requiring hospitalization. The patient had elevated serum amylase and lipase levels. Laboratory test results for other possible etiologies were negative. His symptoms resolved when dapsone was discontinued. Dapsone was reintroduced for exacerbation of dermatitis herpetiformis 4 months later. The patient again had severe abdominal pain with high amylase and lipase levels. Again, symptoms resolved following dapsone discontinuation. DISCUSSION: Only 1 other case of pancreatitis associated with dapsone was found in a MEDLINE search of the literature (1966–June 2003) using the key terms dapsone and pancreatitis. An objective causality assessment revealed dapsone to be a probable cause of acute pancreatitis, based on the Naranjo probability scale. Drugs should always be considered as causative factors for pancreatitis in patients without known risk factors. Dapsone is increasingly used as a second line of treatment of Pneumocystis carinii pneumonia (PCP). The recognition of dapsone-induced pancreatitis is of particular importance in these patients. CONCLUSIONS: While dapsone is traditionally used for the treatment of leprosy and dermatitis herpetiformis, its use for PCP prophylaxis, malaria, brown recluse spider bites, and acne is not uncommon. Pancreatitis is an uncommon adverse effect of dapsone, and greater awareness of this association will prompt a high index of suspicion in an appropriate clinical setting. Further reporting of cases and clinical research of drug-induced pancreatitis is indicated.—Authors' Abstract

Lowary, T. L. Recent progress towards the identification of inhibitors of mycobacterial cell wall polysaccharide biosynthesis. Mini. Rev. Med. Chem. **3(7)** (2003) 689–702.

Mycobacterial infections have recently attracted significant attention from international health agencies due to the resurgence of these diseases worldwide. This review summarizes the recent work directed towards the identification of new antituberculosis agents that act by inhibiting mycobacterial cell wall polysaccharide biosynthesis.—Author's Abstract

Man, H. W., Corral, L. G., Stirling, D. I., and Muller, G. W. Alpha-fluoro-substituted thalidomide analogues. Bioorg. Med. Chem. Lett. **13(20)** (2003) 3415–3417.

Thalidomide, (1), has made a remarkable comeback from its days of a sedative with teratogenic properties due to its ability to selectively inhibit TNF-alpha, a key pro-inflammatory cytokine and its clinical benefit in the treatment of cancer. Thalidomide contains one chiral center and is known to be chirally unstable under *in vitro* and *in vivo* conditions. It has been hypothesized that different biological properties are associated with each isomer. Thus, chirally stable analogues of thalidomide, alpha-fluorothalidomide, (3) and alpha-fluoro-4-aminothalidomide (4) were prepared by electrophilic fluorination. Analogue 3 was found to be cytotoxic and did not inhibit TNF-alpha production in LPS stimulated hPBMC below toxic concentrations. On the other hand, 4 was non-cytotoxic at the tested concentrations and was found to be 830-fold more potent than thalidomide as TNF-alpha inhibitor.—Authors' Abstract

Mani, C., Selvakumar, N., Gajendiran, N., Panigrahi, B., Venkatesan, P., and Narayanan, P. R. Standardization and evaluation of DNA-lanthanide fluorescence spectroscopy for determining rifampicin resistance in *Mycobacterium tuberculosis* clinical isolates. Int. J. Tuberc. Lung Dis. **7(9)** (2003) 873–878.

SETTING: Tuberculosis Research Centre, Chennai. OBJECTIVE: To rapidly identify multidrug-resistant *Mycobacterium tuberculosis* using a novel method. DESIGN: A new assay, based on DNA-lanthanide fluorescence, was standardized and evaluated using 93 each of coded rifampicin-resistant and rifampicin-sensitive *M. tuberculosis* clinical isolates for the correct identification of rifampicin resistance. The results obtained by the new assay were compared with the conventional results. RESULTS AND CONCLUSION: The new assay gave a sensitivity and specificity of 88% and 85%, respectively. It is simple, easy to perform and requires 48 hours for the drug susceptibility results to be available after obtaining the primary culture.—Authors' Abstract

Niemi, M., Backman, J. T., Neuvonen, M., and Neuvonen, P. J. Effect of rifampicin on the pharmacokinetics and pharmacodynamics of nateglinide in healthy subjects. Br. J. Clin. Pharmacol. **56(4)** (2003) 427–432.

AIMS: Our aim was to investigate the effects of rifampicin on the pharmacokinetics and pharmacodynamics of nateglinide, a novel short-acting antidiabetic drug. METHODS: In a randomized crossover study with two phases, 10 healthy volunteers took 600 mg rifampicin or placebo orally once daily for 5 days. On day 6 of both phases, they ingested a single 60 mg dose of nateglinide. Plasma nateglinide and blood glucose concentrations were measured for up to 7 h postdose. RESULTS: Rifampicin decreased the mean AUC(0,7 h) of nateglinide by 24% (range 5–53%; $p = 0.0009$) and shortened its half-life ($t(1/2)$) from 1.6 to 1.3 h ($p = 0.001$). However, the peak plasma nateglinide concentration (Cmax) remained unchanged. The AUC(0,7 h) of the M7 metabolite of nateglinide was decreased by 19% ($p = 0.002$) and its $t(1/2)$ was shortened from 2.1 to 1.6 h by rifampicin ($p = 0.008$). Rifampicin had no significant effect on the blood glucose-lowering effect of nateglinide. CONCLUSIONS: Rifampicin modestly decreased the plasma concentrations of nateglinide proba-

bly by inducing its oxidative biotransformation. In some patients, rifampicin may reduce the blood glucose-lowering effect of nateglinide.—Authors' Abstract

Park, J. Y., Kim, K. A., Park, P. W., Park, C. W., and Shin, J. G. Effect of rifampin on the pharmacokinetics and pharmacodynamics of gliclazide. Clin. Pharmacol. Ther. **74(4)** (2003) 334–340.

OBJECTIVE: Our objective was to investigate the effect of rifampin (INN, rifampicin) on the pharmacokinetics and pharmacodynamics of gliclazide, a sulfonylurea antidiabetic drug. METHOD: In a randomized 2-way crossover study with a 4-week washout period, 9 healthy Korean subjects were treated once daily for 6 days with 600 mg rifampin or with placebo. On day 7, a single dose of 80 mg gliclazide was administered orally. Plasma gliclazide, blood glucose, and insulin concentrations were measured. RESULTS: Rifampin decreased the mean area under the plasma concentration-time curve for gliclazide by 70% ($p < 0.001$) and the mean elimination half-life from 9.5 to 3.3 hours ($p < 0.05$). The apparent oral clearance of gliclazide increased about 4-fold after rifampin treatment ($p < 0.001$). A significant difference in the blood glucose response to gliclazide was observed between the placebo and rifampin phases. CONCLUSION: The effect of rifampin on the pharmacokinetics and pharmacodynamics of gliclazide suggests that rifampin affects the disposition of gliclazide in humans, possibly by the induction of cytochrome P450 2C9. Concomitant use of rifampin with gliclazide can considerably reduce the glucose-lowering effects of gliclazide.—Authors' Abstract

Peng, J., Hu, J. F., Kazi, A. B., Li, Z., Avery, M., Peraud, O., Hill, R. T., Franzblau, S. G., Zhang, F., Schinazi, R. F., Wirtz, S. S., Tharnish, P., Kelly, M., Wahyuono, S., and Hamann, M. T. Manadomanzamines A and B: a novel alkaloid ring system with potent activity against mycobacteria and HIV-1. J. Am. Chem. Soc. **125(44)** (2003) 13382–13386.

Two novel alkaloids, named manadomanzamines A (1) and B (2), were isolated from an Indonesian sponge *Acanthostrongylophora* sp. (Haplosclerida: Petrosiidae). Their structures were elucidated and shown to be a novel organic skeleton related to the manzamine type alkaloids. Their absolute configuration and conformation were determined by CD, NOESY, and molecular modeling analysis. The microbial community analysis for the sponge that produces these unprecedented alkaloids has also been completed. Manadomanzamines A (1) and B (2) exhibited strong activity against *Mycobacterium tuberculosis* (Mtb) with MIC values of 1.9 and 1.5 mug/mL, respectively. Manadomanzamines A and B also exhibit activities against human immunodeficiency virus (HIV-1) and AIDS opportunistic fungal infections.—Authors' Abstract

Phetsuksiri, B., Jackson, M., Scherman, H., McNeil, M., Besra, G. S., Baulard, A. R., Slayden, R. A., DeBarber, A. E., Barry, C. E., 3rd, Baird, M. S., Crick, D. C., and Brennan, P. J. Unique mechanism of action of the thiourea drug isoxyl on *Mycobacterium tuberculosis*. J. Biol. Chem. **278(52)** (2003) 53123–53130.

The thiourea isoxyl (thiocarlide; 4,4′-diisoamyloxydiphenylthiourea) is known to be an effective anti-tuberculosis drug, active against a range of multidrug-resistant strains of *Mycobacterium tuberculosis* and has been used clinically. Little was known of its mode of action. We now demonstrate that isoxyl results in a dose-dependent decrease in the synthesis of oleic and, consequently, tuberculostearic acid in *M. tuberculosis* with complete inhibition at 3 microg/ml. Synthesis of mycolic acid was also affected. The anti-bacterial effect of isoxyl was partially reversed by supplementing growth medium with oleic acid. The specificity of this inhibition pointed to a Delta9-stearoyl desaturase as the drug target. Development of a cell-free assay for Delta9-desaturase activity allowed direct demonstration of the inhibition of oleic acid synthesis by isoxyl. Interestingly, sterculic acid, a known inhibitor of Delta9-desaturases, emulated the effect of isoxyl

on oleic acid synthesis but did not affect mycolic acid synthesis, demonstrating the lack of a relationship between the two effects of the drug. The three putative fatty acid desaturases in the *M. tuberculosis* genome, desA1, desA2, and desA3, were cloned and expressed in *Mycobacterium bovis* BCG. Cell-free assays and whole cell labeling demonstrated increased Delta9-desaturase activity and oleic acid synthesis only in the desA3-overexpressing strain and an increase in the minimal inhibitory concentration for isoxyl, indicating that DesA3 is the target of the drug. These results validate membrane-bound Delta9-desaturase, DesA3, as a new therapeutic target, and the thioureas as anti-tuberculosis drugs worthy of further development.—Authors' Abstract

Queiroz, R. H., Pereira, R. C., Gotardo, M. A., Cordeiro, D. S., and Melchior, E., Jr. Determination of clofazimine in leprosy patients by high-performance liquid chromatography. J. Anal. Toxicol. **27(6)** (2003) 377–380.

An original, simple, specific, and rapid high-performance liquid chromatography assay for the determination of clofazimine in human plasma is presented. The procedure consists of extracting the drug and the internal standard (medazepam) from 0.5 mL plasma with dichloromethane/diisopropyl ether (1:1, v/v) at pH 3.0, after precipitating the proteins with methanol. The drugs were then quantitated on a reversed-phase C8 using a mobile phase consisting of a mixture of methanol/0.25 N sodium acetate buffer at pH 3.0 (74:26, v/v). The flow-rate and wavelength were set at 1 mL/min and 286 nm, respectively. The precision, linearity, and limit of quantitation of the method were within acceptable limits. The method was considered adequate and could be applied in studies involving blood level monitoring and pharmacokinetics in leprosy patients.—Authors' Abstract

Salamat, A., and Watson, H. G. Drug-induced methaemoglobinaemia presenting with angina following the use of dapsone. Clin. Lab. Haematol. **25(5)** (2003) 327–328.

Anemia may result in tissue hypoxia which may induce or exacerbate symptoms of ischemia. Tissue hypoxia may however also result from the presence of hemoglobin with altered oxygen-binding characteristics. Drug-induced methemoglobinaemia in which oxygen is irreversibly bound to hemoglobin may complicate the use of some common drugs. This condition may result in severe tissue hypoxia, which is rapidly and cheaply reversed by methylene blue.—Authors' Abstract

Walter, M. C., Lochmuller, H., Schlotter-Weigel, B., Meindl, T., and Muller-Felber, W. Successful treatment of muscle sarcoidosis with thalidomide. Acta Myol. **22(1)** (2003) 22–25.

A 36-year-old male patient suffered from therapy resistant sarcoidosis with long-standing contractures, myopathy, skin lesions and pulmonary changes. Low-dose therapy with thalidomide (50 mg/day) was well tolerated, and the patient rapidly improved. Thalidomide was effective for muscular, cutaneous, and pulmonary involvement in our patient. This is the first report on the efficacy of thalidomide in muscle sarcoidosis. Therefore, thalidomide may become a second-line agent in patients with severe muscle and skin involvement, but further studies are warranted.—Authors' Abstract

Zhang, Y., Wade, M. M., Scorpio, A., Zhang, H., and Sun, Z. Mode of action of pyrazinamide: disruption of *Mycobacterium tuberculosis* membrane transport and energetics by pyrazinoic acid. J. Antimicrob. Chemother. **52(5)** (2003) 790–795.

Pyrazinamide is an important sterilizing drug that shortens tuberculosis (TB) therapy. However, the mechanism of action of pyrazinamide is poorly understood because of its unusual properties. Here we show that pyrazinoic acid, the active moiety of pyrazinamide, disrupted membrane energetics and inhibited membrane transport function in *Mycobacterium tuberculosis*. The preferential activity of pyrazinamide

against old non-replicating bacilli correlated with their low membrane potential and the disruption of membrane potential by pyrazinoic acid and acid pH. Inhibitors of membrane energetics increased the antituberculous activity of pyrazinamide. These findings shed new light on the mode of action of pyrazinamide and may help in the design of new drugs that shorten therapy.—Authors' Abstract

Clinical Sciences

Chu, N. H., Zhu, L. Z., Yie, Z. Z., Yuan, S. L., Wang, J. Y., Xu, J. L., and Ma, L. P. [A controlled clinical study on the efficacy of recombinant human interleukin-2 in the treatment of pulmonary tuberculosis] Zhonghua Jie He He Hu Xi Za Zhi **26(9)** (2003) 548–551. [Article in Chinese].

OBJECTIVE: To study and evaluate the efficacy and safety of recombinant human interleukin-2 (IL-2) in the treatment of pulmonary tuberculosis. METHODS: Two hundred and nine cases with re-treated *Mycobacterium tuberculosis*-positive pulmonary tuberculosis were randomly divided into a trial group (106 cases, treated with 3PaZ (TH)L(2)VE(AK) + IL-2/4PaL(2)V) and a control group (103 cases, treated with 3PaZ(TH)L(2)VE(AK)/4PaL(2)V). The efficacy of 203 cases was available for evaluation when the course was completed (trial group 103 cases, control group 100 cases). RESULTS: The sputum smear-negative conversion rates at the 1st and the 2nd month of therapy were 33.3% and 69.4% in the trial group, and 7.2% and 44.9% in the control group ($p < 0.01$). At the completion of the therapy, the X-ray resolution rates were 64.1% and 36.0% respectively for the trial and the control groups, the difference being significant ($p < 0.001$). There were significant differences in CD(4) T cells, the ratio of CD(4)/CD(8) and NK cells between the two groups ($p < 0.01$). The level of soluble interleukin-2 receptor (sIL-2R) was significantly different between the two groups after treatment for 3 months ($p < 0.05$). IL-2 associated side effects were rare and mild. CONCLUSION: As an effective and relatively safe biological agent, IL-2 can be added to the standard chemotherapy for pulmonary tuberculosis.—Authors' Abstract

Corbett, E. L., Charalambous, S., Fielding, K., Clayton, T., Hayes, R. J., De Cock, K. M, and Churchyard, G. J. Stable incidence rates of tuberculosis (TB) among human immunodeficiency virus (HIV)-negative South African gold miners during a decade of epidemic HIV-associated TB. J. Infect. Dis. **188(8)** (2003) 1156–1163.

During the last decade, annual tuberculosis (TB) case-notification rates increased 4-fold, to >4000 cases/100,000 person-years, in the study workforce, among whom prevalence of human immunodeficiency virus (HIV) was 30% in 2000. Three separate cohort studies, totalling 6454 HIV-negative participants, were combined and analyzed for time trends. Observed incidence of TB varied between 962 (1991–1994) and 1589 (1999–2000) cases/100,000 person-years ($p = 0.17$, test for trend). There was, however, a progressive increase in age, and, for each period, older age was associated with increased incidence rates of TB ($p < 0.001$). Having adjusted for age differences, there was no significant association between incidence of TB and calendar period ($p = 0.81$, test for trend). Relative to 1991–1994, multivariate-adjusted incidence-rate ratios were 0.94, for 1995–1997, 0.96, for 1998–1999, and 1.05, for 1999–2000. Preventing a secondary epidemic of TB among HIV-negative individuals may be achievable with conventional means, even in settings with a high burden of HIV-associated TB.v.—Authors' Abstract

Da Silva Souza, C., and Bacha, J. T. Delayed diagnosis of leprosy and the potential role of educational activities in Brazil. Lepr. Rev. **74(3)** (2003) 249–258.

This study identifies possible obstacles to the early diagnosis of leprosy. Semi-structured interviews were held with 40 patients at a secondary health service in upstate Sao Paulo, Brazil. The data concerning the sample were: 75% males, age range 13 to 76 years, 85% with elementary school education, 85% multibacillary. Skin lesions associated with sensory alterations had been noticed by 55% of the patients; 32.5% of the patients had been misdiagnosed as having conditions other than leprosy. The diagnosis was made 1 year after the awareness of signs/symptoms in 55% of the patients. In this group, 54% had impairment grade 1, while 23% had no disabilities. Forty-five percent of all patients interviewed had some information about the disease prior to diagnosis. Eleven patients (27.5%) had previous contact with leprosy patients, but this did not prevent late diagnosis in 64%. After the disease was confirmed, about half of the interviewed patients (47.5%) showed mainly positive feelings due to the prospect of treatment and cure. Our results suggest that misdiagnoses and unawareness of the disease were the main factors that influenced the delayed diagnosis. We consider the effective involvement of various segments of society, particularly the integration and partnership of the public health services and health education centres to be valuable tools for the planning and execution of educational activities directed at risk groups and the community.—Authors' Abstract

de Freitas, M. R., Nascimento, O. J., Quaglino, E. A., Oliveira, A., and Hahn, M. D. Small-fiber polyneuropathy in leprosy without skin changes: study of 17 cases. Arq. Neuropsiquiatr. **61(3A)** (2003) 542–546.

Leprosy is one of the most common diseases of the peripheral nerves. In some cases there is only neural involvement without skin changes (neuritic form). The neuropathy has often a distal stocking and glove distribution with thermal and pinprick anesthesia and preservation of proprioception. There is no weakness, the tendon reflexes may be preserved and sometimes the nerves are thickened. We reported 17 patients with a predominantly small-fiber polyneuropathy due to leprosy. All patients had distal temperature and pain anesthesia with different individual variations. The tendon reflexes were normal in seven patients and in eight there was thickening of the nerves. The nerve conduction was normal in three patients. Sural nerve biopsy consisted of: 1) inflammatory infiltrates, 2) vacuolated "foamy" cells, 3) fibrosis of endoneurium, perineurium, and epineurium, 4) partial or total loss of nerve fibers, 5) large number of bacilli. We concluded that in countries where leprosy is frequent, nerve biopsy is an obligatory procedure in patients with predominantly small-fiber polyneuropathy.—Authors' Abstract

Inamadar, A. C., Palit, A., Athanikar, S. B., Sampagavi, V. V., and Deshmukh, N. S. Generalized anetoderma in a patient with HIV and dual mycobacterial infection. Lepr. Rev. **74(3)** (2003) 275–278.

A middle-aged HIV infected man receiving treatment for pulmonary tuberculosis, presented with a febrile illness along with evanescent, erythematous nodular lesions all over the body. On examination, he had features suggestive of lepromatous leprosy with lesions of erythema nodosum leprosum. In addition, there were multiple small, circumscribed areas of slack skin, clinically and histopathologically suggestive of anetoderma. Both leprosy and HIV infection are known to give rise to lesions of anetoderma. Pathogenesis of anetoderma in these infectious conditions is discussed.—Authors' Abstract

Lee, H. N., Embi, C. S., Vigeland, K. M., and White, C. R., Jr. Concomitant pulmonary tuberculosis and leprosy. J. Am. Acad. Dermatol. **49(4)** (2003) 755–757.

Concomitant tuberculosis and leprosy is uncommon, even in endemic countries. We report a patient with borderline lepromatous leprosy and type 1 reversal reaction initially diagnosed while the patient was undergoing treatment for pulmonary tuberculosis. The diagnosis was on the basis of characteristic histopathology and Fite-Farraco stain.—Authors' Abstract

Marques, W., Jr., Foss, N. T., Arruda, A. P., and Barreira, A. A. Near-nerve potential in lepromatous leprosy. Muscle Nerve **28(4)** (2003) 460–463.

In leprosy, sensory action potentials (SAPs) may be normal in spite of clinical sensory loss. This may result from the early involvement of small nerve fibers, which have potentials that are not detected in routine studies. To evaluate this possibility, we used a near-nerve recording technique that records potentials from nerve fibers as small as 4 to 6 microm in diameter. We hypothesized that this technique might increase the sensitivity of nerve conduction studies in detecting leprosy neuropathy. We found the technique to be useful for recording conduction abnormalities in recently diagnosed patients, including those with preserved sensation, suggesting that axonal loss may be the underlying mechanism. Contrary to our hypothesis, however, recording the late SAP components did not improve the sensitivity of nerve conduction studies. We suggest that the late components having normal conduction velocities may be generated by either regenerating or remyelinating abnormal fibers, which have an electrophysiological behavior similar to that of normal 4 to 6-microm-diameter fibers.—Authors' Abstract

Munck, K., and Mandpe, A. H. Mycobacterial infections of the head and neck. Otolaryngol. Clin. North Am. **36(4)** (2003) 569–576.

Mycobacterial infections are grouped into infections caused by *M. tuberculosis* and those caused by the atypical mycobacterial organisms. Tuberculosis is a systemic disease, with cervical lymphadenitis of the head and neck being the most common extrapulmonary manifestation of the disease. It is important to use imaging, histopathologic examination, and culture to differentiate tuberculosis from atypical mycobacterial infections, because treatments differ. Tuberculosis is best treated as a systemic disease, with anti-tuberculosis medication. The atypical infections can be addressed as local infections and are amendable to surgical therapy.—Authors' Abstract

Pimentel, M. I. F., da Costa Nery, J. A., Borges, E., Gonçalves, R. R., and Sarno, E. N. Initial neurological exam of multibacillary leprosy: correlation between the presence of affected nerves and disability present at diagnosis and with the occurence of overt neuritis. An. bras. Dermatol., Rio de Janeiro **78(5)** (2003) 561–568.

BACKGROUND: Disabilities constitute the main problem of leprosy. It is important to identify risk factors involved, so it can be possible the prone patients be followed-up more carefully. OBJECTIVES: To determine if the presence of thick and/or painful peripheral nerves at diagnosis correlates with disabilities already present at the initial examination, as well as with subsequent development of neuritis, during and after multidrug therapy. METHODS: One hundred and three patients with multibacillary forms of leprosy were studied, and we noted the presence of compromised peripheral nerves at diagnosis, the disability grade before treatment (DGBT), and the occurrence of neuritis episodes during and after multibacillary multidrug therapy. RESULTS: The detection of affected peripheral nerves at diagnosis correlated statistically ($p < 0.005$) with the occurrence of disabilities (DGBT >0). It also correlated significantly with the development of neuritis in the follow-up (average of 64.6 months from diagnosis, during and after multidrug therapy). CONCLUSIONS: We emphasize the need of a good examination of peripheral nerve trunks in multibacillary patients at the diagnosis, in order to improve the detection of disabilities already present, and specially to prevent further disabilities. Healthy professionals who deal with leprosy patients must be aware to the initial neurological impairments because those patients are more susceptible to the occurrence of neuritis and neurological sequelae. —Anais Brasileiros de Dermatologia

Tettamanti, A. V., Talanczuk, J. B., Martorano, A., Cahuepé, Massone, C., and Villanueva, C. Tuberculosis verrucosa cutanae. Rev. Argent. Dermatol. **84** (2003) 78–88. [Article in Spanish].

La tuberculosis es una enfermedad infectocontagiosa crónica provocada por el *My-*

cobacterium tuberculosis, el *Mycobacterium bovis* y, en ciertas condiciones, por el bacilo de Calmette-Guerín (BCG), cepa atenuada de *Mycobacterium bovis.*

A partir de la década del 80, hubo un incremento a nivel mundial de los pacientes afectados de tuberculosis pulmonar y extrapulmonar; este aumento en los índices de incidencia se vió asociado a la pandemia provocada por el virus HIV.

La tuberculosis cutánea corresponde al 1% de los casos de tuberculosis extrapulmonar. Esta forma de tuberculosis es de distribución mundial, pero predomina en regiones de clima húmedo y frío. Afecta predominantemente a personas de nivel socioeconómico bajo.

A continuación presentamos un caso de tuberculosis verrucosa cutánea dada su infrecuencia y hacemos una revisión bibliográfica sobre esta patología. —Revista Argentina de Dermatologia

Ustianowski, A. P., and Lockwood, D. N. Leprosy: current diagnostic and treatment approaches. Curr. Opin. Infect. Dis. **16(5)** (2003) 421–427.

PURPOSE OF REVIEW: Leprosy remains an important problem globally and leprosy patients may present to physicians outside leprosy endemic areas. We review the recent biological and clinical advances in leprosy. RECENT FINDINGS: Sequencing the genome has been a major biological advance and will open up new possibilities for research. The three cardinal criteria (anaesthetic skin patches, thickened nerves and acid-fast bacilli in skin smears) have not yet been bettered. Multidrug therapy for leprosy is highly effective with low relapse rates though the optimal duration of therapy for multibacillary patients is unclear. Nerve damage remains a significant problem (in some series only 50% responding to steroid therapy). New treatments for leprosy reactions are needed. Stigma remains a problem but is being combated by patient groups. SUMMARY: Far from being eliminated as a public health problem, leprosy still causes a considerable long-term morbidity in both the developing and developed world. New treatments for leprosy reactions are needed and the optimal length of multidrug therapy required further research.—Authors' Abstract

Immunopathology

Feng, C. G., Scanga, C. A., Collazo-Custodio, C. M., Cheever, A. W., Hieny, S., Caspar, P., and Sher, A. Mice lacking myeloid differentiation factor 88 display profound defects in host resistance and immune responses to *Mycobacterium avium* infection not exhibited by toll-like receptor 2 (TLR2)- and TLR4-deficient animals. J. Immunol. **171(9)** (2003) 4758–4764.

To assess the role of Toll-like receptor (TLR) signaling in host resistance to *Mycobacterium avium* infection, mice deficient in the TLR adaptor molecule myeloid differentiation factor 88 (MyD88), as well as TLR2(–/–) and TLR4(–/–) animals, were infected with a virulent strain of *M. avium*, and bacterial burdens and immune responses were compared with those in wild-type (WT) animals. MyD88(–/–) mice failed to control acute and chronic *M. avium* growth and succumbed 9–14 wk postinfection. Infected TLR2(–/–) mice also showed increased susceptibility, but displayed longer survival and lower bacterial burdens than MyD88(–/–) animals, while TLR4(–/–) mice were indistinguishable from their WT counterparts. Histopathological examination of MyD88(–/–) mice revealed massive destruction of lung tissue not present in WT, TLR2(–/–), or TLR4(–/–) mice. In addition, MyD88(–/–) and TLR2(–/–), but not TLR4(–/–), mice displayed marked reductions in hepatic neutrophil infiltration during the first 2 h of infection. Although both MyD88(–/–) and TLR2(–/–) macrophages showed profound defects in IL-6, TNF, and

IL-12p40 responses to *M. avium* stimulation *in vitro*, *in vivo* TNF and IL-12p40 mRNA induction was impaired only in infected MyD88(–/–) mice. Similarly, MyD88(–/–) mice displayed a profound defect in IFN-gamma response that was not evident in TLR2(–/–) or TLR4(–/–) mice or in animals deficient in IL-18. These findings indicate that resistance to mycobacterial infection is regulated by multiple MyD88-dependent signals in addition to those previously attributed to TLR2 or TLR4, and that these undefined elements play a major role in determining bacterial induced proinflammatory as well as IFN-gamma responses.—Authors' Abstract

Fremond, C. M., Nicolle, D. M., Torres, D. S., and Quesniaux, V. F. Control of *Mycobacterium bovis* BCG infection with increased inflammation in TLR4-deficient mice. Microbes Infect. **5(12)** (2003) 1070–1081.

Live mycobacteria have been reported to signal through several pattern recognition receptors (PRR), among them toll-like receptor 4 (TLR4) and TLR2 *in vitro*. Here, we investigated the role of TLR4 in host resistance to *Mycobacterium bovis* (BCG) infection *in vivo*. *In vitro*, macrophages of TLR4 mutant C3H/HeJ mice infected with BCG expressed lower levels of TNF than controls, and TNF release was further decreased, although not completely absent, in the absence of TLR2. *In vivo*, TLR4 mutant C3H/HeJ and control C3H/HeOUJ mice were infected with BCG ($2 \times 10(6)$ CFU i.v.). Both TLR4 mutant and wild-type mice were able to control the infection and survived 8 months post-BCG infection. Macrophage activation with abundant acid-fast bacilli and expression of inducible nitric oxide synthase (iNOS) and MHC class II antigens was seen in both groups of mice. However, TLR4 mutant mice experienced an arrest of body weight gain and showed signs of increased inflammation, with persistent splenomegaly, increase in granuloma number and augmented neutrophil infiltration. Infection of TLR4-deficient mice with higher doses of BCG (1 and $3 \times 10(7)$ CFU, i.v.) increased the inflammation in spleen and liver, associated with a transient, higher bacterial load in the liver. In summary, TLR4 mutant mice show normal macrophage recruitment and activation, granuloma formation and control of the BCG infection, but this is associated with persistent inflammation. Therefore, TLR4 signaling is not essential for early control of BCG infection, but it may have a critical function in fine tuning of inflammation during chronic mycobacterial infection.—Authors' Abstract

Hasan, Z., Shah, B. H., Mahmood, A., Young, D. B., and Hussain, R. The effect of mycobacterial virulence and viability on MAP kinase signalling and TNF alpha production by human monocytes. Tuberculosis (Edinb). **83(5)** (2003) 299–309.

SETTING: The success of *Mycobacterium tuberculosis* as a human pathogen depends on its ability to tolerate and perhaps manipulate host defense mechanisms. OBJECTIVE: To determine the induction of tumour necrosis factor-alpha (TNF alpha), a central mediator of immunity, by human monocytes infected with virulent *M. tuberculosis*, *M. leprae* and attenuated *M. bovis* BCG. DESIGN: Mycobacteria-induced cellular activation pathways of TNF alpha production was investigated using an inhibitor of protein tyrosine kinase (PTKs) and an inhibitor of mitogen-activated protein (MAP) kinases. RESULTS: TNF alpha production was significantly lower during infection with virulent *M. tuberculosis* than with BCG and this differential response was independent of mycobacterial viability. TNF alpha production involved the PTK and MAP kinase pathways. Reduced TNF alpha induction by *M. tuberculosis* was associated with a reduction in the extent and duration of phosphorylation of extracellular-signal regulated kinases (ERK 1/2). Infection with *M. leprae* triggered low and transient ERK 1/2 activation as well as low TNF alpha production. CONCLUSION: Maintenance of the differential response in both live and heat-killed preparations suggests that the reduced TNF alpha response associated with virulent mycobacteria is due to differences in the presence of components capable of triggering host pattern recognition re-

ceptors, rather than events associated with phagosome trafficking or the active release of intracellular modulators.—Authors' Abstract

Hestvik, A. L., Hmama, Z., and Av-Gay, Y. Kinome analysis of host response to mycobacterial infection: a novel technique in proteomics. Infect. Immun. **71(10)** (2003) 5514–5522.

An array of mammalian phospho-specific antibodies was used to screen for a host response upon mycobacterial infection, reflected as changes in host protein phosphorylation. Changes in the phosphorylation state of 31 known signaling molecules were tracked after infection with live or heat killed *Mycobacterium bovis* BCG or after incubation with the mycobacterial cell wall component lipoarabinomannan (LAM). Mycobacterial infection triggers a signaling cascade leading to activation of stress-activated protein kinase and its subsequent downstream target, c-Jun. Mycobacteria were also shown to inhibit the activation of protein kinase C varepsilon and to induce phosphorylation of proteins not yet known to be involved in mycobacterial infection, such as the cytoskeletal protein alpha-adducin, glycogen synthase kinase 3beta, and a receptor subunit involved in regulation of intracellular Ca(2+) levels. The mycobacterial cell wall component LAM has been identified as a trigger for some of these modulation events.—Authors' Abstract

Kurabachew, M., Enger, O., Sandaa, R. A., Lemma, E., and Bjorvatn, B. Amplified ribosomal DNA restriction analysis in the differentiation of related species of mycobacteria. J. Microbiol. Methods **55(1)** (2003) 83–90.

This study explores the potential of the amplified ribosomal DNA restriction analysis (ARDRA) for intra- and interspecies identification of the genus Mycobacteria. A set of primers was used to amplify part of the 16S and 23S rDNA as well as the 16S–23S rDNA spacer from 121 isolates belonging to 13 different mycobacterial species. Restriction analysis was carried out with five different restriction enzymes, namely CfoI, HaeIII, RsaI, MspI and TaqI. Restriction digestion of the PCR product using CfoI enabled differentiation between 9 of the 13 mycobacterial species, whereas the remaining four enzymes differentiated between 7 of these 13 species. None of the five enzymes distinguished between different isolates of *Mycobacterium tuberculosis* or between species within the *M. tuberculosis* complex i.e., *M. tuberculosis*, *M. bovis*, *M. bovis* BCG and *M. africanum*. Although ARDRA analysis of the 16S–23S rDNA does not seem to have a potential for intraspecies differentiation, it has proven to be a rapid and technically relatively simple method to recognize strains belonging to the *M. tuberculosis* complex as well as to identify mycobacterial species outside this complex.—Authors' Abstract

Rook, G. A., Martinelli, R., and Brunet, L. R. Innate immune responses to mycobacteria and the downregulation of atopic responses. Curr. Opin. Allergy Clin. Immunol. **3(5)** (2003) 337–342.

PURPOSE OF REVIEW: Exposure to certain environmental microorganisms can promote the induction of T regulatory cells via the innate immune system. This review explores the possibility that reduced exposure to such organisms is leading to increased immunoregulatory disorders in a subset of individuals in whom this regulatory T-cell-inducing pathway is less efficient. We concentrate on mycobacteria and on asthma, because these are well documented. RECENT FINDINGS: The blood cells of the children of farmers, who are partly protected from allergies, express increased levels of messenger RNA encoding CD14 and TLR2, and polymorphisms of CD14 are linked to allergic manifestations in some studies. Polymorphisms of TLR2 (which recognizes mycobacterial components in concert with CD14) are involved in the pattern of response to mycobacteria, and in the type of leprosy that develops. Similarly, polymorphisms of Nramp1, which affect the response to mycobacteria, are linked with the diseases of immunodysregulation that are increasing in parallel with allergic disorders. Moreover, congenic

mice bearing different variants of Nramp1 differ in their allergic responses. These parallels are suggestive, in view of the observation that a saprophytic environmental mycobacterium is a potent inducer of regulatory T cells, and has shown significant effects in several phase I/II studies in man. SUMMARY: The components of the innate immune system that are involved in responses to mycobacteria overlap with those implicated in allergic disorders. Polymorphisms might define the subset of individuals who develop immunoregulatory disorders. Understanding the role of the innate immune system will facilitate the design of clinical trials using microbial products.—Authors' Abstract

Rossouw, M., Nel, H. J., Cooke, G. S., Helden, P. D., and van Hoal, E. G. Association between tuberculosis and a polymorphic NFκB binding site in the interferon γ gene. Lancet (British edition) **361(9372)** (2003) 1871–1872.

Interferon γ is believed to be crucial for host defense against many infections. To test the hypothesis that a polymorphism in the gene for interferon γ (*IFNG*) is associated with susceptibility to tuberculosis, we did two independent investigations. In a case-control study of 313 tuberculosis cases, we noted a significant association between a polymorphism (+874A→T) in *IFNG* and tuberculosis in a South African population ($p = 0.0055$). This finding was replicated in a family-based study, in which the transmission disequilibrium test was used in 131 families ($p = 0.005$). The transcription factor NFκB binds preferentially to the +874T allele, which is over-represented in controls. This preferential binding suggests that genetically determined variability in interferon γ and expression might be important for the development of tuberculosis.—Tropical Diseases Bulletin

Villeneuve, C., Etienne, G., Abadie, V., Montrozier, H., Bordier, C., Laval, F., Daffe, M., Maridonneau-Parini, I., and Astarie-Dequeker, C. Surface-exposed glycopeptidolipids of *Mycobacterium smegmatis* specifically inhibit the phagocytosis of mycobacteria by human macrophages. Identification of a novel family of glycopeptidolipids. J. Biol. Chem. **278(51)** (2003) 51291–51300.

Phagocytosis by macrophages represents the early step of the mycobacterial infection. It is governed both by the nature of the host receptors used and the ligands exposed on the bacteria. The outermost molecules of the nonpathogenic *Mycobacterium smegmatis* were extracted by a mechanical treatment and found to specifically and dose dependently inhibit the phagocytosis of both *M. smegmatis* and the opportunistic pathogen *M. kansasii* by human macrophages derived from monocytes. The inhibitory activity was attributed to surface lipids because it is extracted by chloroform and reduced by alkaline hydrolysis but not by protease treatment. Fractionation of surface lipids by adsorption chromatography indicated that the major inhibitory compounds consisted of phospholipids and glycopeptidolipids (GPLs). Mass spectrometry and nuclear magnetic resonance spectroscopy analyses, combined with chemical degradation methods, demonstrated the existence of a novel family of GPLs that consists of a core composed of the long-chain tripeptidyl amino-alcohol with a di-O-acetyl-6-deoxytalosyl unit substituting the allo-threoninyl residue and a 2-succinyl-3,4-di-O-CH3-rhamnosyl unit linked to the alaninol end of the molecules. These compounds, as well as diglycosylated GPLs at the alaninol end and de-O-acylated GPLs, but not the non-serovar-specific di-O-acetylated GPLs, inhibited the phagocytosis of *M. smegmatis* and *M. avium* by human macrophages at a few nanomolar concentration without affecting the rate of zymosan internalization. At micromolar concentrations, the native GPLs also inhibit the uptake of both *M. tuberculosis* and *M. kansasii*. De-O-acylation experiments established the critical roles of both the succinyl and acetyl substituents. Collectively, these data provide evidence that surface-exposed mycobacterial glycoconjugates are efficient competitors of the interaction between macrophages and mycobacteria and, as such, could represent pharmacological tools for the control of mycobacterial infections.—Authors' Abstract

Viveiros, M., Leandro, C., and Amaral, L. Mycobacterial efflux pumps and chemotherapeutic implications. Int. J. Antimicrob. Agents. **22(3)** (2003) 274–278.

The demonstration of the existence of active efflux pumps in mycobacteria raises the question of whether or not these can increase in number and activity rendering wild-type mycobacteria increasingly resistant to a given antibiotic. This could be a mechanism by which mutated resistant strains become better fit to the selective environment. *Mycobacterium tuberculosis* genome analysis reveals several genes encoding putative drug efflux pumps. During the course of tuberculosis chemotherapy many of these pumps might play a role in the survival of the mycobacterial populations. Compounds capable of inactivating these pumps could improve anti-tuberculous therapeutics.—Authors' Abstract

Immunopathology (Leprosy)

Bleharski, J. R., Li, H., Meinken, C., Graeber, T. G., Ochoa, M. T., Yamamura, M., Burdick, A., Sarno, E. N., Wagner, M., Rollinghoff, M., Rea, T. H., Colonna, M., Stenger, S., Bloom, B. R., Eisenberg, D., and Modlin, R. L. Use of genetic profiling in leprosy to discriminate clinical forms of the disease. Science **301(5639)** (2003) 1527–1530.

See Current Literature, Molecular and Genetic Studies, p. 112.

Shankarkumar, U., Ghosh, K., Badakere, S., and Mohanty, D. Novel HLA Class I Alleles Associated with Indian Leprosy Patients. J. Biomed. Biotechnol. **2003(3)** (2003) 208–211.

Convincing results on HLA Class II associations have been reported, however data on HLA class I association are limited and inconsistent from studies in Leprosy. We present here the HLA A, B, and C allele distribution by molecular high resolution PCR-SSOP technique in 32 leprosy patients compared with the 67 controls, from the same ethnic background. The significant results from the present study were a significant increase in frequency of HLA A*0206, A*1102, B*4016, B*5110, Cw*0407, and Cw*0703 was observed when compared to controls. A striking decrease in the frequency of HLA A*0101, Cw*04011, and Cw*0602 leprosy patients was observed when compared to the controls. Further haplotype A*1102-B*4006-Cw*1502 was significantly increased among the lepromatous leprosy patients when compared to the controls. It seems that HLA class I alleles play vital roles in disease association/pathogenesis with leprosy among Indians.—Authors' Abstract

Sridevi, K., Khanna, N., Chattree, V., Pal, P. C., Haq, W., and Rao, D. N. Reversal of T cell anergy in leprosy patients: *in vitro* presentation with *Mycobacterium leprae* antigens using murabutide and Trat peptide in liposomal delivery. Int. Immunopharmacol. **3(12)** (2003) 1589–1600.

Mycobacterium leprae, the causative agent of leprosy resides and multiplies within the host monocytes and macrophages, thereby evading host immune system. Cell-mediated immune response (CMI) plays a vital role as evidenced from the high CMI in BT/TT (borderline and tuberculoid) patients and conversely low in BL/LL (borderline and lepromatous) patients. In the present study, an attempt was made to immunomodulate the anergized T cells of lepromatous leprosy patients by presenting the mycobacterial antigen in combination with T cell adjuvant, murabutide (active analog of muramyl' dipeptide, MDP-BE) and a Trat peptide (T cell epitope of Integral membrane protein (Trat) from Escherichia coli) in particulate form (liposomes) or soluble form (media). PBMNC

of normal, BT/TT and BL/LL were stimulated *in vitro* with five mycobacterial antigens (Ag) in the following formulations, Ag, Ag+murabutide, Ag+murabutide+Trat peptide either in liposomes or in medium. All the five antigen(s) when delivered in liposomes containing murabutide and Trat peptide showed a very high lymphoproliferative response ($p < 0.001$) in all the three groups. IFN-gamma and IL-2 were significantly ($p < 0.001$) high in these culture supernatants compared to IL-10 and IL-4 confirming a shift from CD4+Th2 to Th1 response in leprosy patients with particulate mode of antigen presentation. Interestingly, PBMNC derived from lepromatous patients also showed consistent T cell proliferation with all the formulations. Further, the mechanism of liposomal processing of antigens was studied using different inhibitors that interfere at different stages of antigen presentation. Results indicate that this study may pave way for an immunotherapeutic approach for reverting the anergic T cells of lepromatous patients to proliferating T cells with the release of Th1 cytokines thereby restoring the CMI response in these patients.—Authors' Abstract

Stefani, M. M., Martelli, C. M., Gillis, T. P., Krahenbuhl, J. L.; and the Brazilian Leprosy Study Group. *In situ* type 1 cytokine gene expression and mechanisms associated with early leprosy progression. J. Infect. Dis. **188(7)** (2003) 1024–1031.

We explored the prognostic value of in situ cytokine patterns in 39 patients with single-skin-lesion paucibacillary leprosy before single-dose therapy, with 3 years of follow-up. Interferon (IFN)-gamma, interleukin (IL)-12, IL-10, IL-4, tumor necrosis factor (TNF)-alpha, and macrophage inflammatory protein (MIP)-1alpha mRNA was quantified in skin biopsy samples at diagnosis, and *Mycobacterium leprae* DNA was detected in 51.4% of cases. Type 1 immunity predominance with measurable IFN-gamma and undetectable IL-4, which is indicative of effective cell-mediated immunity, is compatible with both the reversal reactions (33.3%) and the resolution of lesions (64.1%) observed. A positive correlation between IL-12 and IFN-gamma indicated type 1 polarization via IL-12. The TNF-alpha/MIP-1alpha correlation implied the TNF-alpha induction of chemokines, which is important for granuloma formation. Positive correlations between key regulatory cytokines-IL-10 and IFN-gamma, IL-10 and IL-12, and IL-10 and TNF-alpha-suggests that there may be some level of an intralesional pro- or anti-inflammatory mechanism essential in avoiding immunopathology.—Authors' Abstract

Tadesse, A., Taye, E., Sandoval, F., and Shannon, E. J. Thalidomide does not modify the ability of cells in leprosy patients to incorporate [3H]-thymidine when incubated with *M. leprae* antigens. Lepr. Rev. **74(3)** (2003) 206–214.

The immune response in reversal reaction, (RR) and in erythema nodosum leprosum (ENL) is characterized in vitro by an enhancement in lymphocyte blast transformation against *M. leprae*. As thalidomide is an effective treatment for ENL, this study assessed the effect of this drug on these phenomena. Mononuclear cells from patients attending the clinic at ALERT and from healthy staff were cultured for 5 days with integral *M. leprae* (IMl), or a modified Dharmendra antigen (Dhar), or PPD from *M. tuberculosis*. In one set of cultures, thalidomide was added once at the initiation of the culture; in the other set thalidomide was added a second time (2×), 18 h prior to harvesting the cells. The mononuclear cells, in the absence of thalidomide, from healthy staff, borderline tuberculoid patients (BT) and BT patients in RR (BT/RR) incorporated [3H]-thymidine best when cultured with PPD > Dhar > *M. leprae*. The cells from patients with ENL did not respond well to the *M. leprae* antigens. Thalidomide (2×) enhanced proliferation to Dhar in the BTRR group (Wilcoxon signed rank test, $p < 0.05$). No significant changes occurred for the other groups. Comparing PPD-stimulated cells treated with thalidomide once to those treated with thalidomide twice, thalidomide (2×) suppressed incorporation of [H3]-thymidine by the PPD-stimulated ($p < 0.05$) as well as IMl-stimulated ($p < 0.05$) cells in the healthy

staff group. In the Dhar-stimulated cells from the healthy staff thalidomide significantly suppressed TNF-alpha ($p < 0.05$). A mixed effect was seen within and between the other groups, but there was a trend for thalidomide to suppress TNF-alpha induced by the *M. leprae*, Dhar and PPD antigens.–Authors' Abstract

Immunopathology (Tuberculosis)

Abarca-Rojano, E., Rosas-Medina, P., Zamudio-Cortez, P., Mondragon-Flores, R., and Sanchez-Garcia, F. J. *Mycobacterium tuberculosis* virulence correlates with mitochondrial cytochrome c release in infected macrophages. Scand. J. Immunol. **58(4)** (2003) 419–427.

Mitochondria are at the center of molecular events involved in energy production, cell survival and apoptosis. Mitochondrial membrane potential (Deltapsim) is maintained by cellular catabolic reactions and the electron transport chain of which cytochrome c is a constituent, whereas the proton leak pathway, ATP synthesis and turnover consume it. Mitochondrial alterations such as a drop in Deltapsim, swelling and cytochrome c release have been observed in apoptosis. However, there is a paucity of information concerning mitochondrial function in the course of intracellular infections, a process that must certainly induce stress on the host cell. This work analyses the effect that two strains of mycobacteria of opposing virulence have on the mitochondria of murine macrophages in the early stages of infection. It was found that infection of J774 cells with both *Mycobacterium tuberculosis* H37Ra and *M. tuberculosis* H37Rv readily induced changes in Deltapsim as well as in mitochondrial morphology at the ultrastructural level. In addition, an increase in cytosolic ATP was found at 24 hr post infection with both strains of *M. tuberculosis*. Interestingly, only *M. tuberculosis* H37Rv was able to induce cytochrome c release from mitochondria to the cytosol, thus suggesting the occurrence in *M. tuberculosis* H37Rv of a specific factor(s) capable of regulating cytochrome c translocation. The precise role of cytochrome c release in the context of a mycobacterial infection remains to be elucidated.—Authors' Abstract

Al-Attiyah, R., Mustafa, A. S., Abal, A. T., Madi, N. M., and Andersen, P. Restoration of mycobacterial antigen-induced proliferation and interferon-gamma responses in peripheral blood mononuclear cells of tuberculosis patients upon effective chemotherapy. FEMS Immunol. Med. Microbiol. **38(3)** (2003) 249–256.

Peripheral blood mononuclear cells (PBMC) were obtained from culture-proven tuberculosis (TB) patients before and after 2 and 6 months of chemotherapy with a multi-drug regimen. PBMC were tested for cellular responses in antigen-induced proliferation and interferon-gamma (IFN-gamma) assays in response to complex mycobacterial antigens (whole cell *Mycobacterium bovis* BCG and *M. tuberculosis*, cell walls and short-term culture filtrate [ST-CF] of *M. tuberculosis*), fractionated ST-CF antigens (fractions F1–F10) and ESAT-6. The responses in TB patients before anti-TB treatment were low (median stimulation index (SI) = 1–7, median delta IFN-gamma = 0–12 U ml(–1), and percent responders = 13–67%) to all the antigenic preparations. Following the administration of anti-TB chemotherapy for 2 months, there were significant ($p < 0.05$) improvements in the cellular responses (median SI = 9–76, median delta IFN-gamma = 3–70 U ml(–1), and percent responders = 33–100%) to most of the antigenic preparations tested. However, concanavalin A-induced proliferation responses of PBMC from the same patients before and after 2 months of chemotherapy were high and comparable (median SI = 101 and 114, respectively, $p > 0.05$, 100% responders). A further increase in IFN-gamma responses (median delta IFN-gamma = 14–250 U ml(–1) and percent responders = 43–100%) to mycobacterial antigens was observed in patients

receiving chemotherapy for 6 months. Among the ST-CF fractions, F1 and F2 containing low molecular mass proteins resulted in the highest responses, whereas ESAT-6 showed responses comparable to these fractions only in a minority of the patients. HLA-DR typing of these patients showed heterogeneity in the expression of molecules encoded by HLA-DRB genes. These results show that effective chemotherapy restores cellular responses of TB patients to a large number of *M. tuberculosis* antigens, which could be useful in monitoring the efficacy of anti-TB treatment.—Authors' Abstract

Capuano, S. V. 3rd, Croix, D. A., Pawar, S., Zinovik, A., Myers, A., Lin, P. L., Bissel, S., Fuhrman, C., Klein, E., and Flynn, J. L. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. Infect. Immun. **71(10)** (2003) 5831–5844.

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Chackerian, A. A., and Behar, S. M. Susceptibility to *Mycobacterium tuberculosis*: lessons from inbred strains of mice. Tuberculosis (Edinb). **83(5)** (2003) 279–285.

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Choudhary, R. K., Mukhopadhyay, S., Chakhaiyar, P., Sharma, N., Murthy, K. J., Katoch, V. M., and Hasnain, S. E. PPE antigen Rv2430c of *Mycobacterium tuberculosis* induces a strong B-cell response. Infect. Immun. **71(11)** (2003) 6338–6343.

The variation in sequence and length in the C-terminal region among members of the unique PE (Pro-Glu) and PPE (Pro-Pro-Glu) protein families *of Mycobacterium tuberculosis* is a likely source of antigenic variation, giving rise to the speculation that these protein families could be immunologically important. Based on in silico analysis, we selected a hypothetical open reading frame (ORF) encoding a protein belonging to the PPE family and having epitopes with predictably higher antigenic indexes. Reverse transcriptase PCR using total RNA extracted from *in vitro*-cultured *M. tuberculosis* H37Rv generated an mRNA product corresponding to this gene, indicating the expression of this ORF (Rv2430c) at the mRNA level. Recombinant protein expressed in *Escherichia coli* was used to screen the sera of *M. tuberculosis*-infected patients, as well as those of clinically healthy controls (N = 10), by enzyme-linked immunosorbent assay. The panel of patient sera comprised sera from fresh infection cases (category 1; N = 32), patients with relapsed tuberculosis (category 2; N = 30), and extrapulmonary cases (category 3; N = 30). Category 2 and 3 sera had strong antibody responses to the PPE antigen, equal to or higher than those to other well-known antigens, such as Hsp10 or purified protein derivative (PPD). However, a higher percentage of patients belonging to category 1, as opposed to clinically healthy controls, showed stronger antibody response against the PPE protein when probed with anti-immunoglobulin M (IgM) (71 *vs* 37.5%) or anti-IgG (62.5 versus 28.12%). Our results reveal that this PPE ORF induces a strong B-cell response compared to that generated by *M. tuberculosis* Hsp10 or PPD, pointing to the immunodominant nature of the protein.—Authors' Abstract

Cirilli, M., Zheng, R., Scapin, G., and Blanchard, J. S. The three-dimensional structures of the *Mycobacterium tuberculosis* dihydrodipicolinate reductase-NADH-2,6-PDC and -NADPH-2,6-PDC complexes. Structural and mutagenic analysis of relaxed nucleotide specificity. Biochemistry **42(36)** (2003) 10644–10650.

Dihydrodipicolinate reductase (DHPR) catalyzes the reduced pyridine nucleotide-dependent reduction of the alpha,beta-unsaturated cyclic imine, dihydrodipicolinate, to generate tetrahydrodipicolinate. This enzyme catalyzes the second step in the bacterial biosynthetic pathway that generates meso-diaminopimelate, a component of bacterial cell walls, and the amino acid

L-lysine. The *Mycobacterium tuberculosis* dapB-encoded DHPR has been cloned, expressed, purified, and crystallized in two ternary complexes with NADH or NADPH and the inhibitor 2,6-pyridinedicarboxylate (2,6-PDC). The structures have been solved using molecular replacement strategies, and the DHPR-NADH-2,6-PDC and DHPR-NADPH-2,6-PDC complexes have been refined against data to 2.3 and 2.5 A, respectively. The *M. tuberculosis* DHPR is a tetramer of identical subunits, with each subunit composed of two domains connected by two flexible hinge regions. The N-terminal domain binds pyridine nucleotide, while the C-terminal domain is involved in both tetramer formation and substrate/inhibitor binding. *The M. tuberculosis* DHPR uses NADH and NADPH with nearly equal efficiency based on V/K values. To probe the nature of this substrate specificity, we have generated two mutants, K9A and K11A, residues that are close to the 2′-phosphate of NADPH. These two mutants exhibit decreased specificity for NADPH by factors of 6- and 30-fold, respectively, but the K11A mutant exhibits 270% of WT activity using NADH. The highly conserved structure of the nucleotide fold may permit other enzyme's nucleotide specificity to be altered using similar mutagenic strategies.—Authors' Abstract

Cosma, C. L., Sherman, D. R., and Ramakrishnan, L. The secret lives of the pathogenic mycobacteria. Annu. Rev. Microbiol. **57** (2003) 641–676.

Pathogenic mycobacteria, including the causative agents of tuberculosis and leprosy, are responsible for considerable morbidity and mortality worldwide. A hallmark of these pathogens is their tendency to establish chronic infections that produce similar pathologies in a variety of hosts. During infection, mycobacteria reside in macrophages and induce the formation of granulomas, organized immune complexes of differentiated macrophages, lymphocytes, and other cells. This review summarizes our understanding of Mycobacterium-host cell interactions, the bacterial-granuloma interface, and mechanisms of bacterial virulence and persistence. In addition, we highlight current controversies and unanswered questions in these areas.—Authors' Abstract

Cowley, S. C., and Elkins, K. L. CD4(+) T cells mediate IFN-gamma-independent control of *Mycobacterium tuberculosis* infection both *in vitro* and *in vivo*. J. Immunol. **171(9)** (2003) 4689–4699.

Although IFN-gamma is necessary for survival of *Mycobacterium tuberculosis* infection in people and animal models, it may not be sufficient to clear the infection, and IFN-gamma is not a reliable correlate of protection. To determine whether IFN-gamma-independent mechanisms of immunity exist, we developed a murine *ex vivo* culture system that directly evaluates the ability of splenic or lung lymphocytes to control the growth of *M. tuberculosis* within infected macrophages, and that models *in vivo* immunity to tuberculosis. Surprisingly, CD4(+) T cells controlled >90% of intracellular *M. tuberculosis* growth in the complete absence of IFN-gamma stimulation of macrophages, via a NO-dependent mechanism. Furthermore, bacillus Calmette-Guerin-vaccinated IFN-gamma-deficient mice exhibited significant protection against *M. tuberculosis* challenge that was lost upon depletion of CD4(+) T cells. These findings demonstrate that CD4(+) T cells possess IFN-gamma-independent mechanisms that can limit the growth of an intracellular pathogen and are dominant in secondary responses to *M. tuberculosis*.—Authors' Abstract

Dawes, S. S., Warner, D. F., Tsenova, L., Timm, J., McKinney, J. D., Kaplan, G., Rubin, H., and Mizrahi, V. Ribonucleotide reduction in *Mycobacterium tuberculosis*: function and expression of genes encoding class Ib and class II ribonucleotide reductases. Infect. Immun. **71(11)** (2003) 6124–6131.

Mycobacterium tuberculosis, the causative agent of tuberculosis, possesses a class Ib ribonucleotide reductase (RNR), encoded by the nrdE and nrdF2 genes, in ad-

dition to a putative class II RNR, encoded by nrdZ. In this study we probed the relative contributions of these RNRs to the growth and persistence of *M. tuberculosis*. We found that targeted knockout of the nrdF2 gene could be achieved only in the presence of a complementing allele, confirming that this gene is essential under normal, in vitro growth conditions. This observation also implied that the alternate class Ib small subunit encoded by the nrdF1 gene is unable to substitute for nrdF2 and that the class II RNR, NrdZ, cannot substitute for the class Ib enzyme, NrdEF2. Conversely, a DeltanrdZ null mutant of *M. tuberculosis* was readily obtained by allelic exchange mutagenesis. Quantification of levels of nrdE, nrdF2, nrdF1, and nrdZ gene expression by real-time, quantitative reverse transcription-PCR with molecular beacons by using mRNA from aerobic and O(2)-limited cultures showed that nrdZ was significantly induced under microaerophilic conditions, in contrast to the other genes, whose expression was reduced by O(2) restriction. However, survival of the DeltanrdZ mutant strain was not impaired under hypoxic conditions in vitro. Moreover, the lungs of B6D2/F(1) mice infected with the DeltanrdZ mutant had bacterial loads comparable to those of lungs infected with the parental wild-type strain, which argues against the hypothesis that nrdZ plays a significant role in the virulence of *M. tuberculosis* in this mouse model.—Authors' Abstract

Ehlers, S. Role of tumour necrosis factor (TNF) in host defence against tuberculosis: implications for immunotherapies targeting TNF. Ann. Rheum. Dis. **62 Suppl 2** (2003) ii37–42.

Studies in mouse infection models clearly demonstrate tumour necrosis factor (TNF) to be a critical component of both the antibacterially protective and the inflammatory immune response to *Mycobacterium tuberculosis*. It is therefore not surprising that treatment of patients—for example, those with rheumatoid arthritis—with biological agents interfering with TNF activity have shown an increased risk of reactivating tuberculosis. However, conceivably, TNF targeting biological agents can be developed that because of their particular mode of action and their specific pharmacodynamics may be less likely to have this side effect.—Author's Abstract

Kipnis, A., Basaraba, R. J., Turner, J., and Orme, I. M. Increased neutrophil influx but no impairment of protective immunity to tuberculosis in mice lacking the CD44 molecule. J. Leukoc. Biol. **74(6)** (2003) 992–997.

Up-regulation of expression of the cell-surface marker CD44 is a major characteristic of T lymphocytes responding in the lungs of mice infected with *Mycobacterium tuberculosis.* These lymphocytes express an activated/memory phenotype as seen by their high expression of the CD44 molecule and low expression of CD62L and CD45RB cell-surface molecules. Based on increasing evidence that the CD44 molecule participates in several aspects of the inflammatory response, we evaluated its role in the response to infection with *M. tuberculosis* using gene-disrupted mice. In this report, we show that CD44 expression is not necessary for the proper trafficking of protective cells to the lungs of mice infected with *M. tuberculosis* or the direct expression of protective immunity leading to control and containment of the bacterial load in this organ. However, although there were no differences in the bacterial load or migration of activated T lymphocytes to the inflamed lung, the absence of the CD44 molecule resulted in a substantially increased accumulation of neutrophils in the lung. These data indicate that loss of CD44 expression does not alter expression of T helper cell type 1 immunity to tuberculosis in the lungs but has major effects on the overall cellular composition of the immunopathological response.—Authors' Abstract

Nagabhushanam, V., Solache, A., Ting, L. M., Escaron, C. J., Zhang, J. Y., and Ernst, J. D. Innate inhibition of adaptive immunity: *Mycobacterium tuberculosis*-induced IL-6 inhibits macrophage responses to IFN-gamma. J. Immunol. **171(9)** (2003) 4750–4757.

In humans and in mice, control of the intracellular pathogen, *Mycobacterium tuberculosis* (Mtb), requires IFN-gamma. Although the adaptive immune response results in production of substantial amounts of IFN-gamma in response to Mtb, the immune response is unable to eradicate the infection in most cases. We have previously reported evidence that Mtb inhibits macrophage responses to IFN-gamma, suggesting that this may limit the ability of IFN-gamma to stimulate macrophages to kill Mtb. We have also observed that uninfected macrophages, adjacent to infected macrophages in culture, exhibit decreased responses to IFN-gamma. Here we report that IL-6 secreted by Mtb-infected macrophages inhibits the responses of uninfected macrophages to IFN-gamma. IL-6 selectively inhibits a subset of IFN-gamma-responsive genes at the level of transcriptional activation without inhibiting activation or function of STAT1. Inhibition of macrophage responses to IFN-gamma by IL-6 requires new protein synthesis, but this effect is not attributable to suppressor of cytokine signaling 1 or 3. These results reveal a novel function for IL-6 and indicate that IL-6 secreted by Mtb-infected macrophages may contribute to the inability of the cellular immune response to eradicate infection.—Authors' Abstract

Okkels, L. M., Brock, I., Follmann, F., Agger, E. M., Arend, S. M., Ottenhoff, T. H., Oftung, F., Rosenkrands, I., and Andersen, P. PPE protein (Rv3873) from DNA segment RD1 of *Mycobacterium tuberculosis*: strong recognition of both specific T-cell epitopes and epitopes conserved within the PPE family. Infect. Immun. **71(11)** (2003) 6116–6123.

Proteins encoded by DNA segment RD1 of *Mycobacterium tuberculosis* have recently been demonstrated to play important roles in bacterial virulence, vaccine development, and diagnostic reagent design. Previously, we characterized two immunodominant T-cell antigens, the early secreted antigen target (ESAT-6) and the 10-kDa culture filtrate protein (CFP10), which are encoded by the esx-lhp operon in this region. In the present study we characterized a third putative open reading frame in this region, rv3873, which encodes a PPE protein. We found that the rv3873 gene is expressed in *M. tuberculosis* H37Rv and that the native protein, Rv3873, is predominantly associated with the mycobacterial cell or wall. When tested as a His-tagged recombinant protein, Rv3873 stimulated high levels of gamma interferon secretion in peripheral blood mononuclear cells isolated from tuberculosis (TB) patients, as well as from healthy tuberculin purified protein derivative-positive donors. In contrast to other RD1-encoded antigens, Rv3873 was also found to be recognized by a significant proportion of *Mycobacterium bovis* BCG-vaccinated donors. Epitope mapping performed with overlapping peptides revealed a broad pattern of T-cell recognition comprising both TB-specific epitopes and epitopes also recognized by BCG-vaccinated donors. The immunodominant epitope (residues 118 to 135) for both TB patients and BCG-vaccinated individuals was found to be highly conserved among a large number of PPE family members.—Authors' Abstract

Seiler, P., Aichele, P., Bandermann, S., Hauser, A. E., Lu, B., Gerard, N. P., Gerard, C., Ehlers, S., Mollenkopf, H. J., and Kaufmann, S. H. Early granuloma formation after aerosol *Mycobacterium tuberculosis* infection is regulated by neutrophils via CXCR3-signaling chemokines. Eur. J. Immunol. **33(10)** (2003) 2676–2686.

Among the first cells to invade a site of infection, polymorphonuclear neutrophils (PMN) play an important role in the control of numerous infections. While PMN are considered critical for control of acute infections, their role in chronic infections remains less well understood. Here we report that PMN are essential for accurate early granuloma formation during chronic *M. tuberculosis* infection without influencing mycobacterial growth restriction. The PMN-mediated regulation of granuloma formation depended on chemokines signaling through CXCR3, in particular MIG, as indicated by immune histochemical analysis of lung sections from C57BL/6 wild-type and CXCR3(–/–) mutant mice and sup-

ported by microarray transcriptome analysis. Hence, PMN play a central role in regulating the focal granulomatous response in the lung, and this early granuloma formation can be segregated from long-term protection against pulmonary *M. tuberculosis* infection.—Authors' Abstract

Shams, H., Barnes, P. F., Weis, S. E., Klucar, P., and Wizel, B. Human CD8+ T cells recognize epitopes of the 28-kDa hemolysin and the 38-kDa antigen of *Mycobacterium tuberculosis*. J. Leukoc. Biol. **74(6)** (2003) 1008–1014.

Mycobacterium tuberculosis antigens that are recognized by human CD8+ T cells are potentially important vaccine target molecules. We used a motif-based strategy to screen selected proteins of *M. tuberculosis* for peptides predicted to bind to human leukocyte antigen (HLA)-A*0201. We identified two 10 amino acid peptides that elicited cytolytic T lymphocyte activity and interferon-gamma production by CD8+ T cells from HLA-A*0201+ healthy tuberculin reactors. These peptides were derived from the 38-kDa antigen and the 28-kDa hemolysin, the latter being a novel target for CD8+ T cells. We speculate that hemolysins may alter the phagosomal membrane surrounding intracellular *M. tuberculosis*, allowing themselves and other antigens to gain access to the major histocompatibility complex class I processing pathway.—Authors' Abstract

Shi, S., Nathan, C., Schnappinger, D., Drenkow, J., Fuortes, M., Block, E., Ding, A., Gingeras, T. R., Schoolnik, G., Akira, S., Takeda, K., and Ehrt, S. MyD88 primes macrophages for full-scale activation by interferon-gamma yet mediates few responses to *Mycobacterium tuberculosis*. J. Exp. Med. **198(7)** (2003) 987–997.

Macrophages are activated from a resting state by a combination of cytokines and microbial products. Microbes are often sensed through Toll-like receptors signaling through MyD88. We used large-scale microarrays in multiple replicate experiments followed by stringent statistical analysis to compare gene expression in wild-type (WT) and MyD88–/– macrophages. We confirmed key results by quantitative reverse transcription polymerase chain reaction, Western blot, and enzyme-linked immunosorbent assay. Surprisingly, many genes, such as inducible nitric oxide synthase, IRG-1, IP-10, MIG, RANTES, and interleukin 6 were induced by interferon (IFN)-gamma from 5- to 100-fold less extensively in MyD88–/– macrophages than in WT macrophages. Thus, widespread, full-scale activation of macrophages by IFN-gamma requires MyD88. Analysis of the mechanism revealed that MyD88 mediates a process of self-priming by which resting macrophages produce a low level of tumor necrosis factor. This and other factors lead to basal activation of nuclear factor kappaB, which synergizes with IFN-gamma for gene induction. In contrast, infection by live, virulent *Mycobacterium tuberculosis* (Mtb) activated macrophages largely through MyD88-independent pathways, and macrophages did not need MyD88 to kill Mtb in vitro. Thus, MyD88 plays a dynamic role in resting macrophages that supports IFN-gamma-dependent activation, whereas macrophages can respond to a complex microbial stimulus, the tubercle bacillus, chiefly by other routes.—Authors' Abstract

Shoyama, Y., Tsuji, C., Shioya, S., Fukuyama, N., and Nakazawa, H. Anti-inflammatory effect of *Pelteobagrus nudiceps* extract on rat model of CFA-induced pulmonary tuberculous granuloma. Pathophysiology **9(2)** (2003) 89–95.

We investigated the effectiveness of supportive therapy with a fish-oil extract called repair tuberculosis (RTB) in anti-tuberculosis treatment, and the underlying mechanism of action. The active component of RTB is the unsaturated fatty acid docosatetraenoic acid (C(22)H(36)O(2)), which was reported to induce the resorption and healing of pulmonary lesions in patients with severe pulmonary tuberculosis. We administered RTB to a rat model of CFA-induced pulmonary tuberculous granuloma (RTB group), and

compared the results with those in a control group, which did not receive RTB. Histological examination of the lungs showed a significantly smaller area of granuloma in the RTB group than in the control group. IFN-gamma levels in bronchoalveolar lavage fluid (BALF) were higher in the RTB group than in the control group, suggesting that Th1-type immune reaction is activated in the RTB group. Moreover, significantly enhanced expression of inducible nitric oxide synthase mRNA in lung tissue was observed in the RTB group. Superoxide production by cells recovered from BALF was attenuated in the RTB group. There were no difference in IL-4 levels in BALF, or in expression of TNF-alpha mRNA in lung tissue between the RTB and control groups. The above results suggest that RTB activates Th1-type cellular immune reaction, promotes absorption of lesions, and inhibits the generation of cytotoxic substances.—Authors' Abstract

Singh, B., Singh, G., Trajkovic, V., and Sharma, P. Intracellular expression of *Mycobacterium tuberculosis*-specific 10-kDa antigen down-regulates macrophage B7.1 expression and nitric oxide release. Clin. Exp. Immunol. **134(1)** (2003) 70–77.

To explore the role of the 10-kDa *Mycobacterium tuberculosis*-specific secreted antigen (MTSA-10 or CFP-10) in modulation of macrophage function, J774 macrophages were transfected stably with DNA encoding MTSA-10. Compared to normal or mock-transfected controls, MTSA-10-expressing macrophages had markedly lower levels of co-stimulatory molecule B7.1 on their surface, while the expression of B7.2 and ICAM-1 was not affected. MTSA-transfected cells also produced significantly less microbicidal free radical nitric oxide (NO) upon stimulation with interferon (IFN)-gamma, lipopolysaccharide or *M. tuberculosis* cell lysate. Western blot analysis revealed the absence of tyrosine-phosphorylated protein slightly larger than 112 kDa in MTSA-transfected macrophages. Moreover, the treatment of control J774 cells with protein tyrosine kinase inhibitor genistein completely mimicked the effects of transfection with MTSA-10, selectively down-regulating NO and B7.1, but not B7.2 or ICAM-1 expression. The observed MTSA-10-mediated block of B7.1 expression and NO release might contribute to the suppression of antimycobacterial response in tuberculosis.—Authors' Abstract

Song, C. H., Lee, J. S., Kim, H. J., Park, J. K., Paik, T. H., and Jo, E. K. Interleukin-8 is differentially expressed by human-derived monocytic cell line U937 infected with *Mycobacterium tuberculosis* H37Rv and *Mycobacterium marinum*. Infect. Immun. **71(10)** (2003) 5480–5487.

Although *Mycobacterium marinum* is closely related to *Mycobacterium tuberculosis* H37Rv genomically, the clinical outcome in humans is quite different for *M. marinum* and *M. tuberculosis* infections. We investigated possible factors in the host macrophages for determining differential pathological responses to *M. tuberculosis* and *M. marinum* using an *in vitro* model of mycobacterial infection. Using suppression-subtractive hybridization, we identified 12 differentially expressed genes in the human monocytic cell line U937 infected with *M. tuberculosis* and *M. marinum*. Of those genes, the most frequently recovered transcript encoded interleukin-8 (IL-8). Northern hybridization revealed that IL-8 mRNA was highly upregulated in *M. tuberculosis*-infected U937 cells compared with *M. marinum*-infected cells. In addition, enzyme-linked immunosorbent assay showed that IL-8 protein secretion was significantly elevated in *M. tuberculosis*-infected U937 cells, human primary monocytes, and monocyte-derived macrophages compared with that in *M. marinum*-infected cells. The depressed IL-8 expression was unique in *M. marinum*-infected cells compared with cells infected with other strains of mycobacteria, including *M. tuberculosis* H37Ra, *Mycobacterium bovis* BCG, or *Mycobacterium smegmatis*. When the expression of NF-kappaB was assessed in mycobacterium-infected U937 cells, IkappaBalpha proteins were significantly degraded in *M. tuberculosis*-infected cells compared with *M. marinum*-infected cells. Collectively,

these results suggest that differential IL-8 expression in human macrophages infected with *M. tuberculosis* and *M. marinum* may be critically associated with distinct host responses in tuberculosis. Additionally, our data indicate that differential signal transduction pathways may underlie the distinct patterns of IL-8 secretion in cells infected by the two mycobacteria.—Authors' Abstract

Microbiology

Alexander, D. C., Jones, J. R., and Liu, J. A rifampin-hypersensitive mutant reveals differences between strains of *Mycobacterium smegmatis* and presence of a novel transposon, IS1623. Antimicrob. Agents Chemother. **47(10)** (2003) 3208–3213.

Rifampin is a front-line antibiotic for the treatment of tuberculosis. Infections caused by rifampin- and multidrug-resistant *Mycobacterium tuberculosis* strains are difficult to treat and contribute to a poor clinical outcome. Rifampin resistance most often results from mutations in rpoB. However, some drug-resistant strains have rpoB alleles that encode the phenotype for susceptibility. Similarly, non-*M. tuberculosis* mycobacteria exhibit higher levels of baseline resistance to rifampin, despite the presence of rpoB alleles that encode the phenotype for susceptibility. To identify other genes involved in rifampin resistance, we generated a library of *Mycobacterium smegmatis* mc(2)155 transposon insertion mutants. Upon screening this library, we identified one mutant that was hypersensitive to rifampin. The transposon insertion was localized to the arr gene, which encodes rifampin ADP ribosyltransferase, an enzyme able to inactivate rifampin. Sequence analysis revealed differences in the arr alleles of *M. smegmatis* strain mc(2)155 and previously described strain DSM 43756. The arr region of strain mc(2)155 contains a second, partial copy of the arr gene plus a novel insertion sequence, IS1623.—Authors' Abstract

Broccolo, F., Scarpellini, P., Locatelli, G., Zingale, A., Brambilla, A. M., Cichero, P., Sechi, L. A., Lazzarin, A., Lusso, P., and Malnati, M. S. Rapid diagnosis of mycobacterial infections and quantitation *of Mycobacterium tuberculosis* load by two real-time calibrated PCR assays. J. Clin. Microbiol. **41(10)** (2003) 4565–4572.

Sensitive and specific techniques to detect and identify *Mycobacterium tuberculosis* directly in clinical specimens are important for the diagnosis and management of patients with tuberculosis (TB). We developed two real-time PCR assays, based on the IS6110 multicopy element and on the senX3-regX3 intergenic region, which provide a rapid method for the diagnosis of mycobacterial infections. The sensitivity and specificity of both assays were established by using purified DNA from 71 clinical isolates and 121 clinical samples collected from 83 patients, 20 of whom were affected by TB. Both assays are accurate, sensitive, and specific, showing a complementary pattern of Mycobacterium recognition: broader for the IS6110-based assay and restricted to the *M. tuberculosis* complex for the senX3-regX3-based assay. Moreover, the addition of a synthetic DNA calibrator prior to DNA extraction allowed us to measure the efficiency of DNA recovery and to control for the presence of PCR inhibitors. The mycobacterial burden of the clinical samples, as assessed by direct microscopy, correlates with the *M. tuberculosis* DNA load measured by the senX3-regX3-based assay. In addition, reduced levels of *M. tuberculosis* DNA load are present in those patients subjected to successful therapy, suggesting a potential use of this assay for monitoring treatment efficacy. Therefore, these assays represent a fully controlled high-throughput system for the evaluation of mycobacterial burden in clinical specimens.—Authors' Abstract

Pauls, R. J., Turenne, C. Y., Wolfe, J. N., and Kabani, A. A high proportion of novel mycobacteria species identified by 16S rDNA analysis among slowly growing AccuProbe-negative strains in a clinical setting. Am. J. Clin. Pathol. **120(4)** (2003) 560–566.

Sequencing of the 16S ribosomal DNA (rDNA) for identification of nontuberculous mycobacteria (NTM) has contributed to the establishment of more than 35 new species during the last decade. Increasingly, NTM are accepted as potential or proven pathogens. We identified, by 16S rDNA sequence analysis, slowly growing NTM isolates negative by AccuProbe (GenProbe, San Diego, CA) that previously were identified by using conventional biochemical techniques, to determine the accuracy of reporting AccuProbe-negative NTM prior to sequence-based identification. Of 82 strains, 30 were deemed novel. An attempt was made to determine the clinical importance of previously misidentified novel species. Clinical cases are described for a number of strains previously identified as *Mycobacterium terrae* complex, *Mycobacterium scrofulaceum*, and *Mycobacterium avium* complex. As sequence-based identification methods become more commonplace in clinical microbiology laboratories, there is a need to understand the significance of previously undescribed species, which often mimic and subsequently are identified as well-established species.—Authors' Abstract

Sassetti, C. M., and Rubin, E. J. Genetic requirements for mycobacterial survival during infection. Proc. Natl. Acad. Sci. U. S. A. **100(22)** (2003) 12989–12994.

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Sha, W., Weng, X. H., Xiao, H. P., and He, G. J. [Investigation of drug-resistance to rifampin and rpoB gene sequence analysis of *Mycobacterium abscessus*]. Zhonghua Jie He He Hu Xi Za Zhi. **26(9)** (2003) 544–547. [Article in Chinese].

OBJECTIVE: To observe the resistance of *Mycobacterium abscessus* to rifampin and to investigate if there is any mutation of rpoB gene in strains with high minimal inhibitory concentration (MIC). METHODS: *Mycobacterium abscessus* was identified with both biochemical methods and PCR-RFLP. The MICs of rifampin to all the clinical strains and the type strain ATCC19977 were determined. DNA sequences were obtained from a 1272 bp fragment of the rpoB gene from either low or high rifampin MIC strains using PCR amplification. RESULTS: Only one strain had low MICs of 4 micro g/ml. MICs of all the other 14 strains, as well as the type stain were above 128–256 micro g/ml. Although there were some differences in nucleotide sequence of rpoB gene, all strains had the same amino acid sequence without any mutations. CONCLUSIONS: *Mycobacterium abscessus* showed a high resistance to rifampin. Mutations of rpoB gene do not seem to be the responsible mechanism.—Authors' Abstract

Zhang, N., Torrelles, J. B., McNeil, M. R., Escuyer, V. E., Khoo, K. H., Brennan, P. J., and Chatterjee, D. The Emb proteins of mycobacteria direct arabinosylation of lipoarabinomannan and arabinogalactan via an N-terminal recognition region and a C-terminal synthetic region. Mol. Microbiol. **50(1)** (2003) 69–76.

See Current Literature, Molecular and Genetic Studies, p. 115.

Microbiology (Leprosy)

Williams, D. L., Oby-Robinson, S., Pittman, T. L., and Scollard, D. M. Purification of *Mycobacterium leprae* RNA for gene expression analysis from leprosy biopsy specimens. Biotechniques **35(3)** (2003) 534–536, 538, 540–541.

Gene expression analysis in *Mycobacterium leprae*, an obligate intracellular pathogen and the etiologic agent of leprosy, has been hampered by the lack of an efficient method to purify RNA from leprosy lesions. Therefore to date, transcripts for only a few genes have been identified. We report the use of a single-tube homogenization/RNA extraction method that produces enough RNA to study the expression of 30 genes from a single skin biopsy specimen of a multibacillary leprosy patient and demonstrate that RNA can be purified after fixation of biopsies in 70% ethanol for up to a year. This represents a major advancement in the ability to study *M. leprae* gene expression directly from biopsy material and should help to define genes that are associated with intracellular survival of this human pathogen.—Authors' Abstract

Microbiology (Tuberculosis)

Calusni, A. L., Roscani, G. N., Villares, M. C., Soini, H., Graviss, E. A., and Ramos, Mde, C. IS6110 restriction fragment length polymorphism of *Mycobacterium tuberculosis* isolated from patients with pulmonary tuberculosis in Campinas, Brazil: evidence of intercontinental distribution of strains. Mem. Inst. Oswaldo Cruz. **98(5)** (2003) 655–658.

Tuberculosis (TB) is a major concern in developing countries. In Brazil, few genotyping studies have been conducted to verify the number of IS6110 copies present in local prevalent strains of *Mycobacterium tuberculosis*, the distribution and clustering of strains. IS6110 DNA fingerprinting was performed on a sample of *M. tuberculosis* isolates from patients with AFB smear-positive pulmonary TB, at a hospital in Brazil. The IS6110 profiles were analyzed and compared to a *M. tuberculosis* database of the Houston Tuberculosis Initiative, Houston, US. Seventy-six fingerprints were obtained from 98 patients. All *M. tuberculosis* strains had an IS6110 copy number between 5–21 allowing for differentiation of the isolates. Human immunodeficiency virus infection was confirmed in nearly half the patients of whom data was available. Fifty-eight strains had unique patterns, while 17 strains were grouped in 7 clusters (2 to 6 strains). When compared to the HTI database, 6 strains matched isolates from El Paso, Ciudad de Juarez, Houston, and New York. Recently acquired infections were documented in 19% of cases. The community transmission of infection is intense, since some clustered strains were recovered during the four-year study period. The intercontinental dissemination of *M. tuberculosis* strains is suspected by demonstration of identical fingerprints in a distant country.—Authors' Abstract

Clark-Curtiss, J. E., and Haydel, S. E. Molecular genetics of *Mycobacterium tuberculosis* pathogenesis. Annu. Rev. Microbiol. **57** (2003) 517–549.

Tuberculosis (TB) has afflicted humankind throughout history. Approximately one third of the world's population is currently infected with *Mycobacterium tuberculosis* and nearly two million people die of TB annually. Although much has been learned about the structure of the tubercle bacillus, the epidemiology of TB, the physiological and immunological responses of the host to infection, and the physiology of *M. tuberculosis* in laboratory broth cultures, much of the basic biology of *M. tuberculo-*

sis in its natural setting (the infected human) remains to be elucidated. Within the past decade, there have been remarkable advances in the development of genetic and molecular biological tools with which to study *M. tuberculosis*. This review discusses the approaches that have been employed and the progress that has been made in discovering how *M. tuberculosis* has achieved its prowess as a successful pathogen.—Authors' Abstract

Gopaul, K. K., Brooks, P. C., Prost, J. F., and Davis, E. O. Characterization of the two *Mycobacterium tuberculosis* recA promoters. J. Bacteriol. **185(20)** (2003) 6005–60015.

The recA gene of *Mycobacterium tuberculosis* is unusual in that it is expressed from two promoters, one of which, P1, is DNA damage inducible independently of LexA and RecA, while the other, P2, is regulated by LexA in the classical way (E. O. Davis, B. Springer, K. K. Gopaul, K. G. Papavinasasundaram, P. Sander, and E. C. Bottger, Mol. Microbiol. 46:791–800, 2002). In this study we characterized these two promoters in more detail. Firstly, we localized the promoter elements for each of the promoters, and in so doing we identified a mutation in each promoter which eliminates promoter activity. Interestingly, a motif with similarity to *Escherichia coli* sigma(70) –35 elements but located much closer to the –10 element is important for optimal expression of P1, whereas the sequence at the –35 location is not. Secondly, we found that the sequences flanking the promoters can have a profound effect on the expression level directed by each of the promoters. Finally, we examined the contribution of each of the promoters to recA expression and compared their kinetics of induction following DNA damage.—Authors' Abstract

Goulding, C. W., Perry, L. J., Anderson, D., Sawaya, M. R., Cascio, D., Apostol, M. I., Chan, S., Parseghian, A., Wang, S. S., Wu, Y., Cassano, V., Gill, H. S., and Eisenberg, D. Structural genomics of *Mycobacterium tuberculosis*: a preliminary report of progress at UCLA. Biophys. Chem. **105(2–3)** (2003) 361–370.

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Gu, S., Chen, J., Dobos, K. M., Bradbury, E. M., Belisle, J. T., and Chen, X. Comprehensive Proteomic Profiling of the Membrane Constituents of *a Mycobacterium tuberculosis* Strain. Mol. Cell. Proteomics. **2(12)** (2003) 1284–1296.

Mycobacterium tuberculosis is an infectious microorganism that causes human tuberculosis. The cell membranes of pathogens are known to be rich in possible diagnostic and therapeutic protein targets. To compliment the *M. tuberculosis* genome, we have profiled the membrane protein fraction of the *M. tuberculosis* H37Rv strain using an analytical platform that couples one-dimensional SDS gels to a microcapillary liquid chromatography-nanospray-tandem mass spectrometer. As a result, 739 proteins have been identified by two or more distinct peptide sequences and have been characterized. Interestingly, approximately 450 proteins represent novel identifications, 79 of which are membrane proteins and more than 100 of which are membrane-associated proteins. The physicochemical properties of the identified proteins were studied in detail, and then biological functions were obtained by sorting them according to Sanger Institute gene function category. Many membrane proteins were found to be involved in the cell envelope, and those proteins with energy metabolic functions were also identified in this study.—Authors' Abstract

Hingley-Wilson, S. M., Sambandamurthy, V. K., and Jacobs, W. R. Jr. Survival perspectives from the world's most successful pathogen, *Mycobacterium tuberculosis*. Nat. Immunol. **4(10)** (2003) 949–955.

Studying defined mutants of *Mycobacterium tuberculosis* in the mouse model of infection has led to the discovery of attenu-

ated mutants that fall into several phenotypic classes. These mutants are categorized by their growth characteristics compared with those of wild-type *M. tuberculosis*, and include severe growth *in vivo* mutants, growth *in vivo* mutants, persistence mutants, pathology mutants and dissemination mutants. Here, examples of each of these mutant phenotypes are described and classified accordingly. Defining the importance of mycobacterial gene products responsible for *in vivo* growth, persistence and the induction of immunopathology will lead to a greater understanding of the host-pathogen interaction and potentially to new antimycobacterial treatment options.—Authors' Abstract

Kumar, A., Bose, M., and Brahmachari, V. Analysis of expression profile of mammalian cell entry (mce) operons of *Mycobacterium tuberculosis*. Infect. Immun. **71(10)** (2003) 6083–6087.

The sequencing of the complete genome of *M. tuberculosis* H37Rv has resulted in the recognition of four mce operons in its genome by in silico analysis. In an attempt to understand the significance of the redundancy of mce operons, we analyzed the expression profile of mce operons after different periods of growth in culture as well as during *in vivo* infection. Our results strongly suggest that mce1 is expressed as a polycistronic message. In culture from day 8 to day 12, expression of only mce1 was observed, but as the cultures progress towards stationary phase the expression profile of mce operons was altered; the transcripts of the mce1 operon were barely detected while those of the mce4 operon were prominent. In an analysis of the expression of mce operons in tubercle material collected from infected animal tissues, we detected the expression of mce1, -3 and -4. Our results imply that mce operons other than mce1 are also expressed during infection and that it is necessary to examine their role in pathogenesis.—Authors' Abstract

Lewinsohn, D. A., Heinzel, A. S., Gardner, J. M., Zhu, L., Alderson, M. R., and Lewinsohn, D. M. *Mycobacterium tuberculosis*-specific CD8+ T cells preferentially recognize heavily infected cells. Am. J. Respir. Crit. Care Med. **168(11)** (2003) 1346–1352.

Both CD4+ and CD8+ T cells are important for successful immunity to tuberculosis and have redundant effector functions, such as cytolysis and release of potent antimycobacterial cytokines such as interferon-gamma and tumor necrosis factor-alpha. We hypothesized that CD8+ T cells play a unique role in host defense to *Mycobacterium tuberculosis* infection as well. Possibilities include preferential and/or enhanced release of granular constituents and/or preferential recognition of heavily infected cells. Utilizing human, *Mycobacterium tuberculosis*-specific, CD4+ and CD8+ T cell clones, we demonstrate that, after recognition of antigen-presenting cells displaying peptide antigen, CD4+ T cells preferentially release interferon-gamma, whereas CD8+ T cells preferentially lyse antigen-presenting cells. Furthermore, utilizing dendritic cells infected with *Mycobacterium tuberculosis* expressing green fluorescent protein, we show that CD8+ T cells preferentially recognize heavily infected cells that constitute the minority of infected cells. These data support the hypothesis that the central role of CD8+ T cells in the control of infection with *Mycobacterium tuberculosis* may be that of surveillance; in essence, recognition of cells in which the containment of *Mycobacterium tuberculosis* is no longer effective.—Authors' Abstract

Lillebaek, T., Dirksen, A., Vynnycky, E., Baess, I. Thomsen, V. O., and Andersen, A. Stability of DNA patterns and evidence of *Mycobacterium tuberculosis* reactivation occurring decades after the initial infection. J. Infect. Dis. **188(7)** (2003) 1032–1039.

Two hundred three freeze-dried strains of *Mycobacterium tuberculosis* collected during the 1960s were compared with 4102 strains collected during the 1990s, and 14 DNA patterns identified among the "historical strains" were 100% identical to patterns identified among the "recent strains." They were isolated from 41 and 40 patients who

had tuberculosis during the 1960s and 1990s, respectively. The patients' mean age differed by >30 years, a finding strongly suggesting that the patients from the 1990s experienced reactivation of *M. tuberculosis* infection acquired during the 1960s. The half-life of IS6110 DNA patterns during latency was estimated to be 36 years (95% confidence interval, 25–54 years). Thus, this comparison of historical and recent strains yields molecular epidemiologic evidence of *M. tuberculosis* reactivation spanning decades and suggests that the rate of change of DNA patterns during latency is much longer than that during active disease. This has important implications for the interpretation of clustering, especially for the extent of recent transmission.—Authors' Abstract

Martin, A., Camacho, M., Portaels, F., and Palomino, J. C. Resazurin microtiter assay plate testing of *Mycobacterium tuberculosis* susceptibilities to second-line drugs: rapid, simple, and inexpensive method. Antimicrob. Agents Chemother. **47(11)** (2003) 3616–3619.

The emergence of multidrug-resistant tuberculosis calls for new, rapid drug susceptibility tests. We have tested 150 *Mycobacterium tuberculosis* isolates against the second-line drugs ethionamide, kanamycin, capreomycin, ofloxacin, and para-aminosalicylic acid by the colorimetric resazurin microtiter assay and the proportion method. By visual reading, MICs were obtained after 8 days. A very good correlation between results by the colorimetric resazurin microtiter assay and the proportion method was obtained. The colorimetric resazurin microtiter assay is inexpensive, rapid, and simple to perform, and implementation of the assay is feasible for low-resource countries.—Authors' Abstract

Ruiz, M., Rodriguez, J. C, Rodriguez-Valera, F., and Royo, G. Amplified-fragment length polymorphism as a complement to IS6110-based restriction fragment length polymorphism analysis for molecular typing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **41(10)** (2003) 4820–4822.

The amplified-fragment length polymorphism (AFLP) technique was applied to clusters of *Mycobacterium tuberculosis* clinical isolates obtained by using IS6110-based restriction fragment length polymorphism (RFLP). Ten of the RFLP clusters showed identical AFLP patterns also, but the other 13 could be resolved into subclusters by AFLP. Our results suggest that some RFLP clusters may not be due to recent transmission and that AFLP may be a useful complementary technique.—Authors' Abstract

Seiler, P., Ulrichs, T., Bandermann, S., Pradl, L., Jorg, S., Krenn, V., Morawietz, L., Kaufmann, S. H., and Aichele, P. Cell-wall alterations as an attribute of *Mycobacterium tuberculosis* in latent infection. J. Infect. Dis. **188(9)** (2003) 1326–1331.

Ziehl-Neelsen (ZN) staining is the key technique for diagnosis of mycobacterial infections; however, a high percentage of patients exhibit positive signs of tuberculosis, as indicated by pathology, culture of mycobacteria, and polymerase chain-reaction analysis, and yet show negative results on ZN staining. In this report we present evidence that such ZN-negative specimens represent *Mycobacterium tuberculosis* bacilli in a dormant state with distinct cell-wall alterations: the classical cell-wall composition-dependent ZN staining of *M. tuberculosis* in lung sections gradually discontinued with persistence of infection, both in mice and in human patients; in contrast, detection of mycobacteria by cell-wall composition-independent staining using a polyclonal anti-*M. bovis* Bacille-Calmette-Guerin serum continued with persistence of infection. These findings have important implications for diagnosis, as well as for both chemotherapy and development of vaccine strategies.'—Authors' Abstract

Sinha, I., Boon, C., and Dick, T. Apparent growth phase-dependent phosphorylation of malonyl coenzyme A:acyl carrier protein transacylase (MCAT), a major fatty acid synthase II component in *Mycobacterium bovis* BCG. FEMS Microbiol. Lett. **227(1)** (2003) 141–147.

Probing protein extracts from exponentially growing and stationary phase cultures of *Mycobacterium bovis* BCG with anti-phospho amino acid antibodies revealed a 31-kDa anti-phospho threonine antibody-reactive protein specific to growing culture. The corresponding protein was purified via two-dimensional gel electrophoresis and identified via mass spectrometry to be malonyl coenzyme A:acyl carrier protein transacylase (MCAT), a component of the fatty acid biosynthetic pathway. MCAT tagged with histidine reacted with anti-phospho threonine antibody and was positive in an in-gel chemical assay for phospho proteins. Analysis of the growth phase dependence of MCAT-His phosphorylation and protein levels showed that phosphorylated MCAT-His can be detected only in growing culture. In contrast, MCAT-His protein level was growth phase-independent. These results suggest that MCAT may be a substrate of a protein kinase and phosphatase, and that aspects of fatty acid synthesis in tubercle bacilli are regulated by protein phosphorylation.—Authors' Abstract

Stanley, S. A., Raghavan, S., Hwang, W. W., and Cox, J. S. Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system. Proc. Natl. Acad. Sci. U.S.A. **100(22)** (2003) 13001–13006.

Although many bacterial pathogens use specialized secretion systems for virulence, no such systems have been described for *Mycobacterium tuberculosis*, a major pathogen of humans that proliferates in host macrophages. In a screen to identify genes required for virulence of *M. tuberculosis*, we have discovered three components and two substrates of the first Sec-independent secretion pathway described in *M. tuberculosis*, which we designate the Snm pathway. Here we demonstrate that the proteins Snm1, -2, and -4 are required for the secretion of ESAT-6 and CFP-10, small proteins previously identified as major T cell antigens. Snm2, a member of the AAA ATPase family, interacts with substrates and with Snm1, another AAA ATPase. We show that *M. tuberculosis* mutants lacking either the Snm system or these substrates exhibit defects in bacterial growth during the acute phase of a mouse infection and are attenuated for virulence. Strikingly, snm mutants fail to replicate in cultured macrophages and to inhibit macrophage inflammatory responses, two well established activities of wild-type *M. tuberculosis* bacilli. Thus, the Snm secretion pathway works to subvert normal macrophage responses and is a major determinant of *M. tuberculosis* virulence.—Authors' Abstract

van Doorn, H. R., Claas, E. C., Templeton, K. E., van der Zanden, A. G., te Koppele Vije, A., de Jong, M. D., Dankert, J., and Kuijper, E. J. Detection of a point mutation associated with high-level isoniazid resistance in *Mycobacterium tuberculosis* by using real-time PCR technology with 3′-minor groove binder-DNA probes. J. Clin. Microbiol. **41(10)** (2003) 4630–4635.

Tuberculosis remains one of the leading infectious causes of death worldwide. The emergence of drug-resistant strains of *Mycobacterium tuberculosis* is a serious public health threat. Resistance to isoniazid (INH) is the most prevalent form of resistance in *M. tuberculosis* and is mainly caused by mutations in the catalase peroxidase gene (katG). Among high-level INH-resistant isolates (MIC > or = 2), 89% are associated with a mutation at codon 315 of katG. There is a need to develop rapid diagnostic tests to permit appropriate antibiotic treatment and to improve clinical management. Therefore, a single-tube real-time PCR, using a novel kind of probe (3′-minor groove binder-DNA probe), was developed to detect either the wild-type or the mutant codon directly in Ziehl-Neelsen-positive sputum samples. The detection limit of the assay for purified DNA was 5 fg per well (one mycobacterial genome), and with spiked sputum samples, it was 20 copies per well, corresponding to 10(3) mycobacteria per ml of sputum. Sputum samples from 20 patients living in Kazakhstan or Moldova and infected with monodrug- or multidrug-resistant *M. tuberculosis* and 20 sputum samples from patients infected with INH-susceptible *M. tuberculosis* were tested. The sensitivities and specificities of

the probes were 70 and 94% for the wild-type probe and 82 and 100% for the mutant probe. Binding to either probe was nonambiguous. This real-time PCR allows the rapid identification of a mutant katG allele and can easily be implemented in a clinical microbiology laboratory.—Authors' Abstract

Wong, D. A., Yip, P. C., Tse, D. L., Tung, V. W., Cheung, D. T., and Kam, K. M. Routine use of a simple low-cost genotypic assay for the identification of mycobacteria in a high throughput laboratory. Diagn. Microbiol. Infect. Dis. **47(2)** (2003) 421–426.

A novel polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis (PRA) of the hsp65 gene was used for the routine identification of mycobacteria in a high throughput clinical laboratory. A total of 2036 clinical isolates were tested by PRA in conjunction with other methods. The PRA identification of *M. tuberculosis* complex was 100% sensitive and specific, and 74.5% of nontuberculous mycobacteria (NTM) were correctly identified. It gave highly consistent results for *Mycobacterium avium* complex (MAC) species and for most isolates of *M. fortuitum*, *M. chelonae*, and *M. kansasii*. It had proven to be highly robust and stable despite usage on such a large-scale and is thus particularly suitable for use in high throughput laboratories in areas with a high incidence of tuberculosis.—Authors' Abstract

Experimental Infections

Capuano, S. V. 3rd, Croix, D. A., Pawar, S., Zinovik, A., Myers, A., Lin, P. L., Bissel, S., Fuhrman, C., Klein, E., and Flynn, J. L. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. Infect. Immun. **71(10)** (2003) 5831–5844.

Nonhuman primates were used to develop an animal model that closely mimics human *Mycobacterium tuberculosis* infection. Cynomolgus macaques were infected with low doses of virulent *M. tuberculosis* via bronchoscopic instillation into the lung. All monkeys were successfully infected, based on tuberculin skin test conversion and peripheral immune responses to *M. tuberculosis* antigens. Progression of infection in the 17 monkeys studied was variable. Active-chronic infection, observed in 50 to 60% of monkeys, was characterized by clear signs of infection or disease on serial thoracic radiographs and in other tests and was typified by eventual progression to advanced disease. Approximately 40% of monkeys did not progress to disease in the 15 to 20 months of study, although they were clearly infected initially. These monkeys had clinical characteristics of latent tuberculosis in humans. Low-dose infection of cynomolgus macaques appears to represent the full spectrum of human *M. tuberculosis* infection and will be an excellent model for the study of pathogenesis and immunology of this infection. In addition, this model will provide an opportunity to study the *latent M. tuberculosis* infection observed in approximately 90% of all infected humans.—Authors' Abstract

Chackerian, A. A., and Behar, S. M. Susceptibility to *Mycobacterium tuberculosis*: lessons from inbred strains of mice. Tuberculosis (Edinb). **83(5)** (2003) 279–285.

Inbred strains of mice exhibit varied patterns of susceptibility following infection with virulent *Mycobacterium tuberculosis*. Susceptible mice have progressive fulminate disease resulting in their premature death; in contrast, resistant mice are able to control bacterial replication, limit lung injury and survive longer. The use of these mouse strains in experimental infection has allowed the identification of immunological correlates of protective versus unsuccessful

host responses to tuberculosis, and the identification of susceptibility loci by combining classical and molecular genetics. These immunological and genetic features that distinguish susceptible and resistant inbred mouse strains may allow us to better understand susceptibility to tuberculous disease in people. A possible benefit would be the delineation of markers of protective immunity for use in vaccine development. This is a review of recent insights into the genetics and immunology of resistance and susceptibility to virulent *M. tuberculosis* using genetically intact mice.—Authors' Abstract

Dasgupta, S., Bhinge, A., Chandran, V., Sewlikar, S., Nimbkar, A., and Datta, D. Role of L-lysine HCl in immunopotentiation towards development of suitable tuberculosis vaccination. Vaccine **21(32)** (2003) 4722–4727.

L-Lysine HCl is being proposed to be a possible biocompatible adjuvant to enhance immune response by virtue of its probable non-specific bridging action and cellular proliferation properties. This proposal has been tried to be substantiated by carrying out experimentation where L-lysine HCl has been used as an adjuvant (various groups based on mode of application and frequency of booster dose were designed) in tuberculosis vaccination experiments with heat killed *Mycobacterium tuberculosis* (MTB) and Bacille Calmette Guerin (BCG). Antibody titre has been followed in all the experiments as a measure of immune response. Amongst the various groups designed, group 1A (L-lysine HCl was given at a separate site as that of the antigen; lysine booster was given to this group intermittently, i.e. lysine given on 0th, 7th, 14th, 21st days of immunization) came out as the stand-alone leader. This mode and frequency of application was then compared with a group which received a standard adjuvant, viz. alhydrogel. Results were obtained which showed the following order in terms of decreasing antibody titre: alhydrogel group > lysine group > control group. Considering the biocompatible nature of lysine in comparison with the reportedly hazardous nature of alum adjuvants, we propose L-lysine HCl as a probable adjuvant in vaccination.—Authors' Abstract

Glatman-Freedman, A. Advances in antibody-mediated immunity against *Mycobacterium tuberculosis*: implications for a novel vaccine strategy. FEMS Immunol. Med. Microbiol. **39(1)** (2003) 9–16.

Cell-mediated immunity is considered to be the major component of the host response against *Mycobacterium tuberculosis*, whereas antibody-mediated immunity historically has been considered inconsequential. In recent years, studies from several groups have challenged the traditional dogma and demonstrated that monoclonal antibodies can modify various aspects of mycobacterial infections. This review describes the experimental evidence supporting a role for antibodies in defense against mycobacterial infections and outlines future challenges to the field of antibody-mediated immunity against *M. tuberculosis*, with particular emphasis on the implications of these findings for a novel vaccine strategy. —Author's Abstract

Manabe, Y. C., Dannenberg, A. M., Jr., Tyagi, S. K., Hatem, C. L., Yoder, M., Woolwine, S. C., Zook, B. C., Pitt, M. L., and Bishai, W. R. Different strains of *Mycobacterium tuberculosis* cause various spectrums of disease in the rabbit model of tuberculosis. Infect. Immun. **71(10)** (2003) 6004–6011.

The rabbit model of tuberculosis has been used historically to differentiate between *Mycobacterium tuberculosis* and *Mycobacterium bovis* based on their relative virulence in this animal host. *M. tuberculosis* infection in market rabbits is cleared over time, whereas infection with *M. bovis* results in chronic, progressive, cavitary disease leading to death. Because of the innate resistance of commercial rabbits to *M. tuberculosis*, 320 to 1,890 log-phase, actively growing inhaled bacilli were required to form one grossly visible pulmonary tubercle at 5 weeks. The range of inhaled doses required to make one tubercle allows us to determine the relative pathogenicities of different strains. Fewer inhaled organisms of the *M. tuberculosis* Erdman strain were required than of *M. tuberculosis* H37Rv to produce a visible lesion at 5 weeks. Fur-

thermore, with the Erdman strain, only 7 of 15 rabbits had healed lesions at 16 to 18 weeks; among the other animals, two had chronic, progressive cavitary disease, a phenotype usually seen only with *M. bovis* infection. Genotypic investigation of the Erdman strain with an H37Rv-based microarray identified gene differences in the RD6 region. Southern blot and PCR structural genetic analysis showed significant differences between *M. tuberculosis* strains in this region. Correlation of the relative pathogenicity, including disease severity, in the rabbit model with the strain genotype may help identify stage-specific *M. tuberculosis* genes important in human disease. —Authors' Abstract

Mederle, I., Le Grand, R., Vaslin, B., Badell, E., Vingert, B., Dormont, D., Gicquel, B., and Winter, N. Mucosal administration of three recombinant *Mycobacterium bovis* BCG-SIVmac251 strains to cynomolgus macaques induces rectal IgAs and boosts systemic cellular immune responses that are primed by intradermal vaccination. Vaccine **21(27–30)** (2003) 4153–4166.

The widely administered *Mycobacterium bovis* BCG is an attractive live vector for the development of AIDS vaccines. We explored immune responses induced in cynomolgus macaques to rBCG-SIV(3), a mixture of three recombinant BCG strains expressing the SIVmac251 nef, gag and env genes. After a single intradermal (ID) inoculation, circulating blood cells from rBCG-SIV(3)-vaccinated monkeys exhibited CTL responses targeted against the three antigens and interferon-gamma (IFNgamma) secretion was observed. A rectal or oral boosting dose of rBCG-SIV(3) elicited anti-SIV IgAs in the rectum of vaccinated monkeys and increased IFNgamma secretion by circulating blood cells. Despite a good response against the vector, rBCG-SIV(3) administration did not induce IgG antibody responses or lymphoproliferation against the SIV antigens in blood. This could be due to the lack of *in vivo* persistence of the recombinant BCG strains that were used. Rectal challenge with fully pathogenic SIVmac251-infected all animals. However, after viral challenge, anti-SIV cellular and antibody responses were higher in rBCG-SIV(3) monkeys than in controls indicating that the vaccine induced anti-SIV CD4(+) T-cell memory.—Authors' Abstract

Rao, V., Dhar, N., and Tyagi, A. K. Modulation of host immune responses by overexpression of immunodominant antigens of *Mycobacterium tuberculosis* in bacille Calmette-Guerin. Scand. J. Immunol. **58(4)** (2003) 449–461.

See Current Literature, Molecular and Genetic Studies, p. 112.

Sander, P., Bottger, E. C., Springer, B., Steinmann, B., Rezwan, M., Stavropoulos, E., and Joseph Colston, M. A recA deletion mutant of *Mycobacterium bovis* BCG confers protection equivalent to that of wild-type BCG but shows increased genetic stability. Vaccine **21(27–30)** (2003) 4124–4127.

The widely used vaccine against tuberculosis, BCG, shows evidence of genetic instability. It has undergone major genetic rearrangements resulting in deletion and duplication of segments of its chromosome. In order to produce a BCG strain with more favorable genetic properties, we inactivated the recA gene. Targeted deletion of the recA gene of BCG resulted in a complete loss of recombination between homologous, chromosomally-located sequences, as well as between plasmid- and chromosomally-located sequences. The deltarecA mutant BCG was as effective as the wild-type in conferring protection in mice against an intravenous challenge with virulent *Mycobacterium tuberculosis*, indicating that the loss of an SOS response-mediated DNA repair mechanism did not compromise the immunological properties of BCG. The availability of a genetically stable, fully immunogenic BCG is important for the future development of BCG as a live vaccine.—Authors' Abstract

Santucci, M. B., Ciaramella, A., Mattei, M., Sumerska, T., and Fraziano, M. Batimastat reduces *Mycobacterium tuberculosis*-induced apoptosis in macro-

phages. Int. Immunopharmacol. **3(12)** (2003) 1657–1665.

In this study, we report evidences that *Mycobacterium tuberculosis* (MTB)-induced apoptosis in macrophages is reduced by a broad-spectrum hydroxamic acid-based matrix metalloproteinase (MMP) inhibitor, Batimastat (BB-94). In particular, we show that BB-94 administration to MTB-infected macrophages inhibits apoptosis and the downmodulation of membrane CD14 expression. Moreover, the addition of broad spectrum matrix metalloproteinase inhibitor to cell culture, during MTB infection, decreases the release of soluble TNF-alpha and leads to a simultaneous increase of membrane TNF-alpha. These results show that MTB-induced apoptosis in macrophages is reduced by a MMP inhibitor and most probably is related to TNF-alpha release. This identifies BB-94 as a simultaneous anti-apoptotic and anti-inflammatory molecule during MTB infection.—Authors' Abstract

Umemura, M., Nishimura, H., Saito, K., Yajima, T., Matsuzaki, G., Mizuno, S., Sugawara, I., and Yoshikai, Y. Interleukin-15 as an immune adjuvant to increase the efficacy of *Mycobacterium bovis* bacillus Calmette-Guerin vaccination. Infect. Immun. **71(10)** (2003) 6045–6048.

Interleukin-15 (IL-15) transgenic mice which had been inoculated with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) 24 weeks previously showed resistance against airborne infection with *Mycobacterium tuberculosis* H37Rv accompanied by an increased CD8(+)-Tc1-cell response. IL-15 may be used as an immune adjuvant given with BCG vaccination to enhance its biologic efficacy.—Authors' Abstract

Vuola, J. M., Ristola, M. A., Cole, B., Jarviluoma, A., Tvaroha, S., Ronkko, T., Rautio, O., Arbeit, R. D., and Reyn, C. F. Immunogenicity of an inactivated mycobacterial vaccine for the prevention of HIV-associated tuberculosis: a randomized, controlled trial. AIDS **17(16)** (2003) 2351–2355.

SUMMARY: OBJECTIVE: Prior to the widespread use of *Mycobacterium bovis*, Bacille Calmette-Guerin (BCG), inactivated whole cell mycobacterial vaccines had been shown effective in the prevention of tuberculosis. The present study was conducted to determine the safety and immunogenicity of an inactivated whole cell mycobacterial vaccine in persons with HIV infection. DESIGN: Randomized, controlled trial. METHODS: A total of 39 HIV-positive patients with prior BCG immunization and CD4 cell counts $\geq 200 \times 106$ cells/l were randomized to five doses of inactivated *Mycobacterium vaccae* (MV) vaccine or control vaccine (CV). Lymphocyte proliferation (LPA) and interferon gamma (IFN-gamma) responses to mycobacterial antigens were assayed at baseline, after three and five doses of vaccine and >1 year later. Parallel studies were conducted in 10 HIV-negative subjects with prior BCG immunization. RESULTS: Among HIV-positive patients, 19 MV recipients had higher LPA and IFN-gamma responses to MV sonicate than 20 CV recipients after three and five doses of vaccine and >1 year later. LPA responses to *Mycobacterium tuberculosis* whole cell lysate increased over time in both groups consistent with prior BCG immunization and current antiretroviral therapy; after three doses, responses were boosted to higher levels in MV subjects than CV subjects. LPA responses to WCL were also boosted in HIV-negative MV recipients. Immunization was safe and had no adverse effects on HIV viral load or CD4 cell count. CONCLUSIONS: In BCG-primed, HIV-positive and HIV-negative subjects, MV induces durable cellular immune responses to a new mycobacterial antigen and boosts pre-existing responses to WCL. MV is a candidate for clinical trials for the prevention of HIV-associated tuberculosis.—Authors' Abstract

Waters, W. R., Nonnecke, B. J., Foote, M. R., Maue, A. C., Rahner, T. E., Palmer, M. V., Whipple, D. L., Horst, R. L., and Estes, D. M. *Mycobacterium bovis* bacille Calmette-Guerin vaccination of

cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. Tuberculosis (Edinb.) **83(5)** (2003) 287–297.

SETTING: 1,25-dihydroxyvitamin D3 (1,25(OH)(2)D(3)) is a potent modulator of immune responses and may be beneficial in the treatment of tuberculosis. Recent evidence suggest that 1,25(OH)(2)D(3) may affect T-dependent responses in cattle; however, mechanisms by which this vitamin modulates activation of bovine T cells are unclear. OBJECTIVE: Determine the effects of 1,25(OH)(2)D(3) on the expression of CD25, CD44, and CD62L by bovine T cell subsets proliferating in response to antigen stimulation. DESIGN: Antigenspecific recall responses of *Mycobacterium bovis* bacille Calmette-Guerin (BCG) vaccinated cattle were used as a model system to evaluate effects of 1,25(OH)(2)D(3) on the proliferation and activation of bovine T cell subsets. RESULTS: CD4(+) and gamma delta TCR(+) cells were the predominant T cell subsets responding to soluble crude *M. bovis*-derived antigens (i.e., purified protein derivative and a BCG whole cell sonicate) by proliferation and activation-induced alterations in phenotype. These subsets exhibited increased CD25 and CD44 mean fluorescence intensity (mfi) and decreased CD62L mfi upon antigen stimulation. Addition of 1,25(OH)(2)D(3) inhibited proliferation of CD4(+) cells and decreased the expression of CD44 on responding (i.e., proliferating) CD4(+) and gamma delta TCR(+) cells. CONCLUSION: These findings suggest that the production of 1,25(OH)(2)D(3) by macrophages within tuberculous lesions would inhibit proliferation and CD44 expression by co-localized CD4(+) and gamma delta TCR(+) cells.—Authors' Abstract

Epidemiology

de Aquino, D. M. C., Santos, J. S., and Costa, J. M. L. [Assessment of a leprosy control program in a hyperendemic county in the State of Maranhão, Brazil, 1991–1995.] Cadernos de Saúde Pública **19(1)** (2003) 119–125. [Article in Spanish].

This is a descriptive study to assess the leprosy control program in the municipality of Buriticupu in Maranhão State, Brazil. The records of 214 patients with different forms of leprosy were studied. Patients were treated at a health center of the Federal University in Maranhão located in the above-mentioned municipality. The study population was comprised of 110 cases with paucibacillary leprosy (PB) and 104 with multibacillary leprosy (MB). The patients were registered between January 1991 and December 1995. Data on the form of the disease, number of contacts registered, examined, and assessed, degree of disability at the beginning and end of treatment, and the register's status were collected on a form designed specifically for this purpose. Analysis of results was based on operational guidelines developed by the Ministry of Health. There was a slight predominance of the PB form. Observation of patients with physical disabilities at the beginning and end of treatmnet was low, as were levels of successful treatment and examined contacts. There was a high dropout level. The program showed "low-level performance" for all indicators used in the study. —Tropical Diseases Bulletin

Dourado, I., Rios, M. H., Pereira, S. M. M., Cunha, S. S., Ichihara, M. Y., Goes, J. C. L., Rodrigues, L. C., Bierrenbach, A. L., and Barreto, M. L. Rates of adverse reactions to first and second doses of BCG vaccination: results of a large community trial in Brazilian school children. **7(4)** (2003) 399–402.

OBJECTIVE: To evaluate the incidence of adverse reactions to 1st and 2nd BCG vaccination in school children. SETTING AND DESIGN: Enhanced surveillance in a Brazilian trail. Suspected reactions were reported to a nurse who visited cases and

completed a standard form. RESULTS: Among 71,341 school children studied, 33 reactions were reported. Of these, 25 fulfilled the criteria, resulting in a rate of one per 2854 vaccinations, with no deaths or BCG adverse effects. Reactions to 2nd doses were more common than to 1st BCG vaccinations, but this difference was not statistically significant. CONCLUSIONS: Adverse reactions to a 2nd dose of BCG may be more frequent than reactions to a 1st dose, but they are still rare events. —Tropical Diseases Bulletin

Figueiredo, I. A., and Silva, A. A. M. [Increase in leprosy detection rates in São Luis, Maranhão, Brazil, from 1993 to 1998. Is the endemic expanding?] Cadernos de Saúde Pública **19(2)** (2003) 439–445.

A descriptive epidemiological study on the detection of new leprosy cases was conducted in São Luís, Maranhão, Brazil, during 1993–98. A database was created for the purpose, covering 2796 reported cases. General detection rates were calculated, as well as specific rates by gender, clinical type, and age group. Linear, exponential, geometric, and log adjustment models were performed to analyse time trends in the disease. An increase in detection was observed, involving mostly female and paucibacillary cases, mainly of tuberculoid leprosy. The increase in detection was most evident in the age group 15–19 years. The percentage of detection under 15 years indicated the need for active case search in this group. —Tropical Diseases Bulletin

Phaff, C., Van Den Broek, J., MacArthur, A., Jr., Ndeve, A., and Stuip, Y. Characteristics and treatment outcomes of leprosy patients detected during a leprosy elimination campaign in Mozambique compared with routinely detected patients. Lepr. Rev. **74(3)** (2003) 229–239.

The objective of this study is to assess whether the case-finding method is a determinant for diagnostic characteristics and treatment outcome of newly diagnosed leprosy patients in Northern Mozambique. This is a retrospective cohort study of 3202 patients on the differences between entrance characteristics and treatment outcome in self-reporting patients and patients detected during a leprosy elimination campaign (LEC) in 1999 in Northern Mozambique. As a consequence of LEC activities, 3 times more patients were found compared with the same period 1 year earlier. After the LEC, case detection remained higher in the years 2000–2002 compared with the years preceding the LEC. More young (<15 years) paucibacillary (PB) cases were diagnosed during LEC activities with, surprisingly, equal percentage of disability grades. No gender imbalance was found in diagnosed LEC patients contrary to self-reporting patient groups. Comparing patients detected during a LEC in 1999 with the passive group of 1998 and 1999 showed a slight but statistically significant better treatment result for the passive group. The classification of leprosy (in favor of PB) and age (in favor of older age groups) were also determinants for favorable treatment outcomes. Volunteers had a significantly better result of treatment compared with trained nurses and regardless of detection method. LEC proved to be a useful addition to the National Leprosy and Tuberculosis Programme in Northern Mozambique. As a result, many new cases were diagnosed and put on treatment and their treatment results were very satisfactory. LEC had a lasting impact on case finding. Volunteers make a valuable contribution to leprosy control in Mozambique because they have consistently better treatment results compared with nurses.—Authors' Abstract

Shin, S. S., Hyson, A. M., Castañeda, C., Sánchez, E., Alcántara, F., Mitnick, C. D., Fawzi, M. C. S., Bayona, J., Farmer, P. E., Kim, J. Y., and Furin, J. J. Peripheral Neuropathey associated with treatment for multidrug-resistant tuberculosis. Int. J. Tuberc. Lung Dis. **7(4)** (2003) 347–353.

OBJECTIVE: To review the incidence and management of peripheral neuropathy in patients receiving therapy for multiple drug resistant tuberculosis (MDR-TB). METHODS: A case series with retrospective chart review of 75 patients who initiated individualized therapy for MDR-TB in Lima, Peru, between 1 August 1996 and 31 January 1999. RESULTS: All patients had

confirmed MDR-TB and were receiving individualized therapy, comprised of an average of 6 drugs. 10 (13%) of these patients presented with symptoms of peripheral neuropathy, confirmed by electromyography. All symptoms were reported in the lower extremities, and all were sensory in nature. Median time to presentation from initiation of MDR-TB therapy was 9.1 months. No significant risk factors associated with development of peripheral neuropathy were identified. Management strategies depended on the severity of symptoms and included the treatment of contributing co-morbidities, medications for neuropathic pain, and adjustment of doses of possible offending agents. All patients responded to management: 3 patients were left with mild residual symptoms. Patients whose neuropathy resolved had symptoms for a median of 7 months. CONCLUSIONS: Peripheral neuropathy was encountered in 13% of our cohort of MDR-TB patients. The diagnosis of peripheral neuropathy can be based on clinical presentation alone, and effective management of this side effect is possible without sacrificing MDR-TB treatment efficacy. —Tropical Diseases Bulletin

Taylor, R., King, K., Vodicka, P., Hall, J., and Evans, D. Screening for leprosy in immigrants—a decision analysis model. Lepr. Rev. **74(3)** (2003) 240–248.

Almost all leprosy cases reported in industrialized countries occur amongst immigrants or refugees from developing countries where leprosy continues to be an important health issue. Screening for leprosy is an important question for governments in countries with immigration and refugee programmes. A decision analysis framework is used to evaluate leprosy screening. The analysis uses a set of criteria and parameters regarding leprosy screening, and available data to estimate the number of cases which would be detected by a leprosy screening programme of immigrants from countries with different leprosy prevalences, compared with a policy of waiting for immigrants who develop symptomatic clinical diseases to present for health care. In a cohort of 100,000 immigrants from high leprosy prevalence regions (3.6/10,000), screening would detect 32 of the 42 cases which would arise in the destination country over the 14 years after migration; from medium prevalence areas (0.7/10,000) 6.3 of the total 8.1 cases would be detected, and from low prevalence regions (0.2/10,000) 1.8 of 2.3 cases. Using Australian data, the migrant mix would produce 74 leprosy cases from 10 years intake; screening would detect 54, and 19 would be diagnosed subsequently after migration. Screening would only produce significant case-yield amongst immigrants from regions or social groups with high leprosy prevalence. Since the number of immigrants to Australia from countries of higher endemnicity is not large routine leprosy screening would have a small impact on case incidence.—Authors' Abstract

Other Mycobacterial Diseases

Au, W. Y., Cheng, V. C., Ho, P. L., Yuen, K. Y., Hung, I., Ma, S. Y., Lie, A. K., Liang, R., and Kwong, Y. L. Nontuberculous mycobacterial infections in Chinese hematopoietic stem cell transplantation recipients. Bone Marrow Transplant. **32(7)** (2003) 709–714.

Between 1995 and 2002, nine cases of nontuberculous mycobacterium (NTM) were isolated from 462 allogeneic stem cell transplant (SCT) recipients (1.9%), and none from 139 autologous cases. They included three cases each of *Mycobacterium fortuitum* and *M. chelonae*, and single cases of *M. scrofalaceum*, *M. gordonnae* and *M. avium* complex. Seven cases were respiratory, including five cases requiring treatment, and two involved infected catheters and vascular conduits. Compared with nine cases of *Mycobacterium tuberculosis* (MTB) isolated in the same period, NTM isolation occurred later after HSCT and involved more unrelated donors. Important risk factors for NTM infection included significant aGVHD ($p = 0.043$), leukemia relapse ($p = 0.022$), MUD and mismatch SCT ($p < 0.001$) and existence of BO ($p < 0.001$).

Coinfection with aspergillus was common. Invasive NTM disease required prolonged antimicrobial treatment in five cases due to *M. fortuitum* and *M. chelonae*. With better MTB prophylaxis, intensive immunosuppression and better awareness, NTM has become an emerging threat in oriental allogeneic HSCT recipients. The cutoff between colonization and infection, and the threshold for starting treatment is unclear. NTM isolation is a marker for severe immunosuppression and poor prognosis. When there is doubt over species identity or extent of infection, broad-spectrum cover may be prudent.—Authors' Abstract

Benson, C. A., Williams, P. L., Currier, J. S., Holland, F., Mahon, L. F., MacGregor, R. R., Inderlied, C. B., Flexner, C., Neidig, J., Chaisson, R., Notario, G. F., Hafner, R.; and AIDS Clinical Trials Group 223 Protocol Team. A prospective, randomized trial examining the efficacy and safety of clarithromycin in combination with ethambutol, rifabutin, or both for the treatment of disseminated *Mycobacterium avium* complex disease in persons with acquired immunodeficiency syndrome. Clin. Infect. Dis. **37(9)** (2003) 1234–1243.

This multicenter, randomized, open-label phase 3 clinical trial compared the safety and efficacy of 3 clarithromycin-containing combination regimens for the treatment of disseminated *Mycobacterium avium* complex (MAC) disease in persons with acquired immunodeficiency syndrome. A total of 160 eligible patients with bacteremic MAC disease were randomized to receive clarithromycin with either ethambutol (C+E), rifabutin (C+R), or both (C+E+R) for 48 weeks. After 12 weeks of treatment, the proportion of subjects with a complete microbiologic response was not statistically significantly different among treatment arms: the proportion was 40% in the C+E group, 42% in the C+R group, and 51% in the C+E+R group ($p = 0.454$). The proportion of patients with complete or partial responses who experienced a relapse while receiving C+R (24%) was significantly higher than that of patients receiving C+E+R (6%; $p = 0.027$) and marginally higher than that of patients receiving C+E (7%; $p = 0.057$). Subjects in the C+E+R group had improved survival, compared with the C+E group (hazard ratio [HR], 0.44; 95% confidence interval [CI], 0.23–0.83) and the C+R group (HR, 0.49; 95% CI, 0.26–0.92).—Authors' Abstract

Blaas, S. H., Bohm, S., Martin, G., Erler, W., Gluck, T., Lehn, N., and Naumann, L. Pericarditis as primary manifestation of *Mycobacterium bovis* SSP. caprae infection. Diagn. Microbiol. Infect. Dis. **47(2)** (2003) 431–433.

A 76-year-old white male presented with progressive malaise, weight loss and dyspnea at rest. Echocardiography revealed a circular pericardial effusion and global hypokinesia. Pericardiocentesis showed a purulent exudate and microbiologic examination revealed *Mycobacterium bovis* fully sensitive to isoniazid, streptomycin, ethambutol, rifampin, and pyrazinamide. By spoligotyping the isolate could be further differentiated to *M. bovis* ssp. caprae. Antimycobacterial therapy was initiated but 3 weeks later the patient's circulation and renal function deteriorated and he died with clinical signs of sepsis despite intensive care treatment. Pericarditis is a rare manifestation of tuberculosis and can be fatal even when diagnosed and treated appropriately. In low incidence countries diagnosis is often delayed and even overlooked.—Authors' Abstract

Causero, A., Screm, C., Beltrame, A., and Mastidoro, L. *Mycobacterium marinum*: a case of skin granuloma complicated by tenosynovitis of the extensors. Chir. Organi. Mov. **88(1)** (2003) 93–97. [Article in English, Italian].

The authors report the case of a male patient who owned a tropical aquarium and who developed a *M. marinum* skin infection of the wrist. The clinical findings and microbiological features of the case are described, as are the difficulty in providing a prompt diagnosis, and the need for surgical treatment and the use of antibiotics to treat the infection.—Authors' Abstract

Cousins, D. V., Bastida, R., Cataldi, A., Quse, V., Redrobe, S., Dow, S., Duignan, P., Murray, A., Dupont, C., Ahmed, N., Collins, D. M., Butler, W. R., Dawson, D., Rodriguez, D., Loureiro, J., Romano, M. I., Alito, A., Zumarraga, M., and Bernardelli, A. Tuberculosis in seals caused by a novel member of the Mycobacterium tuberculosis complex: *Mycobacterium pinnipedii* sp. nov. Int. J. Syst. Evol. Microbiol. **53(Pt 5)** (2003) 1305–1314.

A comparison of *Mycobacterium tuberculosis* complex isolates from seals (pinnipeds) in Australia, Argentina, Uruguay, Great Britain and New Zealand was undertaken to determine their relationships to each other and their taxonomic position within the complex. Isolates from 30 cases of tuberculosis in six species of pinniped and seven related isolates were compared to representative and standard strains of *the M. tuberculosis* complex. The seal isolates could be distinguished from other members of the *M. tuberculosis* complex, including the recently defined '*Mycobacterium canettii*' and '*Mycobacterium caprae,*' on the basis of host preference and phenotypic and genetic tests. Pinnipeds appear to be the natural host for this 'seal bacillus,' although the organism is also pathogenic in guinea pigs, rabbits, humans, Brazilian tapir (Tapirus terrestris) and, possibly, cattle. Infection caused by the seal bacillus is predominantly associated with granulomatous lesions in the peripheral lymph nodes, lungs, pleura, spleen and peritoneum. Cases of disseminated disease have been found. As with other members of the *M. tuberculosis* complex, aerosols are the most likely route of transmission. The name *Mycobacterium pinnipedii* sp. nov. is proposed for this novel member of *the M. tuberculosis* complex (the type strain is 6482(T)=ATCC BAA-688(T)=NCTC 13288(T)).—Authors' Abstract

Evans, M. R., Phillips, R., Etuaful, S. N., Amofah, G., Adomako, J., Adjei, O., Dennis-Antwi, J., Lucas, S. B., and Wansbrough-Jones, M. H. An outreach education and treatment project in Ghana for the early stage of *Mycobacterium ulcerans* disease. Trans. R. Soc. Trop. Med. Hyg. **97(2)** (2003) 159–160.

Mycobacterium ulcerans disease starts as a painless, subcutaneous nodule, excision of which prevents the development of large Buruli ulcers. An outreach programme was set up in Ghana to promote nodule recognition and excision. The programme was cost-effective and shifted the pattern of disease presentation. This could from a model for other countries.—Authors' Abstract

Goldschmidt, N., Nusair, S., Gural, A., Amir, G., Izhar, U., and Laxer, U. Disseminated *Mycobacterium kansasii* infection with pulmonary alveolar proteinosis in a patient with chronic myelogenous leukemia. Am. J. Hematol. **74(3)** (2003) 221–223.

A 64-year-old woman with chronic myelogenous leukemia (CML) was admitted due to prolonged fever and lung infiltrates. An open lung biopsy was required to make the diagnosis of pulmonary alveolar proteinosis (PAP) and infection *with Mycobacterium kansasii.* She was treated successfully with combined antimycobacterial therapy for 14 months. However, the leukemia progressed and the patient developed recurrent bilateral lung infiltrates. Blood and bronchoalveolar fluid cultures yielded growth of Acinetobacter. She died shortly thereafter due to septic shock. The relationship between *M. kansasii* infection, PAP, and abnormal host defense in CML is discussed. Copyright 2003 Wiley-Liss, Inc.—Authors' Abstract

Griffith, D. E., Brown-Elliott, B. A., and Wallace, R. J. Jr. Thrice-weekly clarithromycin-containing regimen for treatment of *Mycobacterium kansasii* lung disease: results of a preliminary study. Clin. Infect. Dis. **37(9)** (2003) 1178–1182.

We initiated a prospective trial of an intermittent clarithromycin-containing regimen for the treatment of patients with *Mycobacterium kansasii* lung disease. Eighteen patients (10 men and 8 women) with *M. kansasii* lung disease received a regimen consisting of 500–1000 mg of clar-

ithromycin, 25 mg/kg ethambutol, and 600 mg of rifampin 3 times per week. The primary treatment end point was a 12-month period during which sputum cultures were sterile while the patient was receiving therapy. Four male patients were lost to follow-up, but all of the remaining patients successfully completed therapy without significant drug-related adverse events. The mean time (±standard deviation [S.D.]) to sputum conversion was 1.0 ± 0.9 months, and the mean duration (±S.D.) of therapy was 13.4 ± 0.9 months. No patient who successfully completed therapy had relapsed after a mean (±S.D.) of 46 ± 8.0 months. Clarithromycin- and rifampin-containing regimens offer the possibility of effective short-course and intermittent treatment of *M. kansasii* lung disease.—Authors' Abstract

Hung, G. U., Lan, J. L., Yang, K. T., Lin, W. Y., and Wang, S. J. Scintigraphic findings of *Mycobacterium avium* complex tenosynovitis of the index finger in a patient with systemic lupus erythematosus. Clin. Nucl. Med. **28(11)** (2003) 936–938.

The presented case is a 36-year-old woman with a history of systemic lupus erythematosus for 10 years. She had progressively painful swelling of the right index finger that later proved to be a rare case of tenosynovitis caused *by Mycobacterium avium* complex. Serial images of 3-phase bone scans, gallium scan, and magnetic resonance imaging demonstrate the area of involvement.—Authors' Abstract

Ito, K., Hashimoto, K., and Ogata, H. [Activity of cephems and carbapenems against clinically isolated *Mycobacterium abscessus*]. Kekkaku **78(9)** (2003) 587–590. [Article in Japanese].

To screen effective useful drugs for disease due to *M. abscessus,* we determined MIC of 3 cephems [ceftazidime (CAZ), cefoxitin (CFX), flomoxef (FMOX)] and 3 carbapenems [imipenem (IPM), panipenem (PAPM), meropenem (MEPM)] for 8 strains of clinically isolated *M. abscessus* by micro-dilution method using MGIT system. In all the 8 strains, MICs of CAZ are higher than 32 micrograms/ml. MIC50, MIC90, MIC range of CFX are 32 micrograms/ml, >32 micrograms/ml and 16 ≥32 micrograms/ml respectively, and for FMOX, 16 micrograms/ml, 32 micrograms/ml and 16–32 micrograms/ml; for IPM, 8 micrograms/ml, 16 micrograms/ml and 8–16 micrograms/ml; for PAPM, 4 micrograms/ml, 16 micrograms/ml and 4–16 micrograms/ml; for MEPM, 8 micrograms/ml, 16 micrograms/ml and 8–16 micrograms/ml. From this study, it is concluded that FMOX, IPM, PAPM and MEPM can be clinically useful drugs in the treatment of the disease due to *M. abscessus*.—Authors' Abstract

Kerbiriou, L., Ustianowski, A., Johnson, M. A., Gillespie, S. H., Miller, R. F., and Lipman, M. C. Human immunodeficiency virus type 1-related pulmonary *Mycobacterium xenopi* infection: a need to treat? Clin. Infect. Dis. **37(9)** (2003) 1250–1254.

We report treatment decisions and outcomes for 20 patients who were infected with human immunodeficiency virus type 1 (HIV-1) and were receiving highly active antiretroviral therapy (HAART) who had respiratory symptoms and from whom *Mycobacterium xenopi* was isolated. All patients also had coexisting pulmonary pathologic conditions. The median blood T cell CD4 count was 37 cells/microL (range, 2–480 cells/microL). Fifteen of 20 patients received no antimycobacterial therapy and remain healthy after a median of approximately 4 years of follow-up, and 2 patients required treatment specifically for *M. xenopi* infection, both showing clinical improvement. We conclude that pulmonary *M. xenopi* isolation in HIV-1 patients receiving HAART does not usually require specific treatment.—Authors' Abstract

Kohnen, T., Schopfer, D., Buhren, J., and Hunfeld, K. P. [Flap Amputation in *Mycobacterium chelonae* Keratitis after Laser-in-situ Keratomileusis (LASIK)]. Klin. Monatsbl. Augenheilkd. **220(9)** (2003) 634–637. [Article in German].

BACKGROUND: Keratitis due to *Mycobacterium chelonae* after laser-in-situ ker-

atomileusis (LASIK) is a rare, but severe complication. In the following report, we present clinical findings, microbiological investigation, treatment and outcome of the first case of *Mycobacterium chelonae* reported in Europe. PATIENT AND METHODS: A 52-year old woman presented with atypical unilateral keratitis after LASIK. *Mycobacterium chelonae* keratitis was diagnosed by microbiological investigation. Interface irrigation and treatment with topical and oral antibiotics was performed. RESULTS: Despite intensive treatment, flap removal was necessary to control the infection. Best-corrected visual acuity dropped from preoperatively 1.0 to postoperatively 0.2. CONCLUSION: The diagnosis of mycobacterial keratitis after laser-in-situ keratomileusis is often delayed due to atypical clinical appearance. Therefore consideration of atypical pathogens and rapid microbiological diagnosis is necessary to provide adequate treatment.—Authors' Abstract

Li, X. J., Wu, Q. X., and Zeng, X. S. Nontuberculous mycobacterial cutaneous infection confirmed by biochemical tests, polymerase chain reaction-restriction fragment length polymorphism analysis and sequencing of hsp65 gene. Br. J. Dermatol. **149(3)** (2003) 642–646.

We report a woman in whom a slow-growing scotochromogenic strain of Mycobacterium was cultured from skin lesions. According to its phenotypic and biochemical characteristics we could predict only that it might be *M. szulgai*, *M. scrofulaceum* or *M. gordonae*. Polymerase chain reaction amplification of the hsp65 gene and subsequent restriction fragment length polymorphism analysis on the isolated strain showed that its restriction pattern differed from both *M. scrofulaceum* and other scotochromogenic species. Ninety-nine per cent similarity was detected between the isolated strain *and M. gordonae* by sequencing of the hsp65 gene. This result suggests that the isolated strain may be either a slow-growing scotochromogenic Mycobacterium most *resembling M. gordonae* or a novel mycobacterial species.—Authors' Abstract

Manfredi, R., Nanetti, A., Tadolini, M., Calza, L., Morelli, S., Ferri, M., and Marinacci, G. Role of *Mycobacterium xenopi* disease in patients with HIV infection at the time of highly active antiretroviral therapy (HAART). Comparison with the pre-Haart period. Tuberculosis (Edinb.) **83(5)** (2003) 319–328.

BACKGROUND AND SETTING: A reliable and timely clinical, radiological, and bacteriological diagnosis, and an optimal treatment of non-tubercular mycobacteriosis (including *Mycobacterium xenopi* disease), remain an unanswered challenge for clinicians facing immunocompromised patients, including those with HIV infection. OBJECTIVE: The aim of our survey is to report the frequency, and the epidemiological, immunological, microbiological, clinical, and therapeutic features of all confirmed HIV-associated *M. xenopi* disease observed from 1993–2002, with special attention paid to eventual differences that emerged after the introduction of potent antiretroviral therapy (highly active antiretroviral therapy, HAART), on the basis of an international literature update. DESIGN AND RESULTS: Our series of 17 consecutive confirmed *M. xenopi* infections retrieved in 14 out of 3000 patients followed for HIV disease complications raises a broad series of clinical, diagnostic, therapeutic, and prophylactic concerns. The great majority of *M. xenopi* disease involved the lower respiratory tract, but atypical features including cavitation and prominent exudative features became apparent in patients successfully treated with HAART, pointing out the possible role of the so-called immune reconstitution syndrome in these episodes. CONCLUSIONS: Diagnostic problems represented by late or missed identification due to slow culture and frequently concomitant opportunistic disorders, join therapeutic difficulties due to the unpredictable in vitro antimicrobial susceptibility profile of these organisms, selection of treatment and chemoprophylaxis according with clinical-radiological and microbiological suspicion, and concomitantly administered medications.—Authors' Abstract

Marsollier, L., Aubry, J., Saint-Andre, J. P., Robert, R., Legras, P., Manceau, A. L., Bourdon, S., Audrain, C., and Carbonnelle, B. [Ecology and transmission

of *Mycobacterium ulcerans*]. Pathol. Biol. (Paris) **51(8–9)** (2003) 490–495. [Article in French].

Mycobacterium ulcerans is an environmental pathogen concerning mainly the tropical countries; it is the causative agent of Buruli ulcer, which has become the third most important mycobacterial disease. In spite of water-linked epidemiological studies to identify the sources of *M. ulcerans*, the reservoir and the mode of transmission of this organism remain elusive. To determine the ecology and the mode of transmission *of M. ulcerans* we have set up an experimental model. This experimental model demonstrated that water bugs were able to transmit *M. ulcerans* by bites. In insects, the bacilli were localized exclusively within salivary glands, where it could both multiply contrary to other mycobacteria species. In another experimental study, we report that the crude extracts from aquatic plants stimulate in vitro the growth of *M. ulcerans* as much as the biofilm formation by *M. ulcerans* has been observed on aquatic plants. Given that the water bugs are essentially carnivorous, it is difficult to imagine a direct contact in the contamination of aquatic bugs and plants. It seems very likely that an intermediate host exists. In an endemic area of Daloa in Cote d'Ivoire, our observations were confirmed.—Authors' Abstract

Panunto, A. C., Villares, M. C., and Ramos, M. C. IS1245 restriction fragment length polymorphism typing of *Mycobacterium avium* from patients admitted to a reference hospital in Campinas, Brazil. Braz. J. Med. Biol. Res. **36(10)** (2003) 1397–1401.

Mycobacterium avium is an important pathogen among immunodeficient patients, especially patients with AIDS. The natural history of this disease is unclear. Several environmental sources have been implicated as the origin of this infection. Polyclonal infection with this species is observed, challenging the understanding of its pathogenesis and treatment. In the present study 45 *M. avium* strains were recovered from 39 patients admitted to a reference hospital between 1996 and 1998. Species identification was performed using a species-specific nucleic acid hybridization test (AccuProbe) from Gen-Probe. Strains were genotyped using IS1245 restriction fragment length polymorphism typing. Blood was the main source of the organism. In one patient with disseminated disease, *M. avium* could be recovered more than once from potentially sterile sites. Strains isolated from this patient had different genotypes, indicating that the infection was polyclonal. Four patient clones were characterized in this population, the largest clone being detected in eight patients. This finding points to a common-source transmission of the organism.—Authors' Abstract

Smith, S., Taylor, G. D., and Fanning, E. A. Chronic cutaneous Mycobacterium haemophilum infection acquired from coral injury. Clin. Infect. Dis. **37(7)** (2003) e100–101.

A 61-year-old previously healthy man developed chronic dermal granulomata in his right arm after receiving a coral injury in Thailand. After 7 biopsies, infection caused by *Mycobacterium haemophilum* was diagnosed. This case highlights the difficulty of isolating this fastidious organism in the laboratory and suggests that seawater or coral was the source of the infection.—Authors' Abstract

Sungkanuparph, S., Sathapatayavongs, B., and Pracharktam, R. Infections with rapidly growing mycobacteria: report of 20 cases. Int. J. Infect. Dis. **7(3)** (2003) 198–205.

OBJECTIVES: A series of cases infected with rapidly growing mycobacteria was studied to determine the spectrum of disease, antimicrobial susceptibility, treatment, and outcome. METHODS: The cases identified as infections with rapidly growing mycobacteria in Ramathibodi Hospital from January 1993 to December 1999 were retrospectively studied. RESULTS: Most of the cases had no underlying disease. Only two cases were HIV-infected patients. The presenting clinical features were lym-

phadenitis (seven cases), skin and/or subcutaneous abscess (seven cases), localized eye infection (four cases), pulmonary infection (one case), and chronic otitis media (one case). Four of seven cases with lymphadenitis had Sweet's syndrome, and one had psoriasis as an associated skin manifestation. Anemia was present in five cases, and improved with treatment of the primary disease. The organisms *were Mycobacterium chelonae*/abscessus group (17 cases) and *Mycobacterium fortuitum* group (three cases). Susceptibility patterns of the organisms showed susceptibility to amikacin, netilmicin, and imipenem. *M. fortuitum* group was susceptible to more antibiotics than *M. chelonae*/abscessus group. The clinical responses corresponded to the antimicrobial susceptibility. Combinations of two or more drugs were used for the medical treatment. Surgical resection was performed where possible, to reduce the load of the organism, especially in cases with very resistant organisms. CONCLUSIONS: Infections with rapidly growing mycobacteria can occur in apparently normal hosts. The clinical syndrome is variable. The pathology is nonspecific. Clinical responses varied, but seemed to correlate with the in vitro susceptibility result. More studies are needed to enable us to deal with this infection effectively.—Authors' Abstract

Sungkanuparph, S., Sathapatayavongs, B., and Pracharktam, R. Infections with rapidly growing mycobacteria: report of 20 cases. Int. J. Infect. Dis. **7(3)** (2003) 198–205.

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Szlavik, J., and Sarvari, C. Pulmonary *Mycobacterium xenopi* infection in AIDS patients treated with HAART in Hungary. Eur. J. Clin. Microbiol. Infect. Dis. **22(11)** (2003) 701–703.

Reported here are three cases of pulmonary *Mycobacterium xenopi* infection that occurred in AIDS patients in Hungary shortly after starting highly active antiretroviral therapy. In this country, *Mycobacterium xenopi* is the most common nontuberculous mycobacterial species causing pulmonary mycobacterial infections. Cases of pulmonary *Mycobacterium xenopi* disease have been described in patients infected with the human immunodeficiency virus infection and in patients with other immunodeficiencies; however, only limited information is currently available concerning the connection between nontuberculous *Mycobacterium infection* and AIDS in Hungary. This report thus adds useful information regarding the diagnosis, clinical course, and

treatment regimens of *Mycobacterium xenopi* infections in AIDS patients.—Authors' Abstract

van der Werf, T. S., Stinear, T., Stienstra, Y., van der Graaf, W. T., and Small, P. L. Mycolactones and *Mycobacterium ulcerans* disease. Lancet **362(9389)** (2003) 1062–1064.

CONTEXT: *Mycobacterium ulcerans* causes devastating necrotic lesions in affected individuals. The disease, commonly called Buruli ulcer, is increasing in prevalance in western African countries. Treatment is mainly surgical; no clinical trials have been done to support the use of antimycobacterial drugs. A secreted polyketide toxin, mycolactone, is responsible for the tissue damage; its chemical structure has been elucidated. STARTING POINT: Although the main treatment is surgical, many patients with Buruli ulcer present late because of unusual beliefs about the disease and its treatment. Isabelle Aujoulat and colleagues recently showed, in a study in southern Benin, Africa (Trop Med Int Health 2003; 8: 750–759), that although the ulcer is well recognized, the cause is often seen as environmental or because of witchcraft. In addition, treatment is thought to be destructive, costly, and ineffective. WHERE NEXT? Antimycobacterial drug regimens that hold promise based on animal and preliminary human studies will soon be tested in large well-designed controlled clinical trials. Information gleaned from the genomic sequence of *M. ulcerans* could be used to design more effective vaccines, or new drug targets (e.g., that knock out the enzymes of *M. ulcerans* that synthesize mycolactone species).—Authors' Abstract

Molecular and Genetic Studies

Bleharski, J. R., Li, H., Meinken, C., Graeber, T. G., Ochoa, M. T., Yamamura, M., Burdick, A., Sarno, E. N., Wagner, M., Rollinghoff, M., Rea, T. H., Colonna, M., Stenger, S., Bloom, B. R., Eisenberg, D., and Modlin, R. L. Use of genetic profiling in leprosy to discriminate clinical forms of the disease. Science **301(5639)** 1527–1530.

Leprosy presents as a clinical and immunological spectrum of disease. With the use of gene expression profiling, we observed that a distinction in gene expression correlates with and accurately classifies the clinical form of the disease. Genes belonging to the leukocyte immunoglobulin-like receptor (LIR) family were significantly upregulated in lesions of lepromatous patients suffering from the disseminated form of the infection. In functional studies, LIR-7 suppressed innate host defense mechanisms by shifting monocyte production from interleukin-12 toward interleukin-10 and by blocking antimicrobial activity triggered by Toll-like receptors. Gene expression profiles may be useful in defining clinical forms of disease and providing insights into the regulation of immune responses to pathogens.—Authors' Abstract

Cooksey, R. C., Limor, J., Morlock, G. P., and Crawford, J. T. Identifying Mycobacterium species and strain typing using a microfluidic labchip instrument. Biotechniques **35(4)** (2003) 786–794.

We developed schemes for rapid identification of Mycobacterium species and strain typing using a microfluidic labchip instrument. A 439-bp region of the gene that codes for the 65-kDa heat shock protein (hsp65), which has sequence polymorphisms specific for most mycobacterial species, was examined using PCR-restriction analysis (PRA). We performed PRA in duplicate, using 2 strains each of 12 species, and observed that fragment sizes (bp) determined automatically by the instrument were consistently smaller than the correct sizes for each of the species as determined by sequence analysis (mean variance, <7 bp). *Mycobacterium tuberculosis* isolates were

typed with the labchip instrument using mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing, which determines the number of copies of repeated units at 12 loci in the genome based on product size after PCR amplification. Seven strains with one to six repeat copies at each locus were examined. Sizes were smaller by a mean of 13.47 bp compared with correct sizes predicted by sequence analysis, but could be used to correctly identify all strains types. Isolates of *Mycobacterium chelonae* and *Mycobacterium abscessus* were typed using randomly amplified polymorphic DNA (RAPD) electrophoresis, and patterns obtained using the labchip instrument were compared with multilocus enzyme electrophoresis (MEE) types. Patterns were distinct and reproducible for all strains except those with closely related MEE types. The labchip instrument is a versatile alternative for sizing mycobacterial DNA fragments.—Authors' Abstract

Daugelat, S., Kowall, J., Mattow, J., Bumann, D., Winter, R., Hurwitz, R., and Kaufmann, S. H. The RD1 proteins of *Mycobacterium tuberculosis*: expression in *Mycobacterium smegmatis* and biochemical characterization. Microbes Infect. **5(12)** (2003) 1082–1095.

A 9.5-kb section of DNA called region of deletion 1 (RD1) is present in virulent *Mycobacterium tuberculosis* strains but is deleted in all attenuated *Mycobacterium bovis* BCG vaccine strains. This region codes for at least nine genes. Some or all RD1 gene products may be involved in virulence and pathogenesis, and at least two, ESAT-6 and CFP-10, represent potent T- and B-cell antigens. In order to produce the entire set of RD1 proteins with their natural post-translational modifications, a robust expression system for *M. tuberculosis* proteins in the fast-growing saprophytic strain *Mycobacterium smegmatis* was developed. Our system employs the inducible acetamidase promoter and allows translational fusion of recombinant *M. tuberculosis* proteins with polyhistidine or influenza hemagglutinin epitope tags for affinity purification. Using eGFP as reporter gene, we showed that the acetamidase promoter is tightly regulated in *M. smegmatis* and that this promoter is much stronger than the widely used constitutive groEL2 promoter. We then cloned 11 open reading frames (ORFs) found within RD1 and successfully expressed and purified the respective proteins. Sera from tuberculosis patients and *M. tuberculosis*-infected mice reacted with 10 purified RD1 proteins, thus demonstrating that Rv3871, Rv3872, Rv3873, CFP-10, ESAT-6, Rv3876, Rv3878, Rv3879c and ORF-14 are expressed *in vivo*. Finally, glycosylation of the RD1 proteins was analyzed. We present preliminary evidence that the PPE protein Rv3873 is glycosylated at its C terminus, thus highlighting the ability of *M. smegmatis* to produce *M. tuberculosis* proteins bearing posttranslational modifications.—Authors' Abstract

Goulding, C. W., Perry, L. J., Anderson, D., Sawaya, M. R., Cascio, D., Apostol, M. I., Chan, S., Parseghian, A., Wang, S. S., Wu, Y., Cassano, V., Gill, H. S., and Eisenberg, D. Structural genomics of *Mycobacterium tuberculosis*: a preliminary report of progress at UCLA. Biophys. Chem. **105(2–3)** (2003) 361–370.

The growing list of fully sequenced genomes, combined with innovations in the fields of structural biology and bioinformatics, provides a synergy for the discovery of new drug targets. With this background, the TB Structural Genomics Consortium has been formed. This international consortium is comprised of laboratories from 31 universities and institutes in 13 countries. The goal of the consortium is to determine the structures of over 400 potential drug targets from the genome of *Mycobacterium tuberculosis* and analyze their structures in the context of functional information. We summarize the efforts of the UCLA consortium members. Potential drug targets were selected using a variety of bioinformatics methods and screened for certain physical and species-specific properties to yield a starting group of protein targets for structure determination. Target determination methods include protein phylogenetic profiles and Rosetta Stone methods, and the

use of related biochemical pathways to select genes linked to essential prokaryotic genes. Criteria imposed on target selection included potential protein solubility, protein or domain size, and targets that lack homologs in eukaryotic organisms. In addition, some protein targets were chosen that are specific to *M. tuberculosis,* such as PE and PPE domains. Thus far, the UCLA group has cloned 263 targets, expressed 171 proteins and purified 40 proteins, which are currently in crystallization trials. Our efforts have yielded 13 crystals and eight structures. Seven structures are summarized here. Four of the structures are secreted proteins: antigen 85B; MPT 63, which is one of the three major secreted proteins of *M. tuberculosis;* a thioredoxin derivative Rv2878c; and potentially secreted glutamate synthetase. We also report the structures of three proteins that are potentially essential to the survival of *M. tuberculosis:* a protein involved in the folate biosynthetic pathway (Rv3607c); a protein involved in the biosynthesis of vitamin B5 (Rv3602c); and a pyrophosphatase, Rv2697c. Our approach to the *M. tuberculosis* structural genomics project will yield information for drug design and vaccine production against tuberculosis. In addition, this study will provide further insights into the mechanisms of mycobacterial pathogenesis.—Authors' Abstract

Rao, V., Dhar, N., and Tyagi, A. K. Modulation of host immune responses by overexpression of immunodominant antigens of *Mycobacterium tuberculosis* in bacille Calmette-Guerin. Scand. J. Immunol. **58(4)** (2003) 449–461.

Based on their immunodominant nature and ability to induce appropriate immune responses in the host, several antigens of *Mycobacterium tuberculosis* have shown promise of protection. However, most of the candidate vaccines developed by employing various strategies have afforded protection that is at best comparable with bacillus Calmette-Guerin (BCG) in animal models. Due to the inherent ability of BCG to prime cellular responses in the host, it has become an attractive vehicle for development of a vaccine against intracellular infections. In this study, we have cloned the genes of three immunodominant antigens of *M. tuberculosis* viz. the ESAT6 (Rv3875), the 19 kDa lipoprotein (Rv3763) and the 38 kDa antigen (Pst homolog) (Rv0934) in pSD5 under the transcriptional control of Trrn, a strong mycobacterial promoter, and expressed them in BCG. The19 kDa antigen and the 38 kDa antigen were expressed at levels that were approximately five and eightfolds higher in the cytosols of recombinant BCG strains rBCG19T and rBCG38T, respectively, as compared with their corresponding levels in *M. bovis* BCG. Both these antigens were also secreted into the extracellular medium at enhanced levels (19 kDa antigen fourfold and 38 kDa antigen twofold) by rBCG strains in comparison with the wild type BCG. ESAT6 antigen, which is absent in *M. bovis* BCG, was also expressed at a very high level in the cytosol of the rBCG strain (rBCGE6T). Evaluation of immune responses induced by these three rBCG strains in mice shows a markedly different pattern. The rBCG strain overexpressing the 38 kDa antigen exhibited a predominant T helper 1 (Th1) response with high levels of interferon-gamma (IFN-gamma) production, whereas overexpression of the 19 kDa antigen resulted in completely polarized Th2 responses against the BCG sonicate. The rBCG-expressing ESAT6 antigen induced a mixed Th1/Th2 response. Our observations suggest that the 38 kDa antigen may hold excellent promise in the rBCG approach for the development of a vaccine against tuberculosis.—Authors' Abstract

Rivera-Gutierrez, S., Montoro-Cardoso, E., Valdivia, J. A., Cox, R. A., and Gonzalez-y-Merchand, J. A. The number and organization of the rRNA genes of several strains of *Mycobacterium simiae*. FEMS Microbiol. Lett. **227(1)** (2003) 133–139.

The type strain of *Mycobacterium simiae* and four Cuban strains, each representing a group of variants sharing a characteristic pattern of glycopeptidolipids, were investigated. Each of the five strains was found to have a single rRNA (rrn) operon per genome. Each rrn operon was found to be

located downstream from murA. Unusually for slow-growing mycobacteria, three transcription start points were identified for each operon. Gene sequences were established extending from near to the 3′-ends of murA, the intergenic regions and the 5′-ends of the 16S rDNAs. Characteristic strain differences were identified.—Authors' Abstract

Sassetti, C. M., and Rubin, E. J. Genetic requirements for mycobacterial survival during infection. Proc. Natl. Acad. Sci. U. S. A. **100(22)** (2003) 12989–12994.

Despite the importance of tuberculosis as a public health problem, we know relatively little about the molecular mechanisms used by the causative organism, *Mycobacterium tuberculosis*, to persist in the host. To define these mechanisms, we have mutated virtually every nonessential gene of *M. tuberculosis* and determined the effect disrupting each gene on the growth rate of this pathogen during infection. A total of 194 genes that are specifically required for mycobacterial growth *in vivo* were identified. The behavior of these mutants provides a detailed view of the changing environment that the bacterium encounters as infection proceeds. A surprisingly large fraction of these genes are unique to mycobacteria and closely related species, indicating that many of the strategies used by this unusual group of organisms are fundamentally different from other pathogens.—Authors' Abstract

Shankarkumar, U., Ghosh, K., Badakere, S., and Mohanty, D. Novel HLA Class I Alleles Associated with Indian Leprosy Patients. J. Biomed. Biotechnol. **2003(3)** (2003) 208–211.

See Current Literature, Immuno-pathology, Leprosy, p. 82.

Wimmerova, M., Engelsen, S. B., Bettler, E., Breton, C, and Imberty, A. Combining fold recognition and exploratory data analysis for searching for glycosyltransferases in the genome of *Mycobacterium tuberculosis*. Biochimie. **85(7)** (2003) 691–700.

Fold recognition was applied to the systematic analysis of the all sequences encoded by the genome of *Mycoplasma tuberculosis* H37Rv in order to identify new putative glycosyltransferases. The search was conducted against a library composed of all known crystal structures of glycosyltransferases and some related proteins. A clear relationship appeared between some sequences and some folds. It appears necessary to complete the fold recognition approach with a statistical approach in order to identify the relevant data above the background noise. Exploratory data analysis was carried out using several methods. Analytical methods confirmed the validity of the approach, while predictive methods, although very preliminary in the present case, allowed for identifying a number of sequences of interest that should be further investigated. This new approach of combining bioinformatics and chemometrics appears to be a powerful tool for analysis of newly sequenced genomes. Its application to glycobiology is of great interest.—Authors' Abstract

Zhang, N., Torrelles, J. B., McNeil, M. R., Escuyer, V. E., Khoo, K. H., Brennan, P. J., and Chatterjee, D. The Emb proteins of mycobacteria direct arabinosylation of lipoarabinomannan and arabinogalactan via an N-terminal recognition region and a C-terminal synthetic region. Mol. Microbiol. **50(1)** (2003) 69–76.

The arabinans of the mycobacterial cell wall are key structural and immunological polymers in the context of arabinogalactan (AG) and lipoarabinomannan (LAM) respectively. The three homologous membrane proteins EmbA, EmbB and EmbC are known to be involved in the synthesis of arabinan but their biochemical functions are not understood. Herein we show, that synthesis of LAM, but not AG, ceases after inactivation of embC in *Mycobacterium smegmatis* by insertional mutagenesis. LAM synthesis is restored upon complementation with the embC wild-type gene.

Previously we have shown that the synthesis of the arabinan of AG is affected by embA or embB disruption. Thus the Emb proteins are capable of differential recognition of the galactan or mannan acceptors prior to appropriate arabinosylation. In addition, a combination of genetic and biochemical approaches have allowed us to assign some specific functions to the regions of emb gene products. Complementation of the embCmacr; mutant with a hybrid gene encoding the N-terminus of EmbC and the C-terminus of EmbB resulted in LAM with a lower molecular weight than the wild-type LAM. Structural studies involving enzyme digestion, chromatography and mass spectrometry analyses revealed that the arabinan of the 'LAM' formed in the hybrid was of AG kind rather than LAM type of arabinan.—Authors' Abstract