

## CURRENT LITERATURE

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

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## General and Historical

**Botting, J.** The History of Thalidomide. *Drug News Perspect.* **15(9)** (2002) 604–611.

The first paper describing the pharmacological actions of thalidomide was published in 1956. The drug, then designated as K17, was thought to have sedative effects superior to those of comparator drugs and was thought to be virtually nontoxic. Only 2 years after thalidomide's launch as Contergan in Germany, its alleged lack of toxicity came into question, with reports of the drug causing numerous side effects. Shortly thereafter, thalidomide was connected with an epidemic of horrific deformities in children whose mothers had taken the drug during pregnancy. This disaster brought on by thalidomide's teratogenic effects was responsible for the institution of some regulatory bodies, such as the United Kingdom's Committee on the Safety of Drugs, and for the strengthening of others, such as the U.S. Food and Drug Administration. An objective examination of published papers and contemporary accounts confirms that the preclinical tests on thalidomide were super-

ficial, and there is no doubt that it was never administered to pregnant animals prior to its use in patients. Within a short time after its withdrawal from the market due to its suspected association with fetal abnormalities, the drug was shown to produce fetal toxicity in laboratory animals. Had there been more extensive testing on laboratory animals before the drug was launched, the disaster could have been avoided.—Author's Abstract

**Dolev, E.** [The thalidomide story: a new chapter] *Harefuah* **142(3)** (2003) 186–187, 239, 238. [Article in Hebrew]

Thalidomide, a medication the use of which was banned about forty years ago due to its teratogenic effect, has returned into use due to its anti-angiogenic and immunomodulatory virtues. It is used now as a novel treatment in several malignant diseases as well as in the treatment of various inflammatory conditions. Recently it has been found that thalidomide may antagonize corneal blood vessels growth which is

a late results of exposure to mustard gas. The story of thalidomide, which is very unusual, may serve as the snake symbol in medicine, to remind us about the possible double face of every medication, of which every physician should be aware.—Author's Abstract

**Fusegawa, H., Wang, B. H., Sakurai, K., Nagasawa, K., Okauchi, M., and Nagakura, K.** Outbreak of tuberculosis in a 2000-year-old Chinese population. *Kansenshogaku Zasshi*. **77(3)** (2003) 146–149.

The molecular identification of *Mycobacterium tuberculosis* DNA in ancient human remains has been achieved mainly in mummies with macroscopic changes but not in the skeletons without bone tuberculosis. Using polymerase chain reaction studies, we identified mycobacterial DNA in 2000-year-old human skeletons without pathological changes. Our findings suggest that these people suffered from an outbreak of tuberculosis. Molecular examinations for mycobacterial DNA in the bone marrow of skeletons may contribute to the clarification of ancient diseases in old human populations.—Authors' Abstract

**Gomez i Prat, J. and de Souza, S. M.** Prehistoric tuberculosis in America: adding comments to a literature review. *Mem. Inst. Oswaldo Cruz*. **98 (Suppl 1)** (2003) 151–159.

Tuberculosis is a prehistoric American human disease. This paper reviews the literature and discusses hypotheses for origins and epidemiological patterns of prehistoric tuberculosis. From the last decades, 24 papers about prehistoric tuberculosis were published and 133 cases were reviewed. In South America most are isolated case studies, contrary to North America where more skeletal series were analyzed. Disease was usually located at the deserts of Chile and Peru, Central Plains in USA, and Lake Ontario in Canada. Skeletal remains represent most of the cases, but 16 mummies have also been described. Thirty individuals had lung disease, 19 of them diagnosed by the

ribs. More than 100 individuals had osseous tuberculosis and 26 also had it in other organs. As today, transmission of the infection and establishment of the disease were favored by cultural and life-style changes such as sedentarization, crowding, undernutrition, use of dark and insulated houses, and by the frequency of interpersonal contacts. The papers confirm that despite previous perceptions, tuberculosis seems to have occurred in America for millennia. It only had epidemiological expression when special conditions favored its expansion. Occurring as epidemic bursts or low endemic disease, it had differential impact on groups or social segments in America for at least two millennia.—Authors' Abstract

**Mori, S., Kato, S., Yokoyama, H., Tanaka, U., and Kaneda, S.** [The history of Yunosawa village and the policy of leprosy of Japan. II] *Nihon Hansenbyo Gakkai Zasshi*. **72(1)** (2003) 27–44. [Article in Japanese]

There was a village which was called Yunosawa, lots of leprosy patients lived, existed from 1887 to 1941, Kusatu town, Gunma Prefecture, Japan. It was the only place continued securing self-government to the last as area was free from the isolation policy of State in prewar days there. The aim of this study will make clear the dynamism of “The protection from the tension of the society of leprosy patient currently persecuted” to “The defense of the society from the leprosy patient who is a source of infection.” In this study, explained the history of the Yunosawa village and the shift of the policy of leprosy by State had relation to the village. In addition, the effort of residents and Christianity persons' activity are drawn in this paper. Moreover also drew what is desired how it is going to live under adverse circumstances, and showed worth of free medical-treatment area here.—Authors' Abstract

**Nwosu, M. C. and Nwosu, S. N.** Leprosy control in the post leprosaria abolition years in Nigeria: reasons for default and irregular attendance at treatment centres. *West Afr. J. Med.* **21(3)** (2002) 188–191.

A questionnaire was administered to all patients with leprosy seen at the four leprosy clinics in Anambra State in a face to face interview. The questions covered, among other items, the clinic attendance behaviour and the single most important reason, monthly, for absenteeism in the preceding year. The total and individual frequencies of the reasons for absenteeism were determined for the various behavioural subgroups. The differences in frequencies and associations were analysed. Values of  $p < 0.05$  were considered as significant. The results showed that 27 females and 26 males were interviewed. 39.6% of the patients were irregular attenders 735% were defaulters. Attendance at meetings ( $p < .001$ ); work at home ( $p < 0.01$ ) fear/shame/indignation ( $p < 0.05$ ); no confidence in treatment ( $p < 0.025$ ) were significant reasons for absenteeism among irregular attenders inter-current illnesses as reasons for absenteeism did not differ significantly between regular and irregular attendees. The association between clinic attendance behaviour and lesion location (revealed Vs concealed) was not statistically significant ( $X(2)0.3$ ). The findings in this study indicate that in the post leprosia abolition years, default and irregular clinic attendance by patients with leprosy are numerically large and may compound the problems of control programmes, and thus negate the realization of the global goal of intercepting leprosy transmission.—Authors' Abstract

**Sato, H. and Narita, M.** Politics of leprosy segregation in Japan: the emergence, transformation and abolition of the patient segregation policy. *Soc. Sci. Med.* **56(12)** (2003) 2529–2539.

The segregation of leprosy patients, a practice introduced early in the 20th century, was maintained in Japan after World War II. It locked in the viability of subsequent policy choices, and patients' isolation

was sustained long after it was proven to be scientifically unnecessary. For leprologists and leprosarium directors, there was little opportunity to conceptualize and test the epidemiological validity and effectiveness of outpatient services as alternatives to the existing policy, since most of the patients were already hospitalized. Since leprosy was no longer a threat to the general public, bureaucratic officials, as well as legislators, lacked strong incentives to reformulate the overall policy. Within the Ministry of Health and Welfare, daily tasks were largely transferred to the section for leprosarium management, and the search for other options lost importance. For patients, long institutionalization elevated their dependency on life in leprosia. These conditions must be emphasized as policy legacies, the results of past policies, since they posed obstacles to effective policy innovation in accordance with changing scientific knowledge. To make policies reflective of scientific knowledge, it is essential to understand and foresee the effect of policy legacy, when introducing and appraising public health policies.—Authors' Abstract

**Wolf, R. and Wolf, D.** A tattooed butterfly as a vector of atypical *Mycobacteria*. *J. Am. Acad. Dermatol.* **48(5 Suppl)** (2003) S73–S74.

We report the first case of cutaneous inoculation of atypical *Mycobacteria* secondary to tattooing. The diagnosis of atypical *Mycobacteria* infection of the skin was confirmed on the basis of the clinical and histologic appearance, the detection of acid-fast bacilli on Ziehl-Neelsen stain, and positive polymerase chain reaction. The medical complications of tattooing, which are manifold, are briefly summarized. This case emphasizes the need for federal regulation of tattooing, which is an invasive procedure associated with infectious and noninfectious complications.—Authors' Abstract

## Chemotherapy

**Aberg, J. A., Williams, P. L., Liu, T., Lederger, H. M., Hafner, R., Torriani, F. J., Lennox, J. L., Dube, M. P., MacGregor, R. R., Currier, J. S., and AIDS Clinical Trial Group 393 Study Team.** A study of discontinuing maintenance therapy in human immunodeficiency virus-infected subjects with disseminated *Mycobacterium avium* complex: AIDS. *J. Infect. Dis.* **187(8)**(2003) 1346.

The present nonrandomized prospective study evaluated whether antimycobacterial therapy for disseminated *Mycobacterium avium* complex (MAC) could be withdrawn from human immunodeficiency virus-infected subjects who experienced immunologic recovery while receiving highly active antiretroviral therapy (HAART). Eligible subjects had received macrolide-based therapy for least 12 months, were asymptomatic for MAC, had received HAART for at least 16 weeks, and had CD4+ T cell counts >100 cells/microL. Forty-eight subjects were enrolled, with a median CD4+ T cell count of 240 cells/microL at the time of discontinuation of MAC therapy. Forty-seven subjects remained MAC free, whereas 1 subject developed localized MAC osteomyelitis. The median duration of follow-up while not receiving therapy was 77 weeks, and the incidence of MAC infection was 1.44/100 person-years (95% confidence interval, 0.04–8.01). Withdrawal of anti-MAC therapy appears to be safe in patients who have been treated with a macrolide-based regimen for at least 1 year and have an immunologic response on HAART.—Authors' Abstract

**Brown-Elliott, B. A., Crist, C. J., Mann, L. B., Wilson, R. W., and Wallace, R. J. Jr.** *In Vitro* Activity of Linezolid against Slowly Growing Nontuberculous Mycobacteria. *Antimicrob. Agents Chemother.* **47(5)** (2003) 1736–1738.

MICs of linezolid in broth microdilutions were tested against 341 slowly growing nontuberculous mycobacteria (NTM) belonging to 15 species. The proposed line-

zolid susceptibility MICs for all *Mycobacterium marinum*, *Mycobacterium szulgai*, *Mycobacterium kansasii*, *Mycobacterium malmoeense*, and *Mycobacterium xenopi* isolates and for 90% of *Mycobacterium gordonae* and *Mycobacterium triplex* isolates were  $\leq 8$  micro g/ml. Linezolid has excellent therapeutic potential against most species of NTM.—Authors' Abstract

**Das, A. R., Dattagupta, N., Sridhar, C. N., and Wu, W. K.** A novel thiocationic liposomal formulation of antisense oligonucleotides with activity against *Mycobacterium tuberculosis*. *Scand. J. Infect. Dis.* **35(3)** (2003) 168–174.

This study describes the development of a novel thiocationic (OBEHYTOP) lipid-based formulation of phosphorothioate antisense oligonucleotides (PAOs) showing inhibitory activity against *Mycobacterium tuberculosis* (mTB) as measured by an *in vitro* BACTEC 460TB assay. PAOs were designed based on sequences complementary to essential regions of the mycobacterial genome from published nucleic acid databases in GenBank. These included the superoxide dismutase sod A gene (TBS3), catalase-peroxidase katG gene (TBK1, TBK10), RNA polymerase beta-subunit rpo B gene (TBR5) and diaminopimelate decarboxylase lys A gene (TBL5). The effect of PAOs (TBS3, K1, K10, R5 and L5) alone on mTB was not significant compared with the no-drug control over a period of exposure of 150 hr (ranges of –11.8 to +23.58% at 72 hr; 15.26 to +25.82% at 96 hr and –5.51 to +24.00% at 150 hr). Liposomal formulations (10:5:2 OBEHYTOP : oleic acid: vitamin D3) of PAOs resulted in statistically significant ( $p < 0.05$  in all cases) inhibition (ranges of –51.45 to –63.00% at 72 hr; –56.75 to –67.96% at 96 hr; –51.45 to –60.26% at 150 hr) compared with PAOs alone, thiocationic liposomal control and liposomal components. Positive controls of streptomycin and isoniazid used at their minimum inhibitory concentrations of 2.00 and 0.10 microM, respectively, resulted in average % inhibition values of –94% and

–97.36%, respectively, indicating that these thiocationic lipid-formulated PAOs showed inhibitory activity directed against mTB *in vitro*.—Authors' Abstract

**Dhople, A. M. and Namba, K.** Activities of sitafloxacin (DU-6859a), either singly or in combination with rifampin, against *Mycobacterium ulcerans* infection in mice. *J. Chemother.* **15(1)** (2003) 47–52.

Efficacy of a new fluoroquinolone, sitafloxacin (DU-6859a), against *Mycobacterium ulcerans* was evaluated *in vivo* using the mouse footpad system. The growth of *M. ulcerans* in mouse footpads was completely inhibited when mice were fed with sitafloxacin at a dose of 25 mg/kg body weight per day; on the other hand similar effects were observed with ofloxacin at a dose of 100 mg/kg body weight per day. In the presence of rifampin, the above dose of sitafloxacin could be reduced by 75% to achieve total inhibition, while, under similar circumstances, the dose of ofloxacin could be reduced by only 50%. Either used singly or in combination with rifampin, the effects of sitafloxacin were bactericidal. The results suggest that sitafloxacin should be evaluated as a chemotherapeutic agent against *M. ulcerans* infection.—Authors' Abstract

**Dhople, A. M. and Namba, K.** *In-vitro* activity of sitafloxacin (DU-6859a), either singly or in combination with rifampin analogs, against *Mycobacterium leprae*. *J. Infect. Chemother.* **9(1)** (2003) 12–15.

The *in-vitro* antibacterial activity of sitafloxacin (DU-6859a) against *Mycobacterium leprae* was evaluated and compared with those of ofloxacin, levofloxacin, and ciprofloxacin. Two biochemical indicators (intracellular ATP and uptake of [(3)H]-thymidine) were used to measure the *in-vitro* growth of *M. leprae* in Dhople-Hanks (DH) medium. Sitafloxacin was found to be more potent than the other three commonly used fluoroquinolones, with the minimum inhibitory concentration (MIC) against *M. leprae* being 0.1875 microg/ml and the action being bactericidal. The MIC's of

ofloxacin, levofloxacin, and ciprofloxacin were 1.5, 0.75, and 3.0 microg/ml, respectively. Similar to ofloxacin and levofloxacin, sitafloxacin also exhibited synergistic activity when combined with either rifabutin or KRM-1648, but not with rifampin. Thus, further studies on the incorporation of sitafloxacin in multidrug therapy regimens in treating leprosy patients are suggested.—Authors' Abstract

**Gordon, J. N. and Goggin, P. M.** Thalidomide and its derivatives: emerging from the wilderness. *Postgrad. Med. J.* **79(929)** (2003) 127–132.

Forty years on from its worldwide withdrawal, thalidomide is currently undergoing a remarkable renaissance as a novel and powerful immunomodulatory agent. Over the last decade it has been found to be active in a wide variety of inflammatory and malignant disorders where conventional therapies have failed. Recently, considerable progress has been made in elucidating its complex mechanisms of action, which include both anticytokine and antiangiogenic properties. However, in addition to its well known teratogenic potential, it has a significant side effect profile that leads to cessation of treatment in up to 30% of subjects. In response to this, two new classes of potentially safer and non-teratogenic derivatives have recently been developed. This review summarises the biological effects, therapeutic applications, safety profile, and future potential of thalidomide and its derivatives.—Authors' Abstract

**Hall, V. C., El-Azhary, R. A., Bouwhuis, S., and Rajkumar, S. V.** Dermatologic side effects of thalidomide in patients with multiple myeloma. *J. Am. Acad. Dermatol.* **48(4)** (2003) 548–552.

**BACKGROUND:** Thalidomide, an antiangiogenic agent, was approved by the Food and Drug Administration in 1998 for the treatment of erythema nodosum leprosum. Although its teratogenic and neurologic side effects are well known, its dermatologic side effects continue to be defined. **OBJECTIVE:** We report the der-

matologic side effects in 87 patients with multiple myeloma enrolled in a comparative, open-label, clinical trial treated with thalidomide alone (50 patients) or thalidomide and dexamethasone (37 patients). **METHOD:** We reviewed the records of all patients enrolled in the clinical trial. The frequency, type, severity, and time of onset of all skin eruptions that were temporally related to thalidomide treatment were recorded. **RESULTS:** Minor to moderate skin eruptions were noted in 46% of patients taking thalidomide alone and in 43% of those taking thalidomide and dexamethasone. These included morbilliform, seborrheic, maculopapular, or nonspecific dermatitis. Severe skin reactions (exfoliative erythroderma, erythema multiforme, and toxic epidermal necrolysis) that required hospitalization and withdrawal of thalidomide developed in 3 patients receiving thalidomide and dexamethasone. **CONCLUSION:** The prevalence of dermatologic side effects of thalidomide appear to be higher than previously reported. Although in most patients they were minor, in a few patients they were quite severe, particularly when given in conjunction with dexamethasone for newly diagnosed myeloma. Further studies are needed to verify the extent of the interaction between thalidomide and dexamethasone in this group of patients.—Authors' Abstract

**Haslett, P. A., Hanekom, W. A., Muller, G., and Kaplan, G.** Thalidomide and a thalidomide analogue drug costimulate virus-specific CD8+ T cells *in vitro*. *J. Infect. Dis.* **187(6)** (2003) 946–955.

CD8(+) T cell immunity is critical for protection from viral disease, such as that caused by the human immunodeficiency virus (HIV) or cytomegalovirus (CMV). It is therefore important to identify therapies that can boost antiviral immunity. The recent finding that thalidomide acts as a T cell costimulator suggested that this drug may boost antiviral CD8(+) T cell responses. In this *in vitro* study, in a human autologous CD8(+) T cell/dendritic cell (DC) coculture system, thalidomide and a potent thalidomide analogue were shown to enhance virus-specific CD8(+) T cell cytokine pro-

duction and cytotoxic activity. The drug-enhanced antiviral activity was noted in cells from both healthy donors and persons chronically coinfecting with HIV and CMV. This stimulatory effect was directed at CD8(+) T cells, and not DCs. These results suggest an application for thalidomide and the thalidomide analogue as a novel immune-adjuvant therapy in chronic viral infections.—Authors' Abstract

**Hegarty, A., Hodgson, T., and Porter, S.** Thalidomide for the treatment of recalcitrant oral Crohn's disease and orofacial granulomatosis. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **95(5)** (2003) 576–585.

It has been suggested that thalidomide may be effective in the management of Crohn's disease, including the associated oral lesions. We detail the clinical response to low-dose thalidomide of 5 patients with clinical features of orofacial granulomatosis or oral Crohn's disease recalcitrant to recognized immunosuppressant therapy. All patients had clinical resolution of their symptoms and signs. Transient somnolence was the only reported adverse effect. Remission was maintained by extending the period between thalidomide doses. Thalidomide should be considered an effective therapy for the short-term treatment of severe orofacial granulomatosis in appropriately counseled patients.—Authors' Abstract

**Matthews, S. J. and McCoy, C.** Thalidomide: a review of approved and investigational uses. *Clin. Ther.* **25(2)** (2003) 342–395.

**BACKGROUND:** Thalidomide is best known as a major teratogen that caused birth defects in up to 12,000 children in the 1960s. More recently, this agent has been approved by the US Food and Drug Administration for the treatment of erythema nodosum leprosum (ENL) through a restricted-use program. Its immunomodulatory, anti-inflammatory, and antiangiogenic properties are currently under study in a number of clinical conditions. **OBJEC-**

**TIVE:** This article reviews the pharmacology of thalidomide; its approved and off-label uses in dermatologic, oncologic, and gastrointestinal conditions; and adverse events associated with its use. **METHODS:** Relevant articles were identified through searches of MEDLINE (1966–June 2002), International Pharmaceutical Abstracts (1970–June 2002), and EMBASE (1990–June 2002). Search terms included but were not limited to thalidomide, pharmacokinetics, pharmacology, therapeutic use, and teratogenicity, as well as terms for specific disease states and adverse events. Further publications were identified from the reference lists of the reviewed articles. Abstracts of recent symposia were obtained from the American Society of Clinical Oncology Web site. **RESULTS:** Thalidomide is thought to exert its therapeutic effect through the modulation of cytokines, particularly tumor necrosis factor- $\alpha$ . In addition to its approved indication for ENL, thalidomide has been studied in various other conditions, including graft-versus-host disease, discoid lupus erythematosus, sarcoidosis, relapsed/refractory multiple myeloma, Waldenström's macroglobulinemia, myelodysplastic syndromes, acute myeloid leukemia, myelofibrosis with myeloid metaplasia, renal cell carcinoma, malignant gliomas, prostate cancer, Kaposi's sarcoma, colorectal carcinoma, oral aphthous ulcers, Behcet's disease, Crohn's disease, and HIV/AIDS-associated wasting. Adverse events most frequently associated with its use include somnolence, constipation, rash, peripheral neuropathy, and thromboembolism. **CONCLUSIONS:** Use of thalidomide is limited by toxicity, limited efficacy data, and restricted access. Evidence of its efficacy in conditions other than ENL awaits the results of controlled clinical trials.—Authors' Abstract

**Mlambo, G. and Sigola, L. B.** Rifampicin and dexamethasone have similar effects on macrophage phagocytosis of zymosan, but differ in their effects on nitrite and TNF- $\alpha$  production. *Int. Immunopharmacol.* **3(4)** (2003) 513–522.

The antibiotic rifampicin is used extensively in the treatment of mycobacterial and

other infections. It has previously been suggested that rifampicin binds to and activates the glucocorticoid receptor potentially leading to pharmacological glucocorticoid-like effects such as host immunosuppression (Calleja, *et al.*). This study compares the effects of rifampicin with the synthetic glucocorticoid dexamethasone, on macrophage phagocytosis of zymosan particles and production of nitric oxide (NO) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by splenocytes or macrophages. Rifampicin and dexamethasone, partially suppressed zymosan phagocytosis by macrophages, respectively, and both effects were ameliorated by the glucocorticoid receptor antagonist RU486. In other experiments, rifampicin had no effects on NO responses; however, dexamethasone inhibited NO in an RU486-sensitive manner. At high doses, rifampicin moderately suppressed TNF- $\alpha$  while dexamethasone inhibited it in a dose-dependent manner, which was ameliorated by the presence of RU486. These findings suggest that rifampicin has differential immunomodulatory effects on these innate immune mechanisms.—Authors' Abstract

**Okafor, M. C.** Thalidomide for erythema nodosum leprosum and other applications. *Pharmacotherapy.* **23(4)** (2003) 481–493.

Thalidomide, administered as a sedative and antiemetic decades ago, was considered responsible for numerous devastating cases of birth defects and consequently was banned from markets worldwide. However, the drug remarkably has resurfaced with promise of immunomodulatory benefit in a wide array of immunologic disorders for which available treatments were limited. It is approved by the Food and Drug Administration for erythema nodosum leprosum (ENL). Although the relative paucity of leprosy and ENL worldwide may perceivably limit interest in and knowledge about thalidomide, increasing numbers of new and potential uses expand its applicability widely beyond ENL. Thalidomide, an inhibitor of tumor necrosis factor  $\alpha$ , is the best known agent for short-term treatment of ENL skin manifes-

tations, as well as postremission maintenance therapy to prevent recurrence. For this indication, it is effective as monotherapy and as part of combination therapy with corticosteroids. Studies of thalidomide in chronic graft-versus-host disease showed benefit in children and adults as treatment, but not as prophylaxis. The agent has been administered successfully for treatment of cachexia related to cancer, tuberculosis, and human immunodeficiency virus infection, although evidence of efficacy is inconclusive. Thalidomide monotherapy effectively induced objective response in trials in patients with both newly diagnosed and advanced or refractory multiple myeloma. Combination therapy with thalidomide and corticosteroids was also effective in these patients, as well as in treatment of aphthous and genital ulcers. Limited evidence supports the drug's benefit in treatment of Kaposi's sarcoma. Other thalidomide applications include Crohn's disease, rheumatoid arthritis, and multiple sclerosis. Somnolence, constipation, and rash were the most frequently cited adverse effects in studies, but thalidomide-induced neuropathy and idiopathic thromboembolism were critical causes for drug discontinuation. Thalidomide is still contraindicated in pregnant women, women of childbearing age, and sexually active men not using contraception. Clinicians should be conversant with thalidomide in ENL (its primary application) in the natural course of leprosy, as well as in the agent's other applications.—Author's Abstract

**Pannikar, V.** The Return of Thalidomide: New Uses and Renewed Concerns. WHO Pharmaceuticals Newsletter, No. 2, 2003 (available at <http://www.who-umc.org>)

Today, a large number of thalidomide babies continue to be born each year possibly reflecting regulatory insufficiency and widespread use under inadequate supervision. In Brazil, which has more than 1000 registered thalidomide victims, the last officially known case was born in 1995. There is evidence that second generation babies with similar deformities are being born to thalidomide victims. In the US,

Celgene Corporation has had FDA approval to market the drug since 1998 for the cutaneous manifestations of moderate to severe erythema nodosum leprosum. In Europe, the US company Pharmion Corp and French rival Laphal have both secured orphan drug status for thalidomide and have applied to market the drug as a therapy for multiple myeloma and for ENL in the EU. The EU is currently holding discussions on the re-launch of thalidomide. Whatever the outcome of the EU discussions, it cannot be over emphasized that any potential benefit with thalidomide must be balanced with the known toxicity and the accompanying ethical and legal constraints on its use. Experience has shown that it is virtually impossible to develop and implement a fool-proof surveillance mechanism to combat misuse of thalidomide.—Author's Abstract

**Pukrittayakamee, S., Prakongpan, S., Wanwimolruk, S., Clemens, R., Looareesuwan, S., and White, N. J.** Adverse effect of rifampin on quinine efficacy in uncomplicated falciparum malaria. *Antimicrob. Agents Chemother.* **47**(5) (2003) 1509–1513.

The effects of adding rifampin to quinine were assessed in adults with uncomplicated falciparum malaria. Patients were randomized to receive oral quinine either alone (N = 30) or in combination with rifampin (N = 29). Although parasite clearance times were shorter in the quinine-rifampin-treated patients (mean  $\pm$  standard deviation,  $70 \pm 21$  versus  $82 \pm 18$  hr;  $p = 0.023$ ), recrudescence rates were five times higher (N = 15 of 23; 65%) than those obtained with quinine alone (N = 3 of 25; 12%),  $p < 0.001$ . Patients receiving rifampin had significantly greater conversion of quinine to 3-hydroxyquinine and consequently considerably lower concentrations of quinine in their plasma after the second day of treatment (median area under the plasma drug concentration-time curve from day zero to day 7 = 11.7 versus 47.5 micro g/ml. day,  $p < 0.001$ ). Rifampin significantly increases the metabolic clearance of quinine and thereby reduces cure rates. Rifampin should not be combined with quinine for the treatment of malaria,

and the doses of quinine should probably be increased in patients who are already receiving rifampin treatment.—Authors' Abstract

**Schafer, P. H., Gandhi, A. K., Loveland, M. A., Chen, R. S., Man, H. W., Schnetkamp, P. P., Wolbring, G., Govinda, S., Corral, L. G., Payvandi, F., Muller, G. W., and Stirling, D. I.** Enhancement of Cytokine Production and AP-1 Transcriptional Activity in T Cells by Thalidomide-Related Immunomodulatory Drugs. *J. Pharmacol. Exp. Ther.* **305**(3) (2003) 1222–1232.

CC-4047 (ACTIMID(TM)) and CC-5013 (REVIMID(TM)) belong to a class of thalidomide analogs, collectively known as the immunomodulatory drugs (IMiDs(TM)), that are currently being assessed in the treatment of patients with multiple myeloma (MM) and other cancers. IMiDs potently enhance T cell and natural killer (NK) cell responses and inhibit TNF-alpha, IL-1beta, and IL-12 production from LPS-stimulated PBMC. However, the molecular mechanism of action for these compounds is unknown. Herein we report on the ability of the IMiDs to upregulate production of IL-2 from activated human CD4(+) and CD8(+) peripheral blood T cells, production of IL-2 and IFN-gamma from Th1-type cells, and production of IL-5 and IL-10 from Th2-type cells. Elevation of IL-2 production from Jurkat T cells was observed as early as 6 hours post-stimulation and correlated with an increase in IL-2 promoter activity that was dependent upon the proximal but not the distal AP-1 binding site. The IMiDs enhanced AP-1-driven transcriptional activity 2 to 4-fold after 6 hours of T cell stimulation, and their relative potencies for AP-1 activation correlated with their potencies for increased IL-2 production in Jurkat T cells and in CD4(+) or CD8(+) human peripheral blood T cells. The most potent of these IMiDs, CC-4047, had no effect on NFAT transcriptional activity, calcium signaling, or phosphorylation of ERK1/2, JNK1/2, p38 MAPK, or c-Jun/Jun D in Jurkat T cells. These data suggest that IMiDs increase T cell cytokine production by potentiating AP-1 transcriptional activity.—Authors' Abstract

**Valerio, G., Bracciale, P., Manisco, V., Quitadamo, M., Legari, G., and Bellanova, S.** Long-term tolerance and effectiveness of moxifloxacin therapy for tuberculosis: preliminary results. *J. Chemother.* **15**(1) (2003) 66–70.

The resurgence of tuberculosis is a major problem. Increasing multiple resistance to current drugs used for therapy, non-compliance to therapy or co-morbidity are challenging problems that do not allow use of standard therapy in all patients. Quinolones are claimed to be active drugs in TB infection. Moxifloxacin shows the highest intracellular concentration *in vitro* and in experimental animals, but long-term tolerability is unknown. Our aim was to observe in compliant patients, not eligible for standard therapy, the effect of 6 months of therapy with moxifloxacin, isoniazid and rifampin. Nineteen patients, a control group, were observed for the same period under therapy with streptomycin, pirazinamide, rifampin, isoniazid. The patients were affected by indolent miliary pattern and concomitant lymphoma or leukemia in 3 cases; rare nodular involvement with genitourinary diseases in 3 others; segmental to lobar involvement in 4 others with concomitant multidrug resistance, bone localization, hepatitis. The control group was more uniform and showed segmental to lobar nodular involvement with pleuritis in 3 patients, together with hepatitis in 3. Monthly checks of blood gas analysis, chest X-ray, functional testing, serum titers of antibodies against antigen 60, sputum slides and complete chemical analysis were performed. A follow-up visit was performed 1 month after therapy. Patients under moxifloxacin therapy experienced no toxicity, almost complete sterilization and remission of the disease. Sterilization was obtained in 15 days. Patients under standard therapy also had a good clinical outcome, although therapy was delayed in 3 cases because of increased transaminases within the first 15 days of therapy. Moxifloxacin seems to be well tolerated and combination therapy including moxifloxacin for TB seems to be as active as the standard therapy in patients with complex illness.—Authors' Abstract

## Clinical Sciences

**Bari, M. M.** Surgical reconstruction of leprotic foot drop. *Mymensingh Med. J.* **12(1)** (2003) 11–12.

We have operated 152 patients for correction of foot-drop due to leprosy from March 1992 to July 1999. The method used was circumtibial transfer of the tibial is posterior to the tendons of extensor hallucis longus and the extensor digitorum longus in the foot together with lengthening of the Achilles tendon. The results were satisfactory in 135 of these cases as judged by adequate restoration of heel—toe gait and of active dorsiflexion. The follow up period ranged from 6 months to 8 years. Inadequate post-operative physiotherapy was the reason for unsatisfactory results in seventeen cases.—Author's Abstract

**Davis, S. V., Sheno, S. D., Balachandran, C., and Pai, S. B.** A fatal case of erythema necroticans. *Indian J. Lepr.* **74(2)** (2002) 145–149.

Erythema nodosum leprosum (ENL) classically presents as tender, erythematous nodules over the face, arms and legs. Severe ENL can become vesicular or bullous and break-down and is termed erythema necroticans (Jopling and McDougall, 1996) and is treated with corticosteroids. The causes of death in a majority of leprosy patients are the same as in the general population, with the exception of renal damage in lepromatous leprosy. There is possible increased mortality from side-effects of anti-leprosy drugs, steroids, or other drugs used in reactions, from toxemia in severe reactions, and from asphyxia due to glottic oedema (Jopling and McDougall, 1996). We report here a case of erythema necroticans, the cause of death being septicaemia, secondary to skin ulcers and urinary tract infection, precipitated by corticosteroids.—Authors' Abstract

**Girdhar, A., Chakma, J. K., and Girdhar, B. K.** Pulsed corticosteroid therapy in patients with chronic recurrent ENL: a

pilot study. *Indian J. Lepr.* **74(3)** (2003) 233–236.

A pilot study has been undertaken to compare the efficacy of small dose pulsed betamethasone therapy with need based oral steroids in chronic recurrent erythema nodosum leprosum (ENL) patients. Though this mode of therapy was well tolerated, no advantage with intermittent steroid administration was observed. This could have been on account of small dose of steroid given monthly. Treatment of chronic recurrent erythema nodosum leprosum (ENL) patients continues to be unsatisfactory, particularly, because of nonavailability of thalidomide. Though corticosteroids are effective in suppressing all the manifestations and even restoring partially or fully the functional impairment, their side effects and dependence are equally troublesome. Based on (a) the reported efficacy and safety of intermittent use of corticosteroids in several immune complex mediated disorders (Cathcart, *et al.* 1976, Kimberly, *et al.* 1979), Liebling, *et al.* 1981 and Pasricha and Gupta 1984) and (b) ENL (type II) reactions having similar pathology, a pilot study has been undertaken to see the efficacy and the tolerance of pulsed steroids in chronic ENL patients.—Authors' Abstract

**Keita, S., Faye, O., Konare, H. D., Sow, S. O. Ndiaye, H. T., and Traore, I.** [Evaluation of the clinical classification of new cases of leprosy. Study conducted at the Marchoux Institute in Bamako, Mali] *Ann. Dermatol. Venereol.* **130(2)** (2003) 184–186. [Article in French]

**INTRODUCTION:** The difficulties related to the bacilloscopic diagnosis of leprosy, providing a more reliable classification of cases, in 1995 led the WHO to recommend the use of a new classification, in endemic countries, based on clinical criteria alone, in order to simplify the poly-chemotherapeutic regimens. According to our experience in the Marchoux Institute, this classification may lead to errors in diagnosis through overzealous or mis-

interpretation of the two forms of leprosy. The aim of our study was to evaluate the concordance between this clinical classification and that based on a bacilloscopic examination. **PATIENTS AND METHODS:** We conducted a descriptive study of new cases of leprosy seen at the Marchoux Institute, without distinction in gender or age, from January to December 2000. All the patients included underwent clinical examination and a bacilloscopic exploration to provide a double classification. The concordance between the two classifications was assessed using the Kappa test. **RESULTS:** Two hundred new cases of leprosy were included. Out of 126 clinically multi-bacillary cases, 61 were confirmed bacteriologically, and 65 were false positives. Out of 74 clinical cases with few bacilli, 2 were bacteriologically multi-bacilli. The concordance between the two classifications was average (Kappa = 0.40). There was a significant difference between the percentages of multi-bacilli observed in both classifications ( $p < 10^{-8}$ ). **DISCUSSION:** The clinical classification may well overestimate the multi-bacillary form. In the absence of a reliable bacilloscopic apparatus, a more detailed clinical classification of leprosy forms must be developed.—Authors' Abstract

**Khan, T., Awan, A. A., Kazmi, H. S., Shah, A. A., Muhammad, S., and Muhammad, S.** Frequency of ocular complications of leprosy in institutionalized patients in NWFP Pakistan. *J. Ayub. Med. Coll. Abbottabad* **14(4)** (2002) 29–33.

**BACKGROUND:** There is no systemic disease, which so frequently gives rise to disorders of the eye as leprosy does. The study was conducted to determine the prevalence and gravity of ocular complications in institutionalized leprosy patients in NWFP. It is important to provide necessary information to leprosy health workers and general physicians in order to sensitize them to early detection and treatment or referral to appropriate centre. **METHODS:** A prospective study of ocular complications of leprosy patients was conducted at the leprosy centre of Lady Reading Hospital Peshawar and the Leprosy Hospital Bal-

akot, district Mansehra. The study included a record of the name, age, sex, type, duration of disease and completion of multi-drug therapy (MDT). Classification of the patients was done according to Ridley and Jopling 5-group system. Visual acuity was tested by Snellen chart and those patients having a vision of less than 3/60 were labelled as blind. Ocular adnexa were examined by naked eye and lacrimal sac regurgitation test was done. Slit lamp biomicroscopy was done for anterior segment examination and direct ophthalmoscope was used for fundoscopy. **RESULTS:** The authors studied 143 patients in the above mentioned leprosy centres. Out of these, 59 had lepromatous leprosy, 39 borderline tuberculoid leprosy, 9 tuberculoid leprosy, 33 borderline lepromatous leprosy, and 33 borderline leprosy. The majority of patients came from the northern districts of NWFP, including Malakand division and district Mansehra. The male to female ratio was 4:1. The age of the patients ranged from 14 to 80 years and the duration of the disease ranged from 1 year to 48 years. Ocular complications were found in 73% of the patients. These complications included loss of eyebrows in 57 patients, loss of eyelashes in 37, corneal changes (including opacity, ulceration, and/or anaesthesia) in 44, iridocyclitis in 31, lagophthalmos in 36, ectropion in 13, and chronic dacryocystitis in 3. Of the total of 15 (11%) patients who went blind from ocular complications, 16 eyes did so due to corneal opacities, 6 eyes due to cataract, 5 eyes due to chronic anterior uveitis and one eye due to corneal ulcer, panophthalmitis and phthisis bulbi each. **CONCLUSIONS:** A significant number of leprosy patients (73%) have ocular complications. The frequency of ocular complications increases with the increasing age and duration of disease of the patients.—Authors' Abstract

**Malaviya, G. N.** Median nerve palsy following claw finger correction in leprosy: effect of *M. leprae* or a consequence of surgery. *Indian J. Lepr.* **74(3)** (2002) 217–220.

Median nerve palsy, though not a frequent occurrence after claw finger correc-

tion, does exist as a post-operative complication after claw finger correction. A retrospective study was carried out to examine the occurrence of post-operative median palsy, in cases of isolated ulnar palsy, where the transferred motor tendon was routed through the carpal tunnel. We noted that six patients developed median nerve palsy following claw finger correction. Median palsy developed at different times after surgery—the “early onset” type developing within three weeks post-operatively, “reactional” type developed when patient was undergoing physiotherapy exercises and learning to use the transfer and “delayed insidious” type presenting six months or more after operation. We could not succeed to get the true prevalence of such occurrences because all the operated hands could not be re-examined.—Author’s Abstract

**Malaviya, G. N.** Some more about unfavourable results after corrective surgery as seen in leprosy. *Indian J. Lepr.* **74(3)** (2002) 243–257.

The paper describes unfavourable outcomes of some of the commonly performed surgical procedures in leprosy affected persons and the underlying causes. An awareness about unfavourable outcomes of surgery is helpful to the beginners because they can anticipate the problems and take appropriate measures to prevent that and failing which prepare themselves to face and sort that out. Careful pre-operative evaluation of the patient is an important first step.—Author’s Abstract

**Misra, U. K., Kalita, J., Mahadevan, A., and Shankar, S. K.** Pseudoathetosis in a patient with leprosy. *Mov. Disord.* **18(5)** (2003) 598–601.

A 35-year-old man with borderline tuberculoid leprosy developed Type I lepra reaction 12 days after anti-leprosy treatment. There was acute worsening of neuropathic symptoms and skin lesions. He developed severe sensory ataxia and pseudoathetosis resulting in marked disability. His symptoms significantly improved on corticosteroid therapy.—Authors’ Abstract

**Ozkan, T., Ozer, K., Yukse, A., and Gulgonen, A.** Surgical reconstruction of irreversible ulnar nerve paralysis in leprosy. *Lepr. Rev.* **74(1)** (2003) 53–62.

Twenty-five patients with irreversible leprotic ulnar nerve palsy having undergone lumbrical replacement with two different tendon transfer techniques were assessed 6–120 months after surgery. Nineteen patients were reconstructed with the flexor digitorum four-tail procedure (FDS-4T), and six with Zancolli’s lasso procedure (ZLP). Mean paralysis times were 103 months for FDS-4T, and 68 months for ZLP. Mean age of the patients was 36 years (21–57). Grip strength measurements, improvement in active range of motion at the PIP joints, patients’ ability to open and close their hands fully, as well as sequence of phalangeal flexion, were noted. Mean grip strength measurements during follow-up were 76% of the contralateral extremity in the FDS-4T group and 82% in the ZLP group. Comparison of the follow-up grip strength with the preoperative value revealed 1% improvement in the FDS-4T group and 20% in the ZLP group. Claw hand deformity was completely corrected in 12 patients in FDS-4T group, and in five patients in the ZLP group. Residual flexion contracture remained in five patients after surgery. Swan-neck deformity subsequently developed in seven fingers. Age, sex, mean follow-up and surgical technique did not relate statistically to the functional outcome. However, preoperative extensor lag of the PIP joint and mean paralysis time significantly affected the functional outcome. ZLP was found to be a more effective procedure in restoring grip strength, whereas FDS-4T was more effective in correcting claw hand deformity.—Authors’ Abstract

**Ramu, G. and Desikan, K. V.** Reactions in borderline leprosy. *Indian J. Lepr.* **74(2)** (2002) 115–128.

This is a retrospective study of 276 patients consisting of 157 active and 119 reactive patients of borderline leprosy. They were followed up for 10 years after sulphone monotherapy. The presenting symptoms were carefully examined from the

records and systematically presented. Frequency of reactions was least in BT cases and most in BL cases. Risk factors of reaction appear to be the type of leprosy, multiplicity of lesions, high BI and, possibly, psychological stress. Biopsy of skin lesions was performed in all cases initially, and at the subsidence of the disease. Histological findings closely correlated with clinical classification. While all the cases showed clinical subsidence, histological subsidence was found in 200 (73%) cases, and the condition was static in 36 cases (13%). Immunological upgrading was seen in 110%, while 4% showed downgrading. Bacteriological status and lepromin reaction of active and reactive cases were compared. All these factors need to be taken into consideration for instituting prompt and proper treatment.—Authors' Abstract

**Richard, B. M.** Location of the extracranial extent of leprosy facial nerve pathology may allow leprosy facial palsy to be reanimated by free muscle transfer. *Br. J. Plast. Surg.* **56**(1) (2003) 14–19.

Leprosy is a mycobacterial nerve and skin infection, which can be eradicated by antibiotics. Some patients affected by leprosy, once cured, have residual nerve impairment with paralysis and sensory neuropathy. A series of patients with facial nerve paralysis, investigated using clinical, histological and electrophysiological techniques, demonstrated that the nerve pathology was distal to the section of main trunk prior to its bifurcation. Facial reanimation was achieved with a free gracilis-muscle transfer, coapting its motor nerve to the ipsilateral facial nerve trunk proximal to the site of the leprosy pathology, with a moderate clinical result.—Author's Abstract

**Thappa, D. M., Dave, S., Karthikeyan, K., Laxmisha, C., and Jayanthi, S.** Localized lepromatous leprosy presenting as a painful nodule in a muscle. *Indian J. Lepr.* **74**(3) (2002) 237–242.

Lepromatous leprosy is a generalized disease usually presenting with numerous macules, papules, nodules or plaques in-

volving wide areas of the skin. It is generally believed that in India lepromatous leprosy often originates from the borderline spectrum (Jha, *et al.*, 1991). Localized lepromatous or borderline lepromatous disease is a rare variant of multibacillary leprosy (Yoder, *et al.*, 1985; Jha, *et al.*, 1991; Pfaltzgraff and Ramu, 1994; Vijaikumar, *et al.*, 2001). This variant usually presents as a single nodule or a localized area of nodules and papules, while most of the body surface appears normal (Pfaltzgraff and Ramu, 1994; Vijaikumar, *et al.*, 2001). Its occurrence in our case as a single painful nodule in the bicep muscle of left forearm was indeed intriguing, such presentation being rarely reported in the literature.—Authors' Abstract

**Yowan, P., Danneman, K., Koshy, S., Richard, J., and Daniel, E.** Knowledge and practice of eye-care among leprosy patients. *Indian J. Lepr.* **74**(2) (2002) 129–135.

In one hundred and thirty leprosy patients attending the Schieffelin Leprosy Research and Training Center, Karigiri, Tamil Nadu, India, the knowledge, attitude and practice of eye-care were ascertained using a questionnaire developed by Mathews and Mangalam. 74.6% the patients surveyed were aware of the disease, 60% knew about the early signs of leprosy, 74.6% considered leprosy curable and 36.9% knew the duration of treatment with MDT. Less than half of the patients (40.8%) knew that blindness occurred in leprosy and was preventable. More males had this knowledge (46.5%) than females (22.6%) ( $p = 0.001$ ). Knowledge on how to take care of the eyes (26.9%), that eyes become anaesthetic due to leprosy (27.7%), and that precautions should be taken if sensation is lost (27.7%) was very poor. Knowledge on prevention of damage in eyes (57.7%) and the fact that rubbing eyes could cause damage (55.4%) was found in more than half the patients. More males (64.6%) had knowledge on the prevention of damage in eyes than females (35.5%) ( $p = 0.008$ ). Only 25.4% of the patients tried some measures to prevent eye injury, 21.5% used home remedies and all had the help of family members in their

eye-care. More males (26.3%) used home remedies than females (6.5%). The older age group had better knowledge on taking care of the eyes than those aged 40 and below ( $p = 0.026$ ). Although more patients with existing complications knew to take care of their eyes than those who did not have complications, the knowledge and practice of eye-care in both these groups

were poor. Knowledge of leprosy in illiterate patients was not different from those who had some formal schooling, but the practice of eye-care differed significantly ( $p = 0.02$ ). Health education must be undertaken to increase the knowledge of eye-care among leprosy patients, especially among illiterate persons, women and younger patients.—Authors' Abstract

## Immuno-Pathology

**Actor, J. K., Indrigo, J., Beachdel, C. M., Olsen, M., Wells, A., Hunter, Jr. R. L., and Dasgupta, A.** Mycobacterial Glycolipid Cord Factor Trehalose 6,6'-Dimycolate Causes a Decrease in Serum Cortisol during the Granulomatous Response. *Neuroimmunomodulation* **10(5)** (2003) 270–282.

Serum cortisol levels were evaluated in mice following intravenous administration of purified mycobacterial glycolipid trehalose 6,6'-dimycolate (TDM). C57BL/6 mice develop lung granulomas in response to TDM, while A/J mice are deficient in this process. Administration of TDM to C57BL/6 mice led to a rapid reduction in serum cortisol, concurrent with initiation of the granulomatous response and cytokine and chemokine mRNA induction. Cortisol levels were lowest on day 5 after TDM administration, but there was significant production of IL-6, TNF-alpha and IL-1beta messages. Granuloma formation and full immune responsiveness to TDM were only apparent upon a sufficient decrease in levels of systemic cortisol. Treatment of the C57BL/6 mice with hydrocortisone abolished inflammatory responses. Histologically nonresponding A/J mice exhibited higher constitutive serum cortisol and demonstrated different kinetics of cortisol reduction upon administration of TDM. A/J mice demonstrated hyperplastic morphology in the suprarenal gland with a high degree of vacuolization in the medullary region and activation of cells in the zona fasciculata and zona reticularis. The A/J mice were dysregulated with respect to cytokine responses thought to be necessary during

granuloma formation. The high constitutive serum cortisol in the A/J mice may therefore contribute to pulmonary immunoresponsiveness and the establishment of an environment counterproductive to the initiation of granulomatous responses. The identification of a mycobacterial glycolipid able to influence serum cortisol levels is unique and is discussed in relation to immunopathology during tuberculosis disease.—Authors' Abstract

**Bhattacharyya, A., Pathak, S., Basak, C., Law, S., Kundu, M., and Basu, J.** Execution of macrophage apoptosis by *Mycobacterium avium* through apoptosis signal-regulating kinase 1/p38 mitogen-activated protein kinase signaling and caspase 8 activation. *J. Biol. Chem.* **278(29)** (2003) 26517–26525.

Macrophage apoptosis is an important component of the innate immune defense machinery (against pathogenic mycobacteria) responsible for limiting bacillary viability. However, little is known about the mechanism of how apoptosis is executed in mycobacteria-infected macrophages. Apoptosis signal-regulating kinase 1 (ASK1) was activated in *M. avium*-treated macrophages and in turn activated p38 mitogen-activated protein (MAP) kinase. *M. avium*-induced macrophage cell death could be blocked in cells transfected with a catalytically inactive mutant of ASK1 or with dominant negative p38 MAP kinase arguing in favor of a central role of ASK1/p38 MAP kinase signaling in apoptosis of macrophages challenged with *M. avium*. ASK1/p38 MAP ki-

nase signaling was linked to the activation of caspase 8. At the same time, *M. avium* triggered caspase 8 activation and cell death occurred in a Fas-associated death domain (FADD)-dependent manner, suggesting the involvement of death receptor signaling. FADD did not exert its effect through ASK1 activation. The death signal induced upon caspase 8 activation linked to mitochondrial death signaling through the formation of truncated Bid (t-Bid), its translocation to the mitochondria and release of cytochrome c (cyt c). Caspase 8 inhibitor (z-IETD-FMK) could block the release of cytochrome c as well as the activation of caspases 9 and 3. The final steps of apoptosis probably involved caspases 9 and 3, since inhibitors of both caspases could block cell death. Of foremost interest in the present study was the finding that ASK1/p38 signaling was essential for caspase 8 activation linked to *M. avium*-induced death signaling. It provides the first elucidation of a signaling pathway in which ASK1 plays a central role in innate immunity.—Authors' Abstract

**Chiu, B. C., Freeman, C. M., Stolberg, V. R., Komuniecki, E., Lincoln, P. M., Kunkel, S. L., and Chensue, S. W.** Cytokine-Chemokine Networks in Experimental Mycobacterial and Schistosomal Lung Granuloma Formation. *Am. J. Respir. Cell. Mol. Biol.* **29(1)** (2003) 106–116.

Type-1 and type-2 lung granulomas respectively elicited by bead immobilized *Mycobacteria bovis* (PPD) and *Schistosoma mansoni* egg (SEA) Ags display different patterns chemokine expression. This study tested the hypothesis that chemokine expression patterns were related to upstream cytokine signaling. Using quantitative transcript analysis we defined expression profiles for 16 chemokines and then examined the *in vivo* effects of neutralizing antibodies against interferon-gamma (IFN $\gamma$ ), interleukin-4 (IL-4), IL-10, IL-12, and IL-13. Transcripts for CXCL2, 5, 9, 10 and 11 and the CCL chemokines, CCL3 and lymphotactin (XCL1) were largely enhanced by Th1-related cytokines, IFN  $\gamma$  or IL-12. Transcripts for

CCL11, CCL22, CCL17 and CCL1 were enhanced largely by Th2-related cytokines, IL-4, IL-10 or IL-13. Transcripts for CCL4, CCL2, CCL8, CCL7, and CCL12 were potentially induced by either Th1- or Th2-related cytokines although some of these showed biased expression. IFN $\gamma$  and IL-4 enhanced the greatest complement of transcripts and their neutralization had the greatest anti-inflammatory effect on type-1 and type-2 granulomas, respectively. Th1/Th2 cross-regulation was evident since endogenous Th2 cytokines inhibited type-1, whereas Th1 cytokines inhibited type-2 biased chemokines. These findings reveal a complex cytokine-chemokine regulatory network that dictates profiles of local chemokine expression during T cell-mediated granuloma formation.—Authors' Abstract

**Mendez-Samperio, P., Ayala, H., and Vazquez, A.** NF-kappaB Is Involved in Regulation of CD40 Ligand Expression on *Mycobacterium bovis* Bacillus Calmette-Guerin-Activated Human T Cells. *Clin. Diagn. Lab. Immunol.* **10(3)** (2003) 376–382.

Interaction between CD40L (CD154) on activated T cells and its receptor CD40 on antigen-presenting cells has been reported to be important in the resolution of infection by mycobacteria. However, the mechanism(s) by which *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) up-regulates membrane expression of CD40L molecules is poorly understood. This study was done to investigate the role of the nuclear factor kappaB (NF-kappaB) signaling pathway in the regulation of CD40L expression in human CD4(+) T cells stimulated with BCG. Specific pharmacologic inhibition of the NF-kappaB pathway revealed that this signaling cascade was required in the regulation of CD40L expression on the surface of BCG-activated CD4(+) T cells. These results were further supported by the fact that treatment of BCG-activated CD4(+) T cells with these pharmacological inhibitors significantly down-regulated CD40L mRNA. In this study, inhibitor kappaBalpha (IkappaBalpha) and IkappaBbeta protein production

was not affected by the chemical protease inhibitors and, more importantly, BCG led to the rapid but transient induction of NF-kappaB activity. Our results also indicated that CD40L expression on BCG-activated CD4(+) T cells resulted from transcriptional up-regulation of the CD40L gene by a mechanism which is independent of de novo protein synthesis. Interestingly, BCG-induced activation of NF-kappaB and the increased CD40L cell surface expression were blocked by the protein kinase C (PKC) inhibitors 1-[5-isoquinolinesulfonyl]-2-methylpiperazine and salicylate, both of which block phosphorylation of IkappaB. Moreover, rottlerin a Ca(2+)-independent PKC isoform inhibitor, significantly down-regulated CD40L mRNA in BCG-activated CD4(+) T cells. These data strongly suggest that CD40L expression by BCG-activated CD4(+) T cells is regulated via the PKC pathway and by NF-kappaB DNA binding activity.—Authors' Abstract

**Venkatesh, J., Kumar, P., Murali-Krishna, P. S., Manjunath, R., and Varshney, U.** Importance of uracil DNA glycosylase in *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*, G+C-rich bacteria, in mutation prevention, tolerance to acidified nitrite and endurance in mouse macrophages. *J. Biol. Chem.* **278(27)** (2003) 24350–24358.

Uracil DNA glycosylase (Ung or UDG), initiates the excision repair of an unusual base, uracil in DNA. Ung is a highly conserved protein found in all organisms. Paradoxically, loss of this evolutionarily conserved enzyme has not been seen to result in severe growth phenotypes in the cellular life forms. In this study, we chose G+C rich genome containing bacteria (*Pseudomonas aeruginosa* and *Mycobacterium smegmatis*), as model organisms to investigate the biological significance of ung. Ung deficiency was created either by expression of a highly specific inhibitor protein, Ugi and/or by targeted disruption of the ung gene. We show that abrogation of Ung activity in *P. aeruginosa* and *M. smegmatis* confers upon them an increased mutator phenotype and sensitivity to reac-

tive nitrogen intermediates generated by acidified nitrite. Also, in a mouse macrophage infection model, *P. aeruginosa* (Ung<sup>-</sup>) shows a significant decrease in its survival. Infections of the macrophages, with *M. smegmatis*, show an initial increase in the bacterial counts, which remain at this level for up to 48 hr before a decline. Interestingly, abrogation of Ung activity in *M. smegmatis* results in nearly a total abolition of their multiplication and much-decreased residency in macrophages stimulated with IFN. These observations suggest Ung as a useful target to control growth of G+C rich bacteria.—Authors' Abstract

**Venketaraman, V., Dayaram, Y. K., Amin, A. G., Ngo, R., Green, R. M., Talaue, M. T., Mann, J., and Connell, N. D.** Role of glutathione in macrophage control of mycobacteria. *Infect. Immun.* **71(4)** (2003) 1864–1871.

Reactive oxygen and nitrogen intermediates are important antimicrobial defense mechanisms of macrophages and other phagocytic cells. While reactive nitrogen intermediates have been shown to play an important role in tuberculosis control in the murine system, their role in human disease is not clearly established. Glutathione, a tripeptide and antioxidant, is synthesized at high levels by cells during reactive oxygen intermediate and nitrogen intermediate production. Glutathione has been recently shown to play an important role in apoptosis and to regulate antigen-presenting-cell functions. Glutathione also serves as a carrier molecule for nitric oxide, in the form of S-nitrosoglutathione. Previous work from this laboratory has shown that glutathione and S-nitrosoglutathione are directly toxic to mycobacteria. A mutant strain of *Mycobacterium bovis* BCG, defective in the transport of small peptides such as glutathione, is resistant to the toxic effect of glutathione and S-nitrosoglutathione. Using the peptide transport mutant as a tool, we investigated the role of glutathione and S-nitrosoglutathione in animal and human macrophages in controlling intracellular mycobacterial growth.—Authors' Abstract

**Worku, S. and Hoft, D. F.** Differential effects of control and antigen-specific T cells on intracellular mycobacterial growth. *Infect. Immun.* **71(4)** (2003) 1763–1773.

We investigated the effects of peripheral blood mononuclear cells expanded with irrelevant control and mycobacterial antigens on the intracellular growth of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) in human macrophages. More than 90% of the cells present after 1 week of *in vitro* expansion were CD3(+). T cells were expanded from purified protein derivative-negative controls, persons with latent tuberculosis, and BCG-vaccinated individuals. T cells expanded with nonmycobacterial antigens enhanced the intracellular growth of BCG in suboptimal cultures of macrophages. T cells expanded with live BCG or lysates of *Mycobacterium tuberculosis* directly inhibited intracellular BCG. Recent intradermal BCG vaccination significantly enhanced the inhibitory activity of T cells expanded with mycobacterial antigens ( $p < 0.02$ ), consistent with the induction of memory-immune inhibitory T-cell re-

sponses. Selected mycobacterial antigens (Mtb41 > lipoarabinomannan > 38kd > Ag85B > Mtb39) expanded inhibitory T cells, demonstrating the involvement of antigen-specific T cells in intracellular BCG inhibition. We studied the T-cell subsets and molecular mechanisms involved in the memory-immune inhibition of intracellular BCG. Mycobacteria-specific gammadelta T cells were the most potent inhibitors of intracellular BCG growth. Direct contact between T cells and macrophages was necessary for the BCG growth-enhancing and inhibitory activities mediated by control and mycobacteria-specific T cells, respectively. Increases in tumor necrosis factor alpha, interleukin-6, transforming growth factor beta, and vascular endothelial growth factor mRNA expression were associated with the enhancement of intracellular BCG growth. Increases in gamma interferon, FAS, FAS ligand, perforin, granzyme, and granulysin mRNA expression were associated with intracellular BCG inhibition. These culture systems provide *in vitro* models for studying the opposing T-cell mechanisms involved in mycobacterial survival and protective host immunity.—Authors' Abstract

## Immuno-Pathology (Leprosy)

**Buhrer-Sekula, S., Smits, H. L., Gussenhoven, G. C., Van Leeuwen, J., Amador, S., Fujiwara, T., Klatser, P. R., and Oskam, L.** Simple and fast lateral flow test for classification of leprosy patients and identification of contacts with high risk of developing leprosy. *J. Clin. Microbiol.* **41(5)** (2003) 1991–1995.

The interruption of leprosy transmission is one of the main challenges for leprosy control programs since no consistent evidence exists that transmission has been reduced after the introduction of multidrug therapy. Sources of infection are primarily people with high loads of bacteria with or without clinical signs of leprosy. The availability of a simple test system for the detection of antibodies to phenolic glycolipid-I (PGL-I) of *Mycobacterium leprae* to identify these individuals may be important in the prevention of transmission. We have de-

veloped a lateral flow assay, the ML Flow test, for the detection of antibodies to PGL-I which takes only 10 min to perform. An agreement of 91% was observed between enzyme-linked immunosorbent assay and our test; the agreement beyond chance (kappa value) was 0.77. We evaluated the use of whole blood by comparing 539 blood and serum samples from an area of high endemicity. The observed agreement was 85.9% (kappa = 0.70). Storage of the lateral flow test and the running buffer at 28 degrees C for up to 1 year did not influence the results of the assay. The sensitivity of the ML Flow test in correctly classifying MB patients was 97.4%. The specificity of the ML Flow test, based on the results of the control group, was 90.2%. The ML Flow test is a fast and easy-to-perform method for the detection of immunoglobulin M antibodies to PGL-I of *M. leprae*. It does not require any special equipment, and the highly stable reagents make the test ro-

bust and suitable for use in tropical countries.—Authors' Abstract

**Hernandez, M. O., Neves, I., Sales, J. S., Carvalho, D. S., Sarno, E. N., and Sampaio, E. P.** Induction of apoptosis in monocytes by *Mycobacterium leprae* *in vitro*: a possible role for tumour necrosis factor- $\alpha$ . *Immunology*. **109**(1) (2003) 156–164.

A diverse range of infectious organisms, including mycobacteria, have been reported to induce cell death *in vivo* and *in vitro*. Although morphological features of apoptosis have been identified in leprosy lesions, it has not yet been determined whether *Mycobacterium leprae* modulates programmed cell death. For that purpose, peripheral blood mononuclear cells obtained from leprosy patients were stimulated with different concentrations of this pathogen. Following analysis by flow cytometry on 7AAD/CD14+ cells, it was observed that *M. leprae* induced apoptosis of monocyte-derived macrophages in a dose-dependent manner in both leprosy patients and healthy individuals, but still with lower efficiency as compared to *M. tuberculosis*. Expression of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), Bax- $\alpha$ , Bak mRNA and TNF- $\alpha$  protein was also detected in these cultures; in addition, an enhancement in the rate of apoptotic cells (and of TNF- $\alpha$  release) was noted when interferon- $\gamma$  was added to the wells. On the other hand, incubation of the cells with pentoxifylline impaired mycobacterium-induced cell death, the secretion of TNF- $\alpha$ , and gene expression *in vitro*. In addition, diminished bacterial entry decreased both TNF- $\alpha$  levels and the death of CD14+ cells, albeit to a different extent. When investigating leprosy reactions, an enhanced rate of spontaneous apoptosis was detected as compared to the unreactive lepromatous patients. The results demonstrated that *M. leprae* can lead to apoptosis of macrophages through a mechanism that could be at least partially related to the expression of pro-apoptotic members of the Bcl-2 protein family and of TNF- $\alpha$ . Moreover, while phagocytosis may be necessary, it seems not to be crucial to the induction of cell

death by the mycobacteria.—Authors' Abstract

**Kiszewski, A. E., Becerril, E., Baquera, J., Ruiz-Maldonado, R., and Hernandez Pando, R.** Expression of cyclooxygenase type 2 in lepromatous and tuberculoid leprosy lesions. *Br. J. Dermatol.* **148**(4) (2003) 795–798.

**BACKGROUND:** Leprosy is an infectious disease with two polar forms, tuberculoid leprosy (TL) and lepromatous leprosy (LL), which are dominated by T-helper (Th) 1 and Th2 cells, respectively. High concentrations of prostaglandin E2 produced by the inducible enzyme cyclooxygenase type 2 (COX-2) in LL could inhibit Th1 cytokine production, contributing to T-cell anergy. **Objectives** To compare the COX-2 expression in LL and TL. **Methods** Skin biopsies from 40 leprosy patients (LL, N = 20; TL, N = 20) were used to determine by immunohistochemistry and automated morphometry the percentage of COX-2 immunostained cells. **Results** Most COX-2-positive cells were macrophages; their percentages in the inflammatory infiltrate located in the papillary dermis, reticular dermis and periadnexally were significantly higher in LL than TL (p < 0.001 by Student's *t*-test). **CONCLUSIONS:** The high expression of COX-2 in LL may be related to high prostaglandin production contributing to T-cell anergy.—Authors' Abstract

**Krutzik, S. R., Ochoa, M. T., Sieling, P. A., Uematsu, S., Ng, Y. W., Legaspi, A., Liu, P. T., Cole, S. T., Godowski, P. J., Maeda, Y., Sarno, E. N., Norgard, M. V., Brennan, P. J., Akira, S., Rea, T. H., and Modlin, R. L.** Activation and regulation of Toll-like receptors 2 and 1 in human leprosy. *Nat. Med.* **9**(5) (2003) 525–532.

The expression and activation of Toll-like receptors (TLRs) was investigated in leprosy, a spectral disease in which clinical manifestations correlate with the type of immune response mounted toward *Mycobacterium leprae*. TLR2-TLR1 heterodimers mediated cell activation by killed *M. leprae*,

indicating the presence of triacylated lipoproteins. A genome-wide scan of *M. leprae* detected 31 putative lipoproteins. Synthetic lipopeptides representing the 19-kD and 33-kD lipoproteins activated both monocytes and dendritic cells. Activation was enhanced by type-1 cytokines and inhibited by type-2 cytokines. In addition, interferon (IFN)-gamma and granulocyte-macrophage colony-stimulating factor (GM-CSF) enhanced TLR1 expression in monocytes and dendritic cells, respectively, whereas IL-4 downregulated TLR2 expression. TLR2 and TLR1 were more strongly expressed in lesions from the localized tuberculoid form (T-lep) as compared with the disseminated lepromatous form (L-lep) of the disease. These data provide evidence that regulated expression and activation of TLRs at the site of disease contribute to the host defense against microbial pathogens.—Authors' Abstract

**Lee, S. B., Kim, B. C., Jin, S. H., Park, Y. G., Kim, S. K., Kang, T. J., and Chae, G.** Missense mutations of the interleukin-12 receptor beta 1 (IL12RB1) and interferon-gamma receptor 1 (IFNGR1) genes are not associated with susceptibility to lepromatous leprosy in Korea. *Immunogenetics* **55**(3) (2003) 177–181.

Interleukin-12 receptor beta 1 (IL12RB1), interleukin-12 receptor beta 2 (IL12RB2),

and interferon gamma receptor 1 (IFNGR1) perform important roles in the host defense against intracellular pathogens such as Mycobacteria. Several mutations within their genes have been confirmed as associated with increased susceptibility to mycobacterial infection. However, the association between mutations of the IL12RB1, IL12RB2, and IFNGR1 encoding genes and lepromatous leprosy has not been studied. This study screened for polymorphisms within IL12RB1, IL12RB2, and IFNGR1 encoding genes in the Korean populations using polymerase chain reaction (PCR)/single-strand conformation polymorphism (SSCP) DNA sequencing assay, and an association study was performed using the missense mutations of 705 A/G (Q214R), 1196 G/C (G378R), 1637 G/A (A525T), and 1664 C/T (P534S) of the IL12RB1, 83 G/A (V14M), and 1443 T/C (L467P) for the IFNGR1 encoding genes. There were no differences in the genotype and allele frequencies of IL12RB1 and IFNGR1 genes between 93 lepromatous leprosy patients and 94 control subjects. In conclusion, missense mutations of 705 A/G (Q214R), 1196 G/C (G378R), 1637 G/A (A525T), 1664 C/T (P534S) of the IL12RB1, 83 G/A (V14M), and 1443 T/C (L467P) of the IFNGR1 encoding genes have no association with the susceptibility to lepromatous leprosy in the Korean population.—Authors' Abstract

## Immuno-Pathology (Tuberculosis)

**Boshoff, H. I., Reed, M. B., Barry, C. E., and Mizrahi, V.** DnaE2 Polymerase Contributes to *In Vivo* Survival and the Emergence of Drug Resistance in *Mycobacterium tuberculosis*. *Cell*. **113**(2) (2003) 183–193.

The presence of multiple copies of the major replicative DNA polymerase (DnaE) in some organisms, including important pathogens and symbionts, has remained an unresolved enigma. We postulated that one copy might participate in error-prone DNA repair synthesis. We found that UV irradiation of *Mycobacterium tuberculosis* results

in increased mutation frequency in the surviving fraction. We identified dnaE2 as a gene that is upregulated *in vitro* by several DNA damaging agents, as well as during infection of mice. Loss of this protein reduces both survival of the bacillus after UV irradiation and the virulence of the organism in mice. Our data suggest that DnaE2, and not a member of the Y family of error-prone DNA polymerases, is the primary mediator of survival through inducible mutagenesis and can contribute directly to the emergence of drug resistance *in vivo*. These results may indicate a potential new target for therapeutic intervention.—Authors' Abstract

**Brennan, P. J.** Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. Tuberculosis (Edinb). **83**(1–3) (2003) 91–97.

Much of the early structural definition of the cell wall of *Mycobacterium spp.* was initiated in the 1960s and 1970s. There was a long period of inactivity, but more recent developments in NMR and mass spectral analysis and definition of the *M. tuberculosis* genome have resulted in a thorough understanding, not only of the structure of the mycobacterial cell wall and its lipids but also the basic genetics and biosynthesis. Our understanding nowadays of cell-wall architecture amounts to a massive “core” comprised of peptidoglycan covalently attached via a linker unit (L-Rha-D-GlcNAc-P) to a linear galactofuran, in turn attached to several strands of a highly branched arabinofuran, in turn attached to mycolic acids. The mycolic acids are oriented perpendicular to the plane of the membrane and provide a truly special lipid barrier responsible for many of the physiological and disease-inducing aspects of *M. tuberculosis*. Intercalated within this lipid environment are the lipids that have intrigued researchers for over five decades: the phthiocerol dimycocerosate, cord factor/dimycolyl-trehalose, the sulfolipids, the phosphatidylinositol mannosides, etc. Knowledge of their roles in “signaling” events, in pathogenesis, and in the immune response is now emerging, sometimes piecemeal and sometimes in an organized fashion. Some of the more intriguing observations are those demonstrating that mycolic acids are recognized by CD1-restricted T-cells, that antigen 85, one of the most powerful protective antigens of *M. tuberculosis*, is a mycolyltransferase, and that lipoarabinomannan (LAM), when “capped” with short mannose oligosaccharides, is involved in phagocytosis of *M. tuberculosis*. Definition of the genome of *M. tuberculosis* has greatly aided efforts to define the biosynthetic pathways for all of these exotic molecules: the mycolic acids, the mycocerosates, phthiocerol, LAM, and the polyprenyl phosphates. For example, we know that synthesis of the entire core is initiated on a decaprenyl-P with synthesis of the linker unit, and then there is concomitant extension of the galactan and arabinan chains while this intermediate is transported through the cytoplasmic mem-

brane. The final steps in these events, the attachment of mycolic acids and ligation to peptidoglycan, await definition and will prove to be excellent targets for a new generation of anti-tuberculosis drugs.—Author’s Abstract

**Ehlers, S., Holscher, C., Scheu, S., Tertilt, C., Hehlhans, T., Suwinski, J., Endres, R., and Pfeffer, K.** The Lymphotoxin beta Receptor Is Critically Involved in Controlling Infections with the Intracellular Pathogens *Mycobacterium tuberculosis* and *Listeria monocytogenes*. J. Immunol. **170**(10) (2003) 5210–5218.

Containment of intracellularly viable microorganisms requires an intricate cooperation between macrophages and T cells, the most potent mediators known to date being IFN-gamma and TNF. To identify novel mechanisms involved in combating intracellular infections, experiments were performed in mice with selective defects in the lymphotoxin (LT)/LTbetaR pathway. When mice deficient in LTalpha or LTbeta were challenged intranasally with *Mycobacterium tuberculosis*, they showed a significant increase in bacterial loads in lungs and livers compared with wild-type mice, suggesting a role for LTalphabeta heterotrimers in resistance to infection. Indeed, mice deficient in the receptor for LTalpha(1)beta(2) heterotrimers (LTbetaR-knockout (KO) mice) also had significantly higher numbers of *M. tuberculosis* in infected lungs and exhibited widespread pulmonary necrosis already by day 35 after intranasal infection. Furthermore, LTbetaR-KO mice were dramatically more susceptible than wild-type mice to i.p. infection with *Listeria monocytogenes*. Compared with wild-type mice, LTbetaR-KO mice had similar transcript levels of TNF and IFN-gamma and recruited similar numbers of CD3(+) T cells inside granulomatous lesions in *M. tuberculosis*-infected lungs. Flow cytometry revealed that the LTbetaR is expressed on pulmonary macrophages obtained after digestion of *M. tuberculosis*-infected lungs. LTbetaR-KO mice showed delayed expression of inducible NO synthase protein in granuloma macrophages, implicating deficient macrophage activation as the most likely cause for enhanced sus-

ceptibility of these mice to intracellular infections. Since LIGHT-KO mice proved to be equally resistant to *M. tuberculosis* infection as wild-type mice, these data demonstrate that signaling of LTalpha(1)beta(2) heterotrimers via the LTbetaR is an essential prerequisite for containment of intracellular pathogens.—Authors' Abstract

**Fenhalls, G., Squires, G. R., Stevens-Muller, L., Bezuidenhout, J., Amphlett, G., Duncan, K., and Lukey, P. T.** Associations between Toll-like Receptors and IL-4 in the Lungs of Patients with Tuberculosis. *Am. J. Respir. Cell. Mol. Biol.* **29(1)** (2003) 28–38.

Toll-like receptors (TLRs) are implicated in the intracellular killing of *Mycobacterium tuberculosis* and their expression is modulated by interleukin-4 (IL-4) *in vitro*. Our aim was to examine the expression of TLRs at the site of pathology in tuberculous lung granulomas and to explore the effect of the immune response on TLR expression. Immunohistochemistry was performed on lung granulomas from nine patients with tuberculosis undergoing lobectomy for haemoptysis. All nine patients expressed all of the TLRs studied (TLRs1–5 and 9), whereas only five out of the nine patients had any granulomas positive for IL-4. Statistical analysis of TLR and cytokine staining patterns in 183 individual granulomas from the nine patients revealed significant associations between pairs of receptors and IL-4. A positive association between TLR2 and TLR4 ( $p < 0.0001$ ) and a negative association between TLR2 and IL-4 ( $p < 0.0001$ ) was observed. The associations between TLRs 1, 5 and 9 were significantly different in IL-4 negative compared to IL-4 positive patients. In conclusion, TLRs are expressed by various cell types in the human tuberculous lung and their expression patterns are reflected by differences in the immune response.—Authors' Abstract

**Fratti, R. A., Chua, J., Vergne, I., and Deretic, V.** *Mycobacterium tuberculosis* glycosylated phosphatidylinositol causes phagosome maturation arrest. *Proc. Natl.*

*Acad. Sci. U. S. A.* **100(9)** (2003) 5437–5442.

The tubercle bacillus parasitizes macrophages by inhibiting phagosome maturation into the phagolysosome. This phenomenon underlies the tuberculosis pandemic involving 2 billion people. We report here how *Mycobacterium tuberculosis* causes phagosome maturation arrest. A glycosylated *M. tuberculosis* phosphatidylinositol [mannose-capped lipoarabinomannan (ManLAM)] interfered with the phagosomal acquisition of the lysosomal cargo and syntaxin 6 from the trans-Golgi network. ManLAM specifically inhibited the pathway dependent on phosphatidylinositol 3-kinase activity and phosphatidylinositol 3-phosphate-binding effectors. These findings identify ManLAM as the *M. tuberculosis* product responsible for the inhibition of phagosomal maturation.—Authors' Abstract

**Hsiao, P. F., Tzen, C. Y., Chen, H. C., and Su, H. Y.** Polymerase chain reaction based detection of *Mycobacterium tuberculosis* in tissues showing granulomatous inflammation without demonstrable acid-fast bacilli. *Int. J. Dermatol.* **42(4)** (2003) 281–286.

**BACKGROUND:** Cutaneous tuberculosis is especially difficult to distinguish from other granulomatous dermatoses. We used polymerase chain reaction (PCR) to evaluate the incidence of cutaneous tuberculosis and atypical mycobacterial infection in formalin-fixed, paraffin-embedded tissues with unspecified granulomatous inflammation and negative results for acid-fast bacilli (AFB), and analyzed the pattern of cutaneous tuberculosis in this group of patients. **METHODS:** A total of 38 specimens which had been collected from 36 patients and fulfilled the criteria for tissues described above were used in this study. Two different primer pairs targeting the gene encoding for 16S ribosomal RNA (common to all mycobacteria) and the insertion sequence IS6110 (specific for *M. tuberculosis* complex) were used in the PCR assays. The clinical characteristics, histopathologic findings, and culture results of the patients were also analyzed. **RESULTS:** Four specimens were

excluded from the analysis due to the lack of internal control testing. Of the remaining 34 specimens, 22 were PCR positive for the 16S rRNA gene. Among them, 18 specimens were PCR positive for both the 16S rRNA gene and IS6110. Cutaneous tuberculosis could be diagnosed in these 18 cases (56.2%). Out of the 18 cases, there were 8 women and 10 men. The age range was 15–77 years (mean: 44.2 years). After reviewing their clinical presentation, 11 cases were considered as tuberculosis verrucosa cutis, 6 cases as lupus vulgaris, and 1 case as erythema induratum. The remaining 4 cases (12.5%) positive only for 16S rRNA gene were considered as possible atypical mycobacteria infection. **CONCLUSIONS:** These results show that in paucibacillary form of cutaneous tuberculosis with unclassical clinical and histological presentation, this PCR system provides rapid and sensitive detection of *M. tuberculosis* DNA in formalin-fixed, paraffin-embedded specimens. Cutaneous tuberculosis represents a significant proportion in specimens showing granulomatous inflammation. In areas like Taiwan, where prevalence of pulmonary tuberculosis is still high, tuberculosis verrucosa cutis and lupus vulgaris are common forms of cutaneous tuberculosis and are seen more frequently than atypical mycobacterial infection.—Authors' Abstract

**Jung, Y. J., Ryan, L., LaCourse, R., and North, R. J.** Increased interleukin-10 expression is not responsible for failure of T helper 1 immunity to resolve airborne *Mycobacterium tuberculosis* infection in mice. *Immunology* **109**(2) (2003) 295–299.

With a view to determining whether failure of mice to resolve *Mycobacterium tuberculosis* (Mtb) infection is a consequence of downregulation of T helper 1 (Th1) immunity by interleukin (IL)-10, mice deleted of the gene for IL-10 were compared with wild-type (WT) mice in terms of their ability to make IL-10 mRNA, generate Th1-mediated immunity [as measured by synthesis of mRNA for interferon-gamma (IFN-gamma)], IL-12p40 and inducible nitric oxide synthase (iNOS), and to control

lung infection. It was found that the response of WT mice to infection included a substantial and sustained increase in IL-10 mRNA synthesis in the lungs. A Th1 response in the lungs of WT and IL-10<sup>-/-</sup> mice was evidenced by a large and sustained increase in the synthesis of mRNA for IFN-gamma, IL-12p40 and iNOS, with somewhat higher levels of these mRNA species being made in the lungs of IL-10<sup>-/-</sup> mice, particularly at an early stage of infection. However, IL-10<sup>-/-</sup> mice were no more capable than WT mice at combating infection.—Authors' Abstract

**Kaul, D., Coffey, M. J., Phare, S. M., and Kazanjian, P. H.** Capacity of neutrophils and monocytes from human immunodeficiency virus-infected patients and healthy controls to inhibit growth of *Mycobacterium bovis*. *J. Lab. Clin. Med.* **141**(5) (2003) 330–334.

We compared the differences in growth inhibition of *Mycobacterium bovis* by monocytes and neutrophils from human immunodeficiency virus (HIV)-infected persons (N = 12; mean CD4 count = 451/mm<sup>3</sup>) and healthy controls (N = 6). Phagocytes from all HIV-infected patients were incubated with or without exogenous granulocyte-macrophage colony-stimulating factor (GM-CSF; 500–1000 U/mL). In two of the HIV-infected patients, phagocytes were incubated with or without interleukin (IL)-2 or IL-8 (500–1000 U/mL). Compared with that in HIV-infected patients, the reduction of *M. bovis* growth at 24 hours was 81% greater among monocytes and 69% greater among neutrophils from healthy controls (p = .03 and .04, respectively). Among HIV-infected patients, we noted greater mycobacterial reduction in monocytes (49%, p = .04) and neutrophils (42%, p = .05) from the early-stage patients (mean CD4 count = 760/mm<sup>3</sup>) compared with that in late-stage patients (mean CD4 count = 172/mm<sup>3</sup>). Incubation with GM-CSF, IL-2, or IL-8 did not augment mycobactericidal activity. These findings suggest that the capacity of neutrophils and monocytes from HIV-infected patients to inhibit the growth of *M. bovis* is impaired, and this impairment is more pronounced in

later stages of HIV infection.—Authors' Abstract

**Koguchi, Y., Kawakami, K., Uezu, K., Fukushima, K., Kon, S., Maeda, M., Nakamoto, A., Owan, I., Kuba, M., Kudeken, N., Kadota, J. I., Mukae, H., Kohno, S., Uede, T., and Saito, A.** High plasma osteopontin level and its relationship with IL-12-mediated Th1 response in tuberculosis. *Am. J. Respir. Crit. Care Med.* **167(10)** (2003) 1355–1359.

Osteopontin (OPN, also known as Eta-1), a noncollagenous matrix protein produced by macrophages and T lymphocytes, is expressed in granulomatous lesions caused by *Mycobacterium tuberculosis* infection. In the present study, we compared plasma concentrations of OPN in patients with active pulmonary tuberculosis with those of healthy control subjects and patients with sarcoidosis, another disease associated with granuloma formation. Plasma OPN levels were significantly higher in patients with tuberculosis (N = 48) than control subjects (N = 34) and patients with sarcoidosis (N = 20). OPN levels correlated well with severity of pulmonary tuberculosis, as indicated by the size of lung lesions on chest X-ray films. Furthermore, chemotherapy resulted in a significant fall in plasma OPN levels. In patients with tuberculosis, plasma OPN concentrations correlated significantly with those of interleukin (IL)-12. *In vitro* experiments showed that OPN production by peripheral blood mononuclear cells infected with *M. bovis* BCG preceded the synthesis of IL-12 and interferon (IFN)-gamma, and that neutralizing anti-OPN monoclonal antibody significantly reduced the production of IL-12 and IFN-gamma. Our results suggest that OPN may be involved in the pathological process associated with active pulmonary tuberculosis by inducing IL-12-mediated Th1 responses.—Authors' Abstract

**Munoz, S., Hernandez-Pando, R., Abraham, S. N., and Enciso, J. A.** Mast cell activation by *Mycobacterium tuberculosis*: mediator release and role of CD48. *J. Immunol.* **170(11)** (2003) 5590–5596.

Mast cells (MC) are abundant in the lung and other peripheral tissue, where they participate in inflammatory processes against bacterial infections. Like other effector cells of the innate immune system, MC interact directly with a wide variety of infectious agents. This interaction results in MC activation and inflammatory mediator release. We demonstrated that MC interact with *Mycobacterium tuberculosis*, triggering the release of several prestored reagents, such as histamine and beta-hexosaminidase, and de novo synthesized cytokines, such as TNF-alpha and IL-6. A number of *M. tuberculosis* Ags, ESAT-6, MTSa-10, and MPT-63, have been implicated in MC activation and mediator release. A MC plasmalemmal protein, CD48, was implicated in interactions with mycobacteria because CD48 appeared to aggregate in the MC membrane at sites of bacterial binding and because Abs to CD48 inhibited the MC histamine response to mycobacteria. Cumulatively, these findings suggest that MC, even in the absence of opsonins, can directly recognize *M. tuberculosis* and its Ags and have the potential to play an active role in mediating the host's innate response to *M. tuberculosis* infection.—Authors' Abstract

**Natarajan, K., Latchumanan, V. K., Singh, B., Singh, S., and Sharma, P.** Down-regulation of T helper 1 responses to mycobacterial antigens due to maturation of dendritic cells by 10-kDa *Mycobacterium tuberculosis* secretory antigen. *J. Infect. Dis.* **187(6)** (2003) 914–928.

Interactions of 10-kDa *Mycobacterium tuberculosis* secretory antigen (MTSA) with dendritic cells (DCs) were investigated to elucidate the role of secretory antigens in regulating immune responses to *M. tuberculosis* early in the course of infection. MTSA induced the maturation of different DC subsets. The cytokine profiles of these DCs were characteristic to each DC subset. Of interest, coculture of *M. tuberculosis* whole-cell extract (CE)-pulsed, MTSA-matured DCs with CE-specific T cells led to a marked reduction in interleukin (IL)-2 and interferon (IFN)-gamma production, thereby down-regulating

proinflammatory responses to mycobacterial antigens. Attenuation of IL-2 and IFN- $\gamma$  levels of CE-specific T cells also was obtained when *M. tuberculosis* culture filtrate protein-activated DCs were employed as antigen-presenting cells, which suggests that MTSAs induce maturation of DCs at sites of infection, probably to down-regulate proinflammatory immune responses to mycobacteria that may subsequently be released from infected macrophages.—Authors' Abstract

**Rhoades, E., Hsu, F., Torrelles, J. B., Turk, J., Chatterjee, D., and Russell, D. G.** Identification and macrophage-activating activity of glycolipids released from intracellular *Mycobacterium bovis* BCG. *Mol. Microbiol.* **48(4)** (2003) 875–888.

Intracellular mycobacteria release cell wall glycolipids into the endosomal network of infected macrophages. Here, we characterize the glycolipids of *Mycobacterium bovis* BCG (BCG) that are released into murine bone marrow-derived macrophages (BMMO). Intracellularly released mycobacterial lipids were harvested from BMMO that had been infected with <sup>14</sup>C-labelled BCG. Released BCG lipids were resolved by thin-layer chromatography, and they migrated similarly to phosphatidylinositol dimannosides (PIM2), mono- and diphosphatidylglycerol, phosphatidylethanolamine, trehalose mono- and dimycolates and the phenolic glycolipid, mycoside B. Culture-derived BCG lipids that co-migrated with the intracellularly released lipids were purified and identified by electrospray ionization mass spectrometry. When delivered on polystyrene microspheres, fluorescently tagged BCG lipids were also released into the BMMO, in a manner similar to release from viable or heat-killed BCG bacilli. To determine whether the released lipids elicited macrophage responses, BCG lipid-coated microspheres were delivered to interferon  $\gamma$ -primed macrophages (BMMO or thioglycollate-elicited peritoneal macrophages), and reactive nitrogen intermediates as well as tumour necrosis factor- $\alpha$  and monocyte chemoattractant protein-1

production were induced. When fractionated BCG lipids were delivered on the microspheres, PIM2 species reproduced the macrophage-activating activity of total BCG lipids. These results demonstrate that intracellular mycobacteria release a heterogeneous mix of lipids, some of which elicit the production of proinflammatory cytokines from macrophages that could potentially contribute to the granulomatous response in tuberculous diseases.—Authors' Abstract

**Shams, H., Wizel, B., Lakey, D. L., Samten, B., Vankayalapati, R., Valdivia, R. H., Kitchens, R. L., Griffith, D. E., and Barnes, P. F.** The CD14 receptor does not mediate entry of *Mycobacterium tuberculosis* into human mononuclear phagocytes. *FEMS Immunol. Med. Microbiol.* **36(1–2)** (2003) 63–69.

Prior reports have suggested that CD14 mediates uptake of *Mycobacterium tuberculosis* into porcine alveolar macrophages and human fetal microglia, but the contribution of CD14 to cell entry in human macrophages has not been studied. To address this question, we used flow cytometry to quantify uptake by human monocytes and alveolar macrophages of *M. tuberculosis* expressing green fluorescent protein. Neutralizing anti-CD14 antibodies did not affect bacillary uptake and the efficiency of bacillary entry was similar in THP-1 cells expressing low and high levels of CD14. However, most internalized bacteria were found in CD14+ but not in CD14– monocytes because *M. tuberculosis* infection up-regulated CD14 expression. We conclude that: (1) CD14 does not mediate cellular entry by *M. tuberculosis*; (2) *M. tuberculosis* infection upregulates CD14 expression on mononuclear phagocytes, and this may facilitate the pathogen's capacity to modulate the immune response.—Authors' Abstract

**Stein, C. M., Guwatudde, D., Nakakeeto, M., Peters, P., Elston, R. C., Tiwari, H. K., Mugerwa, R., and Whalen, C. C.** Heritability analysis of cytokines as intermediate phenotypes of tuberculosis. *J. Infect. Dis.* **187(11)** (2003) 1679–1685.

Numerous studies have provided support for genetic susceptibility to tuberculosis (TB); however, heterogeneity in disease expression has hampered previous genetic studies. The purpose of this work was to investigate possible intermediate phenotypes for TB. A set of cytokine profiles, including antigen-stimulated whole-blood assays for interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, transforming growth factor (TGF)-beta, and the ratio of IFN to TNF, were analyzed in 177 pedigrees from a community in Uganda with a high prevalence of TB. The heritability of these variables was estimated after adjustment for covariates, and TNF-alpha, in particular, had an estimated heritability of 68%. A principal component analysis of IFN-gamma, TNF-alpha, and TGF-beta reflected the immunologic model of TB. In this analysis, the first component explained >38% of the variation in the data. This analysis illustrates the value of such intermediate phenotypes in mapping susceptibility loci for TB and demonstrates that this area deserves further research.—Authors' Abstract

**Ulrichs, T., Moody, D. B., Grant, E., Kaufmann, S. H., and Porcelli, S. A.** T-cell responses to CD1-presented lipid antigens in humans with *Mycobacterium tuberculosis* infection. *Infect. Immun.* **71**(6) (2003) 3076–3087.

CD1-restricted presentation of lipid or glycolipid antigens derived from *Mycobacterium tuberculosis* has been demonstrated by *in vitro* experiments using cultured T-cell lines. In the present work, the frequency of T-cell responses to natural mycobacterial lipids was analyzed in *ex vivo* studies of peripheral blood lymphocytes from human patients with pulmonary tuberculosis, from asymptomatic individuals with known contact with *M. tuberculosis* documented by conversion of their tuberculin skin tests, and from healthy tuberculin skin test-negative individuals or individuals vaccinated with *Mycobacterium bovis* BCG. Proliferation and gamma interferon enzyme-linked immunospot assays using peripheral blood lymphocytes and autologous CD1(+) immature dendritic cells revealed that T cells from asymptomatic *M.*

*tuberculosis*-infected donors responded with significantly greater magnitude and frequency to mycobacterial lipid antigen preparations than lymphocytes from uninfected healthy donors. By use of these methods, lipid-antigen-specific proliferative responses were minimally detectable or absent in blood samples from patients with active tuberculosis prior to chemotherapy but became detectable in blood samples drawn 2 weeks after the start of treatment. Lipid antigen-reactive T cells were detected predominantly in the CD4-enriched T-cell fractions of circulating lymphocytes, and anti-CD1 antibody blocking experiments confirmed the CD1 restriction of these T-cell responses. Our results provide further support for the hypothesis that lipid antigens serve as targets of the recall response to *M. tuberculosis*, and they indicate that CD1-restricted T cells responding to these antigens comprise a significant portion of the circulating pool of *M. tuberculosis*-reactive T cells in healthy individuals with previous exposure to *M. tuberculosis*.—Authors' Abstract

**Vordermeier, M., Whelan, A. O., and Hewinson, R. G.** Recognition of mycobacterial epitopes by T cells across mammalian species and use of a program that predicts human HLA-DR binding peptides to predict bovine epitopes. *Infect. Immun.* **71**(4) (2003) 1980–1987.

Bioinformatics tools have the potential to accelerate research into the design of vaccines and diagnostic tests by exploiting genome sequences. The aim of this study was to assess whether *in silico* analysis could be combined with *in vitro* screening methods to rapidly identify peptides that are immunogenic during *Mycobacterium bovis* infection of cattle. In the first instance the *M. bovis*-derived protein ESAT-6 was used as a model antigen to describe peptides containing T-cell epitopes that were frequently recognized across mammalian species, including natural hosts for tuberculosis (humans and cattle) and small-animal models of tuberculosis (mice and guinea pigs). Having demonstrated that some peptides could be recognized by T cells from a number of *M. bovis*-infected

hosts, we tested whether a virtual-matrix-based human prediction program (ProPred) could identify peptides that were recognized by T cells from *M. bovis*-infected cattle. In this study, 73% of the experimentally defined peptides from 10 *M. bovis* antigens that were recognized by bovine T cells contained motifs predicted by ProPred. Finally, in validating this observa-

tion, we showed that three of five peptides from the mycobacterial antigen Rv3019c that were predicted to contain HLA-DR-restricted epitopes were recognized by T cells from *M. bovis*-infected cattle. The results obtained in this study support the approach of using bioinformatics to increase the efficiency of epitope screening and selection.—Authors' Abstract

## Microbiology

**Chemlal, K. and Portaels, F.** Molecular diagnosis of nontuberculous mycobacteria. *Curr. Opin. Infect. Dis.* **16(2)** (2003) 77–83.

See *Current Literature, Molecular & Genetic Studies*

**Florio, W., Batoni, G., Esin, S., Bottai, D., Maisetta, G., Pardini, M., and Campa, M.** Identification of novel proteins in culture filtrates of *Mycobacterium bovis* bacillus Calmette-Guerin in the isoelectric point range 6–11. *Proteomics.* **3(5)** (2003) 798–802.

Two-dimensional gel electrophoresis and mass spectrometry were used to identify proteins in the isoelectric point range 6–11 in culture filtrates of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). Twelve proteins were identified, three of which had not been described previously. The expression of the identified proteins was comparatively analyzed in culture filtrates of BCG in different growth phases and culture conditions. For some of these proteins, the relative protein abundance in the different culture filtrate preparations was significantly different. The differential expression of the identified proteins is discussed in relation to their putative localization and/or biological function.—Authors' Abstract

**Lee, H., Bang, H. E., Bai, G. H., and Cho, S. N.** Novel polymorphic region of the rpoB gene containing *Mycobacterium species*-specific sequences and its use in identification of mycobacteria. *J. Clin. Microbiol.* **41(5)** (2003) 2213–2218.

Sequence analysis of a specific region of the mycobacterium rpoB gene in 35 mycobacterial strains representing 26 different mycobacterial species of clinical importance showed that there exists a highly polymorphic region. Based on the sequences of the polymorphic region, the oligonucleotide probes of 14 mycobacterial species with relatively high clinical importance were designed and shown to be specific to their corresponding mycobacterial species by dot blot hybridization. The results showed that the probes designed in this study are highly specific to each mycobacterial species, which suggests that these sequences may be useful for the species identification of mycobacteria.—Authors' Abstract

**Ozbek, A. and Aktas, O.** Identification of three strains of *Mycobacterium species* isolated from clinical samples using fatty acid methyl ester profiling. *J. Int. Med. Res.* **31(2)** (2003) 133–140.

The cellular fatty acid profiles of 67 strains belonging to three different species of the genus *Mycobacterium* were determined by gas chromatography of the fatty acid methyl esters, using the MIDI Sherlock Microbial Identification System (MIS). The species *M. tuberculosis*, *M. xenopi* and *M. avium* complex were clearly distinguishable and could be identified based on the presence and concentrations of 12 fatty acids: 14:0, 15:0, 16:1 omega 7c, 16:1 omega 6c, 16:0, 17:0, 18:2 omega 6.9c, 18:1 omega 9c, 18:0, 10Me-18:0 tuberculostearic acid, alcohol and cyclopropane. Fatty acid analysis showed that there is great homogeneity within and heterogeneity between *Mycobacterium species*.

Thus the MIS is an accurate, efficient and relatively rapid method for the identification of mycobacteria.—Authors' Abstract

**Padilla, E., Gonzalez, V., Manterola, J. M., Lonca, J., Perez, A., Matas, L., Quesada, M. D., and Ausina, V.** Evaluation of two different cell lysis methods for releasing mycobacterial nucleic acids in the INNO-LiPA mycobacteria test. *Diagn. Microbiol. Infect. Dis.* **46(1)** (2003) 19–23.

The INNO-LiPA Mycobacteria Test (Innogenetics, N.V., Belgium) is a PCR-based reverse hybridization assay for the simultaneous identification of several mycobacterial species. We evaluated two simplified lysis methods for mycobacterial DNA release for application in the INNO-LiPA Mycobacteria Test. The two methods were based on either (i) heat treatment or (ii) sonication. Both methods were performed directly on 45 positive liquid cultures (MB-BacT, BioMerieux, Marcy l'Etoile, France) containing 17 different mycobacterial species. These two simple lysis procedures demonstrated similar effectiveness (100%) to that recommended by the manufacturer. They also significantly shortened the time required for mycobacterial DNA release.—Authors' Abstract

**Sasseti, C. M., Boyd, D. H., and Rubin, E. J.** Genes required for mycobacterial growth defined by high density mutagenesis. *Mol. Microbiol.* **48(1)** (2003) 77–84.

*See abstract under Current Literature, Molecular & Genetic Studies*

**Tortoli, E.** Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990's. *Clin. Microbiol. Rev.* **16(2)** (2003) 319–354.

The advancement of genetic techniques has greatly boosted taxonomic studies in recent years. Within the genus *Mycobacterium*, 42 new species have been detected since 1990, most of which were grown from clinical samples. Along with species for which relatively large numbers of strains have been reported, some of the new species

of mycobacteria have been detected rarely or even only once. From the phenotypic point of view, among the new taxa, chromogens exceed nonchromogens while the numbers of slowly and rapidly growing species are equivalent. Whereas conventional identification tests were usually inconclusive, an important role was played by lipid analyses and in particular by high-performance liquid chromatography. Genotypic investigations based on sequencing of 16S rRNA gene have certainly made the most important contribution. The investigation of genetic relatedness led to the redistribution of the species previously included in the classically known categories of slow and rapid growers into new groupings. Within slow growers, the intermediate branch related to *Mycobacterium simiae* and the cluster of organisms related to *Mycobacterium terrae* have been differentiated; among rapid growers, the group of thermotolerant mycobacteria has emerged. The majority of species are resistant to isoniazid and, to a lesser extent, to rifampin. Many of the new species of mycobacteria are potentially pathogenic, and there are numerous reports of their involvement in diseases. Apart from disseminated and localized diseases in immunocompromised patients, the most frequent infections in immunocompetent people involve the lungs, skin, and, in children, cervical lymph nodes. The awareness of such new mycobacteria, far from being a merely speculative exercise, is therefore important for clinicians and microbiologists.—Author's Abstract

**Wang, J., Parsons, L. M., and Derbyshire, K. M.** Unconventional conjugal DNA transfer in mycobacteria. *Nat. Genet.* **34(1)** (2003) 80–84.

Bacterial conjugation is an active process that results in unidirectional transfer of DNA from a donor to a recipient cell. Most transfer systems are plasmid-encoded and require proteins to act at a unique cis-acting site to initiate and complete DNA transfer. By contrast, the *Mycobacterium smegmatis* DNA transfer system is chromosomally encoded. Here we show that multiple cis-acting sequences present on the chromosome can mediate transfer of a non-

mobilizable test plasmid. Moreover, unlike conventional plasmid transfer, recipient recombination functions are required to allow this plasmid, and derivatives of it, to recircularize through a process similar to gap repair. Extended DNA homology with the recipient chromosome is required to facilitate repair, resulting in acquisition of recipient chromosomal DNA by the plasmid. Together, these results show that DNA transfer in *M. smegmatis* occurs by a mechanism different from that of prototypical plasmid transfer systems.—Authors' Abstract

**Yagi, T., Mahapatra, S., Mikuova, K., Crick, D. C., and Brennan, P. J.** Polymerization of mycobacterial Arabinogalactan and ligation to peptidoglycan. *J. Biol. Chem.* 2003 Apr 28 [Epub ahead of print].

The cell wall of *Mycobacterium spp.* consists predominately of arabinogalactan chains linked at the reducing ends to peptidoglycan via a P-GlcNAc-(alpha1-3)-Rha linkage unit (LU) and esterified to a variety of mycolic acids at the non-reducing ends. Several aspects of the biosynthesis of this complex have been defined, includ-

ing the initial formation of the LU on a polyprenyl phosphate (Pol-P) molecule followed by the sequential addition of galactofuranosyl (GalF) units to generate Pol-P-P-LU-(GalF)<sub>1,2,3</sub>, etc. and Pol-P-P-LU-galactan, catalyzed by a bifunctional galactosyltransferase (Rv3808c) capable of adding alternating 5- and 6-linked GalF units. By applying cell-free extracts of *Mycobacterium smegmatis*, containing cell wall and membrane fragments, and differential labeling with UDP-[(14)C]Galp and recombinant UDP Galp mutase as the source of [(14)C]GalF for galactan biosynthesis and 5-P-[(14)C]ribosyl-P-P as a donor of [(14)C]Araf for arabinan synthesis, we now demonstrate sequential synthesis of the simpler Pol-P-P-LU-(GalF)<sub>n</sub> glycolipid intermediates followed by the Pol-P-P-LU-arabinogalactan and, finally, ligation of the P-LU-arabinogalactan to peptidoglycan. This first-time demonstration of *in vitro* ligation of newly synthesized P-LU-arabinogalactan to newly synthesized peptidoglycan is a necessary forerunner to defining the genetics and enzymology of cell wall polymer-peptidoglycan ligation in *Mycobacterium spp.* and examining this step as a target for new anti-bacterial drugs.—Authors' Abstract

## Microbiology (Leprosy)

**Kim, S. K., Lee, S. B., Kang, T. J., and Chae, G. T.** Detection of gene mutations related with drug resistance in *Mycobacterium leprae* from leprosy patients using Touch-Down (TD) PCR. *FEMS Immunol. Med. Microbiol.* **36**(1-2) (2003) 27-32.

The lack of methods to identify *Mycobacterium leprae* with the resistance against multi-drugs quickly and specifically has hindered effective chemotherapy against *M. leprae* infection. To screen *M. leprae* with resistance against multi-drugs, the Touch-Down (TD)-PCR has been used in this study. Sequences of the folP, rpoA, B, and gyrA, B genes were analyzed for isolates of *M. leprae* from leprosy patients in Korea. We amplified designated region of several genes in *M. leprae* involved in drug resistance and could obtain the PCR products of

each gene. The mutations in the particular region of folP, rpoB, and gyrB gene were certified by TD-PCR single-stranded conformational polymorphism and DNA sequencing, respectively.—Authors' Abstract

**Nayak, S. V., Shivarudrappa, A. S., and Mukkamil, A. S.** Role of fluorescent microscopy in detecting *Mycobacterium leprae* in tissue sections. *Ann. Diagn. Pathol.* **7**(2) (2003) 78-81.

We compared the sensitivity of the fluorescent method with that of the modified Fite-Faraco method in the detection of *Mycobacterium leprae* in tissue sections. Fifty-six skin biopsies were obtained from patients having leprosy, particularly the paucibacillary type. Minor alterations were

made in the deparaffinization and staining technique, as compared with Kuper and May's method, to obtain optimum fluorescence. Of 56 biopsies studied, 39 showed organisms by the fluorescent method and only 25 showed organisms by the modified Fite-Faraco method. The fluorescent method was found to be more advantageous than the

modified Fite-Faraco method, particularly in paucibacillary cases. Fluorescent microscopy has the advantage of speed and ease of screening and reduces observer fatigue. Bacillary positivity rates were higher in the fluorescent method than in the modified Fite-Faraco method in each type of leprosy.—Authors' Abstract

## Microbiology (Tuberculosis)

**Abulimiti, A., Qiu, X., Chen, J., Liu, Y., and Chang, Z.** Reversible methionine sulfoxidation of *Mycobacterium tuberculosis* small heat shock protein Hsp16.3 and its possible role in scavenging oxidants. *Biochem. Biophys. Res. Commun.* **305**(1) (2003) 87–93.

*Mycobacterium tuberculosis* (TB) small heat shock protein Hsp16.3 was found to be a major membrane protein that is most predominantly expressed under oxidative stress and is localized to the thickened cell envelope. Gene knock-out studies indicate that the Hsp16.3 protein is required for TB to grow in its host macrophage cells. The physiological function of Hsp16.3 has not yet revealed. Our analyses via mass spectrometry, conformation-dependent trypsin digestion, nondenaturing pore gradient electrophoresis, ANS-binding fluorescence measurements, and circular dichroism demonstrate that the three and only the three methionine residues (cysteine and tryptophan residues, which can also be readily oxidized by such oxidant as H<sub>2</sub>O<sub>2</sub>), are absent in Hsp16.3 can be readily sulfoxidized with H<sub>2</sub>O<sub>2</sub> treatment *in vitro*, and the methionine sulfoxide can be effectively reduced back to the methionine form. Interconversion between the methionine and methioninesulfoxide has been confirmed by selective oxidation and reduction. The sulfoxidation leads to a small degree of conformational change, which in turn results in a significant decrease of the chaperone-like activity. Data presented in this report strongly implicate that reversible sulfoxidation/desulfoxidation of methionine residues may occur in Hsp16.3, which serves as a way to scavenger reactive oxygen or nitrogen species

abundantly present in macrophage cells, thus protecting the plasma membrane and other components of *M. tuberculosis* allowing their survival in such bacteriocidal hosts.—Authors' Abstract

**Milani, M., Savard, P. Y., Ouellet, H., Ascenzi, P., Guertin, M., and Bolognesi, M.** A TyrCD1/TrpG8 hydrogen bond network and a TyrB10TyrCD1 covalent link shape the heme distal site of *Mycobacterium tuberculosis* hemoglobin O. *Proc. Natl. Acad. Sci. U. S. A.* **100**(10) (2003) 5766–5771.

Truncated hemoglobins (Hbs) are small hemoproteins, identified in microorganisms and in some plants, forming a separate cluster within the Hb superfamily. Two distantly related truncated Hbs, trHbN and trHbO, are expressed at different developmental stages in *Mycobacterium tuberculosis*. Sequence analysis shows that the two proteins share 18% amino acid identities and belong to different groups within the truncated Hb cluster. Although a specific defense role against nitrosative stress has been ascribed to trHbN (expressed during the *Mycobacterium* stationary phase), no clear functions have been recognized for trHbO, which is expressed throughout the *Mycobacterium* growth phase. The 2.1-Å crystal structure of *M. tuberculosis* cyano-met trHbO shows that the protein assembles in a compact dodecamer. Six of the dodecamer subunits are characterized by a double conformation for their CD regions and, most notably, by a covalent bond linking the phenolic O atom of TyrB10 to the aromatic ring of TyrCD1, in the heme distal cavity. All 12 subunits display a cyanide ion bound to the heme Fe atom, sta-

bilized by a tight hydrogen-bonded network based on the (globin very rare) TyrCD1 and TrpG8 residues. The small apolar AlaE7 residue leaves room for ligand access to the heme distal site through the conventional "E7 path," as proposed for myoglobin. Different from trHbN, where a 20-A protein matrix tunnel is held to sustain ligand diffusion to an otherwise inaccessible heme distal site, the topologically related region in trHbO hosts two protein matrix cavities.—Authors' Abstract

**Park, H. D., Guinn, K. M., Harrell, M. I., Liao, R., Voskuil, M. I., Tompa, M., Schoolnik, G. K., and Sherman, D. R.** Rv3133c/dosR is a transcription factor that mediates the hypoxic response of *Mycobacterium tuberculosis*. *Mol. Microbiol.* **48(3)** (2003) 833–843.

Unlike many pathogens that are overtly harmful to their hosts, *Mycobacterium tuberculosis* can persist for years within humans in a clinically latent state. Latency is often linked to hypoxic conditions within the host. Among *M. tuberculosis* genes induced by hypoxia is a putative transcription factor, Rv3133c/DosR. We performed targeted disruption of this locus followed by transcriptome analysis of wild-type and mutant bacilli. Nearly all the genes powerfully regulated by hypoxia require Rv3133c/DosR for their induction. Computer analysis identified a consensus motif, a variant of which is located upstream of nearly all *M. tuberculosis* genes rapidly induced by hypoxia. Further, Rv3133c/DosR binds to the two copies of this motif upstream of the hypoxic response gene alpha-crystallin. Mutations within the binding sites abolish both Rv3133c/DosR binding as well as hypoxic induction of a downstream reporter gene. Also, mutation experiments with Rv3133c/DosR confirmed sequence-based predictions that the C-terminus is responsible for DNA binding and that the aspartate at position 54 is essential for function. Together, these results demonstrate that Rv3133c/DosR is a transcription factor of the two-component response regulator class, and that it is the primary mediator of a hypoxic signal within *M. tuberculosis*.—Authors' Abstract

**Ramachandran, G. and Gurumurthy, P.** Effect of rifampicin & isoniazid on cytochrome P-450 in mycobacteria. *Indian J. Med. Res.* **116** (2002) 140–144.

**BACKGROUND & OBJECTIVES:** Rifampicin and isoniazid are the most important first line drugs used in the treatment of tuberculosis. These drugs are also used in combination with other medications to treat co-infections. It, therefore, becomes important to study the effect of these drugs on the drug metabolizing system, namely, cytochrome P-450, not only in the host but also in the bacteria. We report the effect of rifampicin and isoniazid on the cytochrome P-450 activity in *Mycobacterium smegmatis* and *M. tuberculosis* H37Rv. **METHODS:** Subinhibitory concentrations of rifampicin and isoniazid were added to the organisms after they had attained the growth phase and cytochrome P-450 activity was estimated in the membranous fractions of the bacteria at different time points. **RESULTS:** Rifampicin was able to significantly enhance cytochrome P-450 in both *M. smegmatis* and *M. tuberculosis* H37Rv. Isoniazid was found to inhibit cytochrome P-450 in *M. tuberculosis* H37Rv, while there seemed to be no effect in *M. smegmatis*. **INTERPRETATION & CONCLUSION:** We report here the effect of rifampicin and isoniazid on mycobacterial cytochrome P-450. These findings are similar to those found in eukaryotic organisms. The role of mycobacterial cytochrome P-450 in the metabolism of drugs within the bacteria needs to be elucidated.—Authors' Abstract

**Torres, M. J., Criado, A., Ruiz, M., Llanos, A. C., Palomares, J. C., and Aznar, J.** Improved real-time PCR for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates. *Diagn. Microbiol. Infect. Dis.* **45(3)** (2003) 207–212.

In this study we designed two pairs of probes for the detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* with real-time PCR procedures. One pair of probes spans the region between codon 510 and 528 of the rpoB gene, and the other one screens for mutation at the

regulatory region of the *inhA* gene. We have evaluated these probes in combination with two other pairs of probes previously described to detect mutations in 20 susceptible and 53 unique resistant *M. tuberculosis* clinical isolates. We were able to detect

nine different mutations affecting five codons of the *rpoB* gene, two different mutations at codon 315 of the *katG* gene and a nucleotide substitution (C209T) in the regulatory region of the *inhA* gene within two hours turnaround.—Authors' Abstract

## Experimental Infections

**Dionne, M. S., Ghorri, N., and Schneider, D. S.** *Drosophila melanogaster* Is a Genetically Tractable Model Host for *Mycobacterium marinum*. *Infect. Immun.* **71(6)**(2003) 3540–3550.

*Mycobacterium marinum* is a pathogenic mycobacterial species that is closely related to *Mycobacterium tuberculosis* and causes tuberculosis-like disease in fish and frogs. We infected the fruit fly *Drosophila melanogaster* with *M. marinum*. This bacterium caused a lethal infection in the fly, with a 50% lethal dose (LD(50)) of 5 CFU. Death was accompanied by widespread tissue damage. *M. marinum* initially proliferated inside the phagocytes of the fly; later in infection, bacteria were found both inside and outside host cells. Intracellular *M. marinum* blocked vacuolar acidification and failed to colocalize with dead *Escherichia coli*, similar to infections of mouse macrophages. *M. marinum* lacking the *mag24* gene were less virulent, as determined both by LD(50) and by death kinetics. Finally, in contrast to all other bacteria examined, mycobacteria failed to elicit the production of antimicrobial peptides in DROSOPHILA: We believe that this system should be a useful genetically tractable model for mycobacterial infection.—Authors' Abstract

**Gonzalez-Juarrero, M., Turner, J., Basaraba, R. J., Belisle, J. T., and Orme, I. M.** Florid pulmonary inflammatory responses in mice vaccinated with Antigen-85 pulsed dendritic cells and challenged by aerosol with *Mycobacterium tuberculosis*. *Cell. Immunol.* **220(1)** (2002) 13–19.

Mice immunized by the intranasal route with dendritic cells harvested from the lungs

and then pulsed with Ag85 (LDC-Ag85) were able to prime naive CD4(+) T cells *in vivo*. As a result splenic CD4(+) T cells from these immunized mice were able to produce IFN $\gamma$  following culture with *Mycobacterium tuberculosis*-infected antigen presenting cells. Hematoxylin and eosin stained lung sections from LDC-Ag85 immunized mice after they had been exposed to aerosol challenge with *M. tuberculosis* showed a florid infiltration of macrophages and lymphocytes into granulomas and parenchymal tissues when compared to lung sections from control groups implanted with dendritic cells pulsed with ovalbumin. In addition, using immunohistochemistry, these tissues appeared to have more CD4(+) and CD8(+) cells than the control groups. This was confirmed by flow cytometric analysis which showed that lung cell digests contained increased numbers of CD4 and CD8 interferon $\gamma$  secreting cells. Despite this increase however, no evidence was seen that indicated that the LDC-Ag85 immunized mice were more resistant to *M. tuberculosis* infection than mice immunized with LDC pulsed with an irrelevant protein. Instead, the potent inflammatory response in the LDC-Ag85 resulted in serious consolidation of the lung tissue.—Authors' Abstract

**Hewinson, R. G., Vordermeier, H. M., and Buddle, B. M.** Use of the bovine model of tuberculosis for the development of improved vaccines and diagnostics. *Tuberculosis (Edinb.)* **83(1–3)** (2003) 119–130.

Over the past few years there has been a resurgence in research into bovine tuberculosis due to the sharp rise of the disease in countries such as Great Britain and to the continuing problem of wild-life reservoirs in countries such as New Zealand. One of the

goals of this research is to develop cattle vaccines against TB. The initial testing of candidate vaccines is carried out in laboratory animals, initially mice and subsequently guinea pigs. A unique feature of the cattle vaccination programme is that candidate vaccines which show promise in laboratory models can then be tested in the natural host species, cattle, before progressing to clinical trials. This is a major advantage over the strategy for developing a vaccine for human tuberculosis where, of course, it is impossible to test a candidate vaccine by experimentally challenging the host species with the pathogen. The most commonly used model for testing vaccine candidates in cattle consists of an intra-tracheal challenge of between 10(3) and 10(4) colony forming units of *Mycobacterium bovis*. The pathology observed following challenge is similar to human tuberculosis giving rise to a marked granulomatous reaction and a predominantly cellular immune response. Using this model we have been able to make a number of significant advances towards a bovine TB vaccine. First we have developed antigen cocktails that, when used in a whole blood gamma interferon assay, can differentiate between *M. bovis* infected and BCG vaccinated animals. Next we have developed immune correlates of pathology, which allow us to assess whether the vaccine is protecting animals against challenge before post mortem examination. Finally we have been able to use the model to develop a vaccine that improves the efficacy of BCG against *M. bovis* challenge.—Authors' Abstract

**Hovav, A. H., Mullerad, J., Davidovitch, L., Fishman, Y., Bigi, F., Cataldi, A., and Bercovier, H.** The *Mycobacterium tuberculosis* Recombinant 27-Kilodalton Lipoprotein Induces a Strong Th1-Type Immune Response Deleterious to Protection. *Infect. Immun.* **71(6)** (2003) 3146–3154.

Th1 immune response is essential in the protection against mycobacterial intracellular pathogens. Lipoproteins trigger both humoral and cellular immune responses and may be candidate protective antigens. We studied in BALB/c mice the immunogenicity and the protection offered by the recombinant 27-

kDa *Mycobacterium tuberculosis* lipoprotein and the corresponding DNA vaccine. Immunization with the 27-kDa antigen resulted in high titers of immunoglobulin G1 (IgG1) and IgG2a with a typical Th1 profile and a strong delayed hypersensitivity response. A strong proliferation response was observed in splenocytes, and significant nitric oxide production and gamma interferon secretion but not interleukin 10 secretion were measured. Based on these criteria, the 27-kDa antigen induced a typical Th1-type immune response thought to be necessary for protection. Surprisingly, in 27-kDa-vaccinated mice (protein or DNA vaccines) challenged by *M. tuberculosis* H37Rv or BCG strains, there was a significant increase in the numbers of CFU in the spleen compared to that for control groups. Furthermore, the protection provided by BCG or other mycobacterial antigens was completely abolished once the 27-kDa antigen was added to the vaccine preparations. This study indicates that the 27-kDa antigen has an adverse effect on the protection afforded by recognized vaccines. We are currently studying how the 27-kDa antigen modulates the mouse immune response.—Authors' Abstract

**Lima, K. M., Santos, S. A., Lima, V. M., Coelho-Castelo, A. A., Rodrigues, J. M. Jr., and Silva, C. L.** Single dose of a vaccine based on DNA encoding mycobacterial hsp65 protein plus TDM-loaded PLGA microspheres protects mice against a virulent strain of *Mycobacterium tuberculosis*. *Gene Ther.* **10(8)** (2003) 678–685.

The high incidence of tuberculosis around the world and the inability of BCG to protect certain populations clearly indicate that an improved vaccine against tuberculosis is needed. A single antigen, the mycobacterial heat shock protein hsp65, is sufficient to protect BALB/c mice against challenge infection when administered as DNA vaccine in a three-dose-based schedule. In order to simplify the vaccination schedule, we coencapsulated hsp65-DNA and trehalose dimicolate (TDM) into biodegradable poly(DL-lactide-co-glycolide) (PLGA) microspheres. BALB/c mice immunized with a single dose of DNA-hsp65/TDM-loaded microspheres produced high levels of IgG2a subtype anti-

body and high amounts of IFN-gamma in the supernatant of spleen cell cultures. DNA-hsp65/TDM-loaded microspheres were also able to induce high IFN-gamma production in bulk lung cells from challenged mice and confer protection as effective as that attained after three doses of naked DNA administration. This new formulation also allowed a ten-fold reduction in the DNA dose when compared to naked DNA. Thus, this combination of DNA vaccine and adjuvants with immunomodulatory and carrier properties holds the potential for an improved vaccine against tuberculosis.—Authors' Abstract

**Martin, E., Kamath, A. T., Briscoe, H., and Britton, W. J.** The combination of plasmid interleukin-12 with a single DNA vaccine is more effective than *Mycobacterium bovis* (bacille Calmette-Guerin) in protecting against systemic *Mycobacterium avium* infection. *Immunology*. **109(2)** (2003) 308–314.

Sub-unit vaccines utilizing purified mycobacterial proteins or DNA vaccines induce partial protection against mycobacterial infections. For example, immunization with DNA vaccines expressing the gene for the immunodominant 35000 MW protein, common to *Mycobacterium avium* and *Mycobacterium leprae* but absent from the *Mycobacterium tuberculosis* complex, conferred significant protection against infection with either virulent *M. avium* or *M. leprae* in mice. However, the level of protection was equivalent to that obtained with the viable, attenuated vaccine, *Mycobacterium bovis*, bacille Calmette-Guerin (BCG). The cytokine, interleukin (IL)-12, is essential for priming naive CD4+ T lymphocytes to differentiate into interferon-gamma (IFN-gamma)-secreting T cells. We have used a novel self-splicing vector expressing both chains of murine IL-12 to determine if plasmid IL-12 would increase the efficacy of a vaccine expressing the *M. avium* 35000 MW protein (DNA-Av35). Co-immunization with p2AIL-12 and DNA-Av35 led to a significant increase in the number of antigen-specific IFN-gamma secreting cells and total amount of IFN-gamma released, but a concomitant fall in the antibody response to the 35000 MW protein. This pat-

tern of response was associated with enhanced clearance of *M. avium* from the liver and spleen of coimmunized mice, and was significantly more effective than BCG or DNA-Av35. alone. Following *M. avium* challenge there was significant increase in the expansion of the 35000 MW antigen-reactive T cells in the coimmunized mice. Therefore, plasmid-delivered IL-12 acts as an effective adjuvant to increase the protective efficacy of a single DNA vaccine against *M. avium* infection above that achieved by BCG, and this strategy may improve the efficacy of subunit vaccines against *M. leprae* and *M. tuberculosis*.—Authors' Abstract

**Pym, A. S., Brodin, P., Majlessi, L., Brosch, R., Demangel, C., Williams, A., Griffiths, K. E., Marchal, G., Leclerc, C., and Cole, S. T.** Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. *Nat. Med.* **9(5)** (2003) 533–539.

The live tuberculosis vaccines *Mycobacterium bovis* BCG (bacille Calmette-Guerin) and *Mycobacterium microti* both lack the potent, secreted T-cell antigens ESAT-6 (6-kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein). This is a result of independent deletions in the region of deletion-1 (RD1) locus, which is intact in virulent members of the *Mycobacterium tuberculosis* complex. To increase their immunogenicity and protective capacity, we complemented both vaccines with different constructs containing the *esxA* and *esxB* genes, which encode ESAT-6 and CFP-10 respectively, as well as a variable number of flanking genes. Only reintroduction of the complete locus, comprising at least 11 genes, led to full secretion of the antigens and resulted in specific ESAT-6-dependent immune responses; this suggests that the flanking genes encode a secretory apparatus. Mice and guinea pigs vaccinated with the recombinant strain BCG::RD1-2F9 were better protected against challenge with *M. tuberculosis*, showing less severe pathology and reduced dissemination of the pathogen, as compared with control animals immunized with BCG alone.—Authors' Abstract

**Simbirtsev, A., Kolobov, A., Zabolotnych, N., Pigareva, N., Konusova, V., Kotov, A., Variouchina, E., Bokovanov, V., Vinogradova, T., Vasilieva, S., and Tuthill,** Biological Activity of Peptide SCV-07 Against Murine Tuberculosis. *Russ. J. Immunol.* **8(1)** (2003) 11–22.

SCV-07 (gamma-glutamyl-tryptophan) is a new immunomodulatory compound that was developed and patented both for composition and immunomodulatory use. SCV-07 was shown to have a broad spectrum of immunostimulatory activities both *in vitro* and *in vivo*. In the present study we investigated the biological activity of SCV-07 in a murine model of experimental tuberculosis (TB) induced with *M. bovis-bovis* 8 strain. Therapy with SCV-07 at doses of 0.01, 0.1, and 1 mgr/kg (5 daily injections) decreased the lung damage index compared to untreated controls and to those treated with isoniazid alone. The growth of *M. bovis-bovis* 8 in spleen culture was decreased. Cytokine studies showed that on the 24th day after the treatment with SCV-07 the production of IL-2 was restored to the level seen in uninfected animals. Proliferative responses for both thymic and spleen cells were nearly restored to the responses observed in uninfected animals. IFN-gamma production by both thymic and spleen cells, as well as its circulating levels in serum, was increased by the SCV-07 treatment. Concurrently, IL-4 production was decreased in the same cell types and the serum. These changes suggest that SCV-07 is stimulating a shift of T helper cells to a Th1-like immune response. SCV-07 treatment also stimulated the macrophage functions, which had been decreased by tuberculosis infection and isoniazid therapy, with an improved phagocytosis activity of peritoneal macrophage. The obtained results suggest that SCV-07 treatment increases the efficacy of anti-tuberculosis therapy as well as the strength of the immune response. Thus, SCV-07 is a prospective immunomodulator for a complex therapy of TB.—Authors' Abstract

**Skinner, M. A., Ramsay, A. J., Buchan, G. S., Keen, D. L., Ranasinghe, C., Slobbe, L., Collins, D. M., de Lisle, G. W., and Buddle, B. M.** A DNA prime-

live vaccine boost strategy in mice can augment IFN-gamma responses to mycobacterial antigens but does not increase the protective efficacy of two attenuated strains of *Mycobacterium bovis* against bovine tuberculosis. *Immunology.* **108(4)** (2003) 548–555.

The *Mycobacterium bovis* bacille Calmette-Guerin (BCG) vaccine has variable efficacy for both human and bovine tuberculosis. There is a need for improved vaccines or vaccine strategies for control of these diseases. A recently developed prime-boost strategy was investigated for vaccination against *M. bovis* infection in mice. BALB/c and C57BL/6 mice were primed with a DNA vaccine, expressing two mycobacterial antigens, ESAT-6 and antigen 85 A and boosted with attenuated *M. bovis* strains, BCG or WAg520, a newly attenuated strain, prior to aerosol challenge. Before challenge, the antigen-specific production of interferon-gamma (IFN-gamma) was evaluated by ELISPOT and antibody responses were measured. The prime-boost stimulated an increase in the numbers of IFN-gamma producing cells compared with DNA or live vaccination alone, but this varied according to the attenuated vaccine strain, time of challenge and the strain of mouse used. Animals vaccinated with DNA alone generated the strongest antibody response to mycobacterial antigens, which was predominantly IgG1. BCG and WAg520 alone generally gave a 1–2 log<sub>10</sub> reduction in bacterial load in lungs or spleen, compared to non-vaccinated or plasmid DNA only control groups. The prime-boost regimen was not more effective than BCG or WAg520 alone. These observations demonstrate the comparable efficacy of BCG and WAg520 in a mouse model of bovine tuberculosis. However, priming with the DNA vaccine and boosting with an attenuated *M. bovis* vaccine enhanced IFN-gamma immune responses compared to vaccinating with an attenuated *M. bovis* vaccine alone, but did not increase protection against a virulent *M. bovis* infection.—Authors' Abstract

**Walzl, G., Humphreys, I. R., Marshall, B. G., Edwards, L., Openshaw, P. J.,**

**Shaw, R. J., and Hussell, T.** Prior exposure to live *Mycobacterium bovis* BCG decreases *Cryptococcus neoformans*-induced lung eosinophilia in a gamma interferon-dependent manner. *Infect. Immun.* **71(6)** (2003) 3384–3391.

Some common childhood infections appear to prevent the development of atopy and asthma. In some *Mycobacterium bovis* BCG-vaccinated populations, strong delayed-type hypersensitivity responses to mycobacterial antigens are associated with a reduced risk of atopy. Although BCG exposure decreases allergen-induced lung eosinophilia in animal models, little attention has been given to the effect of immunity to BCG on responses against live pathogens. We used the murine *Cryptococcus neoformans* infection model to investi-

gate whether prior BCG infection can alter such responses. The present study shows that persistent pulmonary BCG infection of C57BL/6 mice induced an increase in gamma interferon, a reduction in interleukin-5, and a decrease in lung eosinophilia during subsequent *Cryptococcus* infection. This effect was long lasting, depended on the presence of live bacteria, and required persistence of mycobacterial infection in the lung. Reduction of eosinophilia was less prominent after infection with a mutant BCG strain (DeltahspR), which was rapidly cleared from the lungs. These observations have important implications for the development of vaccines designed to prevent Th2-mediated disease and indicate that prior lung BCG vaccination can alter the pattern of subsequent host inflammation.—Authors' Abstract

## Epidemiology and Prevention

**de Aquino, D. M., Caldas Ad Ade, J., Silva, A. A., and Costa, J. M.** [Profile of the leprosy patients of hiperendemic area Amazonian Maranh o, Brazil] *Rev. Soc. Bras. Med. Trop.* **36(1)** (2003) 57–64. [Article in Portuguese]

An epidemiological cross-sectional study of 207 patients with leprosy disease, was undertaken between August 1998 to november 2000, aiming at evaluating the socioeconomic, demographic and ambiental profiles of the patients as well as physical incapacity due to the disease. The study was performed in the municipality of Buriticupu-Maranh o state, a hiperendemic leprosy area in the Amazonian Maranh o. The level of incapacity was assessed from parameters established by the Brazilian Health Minister. The clinical evaluation and the results of the physical tests were registered in a standardized form. It was observed a predominance of married people (45,9%), with low level of education (56%), being lend workers (40,1%), with familiar income to the minimum wage (76,3%), aged from 14 to 44 years (63,3%), males (60,9%) and brown (67,6%); 44% living in mud huts, 82,6% deposited their excrements in cesspits and 63,8% do not treat the drinking water, 58% utilized well-

water and 51,7% do not use treated water for ingestion. The most affected segments of the body were the feet (62,3%), eyes (51,2%) end hands (7,2%), being the higher percentage of physical incapacities found among the patients bearing the borderline form of the disease (93%) mainly hands and feet, and in the virchowian form greatest frequency of eyes incapacities. It is concluded that the hyperendemicity associated with the precarious socioeconomic conditions and with a high level of physical incapacities may be involved with the living quality of the patients.—Authors' Abstract

**Sahoo, A., Singh, P. C., Pattnaik, S., and Singh, N.** Incidence of leprosy in school-children and their family members in Berhampur. *Indian J. Lepr.* **74(2)** (2002) 137–143.

A school survey, followed by a contact survey, was carried out in Berhampur, a city in southern Orissa. In a study of 8,870 school-children, leprosy was detected in 15, giving a prevalence rate of 16.91 per 10,000 with a male:female ratio of 8:7. Of these, 14 (93.99%) had paucibacillary leprosy. More cases [11 (73.33%)] were seen in the age-group of 10–15 years. Exposed parts, such

as lower limbs, upper limbs and head and neck in that order, were the sites of predilection, accounting for 85.71% of total lesions. Nerve involvement was found in 2 (13.33%) girls with deformity (ulnar claw) in one of them (6.66%). BCG scar was present in 11 (73.33%) cases. Among the vaccinated cases, tuberculoid type was the most common, followed by indeterminate, pure neuritic and borderline, in that order. A

contact survey detected 2 multibacillary cases in two families (13.33%). In each case, the father was the index source. The study revealed that a maximum number of students, 8 (53.3%), belonged to the middle socioeconomic class. Of the 15 affected, 60% were undernourished and the rest well nourished. No other systemic disease was found clinically associated with leprosy.—Authors' Abstract

## Other Mycobacterial Diseases

**Black, W. C. and Berk, S. G.** Cooling towers—a potential environmental source of slow-growing mycobacterial species. *AIHA J* (Fairfax, Va.) **64**(2) (2003) 238–242.

Over the last decade a rise in the frequency of disease caused by nontuberculous mycobacteria (NTM) has occurred, especially among AIDS patients. The lack of evidence for person-to-person transmission indicates the environment is a source of infection. The ecology and environmental sources of NTMs are poorly understood, and many pathogenic strains have not been observed outside of clinical cases. Several species of NTMs have been reported from treated water distribution systems; however, one type of manmade environment that has not been examined for mycobacteria is that of cooling towers of air-conditioning systems. Such environments not only harbor a variety of microbial species, they also disseminate them in aerosols. The present investigation examined nine cooling towers from various locations in the United States. Cooling tower water was concentrated, treated with cetylpyridinium chloride, and plated onto Middlebrook 7H10 agar supplemented with OADC and cycloheximide. Colonies presumed to be mycobacterial species were isolated and acid-fast stained. Identification was made by amplifying and sequencing 1450 bp fragments of the 16S rRNA gene in both directions, and comparing resulting sequences with those in GenBank. Results showed that at least 75% of tower samples contained NTMs, and most of the isolates closely matched known mycobacterial pathogens. Isolates most closely matched the following GenBank sequences:

*Mycobacterium intracellulare*, *M. szulgai*, *M. bohemicum*, *M. gordonae*, *M. nonchromogenicum*, and *M. n. sp.* "Fuerth 1999." This is the first report of specific NTMs in cooling tower water, and the first report of *M. n. sp.* "Fuerth 1999" from any environmental sample. Although cooling towers have a relatively high pH, they may favor the growth and dissemination of such potential pathogens, and future epidemiologic investigations should consider cooling towers as possible environmental sources of mycobacteria.—Authors' Abstract

**Cappelluti, E., Fraire, A. E., and Schaefer, O. P.** A case of "hot tub lung" due to *Mycobacterium avium* complex in an immunocompetent host. *Arch. Intern. Med.* **163**(7) (2003) 845–848.

Pulmonary disease due to *Mycobacterium avium* complex (MAC) typically occurs in patients with impaired cellular immunity or chronic lung disease. Recently, there has been an increase in the number of reports of pulmonary disease caused by MAC occurring in otherwise healthy individuals, including those reporting recent hot tub use. It is not clear if this respiratory illness represents a true infectious process or a hypersensitivity pneumonitis. We report a case of diffuse pulmonary disease caused by MAC in an immunocompetent individual after hot tub use. The patient's clinical course, transbronchial lung biopsy results, and microbiologic examination findings all pointed to a hypersensitivity reaction due to MAC. With avoidance of the hot tub, and no pharmacological treatment, the patient had complete resolution within 2

months. In light of the number of new cases of "hot tub lung" in otherwise healthy individuals, clinicians should advise their patients of the potential risk associated with hot tub use.—Authors' Abstract

**Colebunders, R., Kint, I., Bastian, I., Mortelmans, E., Jacobs, W., and Marck, E. V.** Renal abscess due to *Mycobacterium avium* complex in a human Immunodeficiency virus-positive patient. *Int. J. Infect. Dis.* **6(3)** (2002) 238.

Disseminated *Mycobacterium avium* complex (MAC) infection usually involves tissues of the mononuclear phagocytic system (e.g. lymph nodes, spleen, and liver). In contrast, renal disease due to MAC is rare. We report the first case of an HIV-positive man with a large renal abscess due to MAC infection.—Authors' Abstract

**Frisk, P., Boman, G., Pauksen, K., Petrini, B., and Lonnerholm, G.** Skin infection caused by *Mycobacterium szulgai* after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* **31(6)** (2003) 511–513.

Infections are responsible for a large part of the morbidity and mortality after BMT because of the sustained impairment of host defenses. We report a case of cutaneous infection caused by *Mycobacterium szulgai* in a boy who underwent BMT with marrow from a matched unrelated donor.—Authors' Abstract

**Gooding, T. M., Kemp, A. S., Robins-Browne, R. M., Smith, M., and Johnson, P. D.** Acquired T-helper 1 lymphocyte anergy following infection with *Mycobacterium ulcerans*. *Clin. Infect. Dis.* **36(8)** (2003) 1076–1077.

We describe a patient with acquired T-helper lymphocyte anergy to mycobacteria following infection with *Mycobacterium ulcerans*. Before infection, the patient's peripheral blood mononuclear cells responded to *in vitro* stimulation with *M. ulcerans* by producing Th1 cytokines, but, after she de-

veloped an ulcer, the response was shifted toward production of Th2 cytokines. Immunomodulatory therapy may be an effective intervention for Buruli ulcer.—Authors' Abstract

**Itoh, S., Kazumi, Y., Abe, C., and Takahashi, M.** Heterogeneity of RNA Polymerase Gene (*rpoB*) Sequences of *Mycobacterium gordonae* Clinical Isolates Identified with a DNA Probe Kit and by Conventional Methods. *J. Clin. Microbiol.* **41(4)** (2003) 1656–1663.

In a previous study, we have evaluated genetic identification by using the *rpoB* gene, which was recently introduced by Kim, *et al.* (*J. Clin. Microbiol.* 39: 2102–2109, 2001; *J. Clin. Microbiol.* 37:1714–1720, 1999). In this process, we examined the *rpoB* gene heterogeneity of clinical isolates identified as *Mycobacterium gordonae* with the conventional biological and biochemical tests and/or a commercially available DNA probe kit. Sequencing of the *rpoB* gene of 34 clinical isolates revealed that *M. gordonae* clinical isolates were classified into four major clusters (A, B, C, and D). Interestingly, organisms belonging to cluster D (15 isolates) did not hybridize with *M. gordonae* ATCC 14470 and specifically possessed urease activity. Therefore, it could be considered to be a novel mycobacterium. The identification of *M. gordonae* is known to have ambiguous results sometimes. On the other hand, identification of clinical isolates seems to be inconvenient and unsuitable because of a more than 99% 16S rRNA gene similarity value between clusters. These findings suggest that the existence of *M. gordonae*-like mycobacteria that share similar biochemical and biological characteristics with the 16S rRNA gene of an *M. gordonae* type strain but less similarity at the genomic DNA level may have complicated the identification of *M. gordonae* in many laboratories. Furthermore, compared with *hsp65* PCR restriction analysis (PRA), *rpoB* PRA would have the advantage of producing no ambiguous results because of the intracluster homogeneity of the *rpoB* gene. In this case, *rpoB* would provide clearer results than *hsp65*, even if

PRA analysis was used. We demonstrated that these *M. gordonae*-like mycobacteria were easily distinguished by PRA of the *rpoB* sequence. Additionally, the significance of this *M. gordonae*-like cluster may help to establish the comparison between the *M. gordonae* isolates from a clinical specimen and an infectious process in a given patient and to determine the true incidence of infection with this microorganism.—Authors' Abstract

**Jenkins, P. A.; Campbell, I. A.; and the Research Committee of The British Thoracic Society.** Pulmonary disease caused by *Mycobacterium xenopi* in HIV-negative patients: five year follow-up of patients receiving standardised treatment. *Respir. Med.* **97(4)** (2003) 439–444.

The literature concerning the management of pulmonary disease caused by *Mycobacterium xenopi* is scanty and consists of retrospective reports, mostly of small series of patients. Our aim was to document the clinical features and response to treatment of this rare but challenging disease. Patients were treated in a randomized, multi-centre trial with either rifampicin plus ethambutol or rifampicin, ethambutol and isoniazid. Clinical, bacteriological and radiological progress was monitored at set intervals for 5 years. As no differences emerged between the two groups, the results have been combined to provide this prospective survey. Forty-two patients were studied. Mean age was 65 years, three-quarters were male and two-thirds had other lung disease(s). Sputum was positive on direct smear in 62%. Cavitation was present in 81%, mostly large cavities, and disease was extensive in 38%. Despite good clinical response and little toxicity the death rate was high (69%), but less than 10% died primarily because of the *M. xenopi* disease. The failure of treatment/relapse rate was 12%. Only 11 (26%) were known to be alive at 5 years of whom seven (17%) were known to be cured. There was no correlation between failure of treatment/relapse and *in vitro* resistance. Better methods of susceptibility testing and more effective regimens are needed, but it is also evident

that improved management of concomitant diseases and better general health will play a major part in increasing survival.—Authors' Abstract

**Kobashi, Y. and Matsushima, T.** Clinical analysis of pulmonary *Mycobacterium avium* complex disease in association with corticosteroid treatment. *J. Infect. Chemother.* **9(1)** (2003) 68–74.

We studied the clinical characteristics of nine patients with pulmonary *Mycobacterium avium* complex disease occurring in association with corticosteroid drugs collected from our associated hospitals during the past 6 years. The average age of the nine patients was 62.2 years and the male/female ratio was 3:6. Regarding underlying disease, respiratory diseases existed in four of the patients and nonrespiratory diseases in the other five patients. The duration of corticosteroid treatment ranged from 5 months to 5 years, and the total dose of corticosteroid drugs ranged from 1.78 to 43.20 g. Pulmonary *Mycobacterium avium* complex disease was detected by clinical symptoms during corticosteroid treatment in six patients, and purified protein derivative was positive in three of eight patients tested. Radiological findings showed an infiltration shadow without cavity and bronchiectasis in the lower lung field. Microbiological examination was smear-positive in three patients, and the isolated mycobacterium was *Mycobacterium intracellulare* in five patients and *Mycobacterium avium* in four. Tolerance was shown to all antituberculous drugs, except for clarithromycin, in all patients. Although treatment including clarithromycin was performed for seven patients, the sputum conversion rate was 33% and an improved clinical effect was noted in only one patient. No change occurred in four and worsening occurred in four. Attention should be paid to the clinical symptoms and radiological findings of patients who have received corticosteroid drugs over a long period of time, because pulmonary *Mycobacterium avium* complex is characterized by atypical radiographic findings with no relationship to the total dose or duration of the administered corticosteroid drugs.—Authors' Abstract

**Mahaisavariya, P., Chaiprasert, A., Khemngern, S., Manonukul, J., Gengviniij, N., Ubol, P. N., and Pinitugsorn, S.** Nontuberculous mycobacterial skin infections: clinical and bacteriological studies. *J. Med. Assoc. Thai.* **86(1)** (2003) 52–60.

**OBJECTIVE:** Nontuberculous mycobacterial (NTM) skin infections were analysed in terms of clinical manifestation in different species to provide clues for the clinical diagnosis and sensitivity patterns of these species were studied for planning appropriate therapy. **DESIGN:** A retrospective study was performed in 123 suspected cases of NTM infections from January 1994 to December 2000. NTM infection was documented by culture result of the infected tissue obtained by skin biopsy. Drug susceptibility test was done as requested. **RESULT:** Rapid growers (*M. fortuitum-chelonae*) were found in 26 cases (65%) and *M. marinum* was responsible for 12 cases (30%) and caused only localized skin lesions on arms or legs as indurated plaque. Disseminated skin infections manifested as multiple abscesses were found in 2 cases caused by *M. avium* in an HIV-infected male patient and mixed infection of *M. szulgai* and *M. terrae* in an immunocompetent female patient after a dental procedure. Both sexes were affected equally in overall number but male predominated in *M. marinum* infection and females predominated in rapid growers. All ages can be affected but most cases were middle aged. Scrofuloderma-like cervical lymphadenitis and cutaneous abscesses were the common manifestation of rapid grower infections. Hyperkeratotic verrucous plaques (tuberculosis verrucosa cutis-like) and sporotrichoid lesions were the common manifestations of *M. marinum* infection. *M. marinum* is sensitive to minocyclin, clarithromycin, amikacin, rifampicin and ethambutol and a good clinical response was obtained with doxycyclin 100 mg orally twice a day for 3 months. Clarithromycin and amikacin showed *in vitro* activity against the same strain of *M. fortuitum* but most strains of rapid growers resisted antituberculous drugs and also various antibiotics. **CONCLUSION:** Clinical manifestations can be used as clues for diagnosis. Medical therapy is recommended for *M. marinum* infection and surgical treat-

ment is recommended for rapid growers.—  
Authors' Abstract

**Matsunaga, S., Kurashima, A., Nagai, H., Akagawa, S., Machida, K., Yotsumoto, H., Mouri, M., and Hebisawa, A.** [A *Mycobacterium abscessus* infection complicated with lipoid pneumonia] *Nihon Kokyuki Gakkai Zasshi.* **41(1)** (2003) 14–8. [Article in Japanese]

A 64-year-old man was admitted to our hospital because of productive cough and fever. Chest radiography on admission revealed air space consolidation in the right middle and lower lung fields and ground-glass opacity in the left middle lung field. He had been constipated for several years and had taken mineral oil for about a year. Sputum smears demonstrated acid bacilli, and cultures disclosed *Mycobacterium abscessus*. The transbronchial lung biopsy specimen showed granulomatous inflammation and numerous lipoid-laden macrophages in the alveolar spaces. Mycobacteria were present within the mineral oil and lipid-laden macrophages. It is likely that the mineral oil increased the pathogenicity of the mycobacteria.—Authors' Abstract

**Mauss, S., Wolf, E., and Hans, J.** Changing incidence of mycobacterial diseases in German patients with HIV infection. *AIDS Read.* **9(6)** (1999) 386, 391–392.

Before the introduction of potent anti-retroviral therapy, disease caused by atypical mycobacteria was a frequent diagnosis in patients with fewer than 100 CD4+ cells/microL and could affect virtually every organ. The diagnosis was associated with a survival of usually less than 1 year, and antimycobacterial treatment could extend the life span by only several months. However, the clinical face of the HIV epidemic has changed profoundly since 1996, most probably due to the antiretroviral combination strategies. In this discussion, we assess the influence of the introduction of antiretroviral combination therapies on the incidence of mycobacterial infections from 1994 to 1998 in a cohort of German

HIV-seropositive patients treated at an HIV outpatient clinic.—Authors' Abstract

**Myojo, M., Fujiuchi, S., Matsumoto, H., Yamazaki, Y., Takahashi, M., Satoh, K., Takeda, A., Nishigaki, Y., Okamoto, K., Fujita, Y., Fujikane, T., and Shimizu, T.** Disseminated *Mycobacterium avium* complex (DMAC) in an immunocompetent adult. *Int. J. Tuberc. Lung Dis.* **7(5)** (2003) 498–501.

A female patient with multiple osteomyelitis and pulmonary *Mycobacterium avium* disease visited an orthopaedic clinic with back pain. Systemic bone scan showed multiple sites of increased radioactivity in the vertebral bodies, right scapula, femurs and ribs. *M. avium* was isolated from sputum and a sample aspirated from the right scapula. The route of infection was unknown as there was no history of trauma or surgery. HIV testing was negative. As there was no underlying immunological disease she was diagnosed as disseminated *M. avium* complex (DMAC) disease in an immunocompetent adult. Cytokine production on several stimuli from peripheral blood mononuclear cells was similar to that in pulmonary *M. avium* patients. Sequence analysis of IFN-gamma receptor revealed no nucleotide substitution. We detected serotypes 1, 2 and 4 from mycobacteria cultured from the right scapula, and conclude that this case could be the result of undetected immune deficiency and/or unrecognised virulence of the infecting isolate.—Authors' Abstract

**Smith, M. B., Molina, C. P., Schnadig, V. J., Boyars, M. C., and Aronson, J. F.** Pathologic features of *Mycobacterium kansasii* infection in patients with acquired immunodeficiency syndrome. *Arch. Pathol. Lab. Med.* **127(5)** (2003) 554–560.

CONTEXT: *Mycobacterium kansasii* is a slow-growing photochromogenic mycobacterium that may infect patients with human immunodeficiency virus (HIV) late in the course of acquired immunodeficiency syndrome (AIDS). The clinical features of pul-

monary and extrapulmonary infections have been described in the literature; however, the pathology of infection has not been adequately addressed. OBJECTIVE: This report describes the pathologic features of 12 cases of *M. kansasii* infection in patients with AIDS. DESIGN: The medical records, autopsy protocols, cytologic material, and histologic material from patients with AIDS and concomitant *M. kansasii* infection at a tertiary-care medical center during 1990–2001 were reviewed. RESULTS: Twelve cases were identified, 6 by autopsy, 5 of which were diagnosed postmortem. Four of the 12 cases had cytologic material and 4 cases had histologic biopsies available for review. Pulmonary infection was most common (9/12), and all patients in whom thoracic lymph nodes were assessed showed involvement (7/7). Abdominal infection was less frequent, with only 1 of 6, 2 of 6, and 2 of 6, demonstrating liver, spleen, and abdominal lymph node infection, respectively. Isolated infections without documented pulmonary infection included brain abscess (N = 1), ulnar osteomyelitis (N = 1), and paratracheal mass (N = 1). Cytologic and histologic material showed a wide range of inflammatory reactions, including granulomas with and without necrosis, neutrophilic abscesses, spindle-cell proliferations, and foci of granular eosinophilic necrosis. The *M. kansasii* bacillus was characteristically long, coarsely beaded, and frequently showed folded, bent, or curved ends. Intracellular bacilli were randomly or haphazardly distributed within histiocytes. CONCLUSION: *Mycobacterium kansasii* infection produces predominantly pulmonary infection in late-stage AIDS with a high incidence of thoracic lymph node involvement and a much lower incidence of dissemination to other sites. Infection is manifest as a wide variety of inflammatory reactions on cytology and histology; however, the characteristic appearance of the bacillus on acid-fast bacilli stain and its intracellular arrangement in histiocytes can allow a presumptive identification.—Authors' Abstract

**Sniezek, P. J., Graham, B. S., Busch, H. B., Lederman, E. R., Lim, M. L., Poggemyer, K., Kao, A., Mizrahi, M.,**

**Washabaugh, G., Yakrus, M., and Winthrop, K.** Rapidly growing mycobacterial infections after pedicures. *Arch. Dermatol.* **139**(5) (2003) 629–634.

**BACKGROUND:** Rapidly growing mycobacteria (RGM) can cause a variety of cutaneous and systemic diseases. The causative organisms are typically *Mycobacterium fortuitum* or *Mycobacterium chelonae* (also known as *Mycobacterium abscessus*). Primary cutaneous lesions may develop after a variable latent period, from weeks to several months, and usually result from direct inoculation after trauma, from injections, or during surgery via contaminated medical instruments. Recently, investigators from the Centers for Disease Control and Prevention, Atlanta, Ga, and the California Department of Health Services, Berkeley, documented a large, unprecedented outbreak of community-acquired RGM infection, during which more than 100 patrons of a northern California nail salon contracted furunculosis in their legs as a result of exposure to whirlpool footbaths that were contaminated with *M. fortuitum*. **OBSERVATIONS:** We report the clinical and epidemiological findings in 3 cases of lower extremity RGM infections that occurred after similar whirlpool footbath exposure at several different nail salons in southern California. These infections typically presented as recurrent furunculosis, causing considerable morbidity as a result of scarring, delayed diagnosis, and the need for long-term polymicrobial therapy. **CONCLUSIONS:** Rapidly growing mycobacterial infections related to pedicures may continue to occur in a sporadic fashion. Clinicians should consider the possibility of RGM infection and inquire about recent pedicures in a patient with recurrent lower extremity furunculosis and abscesses that are unresponsive to conventional antibiotic therapy.—Authors' Abstract

**Tiwari, T. S., Ray, B., Jost, K. C. Jr., Rathod, M. K., Zhang, Y., Brown-Elliott, B. A., Hendricks, K., and Wallace, R. J. Jr.** Forty years of disinfectant failure: outbreak of postinjection *Mycobacterium abscessus* infection caused by contamination of benzalkonium chlo-

ride. *Clin. Infect. Dis.* **36**(8) (2003) 954–962.

Benzalkonium chloride (BC) continues to be used as an antiseptic and contributes to serious outbreaks of disease. In July 1999, 6 postinjection joint infections caused by *Mycobacterium abscessus* were reported to the Texas Department of Health (Austin). We investigated this outbreak and identified 12 case patients who had been seen by the same physician and who had received an intra-articular or periarticular steroid injection during the period of 1 April through 31 July 1999. *M. abscessus* was cultured from either joint fluid or periarticular soft-tissue specimens obtained from 10 patients. We cultured environmental samples, and we compared isolates recovered from case patients with environmental isolates by pulsed-field gel electrophoresis and randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). Four environmental samples containing diluted BC yielded *M. abscessus*. Clinical and environmental strains of *M. abscessus* were indistinguishable by RAPD-PCR. The case patients' strain was resistant to BC. The use of BC as an antiseptic should be discontinued.—Authors' Abstract

**Yang, S. C., Hsueh, P. R., Lai, H. C., Teng, L. J., Huang, L. M., Chen, J. M., Wang, S. K., Shie, D. C., Ho, S. W., and Luh, K. T.** High prevalence of antimicrobial resistance in rapidly growing mycobacteria in Taiwan. *Antimicrob. Agents Chemother.* **47**(6) (2003) 1958–1962.

An increasing number of clinical isolations of rapidly growing mycobacteria (RGM) at the National Taiwan University Hospital were noted from 1992 to 2001. Broth microdilution MICs of 15 antimicrobial agents were determined for 200 clinical isolates of RGM, including the *Mycobacterium fortuitum* group (69 isolates), *M. chelonae* (39 isolates), and *M. abscessus* (92 isolates). Our results showed that the resistance rates of these isolates to the currently available agents were remarkably high. Amikacin was active against nearly all RGM isolates. Clarithromycin was usually

active against *M. abscessus* (79% susceptibility) and the *M. fortuitum* group (65% susceptibility). The majority of *M. fortuitum* group isolates were susceptible to ciprofloxacin (62%) and imipenem (61%). The susceptibilities to other conventional anti-RGM agents of these isolates were poor but differed markedly by species. The newer fluoroquinolones (levofloxacin, moxifloxacin, and gatifloxacin) and meropenem showed better *in vitro* activities against the *M. fortuitum* group isolates than against the other two species of RGM. Linezolid had fairly good activity against these RGM isolates, particularly against *M. chelonae* isolates (82% susceptible). Telithromycin had poor activity against these RGM isolates (the MICs at which 50% of the isolates tested are inhibited [MIC(50)s] were 32 to 64 micro g/ml, and the MIC(90)s were >64 micro g/ml).—Authors' Abstract

**Yang, W. K., Fu, L. S., Lan, J. L., Shen, G. H., Chou, G., Tseng, C. F., and Chi, C. S.** *Mycobacterium avium* complex-associated hemophagocytic syndrome in systemic lupus erythematosus patient: report of one case. *Lupus*. **12(4)** (2003) 312–316.

Hemophagocytic syndrome (HPS) in systemic lupus erythematosus (SLE) patients has not commonly been reported. In this case study, we report the first case of *Mycobacterium avium* complex (MAC)-associated hemophagocytic syndrome in a patient with systemic lupus erythematosus (SLE). This SLE patient, a 15-year-old girl, had been on a high dose of prednisolone (> 0.5mg/kg/day) for more than 3 years. She presented with a spiking fever, hepatosplenomegaly, pancytopenia, hyperferritinemia and adult respiratory distress syndrome. Bone marrow examination revealed hemophagocytosis as well as non-caseating granulomatosis. There was no indication of SLE flare-up. She responded poorly to initial treatment with methyl-prednisolone, intravenous immunoglobulin, etoposide, and drugs for *Mycobacterium tuberculosis* including rifampin, ethambutol, isoniazid and pyrazinamide. However, gastric lavage culture revealed MAC. Following treatment with clarithromycin, ciprofloxacin and amikacin, her condition gradually improved and she was discharged 3 months after admission. In SLE patients with pancytopenia and hyperferritinemia, MAC-associated HPS should be considered in the differential diagnosis.—Authors' Abstract

## Molecular & Genetic Studies

**Adindla S and Guruprasad L.** Sequence analysis corresponding to the PPE and PE proteins in *Mycobacterium tuberculosis* and other genomes. *J. Biosci.* **28(2)** (2003) 169–179.

Amino acid sequence analysis corresponding to the PPE proteins in H37Rv and CDC1551 strains of the *Mycobacterium tuberculosis* genomes resulted in the identification of a previously uncharacterized 225 amino acid-residue common region in 22 proteins. The pairwise sequence identities were as low as 18%. Conservation of amino acid residues was observed at fifteen positions that were distributed over the whole length of the region. The secondary structure corresponding to this region is

predicted to be a mixture of  $\alpha$ -helices and  $\beta$ -strands. Although the function is not known, proteins with this region specific to mycobacterial species may be associated with a common function. We further observed another group of 20 PPE proteins corresponding to the conserved C-terminal region comprising 44 amino acid residues with GFxGT and PxxPxxW sequence motifs. This region is preceded by a hydrophobic region, comprising 40–100 amino acid residues, that is flanked by charged amino acid residues. Identification of conserved regions described above may be useful to detect related proteins from other genomes and assist the design of suitable experiments to test their corresponding functions. Amino acid sequence analysis

corresponding to the PE proteins resulted in the identification of tandem repeats comprising 41–43 amino acid residues in the C-terminal variable regions in two PE proteins (Rv0978 and Rv0980). These correspond to the AB repeats that were first identified in some proteins of the *Methanosarcina mazei* genome, and were demonstrated as surface antigens. We observed the AB repeats also in several other proteins of hitherto uncharacterized function in Archaea and Bacteria genomes. Some of these proteins are also associated with another repeat called the C-repeat or the PKD-domain comprising 85 amino acid residues. The secondary structure corresponding to the AB repeat is predicted mainly as 4 b-strands. We suggest that proteins with AB repeats in *Mycobacterium tuberculosis* and other genomes may be associated as surface antigens. The *M. leprae* genome, however, does not contain either the AB or C-repeats and different proteins may therefore be recruited as surface antigens in the *M. leprae* genome compared to the *M. tuberculosis* genome.—Authors' Abstract

**Alland, D., Whittam, T. S., Murray, M., B., Cave, M. D., Hazbon, M. H., Dix, K., Kokoris, M., Duesterhoeft, A., Eisen, J. A., Fraser, C. M., and Fleischmann, R. D.** Modeling bacterial evolution with comparative-genome-based marker systems: application to *Mycobacterium tuberculosis* evolution and pathogenesis. *J. Bacteriol.* **185**(11) (2003) 3392–3399.

The comparative-genomic sequencing of two *Mycobacterium tuberculosis* strains enabled us to identify single nucleotide polymorphism (SNP) markers for studies of evolution, pathogenesis, and epidemiology in clinical *M. tuberculosis*. Phylogenetic analysis using these “comparative-genome markers” (CGMs) produced a highly unusual phylogeny with a complete absence of secondary branches. To investigate CGM-based phylogenies, we devised computer models to simulate sequence evolution and calculate new phylogenies based on an SNP format. We found that CGMs represent a distinct class of phylogenetic markers that depend critically on the ge-

netic distances between compared “reference strains.” Properly distanced reference strains generate CGMs that accurately depict evolutionary relationships, distorted only by branch collapse. Improperly distanced reference strains generate CGMs that distort and reroot outgroups. Applying this understanding to the CGM-based phylogeny of *M. tuberculosis*, we found evidence to suggest that this species is highly clonal without detectable lateral gene exchange. We noted indications of evolutionary bottlenecks, including one at the level of the PHRI “C” strain previously associated with particular virulence characteristics. Our evidence also suggests that loss of IS6110 to fewer than seven elements per genome is uncommon. Finally, we present population-based evidence that KasA, an important component of mycolic acid biosynthesis, develops G312S polymorphisms under selective pressure.—Authors' Abstract

**Chemlal, K. and Portaels, F.** Molecular diagnosis of nontuberculous mycobacteria. *Curr. Opin. Infect. Dis.* **16**(2) (2003) 77–83.

**PURPOSE OF REVIEW:** Diagnosis of infection due to nontuberculous mycobacteria is not easy, as it must be distinguished from colonization or contamination by other nontuberculous mycobacteria. Molecular methods offer many advantages over conventional methods of identification. The results are obtained rapidly, are reliable and reproducible, and even mixed or contaminated cultures can be examined. This review highlights the recent advances in molecular techniques for identification of nontuberculous mycobacteria. **RECENT FINDINGS:** Nontuberculous mycobacteria are ubiquitous towards the environment and have the potential to colonize and cause serious infection. An increasing number of species and clinical presentations are being described, and progress has been made towards the understanding of the underlying predisposing factors. Disease caused by nontuberculous mycobacteria is often associated with various forms of immunosuppression, particularly HIV infection, whereas mild forms of immune

defects have been observed in some patients who, apart from their nontuberculous mycobacterial disease, seem to be healthy on initial examination. Molecular techniques have shown their usefulness for the identification of most mycobacteria. Probes are widely used in clinical laboratories for the identification of the most common mycobacterial species. Because automated DNA sequencing and the programs for analysing sequence data have become technically simpler, polymerase chain reaction-based sequencing is now used in many mycobacterial reference laboratories as a routine method for species identification. SUMMARY Significant advances have been made with molecular tools for diagnosis of mycobacteria. The DNA microarray technique holds great promise for the future because it is easy to perform, it can be readily automated, and it allows the identification of a large number of mycobacterial species in one reaction.—Authors' Abstract

**Hall, L., Doerr, K. A., Wohlfiel, S. L., and Roberts, G. D.** Evaluation of the MicroSeq System for Identification of Mycobacteria by 16S Ribosomal DNA Sequencing and Its Integration into a Routine Clinical Mycobacteriology Laboratory. *J. Clin. Microbiol.* **41(4)** (2003) 1447–1453.

An evaluation of the MicroSeq 500 microbial identification system by nucleic acid sequencing and the Mayo Clinic experience with its integration into a routine clinical laboratory setting are described. Evaluation of the MicroSeq 500 microbial identification system was accomplished with 59 American Type Culture Collection (ATCC) strains and 328 clinical isolates of mycobacteria identified by conventional and 16S ribosomal DNA sequencing by using the MicroSeq 500 microbial identification system. Nucleic acid sequencing identified 58 of 59 (98.3%) ATCC strains to the species level or to the correct group or complex level. The identification results for 219 of 243 clinical isolates (90.1%) with a distance score of <1% were concordant with the identifications made by phenotypic methods. The remaining 85 isolates had dis-

tance scores of >1%; 35 (41.1%) were identified to the appropriate species level or group or complex level; 13 (15.3%) were identified to the species level. All 85 isolates were determined to be mycobacterial species, either novel species or species that exhibited significant genotypic divergence from an organism in the database with the closest match. Integration of nucleic acid sequencing into the routine mycobacteriology laboratory and use of the MicroSeq 500 microbial identification system and Mayo Clinic databases containing additional genotypes of common species and added species significantly reduced the number of organisms that could not be identified by phenotypic methods. The turnaround time was shortened to 24 hr, and results were reported much earlier. A limited number of species could not be differentiated from one another by 16S ribosomal DNA sequencing; however, the method provides for the identification of unusual species and more accurate identifications and offers the promise of being the most accurate method available.—Authors' Abstract

**Huard, R. C., de Oliveira Lazzarini, L. C., Butler, W. R., van Soolingen, D., and Ho, J. L.** PCR-based method to differentiate the subspecies of the *Mycobacterium tuberculosis* complex on the basis of genomic deletions. *J. Clin. Microbiol.* **41(4)** (2003) 1637–1650.

The classical *Mycobacterium tuberculosis* complex (MtbC) subspecies include *Mycobacterium tuberculosis*, *Mycobacterium africanum* (subtypes I and II), *Mycobacterium bovis* (along with the attenuated *M. bovis* bacillus Calmette-Guerin [BCG]), and *Mycobacterium microti*; increasingly recognized MtbC groupings include *Mycobacterium bovis* subsp. *caprae* and “*Mycobacterium tuberculosis* subsp. *canettii*.” Previous investigations have documented each MtbC subspecies as a source of animal and/or human tuberculosis. However, study of these organisms is hindered by the lack of a single protocol that quickly and easily differentiates all of the MtbC groupings. Towards this end we have developed a rapid, simple, and reliable PCR-based MtbC typing method

that makes use of MtbC chromosomal region-of-difference deletion loci. Here, seven primer pairs (which amplify within the loci 16S rRNA, Rv0577, IS1561', Rv1510, Rv1970, Rv3877/8, and Rv3120) were run in separate but simultaneous reactions. Each primer pair either specifically amplified a DNA fragment of a unique size or failed, depending upon the source mycobacterial DNA. The pattern of amplification products from all of the reactions, visualized by agarose gel electrophoresis, allowed immediate identification either as MtbC composed of *M. tuberculosis* (or *M. africanum* subtype II), *M. africanum* subtype I, *M. bovis*, *M. bovis* BCG, *M. caprae*, *M. microti*, or "*M. canettii*" or as a Mycobacterium other than MtbC (MOTT). This MtbC PCR typing panel provides an advanced approach to determine the subspecies of MtbC isolates and to differentiate them from clinically important MOTT species. It has proven beneficial in the management of Mycobacterium collections and may be applied for practical clinical and epidemiological use.—Authors' Abstract

**Kooyk, Y., Appelmelk, B., and Geijtenbeek, T. B.** A fatal attraction: *Mycobacterium tuberculosis* and HIV-1 target DC-SIGN to escape immune surveillance. *Trends Mol. Med.* **9(4)** (2003) 153–159.

Dendritic cells (DCs) are vital in the defense against pathogens. However, it is becoming increasingly clear that some pathogens subvert DC functions to escape immune surveillance. For example, HIV-1 targets the DC-specific C-type lectin DC-SIGN (DC-specific intercellular-adhesion-molecule-3-grabbing nonintegrin) to hijack DCs for viral dissemination. Binding to DC-SIGN protects HIV-1 from antigen processing and facilitates its transport to lymphoid tissues, where DC-SIGN promotes HIV-1 infection of T cells. Recent studies demonstrate that DC-SIGN is a universal pathogen receptor that also recognizes Ebola, cytomegalovirus and mycobacteria. *Mycobacterium tuberculosis* targets DC-SIGN by a mechanism that is distinct from that of HIV-1, leading to inhibition of the

immunostimulatory function of DC and, hence, promotion of pathogen survival. A better understanding of DC-SIGN-pathogen interactions and their effects on DC function should help to combat infections.—Authors' Abstract

**Mitsos, L. M., Cardon, L. R., Ryan, L., LaCourse, R., North, R. J., and Gros, P.** Susceptibility to tuberculosis: A locus on mouse chromosome 19 (Trl-4) regulates *Mycobacterium tuberculosis* replication in the lungs. *Proc. Natl. Acad. Sci. U. S. A.* **100(11)** (2003) 6610–6615.

The mouse DBA/2 (D2) strain is very susceptible to infection with virulent *Mycobacterium tuberculosis*, whereas C57BL/6 (B6) is much more resistant. Infection of D2 and B6 mice with *M. tuberculosis* H37Rv by the respiratory route is biphasic: during the first 3 weeks, there is rapid bacterial growth in the lung of both strains, whereas beyond this point replication stops in B6 but continues in D2, causing rapidly fatal pulmonary disease. To identify the genes regulating growth of *M. tuberculosis* in the lungs of these two strains, 98 informative (B6 × D2) F2 mice were infected by the respiratory route with *M. tuberculosis* H37Rv ( $2 \times 10^2$  colony-forming units), and the extent of bacterial replication in the lungs at 90 days was used as a quantitative measure of susceptibility in a whole-genome scan. Quantitative trait locus mapping identified a major locus on chromosome 19 (Tuberculosis resistance locus-4, Trl-4; logarithm of odds 5.6), which regulated pulmonary replication of *M. tuberculosis* and accounted for 25% of the phenotypic variance. B6 alleles at Trl-4 were inherited in an incompletely dominant fashion and associated with reduced bacterial replication. An additional effect of a locus (Trl-3), previously shown to affect survival to i.v. infection with *M. tuberculosis*, was also noted. F2 mice homozygous for B6 alleles at both Trl-3 and Trl-4 were as resistant as B6 parents, whereas mice homozygous for D2 alleles were as susceptible as D2 parents. These results suggest a strong genetic interaction between Trl-3 and Trl-4 in regulating pulmonary replication of *M. tuberculosis*.—Authors' Abstract

**Motiwala, A. S., Strother, M., Amonsin, A., Byrum, B., Naser, S. A., Stabel, J. R., Shulaw, W. P., Bannantine, J. P., Kapur, V., and Sreevatsan, S.** Molecular epidemiology of *Mycobacterium avium* subsp. paratuberculosis: evidence for limited strain diversity, strain sharing, and identification of unique targets for diagnosis. *J. Clin. Microbiol.* **41**(5) (2003) 2015–2026.

The objectives of this study were to understand the molecular diversity of animal and human strains of *Mycobacterium avium* subsp. paratuberculosis isolated in the United States and to identify *M. avium* subsp. paratuberculosis-specific diagnostic molecular markers to aid in disease detection, prevention, and control. Multiplex PCR of IS900 integration loci (MPIL) and amplified fragment length polymorphism (AFLP) analyses were used to fingerprint *M. avium* subsp. paratuberculosis isolates recovered from animals (N = 203) and patients with Crohn's disease (N = 7) from diverse geographic localities. Six hundred bacterial cultures, including *M. avium* subsp. paratuberculosis (N = 303), non-*M. avium* subsp. paratuberculosis mycobacteria (N = 129), and other nonmycobacterial species (N = 168), were analyzed to evaluate the specificity of two IS900 integration loci and a newly described *M. avium* subsp. paratuberculosis-specific sequence (locus 251) as potential targets for the diagnosis of *M. avium* subsp. paratuberculosis. MPIL fingerprint analysis revealed that 78% of bovine origin *M. avium* subsp. paratuberculosis isolates clustered together into a major node, whereas isolates from human and ovine sources showed greater genetic diversity. MPIL analysis also showed that the *M. avium* subsp. paratuberculosis isolates from ovine and bovine sources from the same state were more closely associated than were isolates from different geographic regions, suggesting that some of the strains are shared between these ruminant species. AFLP fingerprinting revealed a similar pattern, with most isolates from bovine sources clustering into two major nodes, while those recovered from sheep or humans were clustered on distinct branches. Overall, this study identified a high degree of genetic similarity between *M. avium*

subsp. paratuberculosis strains recovered from cows regardless of geographic origin. Further, the results of our analyses reveal a relatively higher degree of genetic heterogeneity among *M. avium* subsp. paratuberculosis isolates recovered from human and ovine sources.—Authors' Abstract

**Pedulla, M. L., Ford, M. E., Houtz, J. M., Karthikeyan, T., Wadsworth, C., Lewis, J. A., Jacobs-Sera, D., Falbo, J., Gross, J., Pannunzio, N. R., Brucker, W., Kumar, V., Kandasamy, J., Keenan, L., Bardarov, S., Kriakov, J., Lawrence, J. G., Jacobs, W. R. Jr., Hendrix, R. W., and Hatfull, G. F.** Origins of highly mosaic mycobacteriophage genomes. *Cell.* **113**(2) (2003) 171–182.

Bacteriophages are the most abundant organisms in the biosphere and play major roles in the ecological balance of microbial life. The genomic sequences of ten newly isolated mycobacteriophages suggest that the bacteriophage population as a whole is amazingly diverse and may represent the largest unexplored reservoir of sequence information in the biosphere. Genomic comparison of these mycobacteriophages contributes to our understanding of the mechanisms of viral evolution and provides compelling evidence for the role of illegitimate recombination in horizontal genetic exchange. The promiscuity of these recombination events results in the inclusion of many unexpected genes including those implicated in mycobacterial latency, the cellular and immune responses to mycobacterial infections, and autoimmune diseases such as human lupus. While the role of phages as vehicles of toxin genes is well established, these observations suggest a much broader involvement of phages in bacterial virulence and the host response to bacterial infections.—Authors' Abstract

**Rosenberg, N. A., Tsolaki, A. G., and Tanaka, M. M.** Estimating change rates of genetic markers using serial samples: applications to the transposon IS6110 in *Mycobacterium tuberculosis*. *Theor. Popul. Biol.* **63**(4) (2003) 347–363.

In infectious disease epidemiology, it is useful to know how quickly genetic markers of pathogenic agents evolve while inside hosts. We propose a modular framework with which these genotype change rates can be estimated. The estimation scheme requires a model of the underlying process of genetic change, a detection scheme that filters this process into observable quantities, and a monitoring scheme that describes the timing of observations. We study a linear “birth-shift-death” model for change in transposable element genotypes, obtaining maximum-likelihood estimators for various detection and monitoring schemes. The method is applied to serial genotypes of the transposon IS6110 in *Mycobacterium tuberculosis*. The estimated birth rate of 0.0161 (events per copy of the transposon per year) and death rate of 0.0108 are both significantly larger than the estimated shift rate of 0.0018. The sum of these estimates, which corresponds to a “half-life” of 2.4 years for a typical strain that has 10 copies of the element, substantially exceeds a previous estimate of 0.0135 total changes per copy per year. We consider experimental design issues that enable the precision of estimates to be improved. We also discuss extensions to other markers and implications for molecular epidemiology.—Authors’ Abstract

**Sassetti, C. M., Boyd, D. H., and Rubin, E. J.** Genes required for mycobacterial growth defined by high density mutagenesis. *Mol. Microbiol.* **48(1)** (2003) 77–84.

Despite over a century of research, tuberculosis remains a leading cause of infectious death worldwide. Faced with increasing rates of drug resistance, the identification of genes that are required for the growth of this organism should provide new targets for the design of antimycobacterial agents. Here, we describe the use of transposon site hybridization (TraSH) to comprehensively identify the genes required by the causative agent, *Mycobacterium tuberculosis*, for optimal growth. These genes include those that can be assigned to essential pathways as well as many of unknown function. The genes important for the growth of *M. tuberculosis* are largely conserved in the degenerate genome of the leprosy bacillus, *Mycobacterium leprae*, indicating that non-essential functions have been selectively lost since this bacterium diverged from other mycobacteria. In contrast, a surprisingly high proportion of these genes lack identifiable orthologues in other bacteria, suggesting that the minimal gene set required for survival varies greatly between organisms with different evolutionary histories.—Authors’ Abstract