

## CURRENT LITERATURE

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

## General and Historical

**Konomi, N., Lebwohl, E., Mowbray, K., Tattersall, I., and Zhang, D.** Detection of mycobacterial DNA in andean mummies. *J. Clin. Microbiol.* **40(12)** (2002) 4738–4740.

The identification of genetic material from pathogenic organisms in ancient tissues provides a powerful tool for the study of certain infectious diseases in historic populations. We have obtained tissue samples from the genital areas of 12 mummies in the American Museum of Natural History collection in New York, N.Y. The mummies were excavated in the Andes Mountain region of South America, and radiocarbon dating estimates that the mummies date from A.D. 140 to 1200. DNAs were successfully extracted from all tissues and were suitable for PCR analysis. PCRs were carried out to detect *Mycobacterium tuberculosis* complex and mycobacteria other than *M. tuberculosis* (MOTB). *M. tuberculosis* complex was detected in 2 out of 12 samples, and MOTB were detected in 7 samples. This study confirmed the adequate preservation of genetic material in mummified tissues and the existence of mycobacteria, including *M. tuberculosis*, in historic populations in South America.— Authors' Abstract

**Stearns, A. T.** Leprosy: a problem solved by 2000? *Lepr. Rev.* **73(3)** (2002) 215–224.

It is now the year 2001, and in many endemic regions leprosy remains a public health problem by any definition. It is clear that defining leprosy purely by prevalence side-steps some of the real issues. There is still much to do to solve the problem of leprosy. Control programmes require better tests for early diagnosis if leprosy is to be reduced much further. Treatment of the in-

fection and of reactions is still far from ideal, whilst an effective vaccine would be valuable in high-risk regions. Research into the true incidence in each endemic area is essential, and control programs of the future will need a more detailed understanding of the transmission of *M. leprae* to permit new logical interventions. Leprosy remains a devastating disease. Much of the damage that it inflicts is irreversible, and leads to disability and stigmatization. This is perhaps the greatest problem posed. It is easy to dwell on the successes of the elimination campaign, so diverting attention from those populations of 'cured' patients who still suffer from the consequences of infection. Leprosy should be regarded as a problem unsolved so long as patients continue to present with disabilities. WHO has carried out a highly successful campaign in reducing the prevalence of leprosy, and this needs to be acknowledged, but what is happening to the incidence in core endemic areas? Maintaining this success, however, may be an even greater struggle if funding is withdrawn and vertical programmes are absorbed into national health structures. We must take heed of the historian George Santayana, 'those who cannot remember the past are condemned to repeat it'. We should take the example of tuberculosis as a warning of the dangers of ignoring a disease before it has been fully controlled, and strive to continue the leprosy elimination programmes until there are no new cases presenting with disability. The World Health Organisation has shown that leprosy is an eminently treatable disease, and has prepared the ground. The leprosy elimination campaigns truly are 'at a height . . . ready to decline.' Can it be that this is the chance to take leprosy 'at the flood'? If so, perhaps an extension of the elimination programs beyond the year 2001 would indeed 'lead to fortune.'— Author's Abstract

## Chemotherapy

**Alcala, L., Ruiz-Serrano, M. J., Perez-Fernandez Turegano, C., Garcia De Viedma, D., Diaz-Infantes, M., Marin-Arriaza, M., and Bouza, E.** In vitro activities of Linezolid against clinical isolates of *Mycobacterium tuberculosis* that are susceptible or resistant to first-line antituberculous drugs. *Antimicrob. Agents Chemother.* **47(1)** (2003) 416–417.

We evaluated 117 isolates of *Mycobacterium tuberculosis* for susceptibility to linezolid by the proportion and E-test methods. Linezolid showed high *in vitro* activity, with all the strains inhibited by  $\leq 1$  micro g of the drug per ml. E-test MICs were at least 4 dilutions lower than their equivalents by the standard proportion method.—Authors' Abstract

**van Crevel, R., Alisjahbana, B., Lange, W. C. M., de Borst, F., Danusantoso, H., van der Meer, J. W. M., Burger, D., and Nelwan, R. H. H.** Low plasma concentrations of rifampicin tuberculosis patients in Indonesia. *Int. J. Tuberc. Lung Dis.* **6(6)** (2002) 497–502.

Setting: Although rifampicin is a key drug in tuberculosis treatment, little is known about its quality and bioavailability in countries endemic for tuberculosis. High drug levels may lead to increased toxicity, while low drug levels may predispose to treatment failure and relapse. Objective: To investigate possible variations in the bioavailability of plasma rifampicin in tuberculosis patients in Indonesia. Design: Plasma concentrations of rifampicin and the rifampicin content of drug formulations in use were measured among 62 non-selected tuberculosis patients in Jakarta, Indonesia. Results: Plasma concentrations of rifampicin were generally low: 70% of patients had 2-hour plasma concentrations ( $C_{\max}$ ) below 4 mg/liter. No toxic plasma concentrations of rifampicin ( $>20$  mg/liter) were found. The strongest predictive factor for the magnitude of rifampicin concentrations was the drug manufacturer. The rifampicin content of the different drug preparations used was normal

(90.5–103.6% of the reference standard). No association was found between low plasma rifampicin concentrations and delayed sputum conversion or treatment failure. Conclusion: The unexpectedly low plasma concentrations of rifampicin in this setting are most likely due to reduced bioavailability of local drug preparations, as the rifampicin content of the drug preparations was normal. The clinical significance of these findings remains to be determined.—Trop. Dis. Bull.

**Feng, Z. and Barletta, R. G.** Roles of *Mycobacterium smegmatis* D-Alanine: D-Alanine Ligase and D-Alanine Racemase in the mechanisms of action of and resistance to the peptidoglycan inhibitor D-cycloserine. *Antimicrob. Agents Chemother.* **47(1)** (2003) 283–291.

D-Cycloserine (DCS) targets the peptidoglycan biosynthetic enzymes D-alanine racemase (Alr) and D-alanine:D-alanine ligase (Ddl). Previously, we demonstrated that the overproduction of Alr in *Mycobacterium smegmatis* determines a DCS resistance phenotype. In this study, we investigated the roles of both Alr and Ddl in the mechanisms of action of and resistance to DCS in *M. smegmatis*. We found that the overexpression of either the *M. smegmatis* or the *Mycobacterium tuberculosis* *ddl* gene in *M. smegmatis* confers resistance to DCS, but at lower levels than the overexpression of the *alr* gene. Furthermore, a strain overexpressing both the *alr* and *ddl* genes displayed an eightfold-higher level of resistance. To test the hypothesis that inhibition of Alr by DCS decreases the intracellular pool of D-alanine, we determined the alanine pools in *M. smegmatis* wild-type and recombinant strains with or without DCS treatment. Alr-overproducing strain GPM14 cells not exposed to DCS displayed almost equimolar amounts of L- and D-alanine in the steady state. The wild-type strain and Ddl-overproducing strains contained a twofold excess of L- over D-alanine. In all strains, DCS treatment led to a significant accumulation of L-alanine and a concomi-

tant decrease of D-alanine, with approximately a 20-fold excess of L-alanine in the Ddl-overproducing strains. These data suggest that Ddl is not significantly inhibited by DCS at concentrations that inhibit Alr. This study is of significance for the identification of the lethal target(s) of DCS and the development of novel drugs targeting the D-alanine branch of mycobacterial peptidoglycan biosynthesis.— Authors' Abstract

**Itokazu, G. S., Fischer, J. H., Manitpitkul, P., Hariharan, R., and Danziger, L. H.** Lack of effect of nizatidine-induced elevation of gastric pH on the oral bioavailability of dapsone in healthy volunteers. *Pharmacotherapy* **22(11)** (2002) 1420–1425.

**STUDY OBJECTIVE:** To investigate the effect of histamine<sub>2</sub> (H<sub>2</sub>)-receptor antagonist-induced elevation of gastric pH on oral bioavailability of a single dose of dapsone 100 mg. **DESIGN:** Prospective, randomized, crossover, open-label, single-dose pharmacokinetic study. **SETTING:** Teaching hospital. **PATIENTS:** Sixteen men were enrolled in the study; data from 11 subjects were evaluable. **INTERVENTIONS:** Participants received two treatments separated by at least 14 days. Treatment A consisted of a single dose of dapsone 100 mg. Treatment B consisted of a single dose of dapsone 100 mg plus two doses of oral nizatidine 300 mg administered 3–4 hours apart to maintain gastric pH above 6.0. Plasma samples collected before and up to 120 hours after dapsone administration were analyzed for dapsone and monoacetyldapsone (MADDS) by high-performance liquid chromatography. Pharmacokinetic parameters were determined by noncompartmental analysis. **MEASUREMENTS AND MAIN RESULTS:** Gastric pH in the first 6 hours after dapsone administration was above 6.0 for a mean  $\pm$  S.D. of 1.1%  $\pm$  2.9% of the time in the absence of nizatidine and 69.5%  $\pm$  18.0% of the time during nizatidine therapy. The geometric mean dapsone maximum plasma concentration (C<sub>max</sub>) declined by 13% ( $p < 0.01$ ), and median time to C<sub>max</sub> occurred 2 hours later ( $p < 0.01$ ) with nizatidine coadministration compared with dapsone alone. Inclusion of the 90% confi-

dence interval for the mean C<sub>max</sub> ratio within the equivalence interval of 0.8–1.25 demonstrated the lack of clinical significance for this modest decrease in C<sub>max</sub>. Neither the area under the dapsone plasma concentration-time curve from zero to infinity nor the elimination half-life of dapsone were significantly altered by nizatidine. No clinically significant changes were observed in the pharmacokinetics of MADDS with regard to coadministration of nizatidine. **CONCLUSION:** Elevation of gastric pH by H<sub>2</sub>-receptor antagonists, such as nizatidine, does not result in clinically important changes in the rate or extent of oral dapsone absorption.— Authors' Abstract

**Kunichika, N., Miyahara, N., Kotani, K., Takeyama, H., Harada, M., and Tanimoto, M.** Pneumonitis induced by rifampicin. *Thorax* **57(11)** (2002) 1000–1001.

An 81-year-old man was admitted to hospital with pulmonary *Mycobacterium tuberculosis* infection and was treated with rifampicin (RFP), isoniazid (INH), and ethambutol (EB). On day 9 he developed fever and dyspnoea. Chest radiographs showed new infiltration shadows in the right lung. Bronchoalveolar lavage (BAL) was performed and increased numbers of lymphocytes were recovered. Drug induced pneumonitis was suspected so the antituberculous regimen was discontinued and methylprednisolone was administered. The symptoms and infiltration shadows improved. INH and EB were reintroduced without any recurrence of the abnormal shadows. T cell subsets in the BAL fluid and a positive lymphocyte stimulation test for RFP suggest that RFP induced pneumonitis may be related to a complex immunological response.— Authors' Abstract

**Otten, T. F., Solov'eva, N. S., and Vishnevskii, B. I.** [Sensitivity to levofloxacin of various types of non-tuberculosis *Mycobacterium*] *Antibiot. Khimioter* **47(6)** (2002) 34–37.

Activity of levofloxacin (Tavanic) against 10 species of nontuberculosis of mycobacte-

ria was investigated by indirect method of absolute concentrations on Levenstain-Jensen media (levofloxacin concentration 5 and mcg/mL). The investigation was performed on 71 strains of nontuberculosis mycobacteria: *Mycobacterium avium-intracellulare*—24 strains, *M. fortuitum*—17 strains, *M. chelonae*—10 strains, *M. malmoense*—13 strains and 6 other species of mycobacteria. Susceptible to critical levofloxacin concentration were 8 species of 10. Resistance to levofloxacin (10 mcg/mL) was estimated for 16.7 per cent of *M. avium-intracellulare* and 30 per cent of *M. chelonae* strains. It is concluded that levofloxacin may be a drug of choice for management of mycobacteriosis caused by *M. fortuitum*, *M. kansasii*, *M. xenopi*, *M. malmoense*, and in the most of cases due to *M. avium-intracellulare* and *M. chelonae*.—Authors' Abstract

**Park, W. G., Bishai, W. R., Chaisson, R. E., and Dorman, S. E.** Performance of the Microscopic Observation Drug Susceptibility Assay in Drug Susceptibility Testing for *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **40**(12) (2002) 4750–4752.

The drug susceptibility testing performance of a broth-based method with microscopic reading of bacillary growth, the microscopic observation drug susceptibility (MODS) assay, was compared to that of the reference 7H10 agar method of proportion by using 53 isolates of *Mycobacterium tuberculosis* from persons at risk for multidrug-resistant TB. For isoniazid (0.1 micro g/ml) and rifampin (2.0 micro g/ml), there was 100% agreement between MODS results read at day 11 and the reference method. Levels of agreement for ethambutol tested at 2.5 and 7.5 micro g/ml were 70 and 58%, respectively. Levels of agreement for streptomycin tested at 2.0 and 6.0 micro g/ml were 77 and 51%, respectively. For isoniazid and rifampin drug susceptibility testing, MODS is as accurate as and more rapid than the reference method.—Authors' Abstract

**Rawat, M., Newton, G. L., Ko, M., Martinez, G. J., Fahey, R. C., and Av-Gay, Y.** Mycothiol-deficient *Mycobacterium*

*smegmatis* mutants are hypersensitive to alkylating agents, free radicals, and antibiotics. *Antimicrob. Agents Chemother.* **46**(11) (2002) 3348–3355.

Mycothiol (MSH; 1D-myo-inosityl 2-[N-acetyl-L-cysteiny]amido-2-deoxy-alpha-D-glucopyranoside) is the major low-molecular-weight thiol produced by mycobacteria. Mutants of *Mycobacterium smegmatis* mc(2)155 deficient in MSH production were produced by chemical mutagenesis as well as by transposon mutagenesis. One chemical mutant (mutant I64) and two transposon mutants (mutants Tn1 and Tn2) stably deficient in MSH production were isolated by screening for reduced levels of MSH content. The MSH contents of transposon mutants Tn1 and Tn2 were found to be less than 0.1% that of the parent strain, and the MSH content of I64 was found to be 1 to 5% that of the parent strain. All three strains accumulated 1D-myo-inosityl 2-deoxy-alpha-D-glucopyranoside to levels 20- to 25-fold the level found in the parent strain. The cysteine:1D-myo-inosityl 2-amino-2-deoxy-alpha-D-glucopyranoside ligase (MshC) activities of the three mutant strains were  $\leq 2\%$  that of the parent strain. Phenotypic analysis revealed that these MSH-deficient mutants possess increased susceptibilities to free radicals and alkylating agents and to a wide range of antibiotics including erythromycin, azithromycin, vancomycin, penicillin G, rifamycin, and rifampin. Conversely, the mutants possess at least 200-fold higher levels of resistance to isoniazid than the wild type. We mapped the mutation in the chemical mutant by sequencing the mshC gene and showed that a single amino acid substitution (L205P) is responsible for reduced MSH production and its associated phenotype. Our results demonstrate that there is a direct correlation between MSH depletion and enhanced sensitivity to toxins and antibiotics.—Authors' Abstract

**Ridtitid, W., Wongnawa, M., Mahatthanatrakul, W., Punyo, J., and Sunbhanich, M.** Rifampin markedly decreases plasma concentrations of praziquantel in healthy volunteers. *Clin. Pharmacol. Ther.* **72**(5) (2002) 505–513.

**BACKGROUND AND OBJECTIVE:** Praziquantel is extensively metabolized by the hepatic cytochrome P450 (CYP) enzymes. The CYP3A isoforms are likely to be major enzymes responsible for praziquantel metabolism. Rifampin (INN, rifampicin), a potent enzyme inducer of CYP-mediated metabolism (especially CYP2C9, CYP2C19, and CYP3A4), is known to markedly decrease plasma concentrations and effects of a number of coadministered drugs. The aim of this investigation was to study the possible pharmacokinetic interaction between rifampin and praziquantel. **METHODS:** An open, randomized, 2-phase crossover design was used in each study of single or multiple doses. In the single-dose study, 10 healthy Thai male volunteers ingested single doses of 40 mg/kg praziquantel alone (phase 1) or after pretreatment with 600 mg of oral rifampin once daily for 5 days (phase 2). In the multiple-dose study, all participants received multiple doses of 25 mg/kg praziquantel alone (phase 1) or after 5-day pretreatment with 600 mg of oral rifampin once daily (phase 2). Plasma concentrations of praziquantel in each phase were determined by the HPLC method. **RESULTS:** In the single-dose study, rifampin decreased plasma praziquantel concentrations to undetectable levels in 7 of 10 subjects, whereas praziquantel concentrations were reduced by rifampin to undetectable levels in 5 of 10 subjects in the multiple-dose study. In 3 subjects with measurable concentrations in the single-dose study, rifampin significantly decreased the mean maximum plasma concentration (C(max)) and area under the plasma concentration-time curve from 0 to 24 hours [AUC(0–24)] of praziquantel by 81% ( $p < .05$ ) and 85% ( $p < .01$ ), respectively, whereas rifampin significantly decreased the mean C(max) and AUC(0–24) of praziquantel by 74% ( $p < .05$ ) and 80% ( $p < .01$ ), respectively, in 5 subjects with measurable concentrations in the multiple-dose study. The mean C(max) and AUC(0–24) of praziquantel in subjects whose praziquantel concentrations could not be detected in the single-dose study (7 subjects) after rifampin pretreatment were reduced by approximately 99% ( $p < .001$ ) and 94% ( $p < .001$ ), respectively, and in the multiple-

dose study (5 subjects), they were reduced by 98% ( $p < .05$ ) and 89% ( $p < .01$ ), respectively. **CONCLUSIONS:** Rifampin greatly decreased plasma concentrations of single and multiple oral doses of praziquantel to levels lower than that of the minimum therapeutic concentration. Because praziquantel and rifampin are widely used in the treatment of liver flukes (*Opisthorchis viverrini*) and *Mycobacterium tuberculosis*, respectively, in Thailand and in some other countries in southeast Asia, the possibility of one drug influencing the pharmacokinetics of the other must be considered. Therefore simultaneous use of rifampin and praziquantel must be avoided in medical practice to optimize the therapeutic efficacy of praziquantel. — Authors' Abstract

**Sekar, B., Elangeswaran, N., Jayarama, E., Rajendran, M., Kumar, S. S., Vijayaraghavan, R., Anandan, D., and Arunagiri, K.** Drug susceptibility of *Mycobacterium leprae*: a retrospective analysis of mouse footpad inoculation results from 1983 to 1997. *Lepr. Rev.* **73**(3) (2002) 239–244.

We analyzed the results of mouse footpad (MFP) tests performed between 1983 and 1997 in our laboratory for the cases referred with clinical suspicion of relapse/drug resistance. A total of 214 cases, with clinical suspicion of relapse/drug resistance were investigated for susceptibility to the drugs of MDT by MFP inoculation. Among 96 inoculations that showed conclusive results, 81 (84%) were fully sensitive to dapsone, suggesting that most of the clinically suspected relapse is due to drug susceptible *Mycobacterium leprae*. Of the remaining 15 strains (16%) found resistant to dapsone, 13 (87%) were of high grade resistance and one strain each of intermediate grade and low grade dapsone resistance, suggesting that most of the dapsone resistance is secondary in nature. No case of rifampicin resistance was found. Only one case of combined dapsone and unconfirmed clofazimine resistance was found. No other combined multidrug resistance was observed in our analysis. — Authors' Abstract

**Sims, E. J., Goyal, M., and Arnold, C.**

Experimental versus in silico fluorescent amplified fragment length polymorphism analysis of *Mycobacterium tuberculosis*: improved typing with an extended fragment range. *J. Clin. Microbiol.* **40(11)** (2002) 4072–4076.

Whole-genome fingerprinting fluorescent amplified fragment length polymorphism (FAFLP) data were compared with in silico data for the sequenced strains of *Mycobacterium tuberculosis* (H37Rv and CDC1551). For this G+C-rich genome, many predicted fragments were not detected experimentally. For H37Rv, only 108 (66%) of the 163 predicted EcoRI-MseI fragments between 100 and 500 bp were visualized *in vitro*. FAFLP was also used to identify polymorphism in 10 clinical isolates of *M. tuberculosis* characterized previously by IS6110 typing, examining fragments of up to 1000 bp in size rather than

up to 500 bp as was done previously. Five isolates had unique IS6110 profiles and were not known to be epidemiologically related, two isolates were the same single-band IS6110 type but were not known to be epidemiologically related, and the remaining three isolates were epidemiologically related with identical IS6110 profiles. Analysis of fragments in the 500- to 1000-bp range using nonselective primers differentiated better between strains than analysis of fragments in the 50- to 500-bp range using a set of four selective primers. Seventeen polymorphic fragments were identified between 500 and 1000 bp in size compared with nine polymorphic fragments between 50 and 500 bp. Using the 500- to 1000-bp analysis, a level of discrimination similar to that of IS6110 typing was achieved which, unlike the IS6110 typing, was able to differentiate the two *M. tuberculosis* strains, each of which had only a single copy of IS6110.—Authors' Abstract

## Clinical Sciences

**Bastuji-Garin, S., Ochonisky, S., Bouche, P., Gherardi, R. K., Duguet, C., Djeradine, Z., Poli, F., and Revuz, J.** Incidence and risk factors for thalidomide neuropathy: a prospective study of 135 dermatologic patients. *J. Invest. Dermatol.* **119(5)** (2002) 1020–1026.

Thalidomide is effective in several cutaneous diseases. Peripheral neuropathy is the most important adverse event limiting its use. Its incidence rate and its relation to thalidomide doses remain unclear. We prospectively monitored 135 patients treated with thalidomide for various dermatologic diseases for 2 yrs in order to estimate the annual incidence rate and risk factors for neuropathy. Patients underwent standardized neurologic examination and nerve conduction studies prior to, and regularly during treatment. Risk factors for neuropathy were assessed using a Cox proportional-hazards model. Clinical and electrophysiologic evidence of a thalidomide-induced neuropathy were present in 25.2% of the patients; however, when considering all potential cases, this rate

reached 55.6%. The incidence rate was maximal during the first year of treatment (20%). The risk of neuropathy was related to the daily dose whatever the duration of treatment ( $p < 10^{-3}$ ). Considering a daily dose  $\leq 50$  mg per day as reference, the relative risk for thalidomide neuropathy was 8.2 for a daily dose comprised between 50 and 75 mg per day and 20.2 for a daily dose  $> 75$  mg per day ( $p < 10^{-3}$ ). No neuropathy occurred for daily doses  $\leq 25$  mg per day. The neuropathy was subclinical in nearly a quarter of patients with such an adverse event. These data confirm the high rate of thalidomide neuropathy and identify the daily dose as the main risk factor. The risk of neuropathy seems to be negligible for doses less than 25 mg per day, whatever the duration of therapy.—Authors' Abstract

**Chaudhry, V., Cornblath, D. R., Corse, A., Freimer, M., and Simmons-O'Brien, E., and Vogelsang, G.** Thalidomide-induced neuropathy. *Neurology* **59(12)** (2002) 1872–1875.

**BACKGROUND:** Thalidomide is effective for the treatment of some refractory dermatologic and oncologic diseases. Toxic neuropathy limits its use, as embryopathy can be avoided by contraceptive measures. **OBJECTIVE:** To describe the clinical, electrophysiologic, and pathologic features of thalidomide-induced peripheral neuropathy. **METHODS:** Clinical and electrophysiologic examinations were performed in seven patients with thalidomide-induced peripheral neuropathy. Thalidomide was used for graft-vs-host disease, pyoderma gangrenosum, and discoid lupus with dosages ranging from 100 to 1200 mg/day for 5 to 16 months (cumulative dosages of 24 to 384 g). **RESULTS:** All seven patients had clinical and electrophysiologic evidence of a sensory more than motor, axonal, length-dependent polyneuropathy that presented as painful paresthesias or numbness. Sural nerve biopsies, done in three patients, showed evidence of Wallerian degeneration and loss of myelinated fibers. The symptoms, signs, and electrophysiologic data correlated with total cumulative dose of thalidomide. **CONCLUSIONS:** Thalidomide induces a dose-dependent sensorimotor length-dependent axonal neuropathy; it should be judiciously used with close neurologic monitoring.—Authors' Abstract

**Courtright, P., Daniel, E., Sundarrao, R., Ravanes, J., Mengistu, F., Belachew, M., Celloria, R. V., and Ffytche, T.** Eye disease in multibacillary leprosy patients at the time of their leprosy diagnosis: findings from the Longitudinal Study of Ocular Leprosy (LOSOL) in India, the Philippines and Ethiopia. *Lepr. Rev.* 73(3) (2002) 225–238.

Existing prevalence surveys do not provide adequate information to estimate the magnitude of ocular pathology or vision loss in leprosy patients. We sought to determine the prevalence of ocular findings and related risk factors in leprosy patients at the time of their disease diagnosis. We also sought to determine if there were geographic differences and whether these were due to different demographic characteristics of the populations. The study was under-

taken at Schieffelin Leprosy Research & Training Centre (Karigiri, India), Leonard Wood Memorial Leprosy Institute (Cebu, Philippines), and (for 3 yrs only) ALERT (Addis Ababa, Ethiopia). Newly diagnosed multibacillary (MB) leprosy patients as well as MB cases relapsed after dapsone monotherapy were eligible for enrollment. In each study site, the target population was 300. Standardized examinations were conducted between 1991 and 1998. Patient enrollment included 301 patients in Karigiri, 289 patients in Cebu, and 101 patients in Addis Ababa. The age-adjusted prevalence of blindness (< 6/60 in the better eye) and visual impairment (6/24–6/60) was 2.8% and 5.2%, respectively. Lagophthalmos and leprosy related uveal changes were detected in 3.3% (95% CI 2.0–4.7%) and 4.1% (95% CI 2.4–5.7) of patients, respectively. Overall, 11% (95% CI 8.5–13.2%) of newly enrolled MB patients had potentially blinding leprosy related ocular pathology. Lagophthalmos was associated with increasing age, a short duration between onset and diagnosis, and a previous reaction involving the face. Uveal conditions were associated with increasing age. Overall, eye disease was more common in Indian and Ethiopian patients compared to Filipino patients; however, differences were not significant when controlling for age and clinical (non-ocular) factors. Patients with potentially blinding leprosy related pathology were over three times more likely to have other (hand and foot) disabilities than patients without pathology. Differences in the prevalence of blindness and potentially blinding leprosy related ocular pathology between the sites could be accounted for by the differences in age and other clinical factors of the patients at the different sites. Findings suggest that, even in the face of active leprosy control efforts, around 11% of patients will have potentially blinding pathology at the time of their diagnosis and 2.8% will be blind. If those patients with lagophthalmos or blindness are considered appropriate for referral for more detailed assessment, approximately 4% of newly diagnosed leprosy patients will require active follow-up for eye care; including those with reaction involving the face will result in 9.4% of patients requiring active follow-up. These people are likely to be older, with a reaction in-

volving the face, and/or with other disabilities than those not requiring active follow-up.—Authors' Abstract

**Daniel, E., Koshy, S., Rao, G. S., and Rao, P. S.** Ocular complications in newly diagnosed borderline lepromatous and lepromatous leprosy patients: baseline profile of the Indian cohort. *Br. J. Ophthalmol.* **86**(12) (2002) 1336–1340.

**Aim:** To describe ocular manifestations in newly diagnosed borderline lepromatous (BL) and lepromatous leprosy (LL) patients in India. **METHODS:** Ocular complications, at enrollment, occurring in all new borderline lepromatous and lepromatous leprosy patients detected by active case finding within the geographically defined leprosy endemic area of the Gudiyattam Taluk in India from 1991 to 1997 who consented to ocular examinations every 6 months, during and 5 yrs after treatment with multidrug therapy (MDT), were studied. **RESULTS:** Orbicularis oculi weakness (4.62%), lagophthalmos (4.20%), ectropion (0.42%), trichiasis (0.84%), blocked nasolacrimal ducts (1.68%), pterygium (11.34%), impaired corneal sensation (53%), corneal opacity (10.5%), corneal nerve beading (1.68%), punctate keratitis (1.26%), keratic precipitates (4.62%), iris atrophy (1.68%), and cataract (12.6%) were ocular complications seen in the 301 lepromatous patients at enrolment. 4.6% had blind eyes. Increasing age was associated with ocular complications. 80% of patients were skin smear acid fast bacilli (AFB) positive. The LL/BL ratio was 1:6.4. 71% had some limb deformity. 44% had only leprosy related ocular complications (LROC), 28% had only general ocular complications (GOC) while 14% had both LROC and GOC. Ocular complications were significantly related to leg deformities. Corneal nerve beading was seen most in LL patients (100%) having high bacterial content. Lagophthalmos and muscle weakness were associated with reversal reactions. **CONCLUSIONS:** Corneal nerve beading occurs in LL patients with high bacillary count. Patients with reversal reaction are more likely to present with orbicularis oculi weakness and lagophthalmos. Leprosy related ocular

complications and general ocular complications are significant problems in newly diagnosed lepromatous patients. Elderly, deformed, skin smear positive, lepromatous patients are associated with increased ocular morbidity and form a group that require acceptable and accessible eye care.—Authors' Abstract

**Ghorpade, A.** Inoculation (tattoo) leprosy: a report of 31 cases. *J. Eur. Acad. Dermatol. Venereol.* **16**(5) (2002) 494–499.

Thirty-one female patients with leprosy lesions starting over tattoo marks observed over a period of 16 yrs are reported. All the patients belonged to the Chhattisgarh State, which is highly endemic for leprosy. Most of the patients were in the third decade of life. All of them had ornamental tattooing done by roadside tattoo artists, who used unsterile needles for tattooing a large gathering one after another with the same needles. In all of them, the first lesion of leprosy started over a tattoo mark. Twenty-five cases had only single lesion of leprosy exclusively confined to tattoo marks. The duration between tattooing and appearance of first lesion in most of the cases varied from 10 to 20 yrs. Paucibacillary leprosy was the commonest type observed in 29 cases, while two had multibacillary leprosy. The diagnosis was confirmed by histopathology in all cases. The present report supports the hypothesis of transmission of leprosy in these cases through tattooing. To the best of our knowledge, such a large collection of leprosy cases subsequent to tattooing has not been reported so far.—Author's Abstract

**Halim, N. K. and Ogbeide, E.** Haematological alterations in leprosy patients treated with dapsone. *East Afr. Med. J.* **79**(2) (2002) 100–102.

**OBJECTIVE:** To evaluate the hemoglobin concentration (Hb); total white blood cell count (WBC), differential WBC count; platelet count and reticulocyte count in leprosy patients already treated with dapsone. **DESIGN:** A case-control study. **SETTING:** Specialist Hospital Ossiomo, which is a

Leprosarium and Haematology laboratory, University of Benin Teaching Hospital (UBTH), Nigeria. **SUBJECTS:** Seventy six leprosy patients (forty males and thirty six females) age range 13–40 yrs on single dose dapsone. **RESULTS:** The hemoglobin concentration showed a marked decrease while the reticulocyte count was markedly elevated which was suggestive of hemolytic anemia. There was also lymphocytosis in patients during pre and post dapsone therapy. **CONCLUSION:** Leprosy patients on a dosage of 100 mg dapsone, are prone to hemolytic anemia. Leprosy patients should routinely have their Hb, WBC, platelet count and reticulocyte count determined, while on dapsone therapy in order to ascertain the presence of hemolysis.— Authors' Abstract

**Hegazy, A. A., Abdel-Hamid, I. A., Ahmed el, S. F., Hammad, S. M., and Hawas, S. A.** Leprosy in a high-prevalence Egyptian village: epidemiology and risk factors. *Int. J. Dermatol.* **41**(10) (2002) 681–686.

**BACKGROUND:** The epidemiology of leprosy in rural Egypt is unknown. This prospective household survey was conducted in a high-prevalence Egyptian village in order to explore the epidemiologic characteristics of the disease and to determine the possible socioeconomic and HLA genotype risk factors. **METHODS:** The subjects of the study were the residents of Kafr-Tambul village in the Dakahlia governorate, Egypt. There were 10,503 inhabitants of the village, of whom 9643 (91.8%) had a complete visual skin examination, and suspected leprosy patients were subjected to histopathological examination and slit skin smears. Each household was interviewed to record personal data on family members, family size, education, occupation, crowding index at sleep, social score and source of water supply. Human leukocyte antigen (HLA) class II genotypes were analyzed in all leprosy patients and in a number of both household (N = 124) and non-household (N = 30) contacts. **RESULTS:** The overall prevalence of clinical leprosy in the village studied was 24.9/10,000 (95%CI = 16.3–37.6). Individ-

uals above the age of 40 yrs were 4 times more likely to develop leprosy (OR = 4, p = 0.01). The degree of education, crowding index at sleep, social score and source of water supply were found to be unlikely to increase the risk of leprosy (p >0.05). The frequencies of HLA-DR2 and -DQ1 were significantly associated with leprosy (OR = 3.33 and 5.4; CI = 0.95–12.07 and 1.08–30.19, respectively, all p <0.05). **CONCLUSIONS:** Our study provides the first picture of the epidemiology of leprosy in a high-prevalence village in rural Egypt. Leprosy detection campaigns should be initiated and directed towards high-prevalence villages. Provision of leprosy control activities in rural health units is necessary in order to detect new cases. The risk for leprosy is associated with HLA-DR2 and -DQ1 markers, and these markers appear to increase personal susceptibility to leprosy in this village.— Authors' Abstract

**Jain S., Reddy, R. G., Osmani, S. N., Lockwood, D. N., and Suneetha, S.** Childhood leprosy in an urban clinic, Hyderabad, India: clinical presentation and the role of household contacts. *Lepr. Rev.* **73**(3) (2002) 248–253.

A retrospective case note study was done of children below the age of 14 yrs who attended Dhoolpet Leprosy Research Centre (DLRC) over the decade 1990–1999. The aim of the study was to describe the pattern of clinical presentation, the role of household or near neighbour contacts and the incidence of neuritis and reactions. In all, 3118 leprosy patients were registered during this period, of whom 306 were children [182 (60%) male]; 95 children had a single patch, 159 had five or fewer than five patches and 37 had multiple patches. The youngest case detected was 9 months old. The spectrum of leprosy in these children was: TT 62 (20.3%); BT 203 (66.3%); BB 3 (1%); BL 23 (7.5%); LL 5 (1.6%) and PNL 10 (3.3%). Twenty-nine cases (9.4%) were smear positive. Ninety-one children (29.7%) developed a reaction, 86 type I and five type II. A history of contact was present in 119 (38.8%) cases, family contact in 113 (95%) and other than family in six (5%). Classification of the contact was available

in only 60 patients. Among the contacts of the index case, 21 (35%) suffered from PB leprosy and 39 (65%) from MB leprosy. All contacts were from the immediate family. This study shows that childhood leprosy cases continue to present in significant numbers to this outpatient clinic. There is a high level of family contact with leprosy in these cases, strengthening the strategy of screening children in leprosy-affected households. The high incidence of reactions and nerve damage in children emphasizes the importance of early detection and treatment.—Authors' Abstract

**Keita, S., Tiendrebeogo, A., Berthe, D., Faye, O., and N'diaye, H. T.** [Predictive value of consultation reasons in the diagnosis of leprosy in Bamako (Mali)]. *Ann. Dermatol. Venereol.* **129(8-9)** (2002) 1009-1011.

**INTRODUCTION:** One of the weak points in the strategy for eliminating leprosy is the poor quality of screening. To overcome this, the World Health Organization (WHO) encourages endemic countries to run campaigns for the elimination of leprosy by circulating educational messages and mobilizing the medical community for early screening of cases. The aim of our study was to identify the motives for consultation with high predictive value for the diagnosis of leprosy and to determine the late diagnosis factors and hence assist the staff on site to improve the results of their leprosy elimination campaigns. **PATIENTS AND METHODS:** The study consisted, during the second trimester of 1999, in interviewing all the patients consulting for the first time the Marchoux Institute or the units screening for leprosy in the Bamako area. The interview recorded the motives for consultation, the delay before consulting and the reasons for late consulting. To assess their positive predictive value, the motives for consultation were related to the diagnosis retained (leprosy or not). **RESULTS:** One thousand one hundred and seventy seven patients were interviewed. The motive for consulting, "suspected leprosy," scored the highest positive predictive value (PPV) (80 p. 100): 12 cases of leprosy were diagnosed by 15 consultants having sus-

pected leprosy. Neurological problems were the second motive for consultation (PPV = 61.9 p. 100). The most frequent motive for consultation was spots or "macules" (20 p. 100 of consultations), but only provided a positive predictive value of 19 p. 100. Prior consultations and non-specialized treatments were identified as factors of delay in diagnosing leprosy ( $p < 0.001$ ). **CONCLUSIONS:** Diagnosis of leprosy cannot be based on the motives for dermatological consultation alone. The macules are the most apparent signs, but of low predictive value. Nevertheless, they are an early but non-specific sign of leprosy and are often neglected by the patient. Other than macules, attention must be paid to the neurological signs (dysesthesia, motor disorders) when screening for leprosy. These signs may appear early on, or be observed at a late stage in the progression of the disease.—Authors' Abstract

**Lawn, S. D., Wood, C., and Lockwood, D. N.** Borderline tuberculoid leprosy: an immune reconstitution phenomenon in a human immunodeficiency virus-infected person. *Clin. Infect. Dis.* **36(1)** (2003) E5-E6.

Two months after starting highly active antiretroviral treatment (HAART), an individual with human immunodeficiency virus type 1 (HIV-1) infection and profound CD4+ T lymphocytopenia developed several erythematous plaques on his face, which were due to borderline tuberculoid leprosy with reversal reaction. The temporal association between the development of these lesions and changes in blood CD4+ lymphocyte count and plasma HIV-1 load observed during HAART strongly suggests that the presentation of leprosy resulted from immune reconstitution.—Authors' Abstract

**Pan Shu, Pan XiaoFeng, and Liu TongKui.** Analysis on nerve impairment of the upper limb in 641 leprosy patients. *J. Clin. Dermatol.* **31(7)** (2002) 424-425.

Out of 1575 patients from Xinghua, Jiangsu, China, with active and non-active

leprosy, 641 (40.7%) that had nerve impairment in the upper limbs were included in the study. Lateral nerve impairment was seen in 23.17%, which was higher than the incidence of bilateral nerve impairment (17.52%). Nerve impairment was present in 69.23% of active and relapse cases and in 40.46% of non-active cases. 36.63% involved the ulnar nerve, 16.95% involved the median nerve, and 2.35% involved the radial nerve. Claw hand was seen in 73.03% of the cases. Most of the active and relapse cases had single nerve involvement and two-thirds were irreversible. Nerve involvement differs due to delay in diagnosis, different leprosy reactions and different clinical types.—Tropical Diseases Bulletin

**Pattyn, S. and Grillone, S.** Relapse rates and a 10-year follow-up of a 6-week quadruple drug regimen for multibacillary leprosy. *Lepr. Rev.* **73(3)** (2002) 245–247.

Between 1989 and 1993, 136 multibacillary leprosy patients received a 6-week treatment regimen consisting of daily rifampicin 600 mg, ofloxacin 400 mg, clofazimine 100 mg and a weekly dose of 100 mg minocycline. A previous analysis after a mean follow-up of 4–7 yrs revealed a relapse rate of 2%, involving six late (after more than 5 yrs of follow-up) relapses. During the following years, 12 more relapses appeared during years 8–9 of follow-up. A mean follow-up period of 5 yrs is insufficient to evaluate treatment regimens in multibacillary leprosy. The present regimen cannot be recommended.—Authors' Abstract

**Peters, E. S. and Eshiet, A. L.** Male-female (sex) differences in leprosy patients in south eastern Nigeria: females present late for diagnosis and treatment and have higher rates of deformity. *Lepr. Rev.* **73(3)** (2002) 262–267.

A study was undertaken to investigate the possibility that female leprosy patients in South Eastern Nigeria may be at a disadvantage with regard to early presenta-

tion for diagnosis and the prevention of disability. A hospital-based retrospective examination of case notes for the period 1988–1997 was undertaken, totalling 2309 adult patients of whom 1527 (66 degrees/a) were male and 782 (33%) were female (confirming the usual 2:1 male:female ratio for this disease). Data were collected on 1) the clinical type of leprosy, 2) the interval between the onset of symptoms or signs and presentation for diagnosis and treatment and 3) the patterns of physical deformity/disability. The results indicate that in this part of Nigeria, female leprosy patients have a much longer period (duration of illness) between first symptoms or signs and presentation for diagnosis, compared with males; on average, the period before diagnosis in women was almost twice as long as that in men. Furthermore, they suffered a higher proportion of disabilities. There was no evidence to support discrimination against females with leprosy by the health staff or community and female health workers were available in both hospital and primary health care centres to receive and examine female patients. The Discussion refers to the many studies already published on gender issues, identifying a wide range of social, cultural and economic variables attributed by social structure to men and women, and including the impact of stigma, which may be particularly damaging to women in some situations. The main factors that account for late presentation of females with leprosy in this area have however still to be defined. The consequent higher proportion of disability/deformity in women is obviously of considerable concern, underlining the need for further clinical and social research in this part of Nigeria.—Authors' Abstract

**Reichart, P. A., Samaranayake, L. P., Samaranayake, Y. H., Grote, M., Pow, E., and Cheung, B.** High Oral Prevalence of *Candida krusei* in Leprosy Patients in Northern Thailand. *J. Clin. Microbiol.* **40(12)** (2002) 4479–4485.

Although *Candida albicans* is the most common human yeast pathogen, other

*Candida* species such as *C. krusei* are now recognized as emerging agents, especially in patients with human immunodeficiency virus (HIV) disease. *C. krusei* is inherently resistant to the widely used triazole antifungal fluconazole and poses therapeutic problems, especially in systemic candidiasis. In a surveillance study of leprosy patients (with arrested or burnt-out disease) in a leprosarium in northern Thailand, we found a rate of oral carriage of *C. krusei* (36%) significantly ( $p < 0.05$ ) higher than that for a healthy control group (10%). Among the *Candida*-positive patients, 16 of 35 (46%) carried *C. krusei*, while *C. albicans* was the second most common isolate (12 of 35 patients; 34%). The corresponding figures for the control group were 2 of 13 (15%) and 6 of 13 (46%), respectively. Studies of the antifungal resistance of the *C. krusei* isolates from patients indicated that all except one of the isolates were resistant to fluconazole, two isolates were resistant to ketoconazole, and all isolates were sensitive to amphotericin B. Evaluation of their genetic profiles by randomly amplified polymorphic DNA analysis with three different primers and subsequent analysis of the gel profiles by computerized cluster-derived dendrograms revealed that the *C. krusei* isolates from patients belonged to 10 disparate clusters, despite the origin from a single locale. These nascent findings indicate an alarmingly high prevalence of a *Candida* species resistant to a widely used antifungal in a part of the world where HIV disease is endemic.—Authors' Abstract

**Shaw, I. N., Ebenezer, G., and Rao, G. S.**  
Leprosy lesion on the prepuce of the male genitalia: a case report. *Lepr. Rev.* **73**(3) (2002) 276–278.

A case of borderline leprosy in type I reaction with cutaneous lesions on the prepuce is reported. The need to examine the genitalia in all male leprosy patients is stressed.—Authors' Abstract

**Zamir, E., Hudson, H., Ober, R. R., Kumar, S. K., Wang, R. C., Read RW, and Rao, N. A.** Massive mycobacterial choroiditis during highly active antiretroviral therapy: another immune-recovery uveitis? *Ophthalmology* **109**(11) (2002) 2144–2148.

**PURPOSE:** To describe the ocular presentation of disseminated mycobacterial disease occurring during immune-recovery in a patient with acquired immune deficiency syndrome (AIDS). **STUDY DESIGN:** Case report and literature review. **PARTICIPANTS:** A 41-year-old AIDS patient with a prior diagnosis of cytomegalovirus retinitis. **METHODS:** The patient developed progressive, bilateral multifocal choroiditis with panuveitis 2 months after beginning and responding to highly active antiretroviral therapy. His left eye became blind and painful and was enucleated. Pathologic examination revealed massive choroiditis with well-formed, discrete granulomas and multiple intracellular and extracellular acid-fast organisms within the choroidal granulomas. Culture and polymerase chain reaction of vitreous specimens revealed *Mycobacterium avium* complex (MAC). **RESULTS:** Empiric, and later sensitivity-guided, local and systemic antibiotic therapy was used to treat the remaining right eye, but it continued to deteriorate. Despite medical therapy, three vitrectomies and repeated intravitreal injections of amikacin, a total retinal detachment ensued. One week after the third vitrectomy, the patient died from mesenteric artery thrombosis in the setting of disseminated mycobacterial disease. **CONCLUSIONS:** This is the first report of ocular inflammation as the presenting finding in the recently recognized syndrome of immune-recovery MAC disease. Pathogenesis of this entity is related to an enhanced immune response to a prior, subclinical, disseminated infection. The formation of discrete granulomas, normally absent in MAC infections in AIDS, reflects this mechanism.—Authors' Abstract

## Immuno-pathology

**Hostetter, J. M., Steadham, E. M., Haynes, J. S., Bailey, T. B., and Cheville, N. F.** Cytokine effects on maturation of the phagosomes containing *Mycobacteria avium* subspecies *paratuberculosis* in J774 cells. *FEMS Immunol. Med. Microbiol.* **34(2)** (2002) 127–134.

*Mycobacterium avium* subspecies *paratuberculosis* (*M. a. ptb*) is an intracellular pathogen of macrophages. Intracellular survival of several species of pathogenic mycobacteria is dependent on inhibition of maturation of the phagosomes containing these pathogens into functional phagolysosomes. In activated macrophages, however, this capacity is reduced, leading to increased bacterial killing. It is the hypothesis of this study that there is increased acidification and maturation of the phagosome containing *M. a. ptb* in interferon gamma and lipopolysaccharide (IFN-gamma/LPS) activated macrophages. In activated macrophages colocalization of *M. a. ptb* with either a marker of acidic compartments (Lysotracker Red) or compartments containing a late phagosome maturation marker lysosome-associated membrane protein-1 (Lamp-1) were evaluated by laser confocal microscopy. Intracellular survival of *M. a. ptb* in activated macrophages was evaluated directly using differential fluorescent live/dead staining. The results of this study demonstrated increased colocalization of both Lysotracker Red and Lamp-1 with FITC labeled *M. a. ptb*, which correlated with decreased survival of *M. a. ptb* within activated macrophages. — Authors' Abstract

**Jacobs, M., Fick, L., Allie, N., Brown, N., and Ryffel, B.** Enhanced immune response in *Mycobacterium bovis* Bacille Calmette Guerin (BCG)-infected IL-10-deficient mice. *Clin. Chem. Lab. Med.* **40(9)** (2002) 893–902.

The role of the endogenous interleukin-10 (IL-10) in the control of *Mycobacterium bovis* Bacille Calmette Guerin (BCG) in-

fection was assessed using IL-10-deficient (IL-10<sup>-/-</sup>) mice. Similar to wild-type (WT) mice, IL-10<sup>-/-</sup> mice were resistant to intravenous challenge with *Mycobacterium bovis* BCG. Significantly higher plasma concentrations of IL-12 and tumour necrosis factor (TNF) indicated an elevated protective immune response of IL-10<sup>-/-</sup> mice. Determination of bacilli burden in IL-10<sup>-/-</sup> mice showed accelerated clearance in the lungs, spleen and the liver in comparison to WT mice. Enhanced inflammation and a vigorous granulomatous response accompanied accelerated mycobacterial clearance. Immunohistochemical analysis of hepatic granulomas from IL-10<sup>-/-</sup> mice revealed augmented lymphocyte recruitment and macrophage activation, such as increased major histocompatibility complex (MHC) class II and inducible nitric oxide synthase (iNOS) expression. Further, it was found that enlarged granulomas persisted subsequent to mycobacterial clearance and failed to resolve in the absence of IL-10. In conclusion, endogenous IL-10 dampens the cell-mediated immune response to mycobacterial infection. — Authors' Abstract

**Jason, J., Archibald, L. K., Nwanyanwu, O. C., Kazembe, P. N., Chatt, J. A., Norton, E., Dobbie, H., and Jarvis, W. R.** Clinical and immune impact of *Mycobacterium bovis* BCG vaccination scarring. *Infect. Immun.* **70(11)** (2002) 6188–6195.

The World Health Organization recommends *Mycobacterium bovis* BCG vaccination in areas of high tuberculosis prevalence. BCG's clinical and immune effects, not necessarily *Mycobacterium tuberculosis* specific, are unclear. BCG vaccine scarring often is used as a surrogate marker of vaccination or of effective vaccination. We evaluated BCG scarring status in relation to clinical findings and outcome in 700 hospitalized Malawians, of whom 32 had *M. tuberculosis* bloodstream infections (BSI) (10 of whom had cellular immune studies done)

and of whom 48 were infants <6 months old and therefore recently vaccinated (19 of whom had immune studies). In the patients  $\geq 6$  months old, scarring was not related to the presence of pulmonary symptoms (35 versus 30%), chronic cough or fever, mortality, or *M. tuberculosis* BSI. In *M. tuberculosis* BSI patients, scarring was unrelated to mortality, vital signs, or clinical symptoms but those with scarring had higher proportions of memory and activated T cells and more type 2-skewed cytokine profiles. Infants with either BCG scarring (N = 10) or BCG lesional inflammation (N = 5) had no symptoms of sepsis, but 18 of 33 infants without BCG vaccination lesions did. Those with BCG lesions had localized infections more often than did those without BCG lesions. These infants also had lower median percentages of lymphocytes spontaneously making interleukin-4 (IL-4) or tumor necrosis factor alpha (TNF-alpha) and lower ratios of T cells spontaneously making IL-4 to T cells making IL-6. Thus, we found that, in older patients, BCG vaccine scarring was not associated with *M. tuberculosis*-specific or nonspecific clinical protection. Those with *M. tuberculosis* BSI and scarring had immune findings suggesting previous *M. tuberculosis* antigen exposure and induction of a type 2 cytokine pattern with acute reexposure. It is unlikely that this type 2 pattern would be protective against mycobacteria, which require a type 1 response for effective containment. In infants <6 months old, recent BCG vaccination was associated with a non-*M. tuberculosis*-specific, anti-inflammatory cytokine profile. That the vaccinated infants had a greater frequency of localized infections and lesser frequency of sepsis symptoms suggests that this postvaccination cytokine pattern may provide some non-*M. tuberculosis*-specific clinical benefits.—Authors' Abstract

**Oriani, D. S. and Sagardoy, M. A.** Nontuberculous mycobacteria in soils of La Pampa province (Argentina). *Rev. Argent. Microbiol.* **34(3)** (2002) 132–137.

The presence of nontuberculous mycobacteria (NTM) was investigated in forty soil samples belonging to the four physio-

graphic regions (Eastern, Central, Southern and Western) that constitute La Pampa province. The presence of NTM in 67.5% of these soil samples was determined. The density of mycobacteria ranged 25–4500 mycobacteria  $g^{-1}$  dry soil (mean = 516 CFU  $g^{-1}$ ). Significant differences were found in relation to both the investigated regions ( $p < 0.01$ ) and the soil pH ( $r = 0.44^*$ ) ( $p = 0.02$ ). The mycobacteria represented less than 0.00001% of the total aerobic bacteria found in the soils. Twenty-seven isolated mycobacteria were classified according to the culture, biochemical, enzymatic characteristics and antibiotic sensitivity. *Mycobacterium fortitium* was the dominant mycobacterium and was detected in 63% of the positive soils. This species showed ability for living in sandy to sandy loam soils, within a wide pH range (6.5–9.7) and organic matter (4.15–83.63  $g\ kg^{-1}$ ). Two other species were *M. phlei* (range = 50–4500 CFU  $g^{-1}$ ) and *M. kansasii* (range = 50–500 CFU  $g^{-1}$ ).—Authors' Abstract

**Orrell, R. W., King, R. H., Bowler, J. V., and Ginsberg, L.** Peripheral nerve granuloma in a patient with tuberculosis. *J. Neurol. Neurosurg. Psychiatry* **73(6)** (2002) 769–771.

The cause of peripheral neuropathy associated with tuberculosis is controversial. Possibilities include an immune mediated neuropathy, direct invasion of nerves, vasculitic neuropathy, compressive neuropathy, a meningitic reaction, and the toxic effects of antituberculous chemotherapy. This report describes the unusual finding of granulomas in the peripheral nerve of a patient with tuberculosis. The pathological findings were of a delayed hypersensitivity reaction, but with no more specific indications of the mechanism of the neuropathy.—Authors' Abstract

**Sandor, M., Weinstock, J. V., and Wynn, T. A.** Granulomas in schistosome and mycobacterial infections: a model of local immune responses. *Trends Immunol.* **24(1)** (2003) 44–52.

Granulomatous immune responses are interesting models for the effector phase of im-

munity, in that granulomas can be part of both immune protection and disease pathology during the course of various infectious and autoimmune diseases. Focusing mainly on granulomas induced by *Schistosoma* or *Mycobacterium* infection, this article reviews T-cell recruitment, local cytokine networks and the regulation of cytokine networks by neuropeptides in granulomas. In addition, different activation pathways for macrophages residing in granulomas are discussed. Granulomas are unique inflammatory sites that offer a challenging and rewarding model to study immunity.—Authors' Abstract

**Saunders, B. M., Dane, A., Briscoe, H., and Britton, W. J.** Characterization of immune responses during infection with *Mycobacterium avium* strains 100, 101 and the recently sequenced 104. *Immunol. Cell. Biol.* **80(6)** (2002) 544–549.

*Mycobacterium avium* strain 104 was chosen as the *M. avium* isolate to sequence, as it is virulent to humans, stable and readily transfectable. As this strain has not been widely studied we sought to investigate the pattern of 104 infection in mice. Bacterial

growth and the immune response generated were compared with infection with the low virulence *M. avium* strain 100, and the high virulence common laboratory strain, 101. *Mycobacterium avium* strains 104 and 101 grew progressively within mice, while strain 100 was gradually cleared. Strains 104 and 101 induced strong T cell activation and spleen cell cultures produced similar levels of IFN-gamma. In mice infected with strain 100 no significant T cell activation or IFN-gamma production was measured. Further, mice infected with strain 104 or 101 also displayed comparable inflammatory responses and similar granuloma formation, while only minimal inflammation was in mice infected with strain 100. Strains 101 and 104 also grew in a similar fashion in bone-marrow-derived macrophages and induced significant levels of TNF and nitric oxide. Thus infection with *M. avium* strain 104 induced an immunological response comparable to *M. avium* strain 101 and, with the availability of its sequence, should be a useful tool for designing new vaccines or drugs therapies to treat the increasing incidence of *M. avium* infection in humans.—Authors' Abstract

## Immuno-pathology (Leprosy)

**Barbosa, A. A. Jr., Guimaraes, N. S., Follador, I., Sarno, L. S., and Pereira, C. P.** Leprosy combined with elastolytic granuloma. *An. Bras. Dermatol.* **77(5)** (2002) 585–592.

Two cases of leprosy combined with elastolytic giant cell granuloma are reported. Though a coincidental occurrence cannot be excluded, a possible pathogenic relationship between the two conditions is postulated. It is possible that an immunological mechanism plays a role in the elastolytic process, which could also be caused by actinic damage in the skin altered by leprosy.—*An. Bras. de Dermatol.*

**Kang, T. J., Lee, S. B., and Chae, G. T.** A polymorphism in the toll-like receptor 2 is associated with IL-12 production from monocyte in lepromatous leprosy. *Cytokine* **20(2)** (2002) 56–62.

Toll-like receptor 2 (TLR2) is critical in the immune response to mycobacterial infections, and the mutations in the TLR2 have been shown to confer the susceptibility to infection with mycobacteria. We previously reported the detection of TLR2 Arg677Trp mutation in lepromatous leprosy. Here, the events triggered by TLR2 in response to cell lysate of *Mycobacterium leprae* (MLL), the causative agent of lep-

rosy, were investigated. Upon stimulation with MLL, monocytes produced TNF-alpha and Interleukin-12 (IL-12), which play a role in the innate immune response to infection. Anti-TLR2 mAb blocked greater than 50% of the MLL-induced production of IL-12. We also performed the functional study on TLR2 by measurement of IL-12 production in serum and monocytes from leprosy patients with TLR2 mutation (Arg677Trp). The monocytes obtained from patients with the TLR2 mutation, in comparison to the wild-type TLR2, is significantly less responsive to MLL. It was also confirmed that patients with TLR2 mutation showed significantly lower serum levels of IL-12, in comparing with TLR2 wild-type. Our results reveal that innate immune response of monocytes against *M. leprae* is mediated by TLR2, and suggest that the mutation in the intracellular domain of TLR2 gene is associated with IL-12 production in lepromatous leprosy.—Authors' Abstract

**Moudgil, K. D., Gupta, S. K., Naraynan, P. R., Srivastava, L. M., Mishra, R. S., and Talwar, G. P.** Antibody response to phenolic glycolipid I and *Mycobacterium w* antigens and its relation to bacterial load in *M. leprae*-infected mice and leprosy patients. *Clin. Exp. Immunol.* **78(2)** (1989) 214–218.

Twenty-six inbred BALB/cBy mice were infected with live *Mycobacterium leprae* by injecting  $6 \times 10^3$  bacilli in the hind footpad. Bleeds were collected at monthly intervals. After 6 months, acid-fast bacilli (AFB) were harvested monthly from the footpad of mice. The sera were analysed in enzyme immunoassay for antibodies against phenolic glycolipid I (PGL-I) of *M. leprae* and antigens of *Mycobacterium w* (*M. w*); 21 out of 26 (80.7%) mice demonstrated the presence of antibodies against PGL-I and *M. w*. Anti-*M. w* antibodies appeared slightly earlier than did anti-PGL-I antibodies. The titre of anti-*M. w* antibodies was higher than that of anti-PGL-I antibodies. The mice giving a positive antibody response had more than  $7 \times 10^5$  AFB/footpad. The coefficient of correlation (r) between the number of AFB and

antibody titres at the time of harvest was 0.566 for PGL-I and 0.628 for *M. w*. The value of r for bacterial index and antibody titres in 188 leprosy patients was 0.510 for PGL-I and 0.418 for *M. w*; these values were statistically significant ( $p < 0.001$ ). The decrease in bacterial index and antibody titres in treated lepromatous leprosy patients correlated with increase in the duration of chemotherapy. The measurement of anti-PGL-I antibodies of IgM class may serve as an adjunct to skin biopsy and skin-slit smear for serial monitoring of the bacterial load in the course of chemotherapy in leprosy control programs.—Authors' Abstract

**Santos, A. R., Suffys, P. N., Vanderborght, P. R., Moraes, M. O., Vieira, L. M., Cabello, P. H., Bakker, A. M., Matos, H. J., Huizinga, T. W., Ottenhoff, T. H., Sampaio, E. P., and Sarno, E. N.** Role of tumor necrosis factor-alpha and interleukin-10 promoter gene polymorphisms in leprosy. *J. Infect. Dis.* **186(11)** (2002) 1687–1691.

Single-nucleotide polymorphisms within the genes coding for tumor necrosis factor (TNF)-alpha and interleukin (IL)-10 have been associated with several infectious diseases. To determine whether such polymorphisms are associated with leprosy, genotyping was performed at the -308 and -238 positions of the promoter of the TNF-alpha gene in 210 and 191 patients with multibacillary (MB) leprosy, respectively; 90 and 79 patients with paucibacillary; and 92 control subjects. For the -592 and -819 positions within the promoter of the IL-10 gene, 143 patients with MB leprosy, 79 patients with PB leprosy, and 62 control subjects were included in the analysis. TNF2 allele frequency was significantly higher among control subjects than among all patients with leprosy or in the MB group ( $p < 0.05$  and  $p < 0.01$ ). For the IL-10 gene, the frequency of the homozygous -819TT genotype was significantly higher among patients than among control subjects. These data indicate that a relationship exists between TNF-alpha and IL-10 promoter polymorphisms and the development of PB leprosy.—Authors' Abstract

**Teles, R. M., Moraes, M. O., Geraldo, N. T., Salles, A. M., Sarno, E. N., and Sampaio, E. P.** Differential TNFalpha mRNA regulation detected in the epidermis of leprosy patients. *Arch. Dermatol. Res.* **294**(8) (2002) 355–362.

The epidermis is an important site of the immunoinflammatory response in the skin. In the present study, the expression of cytokine and ICAM-1 (intercellular adhesion molecule-1) genes was evaluated by RT-PCR in the epidermis isolated from biopsies from 25 reactional leprosy patients. TNFalpha and IL-6 mRNAs were detected in all individuals during the reactional state (reversal reaction or erythema nodosum leprosum), IL-8 message was detected in 66.6% and 62.5% of the patients, IL-12 mRNA was present in 91.6% and 62.5% and ICAM-1 in 100% and 71.4%, respectively. In addition, when skin biop-

sies were obtained from the same patients before and during the reactional episode, an enhancement in cytokine mRNA, but not in ICAM-1 mRNA, was observed. Seven patients were also evaluated at the onset of reaction and during antiinflammatory treatment. In contrast to a preferential disease in the TNFalpha gene detected in the dermis, during the treatment phase, persistent/enhanced TNFalpha mRNA expression was detected in the epidermis in six out of the seven patients assessed. This peculiar pattern of expression might reflect a differential impact that *in vivo* antiinflammatory therapy has on the epidermis. The present findings indicate that the epidermis plays an important role in the local inflammatory response in leprosy and that the profile of response detected in the epidermis during the reactions may be regulated differently from that in the dermis.— Authors' Abstract

## Immuno-pathology (Tuberculosis)

**Arend, S. M., Van Meijgaarden, K. E., De Boer, K., De Palou, E. C., Van Soolingen, D., Ottenhoff, T. H., and Van Dissel, J. T.** Tuberculin skin testing and *in vitro* T-cell responses to ESAT-6 and culture filtrate protein 10 after infection with *Mycobacterium marinum* or *Mycobacterium kansasii*. *J. Infect. Dis.* **186**(12) (2002) 1797–1807.

T cell responses to ESAT-6 and culture filtrate protein 10 (CFP-10), antigens expressed by *Mycobacterium tuberculosis* but not by *M. bovis* bacille Calmette-Guerin (BCG), were found to discriminate reliably between infection with *M. tuberculosis* and BCG vaccination. Because the *esat-6* and *cfp-10* genes occur in *M. kansasii* and *M. marinum*, T cell responses to ESAT-6 and CFP-10 were investigated in patients infected with *M. kansasii* or *M. marinum*, persons intensively exposed to environmental mycobacteria, and unexposed control subjects. Tuberculin skin tests were performed, and peripheral blood mononuclear cells were cocultured with ESAT-6, CFP-10, peptide mixtures of ESAT-6 and CFP-

10, and control antigens. When enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot assay (ELISPOT) were used to measure interferon-gamma production, most *M. kansasii*- or *M. marinum*-infected patients and several persons exposed to environmental mycobacteria were found to respond to ESAT-6 and/or CFP-10. ELISA and ELISPOT yielded comparable results, as did whole antigen and peptides ( $p < .0001$ ). These results may be relevant for the development of novel assays for diagnosis of tuberculosis.— Authors' Abstract

**Biet, F., Kremer, L., Wolowczuk, I., Delacre, M., and Locht, C.** *Mycobacterium bovis* BCG producing interleukin-18 increases antigen-specific gamma interferon production in mice. *Infect. Immun.* **70**(12) (2002) 6549–6557.

Interleukin-18 (IL-18) and IL-12 play a critical role in the expression of cell-mediated immunity involved in host defense against intracellular pathogens. Both cytokines are

produced by macrophages and act in synergy to induce gamma interferon (IFN-gamma) production by T, B, and natural killer cells. In the present study, we analyzed both cellular and humoral responses upon infection with IL-18-secreting BCG of BALB/c and C3H/HeJ mice, two strains known to differ in their ability to support the growth of BCG. The cDNA encoding mature IL-18 was fused in frame with the alpha-antigen signal peptide-coding sequence, cloned downstream of the mycobacterial hsp60 promoter and expressed in BCG. IL-18 produced by the recombinant BCG strain was functional, as judged by NF-kappaB-mediated luciferase induction in a tissue culture assay. When susceptible mice were infected with IL-18-producing BCG, their splenocytes were found to produce higher amounts of Th1 cytokines after stimulation with mycobacterial antigens than the splenocytes of mice infected with the nonrecombinant BCG. This was most prominent for IFN-gamma, although the mycobacterial antigen-specific secretion of granulocyte-macrophage colony-stimulating factor and IL-10 was also augmented after infection with the recombinant BCG compared to infection with nonrecombinant BCG. In contrast, the immunoglobulin G levels in serum against mycobacterial antigens were lower when the mice were infected with IL-18-producing BCG compared to infection with nonrecombinant BCG. The IL-18 effect was delayed in BALB/c compared to C3H/HeJ mice. These results indicate that the production of IL-18 by recombinant BCG may enhance the immunomodulatory properties of BCG further toward a Th1 profile. This may be particularly useful for immunotherapeutic or prophylactic interventions in which a Th1 response is most desirable.—Authors' Abstract

**Cardoso, F. L., Antas, P. R., Milagres, A. S., Geluk, A., Franken, K. L., Oliveira, E. B., Teixeira, H. C., Nogueira, S. A., Sarno, E. N., Klatser, P., Ottenhoff, T. H., and Sampaio, E. P.** T-cell responses to the *Mycobacterium tuberculosis*-specific antigen ESAT-6 in Brazilian tuberculosis patients. *Infect. Immun.* **70(12)** (2002) 6707–6714.

The *Mycobacterium tuberculosis*-specific ESAT-6 antigen induces highly potent T-cell responses and production of gamma interferon (IFN-gamma), which play a critical role in protective cell-mediated immunity against tuberculosis (TB). In the present study, IFN-gamma secretion by peripheral blood mononuclear cells (PBMCs) in response to *M. tuberculosis* ESAT-6 in Brazilian TB patients was investigated in relation to clinical disease types, such as pleurisy and cavitary pulmonary TB. Leprosy patients, patients with pulmonary diseases other than TB, and healthy donors were assayed as control groups. Sixty percent of the TB patients indeed recognized *M. tuberculosis* ESAT-6, as did 50% of the leprosy patients and 60% of the non-TB controls. Nevertheless, the levels of IFN-gamma in response to the antigen ESAT-6, but not to antigen 85B (Ag85B) and purified protein derivative (PPD), were significantly lower in controls than in patients with treated TB or pleural or cavitary TB. Moreover, according to *Mycobacterium bovis* BCG vaccination status, only 59% of the vaccinated TB patients responded to ESAT *in vitro*, whereas 100% of them responded to PPD. Both CD4 and CD8 T cells were able to release IFN-gamma in response to ESAT. The present data demonstrate the specificity of ESAT-6 of *M. tuberculosis* and its ability to discriminate TB patients from controls, including leprosy patients. However, to obtain specificity, it is necessary to include quantitative IFN-gamma production in response to the antigen as well, and this might limit the use of ESAT-6-based immunodiagnosis of *M. tuberculosis* infection in an area of TB endemicity.—Authors' Abstract

**Chackerian, A., Alt, J., Perera, V., and Behar, S. M.** Activation of NKT cells protects mice from tuberculosis. *Infect. Immun.* **70(11)** (2002) 6302–6309.

The T-cell immune response to *Mycobacterium tuberculosis* is critical in preventing clinical disease. While it is generally accepted that both major histocompatibility complex class I (MHC-I)-restricted CD8(+) and MHC-II-restricted CD4(+) T cells are important for the im-

mune response to *M. tuberculosis*, the role of non-MHC-restricted T cells is still not clearly delineated. We have previously reported that CD1d(-/-) mice do not differ from CD1d(+/+) mice in their survival following infection with *M. tuberculosis*. We now show that, although CD1d-restricted NKT cells are not required for optimum immunity to *M. tuberculosis*, specific activation of NKT cells by the CD1d ligand alpha-galactosylceramide protects susceptible mice from tuberculosis. Treatment with alpha-galactosylceramide reduced the bacterial burden in the lungs, diminished tissue injury, and prolonged survival of mice following inoculation with virulent *M. tuberculosis*. The capacity of activated NKT cells to stimulate innate immunity and modulate the adaptive immune response to promote a potent antimicrobial immune response suggests that alpha-galactosylceramide administration could have a role in new strategies for the therapy of infectious diseases.—Authors' Abstract

**Ciaramella, A., Cavone, A., Santucci, M. B., Amicosante, M., Martino, A., Auricchio, G., Pucillo, L. P., Colizzi, V., and Fraziano, M.** Proinflammatory cytokines in the course of *Mycobacterium tuberculosis*-induced apoptosis in monocytes/macrophages. *J. Infect. Dis.* **186**(9) (2002) 1277–1282.

*Mycobacterium tuberculosis* (MTB) can induce apoptosis in monocytes/macrophages both *in vitro* and *in vivo*, and this phenomenon is associated with mycobacterial survival. The present study addresses the possibility that apoptotic and inflammatory pathways could coexist through a caspase-1-mediated mechanism. In this context, a caspase-1 inhibitor (YVAD), but not caspase-3 (DEVD) or caspase-4 (LEVD) inhibitors, was able to strongly inhibit MTB-induced apoptosis. Moreover, caspase-1 activity was confirmed by the increased maturation of interleukin (IL)-1beta. Of interest, IL-1beta and tumor necrosis factor (TNF)-alpha were produced massively in the course of infection, and both were inhibited by YVAD pretreatment. To determine whether TNF-

alpha was produced actively by apoptotic cells, the intracytoplasmic cytokine content and apoptotic phenotype were analyzed at the single-cell level. Results showed a progressive increase of TNF-alpha production in annexin V-positive cells. These results indicate that MTB-induced apoptosis is associated with pro-inflammatory cytokine production.—Authors' Abstract

**Davis, J. M., Clay, H., Lewis, J. L., Ghorri, N., Herbomel, P., and Ramakrishnan, L.** Real-time visualization of mycobacterium-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. *Immunity* **17**(6) (2002) 693–702.

Infection of vertebrate hosts with pathogenic Mycobacteria, the agents of tuberculosis, produces granulomas, highly organized structures containing differentiated macrophages and lymphocytes, that sequester the pathogen. Adult zebrafish are naturally susceptible to tuberculosis caused by *Mycobacterium marinum*. Here, we exploit the optical transparency of zebrafish embryos to image the events of *M. marinum* infection *in vivo*. Despite the fact that the embryos do not yet have lymphocytes, infection leads to the formation of macrophage aggregates with pathological hallmarks of granulomas and activation of previously identified granuloma-specific Mycobacterium genes. Thus, Mycobacterium-macrophage interactions can initiate granuloma formation solely in the context of innate immunity. Strikingly, infection can redirect normal embryonic macrophage migration, even recruiting macrophages seemingly committed to their developmentally dictated tissue sites.—Authors' Abstract

**Dieli, F., Ivanyi, J., Marsh, P., Williams, A., Naylor, I., Sireci, G., Caccamo, N., Di Sano, C., and Salerno, A.** Characterization of lung gamma delta T-cells following intranasal infection with *Mycobacterium bovis* Bacillus Calmette-Guerin. *J. Immunol.* **170**(1) (2003) 463–469.

The lungs are considered to have an impaired capacity to contain infection by pathogenic mycobacteria, even in the presence of effective systemic immunity. In an attempt to understand the underlying cellular mechanisms, we characterized the gammadelta T cell population following intranasal infection with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). The peak of gammadelta T cell expansion at 7 days postinfection preceded the 30 day peak of alphabeta T cell expansion and bacterial count. The expanded population of gammadelta T cells in the lungs of BCG-infected mice represents an expansion of the resident Vgamma2 T cell subset as well as an influx of Vgamma1 and of four different Vdelta gene-bearing T cell subsets. The gammadelta T cells in the lungs of BCG-infected mice secreted IFN-gamma following *in vitro* stimulation with ionomycin and PMA and were cytotoxic against BCG-infected peritoneal macrophages as well as against the uninfected J774 macrophage cell line. The cytotoxicity was selectively blocked by anti-gammadelta TCR mAb and strontium ions, suggesting a granule-exocytosis killing pathway. Depletion of gammadelta T cells by injection of specific mAb had no effect on the subsequent developing CD4 T cell response in the lungs of BCG-infected mice, but significantly reduced cytotoxic activity and IFN-gamma production by lung CD8 T cells. Thus, gammadelta T cells in the lungs might help to control mycobacterial infection in the period between innate and classical adaptive immunity and may also play an important regulatory role in the subsequent onset of alphabeta T lymphocytes.—Authors' Abstract

**Dieli, F., Sireci, G., Caccamo, N., Di Sano, C., Titone, L., Romano, A., Di Carlo, P., Barera, A., Accardo-Palumbo, A., Krensky, A. M., and Salerno, A.** Selective depression of interferon-gamma and granulysin production with increase of proliferative response by Vgamma9/Vdelta2 T-cells in children with tuberculosis. *J. Infect. Dis.* **186**(12) (2002) 1835–1839.

Vgamma9/Vdelta2 T cells can contribute to protective immune response against

*Mycobacterium tuberculosis*, although the extent to which and mechanisms by which they could actually protect against human tuberculosis remain unclear. We have previously reported that Vgamma9/Vdelta2 T cells from tuberculin purified protein derivative (PPD)-positive children, either healthy or affected by different clinical forms of tuberculosis, strongly proliferate to different phosphoantigens *in vitro*, whereas Vgamma9/Vdelta2 T cells from PPD-negative healthy subjects proliferate very poorly. We report here that Vgamma9/Vdelta2 T cells from tuberculous children have an increased proliferative activity, but decreased interferon (IFN)-gamma production and granulysin expression. After successful chemotherapy, the Vgamma9/Vdelta2 T cell proliferative response strongly decreased, whereas IFN-gamma and granulysin production consistently increased. Disease-associated changes in Vgamma9/Vdelta2 T cell effector functions in patients with tuberculosis are consistent with the possibility that these T cells may play a protective role in immune response against *M. tuberculosis* infection.—Authors' Abstract

**D-Souza, S., Rosseels, V., Romano, M., Tanghe, A., Denis, O., Jurion, F., Castiglione, N., Vanonckelen, A., Palfliet, K., and Huygen, K.** Mapping of murine Th1 helper T-cell epitopes of mycolyl transferases Ag85A, Ag85B, Ag85C from *Mycobacterium tuberculosis*. *Infect. Immun.* **71**(1) (2003) 483–493.

BALB/c (H-2(d)) and C57BL/6 (H-2(b)) mice were infected intravenously with *Mycobacterium tuberculosis* H37Rv or vaccinated intramuscularly with plasmid DNA encoding each of the three mycolyl transferases Ag85A, Ag85B, and Ag85C from *M. tuberculosis*. Th1-type spleen cell cytokine secretion of interleukin-2 (IL-2) and gamma interferon (IFN-gamma) was analyzed in response to purified Ag85 components and synthetic overlapping peptides covering the three mature sequences. Tuberculosis-infected C57BL/6 mice reacted strongly to some peptides from Ag85A and Ag85B but not from Ag85C, whereas tuberculosis-infected BALB/c mice reacted only to peptides from Ag85A. In contrast, spleen cells

from both mouse strains produced elevated levels of IL-2 and IFN-gamma following vaccination with Ag85A, Ag85B, and Ag85C DNA in response to peptides of the three Ag85 proteins, and the epitope repertoire was broader than in infected mice. Despite pronounced sequence homology, a number of immunodominant regions contained component specific epitopes. Thus, BALB/c mice vaccinated with all three Ag85 genes reacted against the same amino acid region, 101 to 120, that was also immunodominant for Ag85A in *M. bovis* BCG-vaccinated and tuberculosis-infected H-2(d) haplotype mice, but responses were completely component specific. In C57BL/6 mice, a cross-reactive T-cell response was detected against two carboxy-terminal peptides spanning amino acids 241 to 260 and 261 to 280 of Ag85A and Ag85B. These regions were not recognized at all in C57BL/6 mice vaccinated with Ag85C DNA. Our results underline the need for comparative analysis of all three Ag85 components in future vaccination studies.—Authors' Abstract

**Duan, L., Gan, H., Golan, D. E., and Remold, H. G.** Critical role of mitochondrial damage in determining outcome of macrophage infection with *Mycobacterium tuberculosis*. *J. Immunol.* **169**(9) (2002) 5181–5187.

Human macrophages (Mphi) respond to *Mycobacterium tuberculosis* (Mtb) infection by undergoing apoptosis, a cornerstone of effective antimycobacterial host defense. Virulent mycobacteria override this reaction by inducing necrosis leading to uncontrolled Mtb replication. Accordingly, Mphi death induced by inoculation with Mtb had the characteristics of apoptosis and necrosis and correlated with moderate increase of mitochondrial permeability transition (MPT), mitochondrial cytochrome c release, and caspase-9 and -3 activation. We hypothesized that changes in intramitochondrial Ca(2+) concentration ( $[Ca(2+)](m)$ ) determine whether Mphi undergo either apoptosis or necrosis. Therefore, we induced mechanism(s) leading to predominant apoptosis or necrosis by modulating  $[Ca(2+)](m)$  and examined their physiological consequences. Adding calcium ionophore A23187

to Mphi inoculated with Mtb further increased calcium flux into the cells which is thought to lead to increased  $[Ca(2+)](m)$ , blocked necrosis, stabilized MPT, decreased mitochondrial cytochrome c release, lowered caspase activation, and accompanied effective antimycobacterial activity. In contrast, Mphi infected with Mtb in presence of the mitochondrial calcium uniporter inhibitor ruthenium red showed increased mitochondrial swelling and cytochrome c release and decreased MPT and antimycobacterial activity. Thus, in Mtb-infected Mphi, high levels of mitochondrial membrane integrity, low levels of caspase activation, and diminished mitochondrial cytochrome c release are hallmarks of apoptosis and effective antimycobacterial activity. In contrast, breakdown of mitochondrial membrane integrity and increased caspase activation are characteristic of necrosis and uncontrolled Mtb replication.—Authors' Abstract

**Feng, C. G., Kullberg, M. C., Jankovic, D., Cheever, A. W., Caspar, P., Coffman, R. L., and Sher, A.** Transgenic mice expressing human interleukin-10 in the antigen-presenting cell compartment show increased susceptibility to infection with *Mycobacterium avium* associated with decreased macrophage effector function and apoptosis. *Infect. Immun.* **70**(12) (2002) 6672–6679.

Interleukin-10 (IL-10) is thought to play an important role in the regulation of microbial immunity. While T-cell-derived IL-10 has been shown to suppress cell-mediated immunity, there has been debate as to whether antigen presenting cell (APC)-derived cytokine can perform the same function *in vivo*. To assess the influence of APC-produced IL-10 on host resistance to mycobacterial infection, transgenic mice expressing human IL-10 under the control of the major histocompatibility complex class II promoter (hu10Tg) were infected with *Mycobacterium avium*, and bacterial burdens and immune responses were compared with those observed in wild-type (wt) animals. Hu10Tg mice harbored substantially higher numbers of *M. avium* and succumbed 16 to 18 weeks postinfection. The granulomas in infected hu10Tg mice

showed marked increases in both acid-fast bacilli and host macrophages. In addition, these animals displayed a dramatic increase in hepatic fibrosis. The increased susceptibility of the hu10Tg mice to *M. avium* infection is independent of T-cell-produced endogenous murine IL-10, since bacterial burdens in mice derived by crossing hu10Tg mice with murine IL-10-deficient mice were not significantly different from those in hu10Tg mice. Importantly, gamma interferon (IFN- $\gamma$ ) responses were not decreased in the infected transgenic animals from those in wt animals, suggesting the normal development of Th1 effector cells. In contrast, mycobacterium-induced macrophage apoptosis as well as production of TNF, nitric oxide, and IL-12p40 were strongly inhibited in hu10Tg mice. Together, these data indicate that APC-derived IL-10 can exert a major inhibitory effect on control of mycobacterial infection by a mechanism involving the suppression of macrophage effector function and apoptosis.—Authors' Abstract

**Greenwell-Wild, T., Vazquez, N., Sim, D., Schito, M., Chatterjee, D., Orenstein, J. M., and Wahl, S. M.** *Mycobacterium avium* infection and modulation of human macrophage gene expression. *J. Immunol.* **169(11)** (2002) 6286–6297.

*Mycobacterium avium* is a facultative intracellular pathogen cleared rapidly via intact host defense mechanisms. In the absence of adequate T cell function, as occurs in HIV-1-induced immunodeficiency, *M. avium* becomes an opportunistic infection with uncontrolled replication and reinfection of macrophage hosts. How *M. avium* infects, survives, and replicates in macrophages without signaling an effective microbicidal counterattack is unresolved. To address whether *M. avium* signals the expression of molecules, which influence mycobacterial survival or clearance, human monocyte-derived macrophage cultures were exposed to *M. avium*. Within minutes, *M. avium*, or its cell wall lipoarabinomannan, binds to the adherent macrophages and induces a spectrum of gene expression. In this innate response, the most abundant genes detected within 2 hr by cDNA ex-

pression array involved proinflammatory chemokines, cytokines including TNF- $\alpha$  and IL-1, and adhesion molecules. Associated with this rapid initial up-regulation of recruitment and amplification molecules was enhanced expression of transcription factors and signaling molecules. By 24 hr, this proinflammatory response subsided, and after 4 days, when some bacteria were being degraded, others escaped destruction to replicate within intracellular vacuoles. Under these conditions, inducible NO synthase was not up-regulated and increased transferrin receptors may facilitate iron-dependent mycobacterial growth. Sustained adhesion molecule and chemokine expression along with the formation of multinucleated giant cells appeared consistent with *in vivo* events. Thus, in the absence of T lymphocyte mediators, macrophages are insufficiently microbicidal and provide a non-hostile environment in which mycobacteria not only survive and replicate, but continue to promote recruitment of new macrophages to perpetuate the infection.—Authors' Abstract

**Harboe, M., Christensen, A., Ahmad, S., Ulvund, G., Harkness, R. E., Mustafa, A. S., and Wiker, H. G.** Cross-reaction between mammalian cell entry (Mce) Proteins of *Mycobacterium tuberculosis*. *Scand. J. Immunol.* **56(6)** (2002) 580–587.

In addition to the previously cloned Mce1A and Mce1E genes of the Mce1 operon of *Mycobacterium tuberculosis* (Ahmed, *et al.* *Scand. J. Immunol.* 1999; 50: 510–8), Mce1B, Mce1D and Mce1F were cloned and expressed as glutathione-S-transferase (GST) fusion proteins in recombinant *Escherichia coli*. Polyclonal antibodies against a predicted B-cell epitope of each of the Mce1 proteins of *M. tuberculosis* were produced by immunizing rabbits with synthetic peptides coupled to keyhole limpet hemocyanin. These antibodies reacted specifically with the corresponding fusion protein, except for GST-Mce1F. A mouse clonal antibody, TB1-5 76C, raised against a synthetic 60-mer peptide corresponding to the residues 106–165 in the N-terminal part of Mce1A, reacted strongly

with GST-Mce1A. The antibody cross-reacted with GST-Mce1F, but not with the other recombinant GST-Mce1 fusion proteins or free GST. Bioinformatic analysis revealed only slight homology between Mce1A and Mce1F, along the length of the polypeptide chains. Higher homology was found between the residues 106–165 of Mce1A and the residues 347–406, further into the mature Mce1F polypeptide chain. There was a striking, localized homology, indicating that the epitope reacting with the monoclonal antibody TB1-5 76C may be narrowed to the KRRITPKD region, the residues 131–138 in Mce1A corresponding to the residues 372–379 in Mce1F. This was confirmed in enzyme-linked immunosorbent assay, showing binding of TB1-5 76C to a 17-mer synthetic peptide containing the KRRITPKD sequence.— Authors' Abstract

**Harmala, L. A., Ingulli, E. G., Curtsinger, J. M., Lucido, M. M., Schmidt, C. S., Weigel, B. J., Blazar, B. R., Mescher, M. F., and Pennell, C. A.** The adjuvant effects of *Mycobacterium tuberculosis* heat shock protein 70 result from the rapid and prolonged activation of antigen-specific CD8+ T cells *in vivo*. *J. Immunol.* **169(10)** (2002) 5622–5629.

Heat shock protein 70 (hsp70) is a potent adjuvant that links innate and adaptive immune responses. To study how hsp70 activates naive CD8(+) T cells *in vivo*, we tracked Ag-specific CD8(+) T cells in mice immunized with a fusion protein containing chicken OVA linked to hsp70 derived from *Mycobacterium tuberculosis* (OVA.TBhsp70). On a molar basis, OVA.TBhsp70 was several hundred times more effective than OVA peptide plus CFA in eliciting specific CD8(+) T cell responses. Immunization with OVA.TBhsp70 activated >90% of detectable OVA-specific CD8(+) T cells within 3 days and led to the persistence of cytotoxic effectors for at least 17 days. These studies demonstrate that the potent adjuvant effect of *M. tuberculosis* hsp70 results from the relatively complete, rapid, and durable activation of Ag-specific CD8(+) T cells.— Authors' Abstract

**Hoft, D. F., Worku, S., Kampmann, B., Whalen, C. C., Ellner, J. J., Hirsch, C. S., Brown, R. B., Larkin, R., Li, Q., Yun, H., and Silver, R. F.** Investigation of the relationships between immune-mediated inhibition of mycobacterial growth and other potential surrogate markers of protective *Mycobacterium tuberculosis* immunity. *J. Infect. Dis.* **186(10)** (2002) 1448–1457.

Tuberculosis (TB) vaccine development is hindered by the lack of clear surrogate markers of protective human immunity to *Mycobacterium tuberculosis*. This study evaluated the hypothesis that immune-mediated inhibition of mycobacterial growth would more directly correlate with protective TB immunity than other immunologic responses. Bacille Calmette-Guerin (BCG) vaccination, known to induce partial protection against TB, was used as a model system to investigate mechanistic relationships among different parameters of antigen-specific immunity. Effects of primary and booster intradermal BCG vaccinations were assessed in 3 distinct assays of mycobacterial inhibition. Correlations between vaccine-induced growth inhibition and other immune responses were analyzed. BCG significantly enhanced all antigen-specific responses. Peak responses occurred at 2 months after boosting. Statistical analyses suggested that each assay measured unique aspects of mycobacterial immunity. Despite previous evidence that type 1 immune responses are essential for TB immunity, interferon-gamma production did not correlate with mycobacterial inhibition. These results have important implications for TB vaccine development.— Authors' Abstract

**Jung, Y. J., LaCourse, R., Ryan, L., and North, R. J.** Evidence inconsistent with a negative influence of T helper 2 cells on protection afforded by a dominant T helper 1 response against *Mycobacterium tuberculosis* lung infection in mice. *Infect. Immun.* **70(11)** (2002) 6436–6443.

Mice incapable of generating an efficient Th2 response because of functional deletion

of the genes for signal transducer and activation of transcription 6 (Stat6), interleukin-4 receptor alpha chain (IL-4Ralpha), or IL-4 plus IL-13 (IL-4/IL-13) were no more resistant than wild-type (WT) mice to airborne infection with virulent *Mycobacterium tuberculosis*. WT mice were able to control infection and hold it at a stationary level following 20 days of log linear *M. tuberculosis* growth. Likewise, infection was kept under control and was held at the same stationary level in IL-4/IL-13(-/-) mice but progressed to a slightly higher level in Stat6(-/-) and IL-4Ralpha(-/-) mice. The onset of stationary-level infection in WT mice was associated with the expression of Th1-mediated immunity, as evidenced by an approximately 100- to 1000-fold increase in the lungs in the synthesis of mRNA for IL-12, gamma interferon (IFN-gamma), and inducible nitric oxide synthase (NOS2) that was sustained for at least 100 days. IL-12 is essential for the induction of Th1 immunity, IFN-gamma is a key Th1 cytokine involved in mediation of immunity, and NOS2 is an inducible enzyme of macrophages and is needed by these cells to express immunity. In response to infection, the lungs of Stat6(-/-) mice showed increases in synthesis of mRNA for IL-12, IFN-gamma, and NOS2 similar to that seen in WT mice. In IL-4/IL-13(-/-) mice, however, synthesis of mRNA for IFN-gamma and NOS2 reached higher levels than in WT mice. These results argue against the notion that a Th2 response is partly or wholly responsible for the inability of Th1-mediated immunity to resolve infection with a virulent strain of *M. tuberculosis*. — Authors' Abstract

**Kawahara, M., Nakasone, T., and Honda, M.** Dynamics of gamma interferon, interleukin-12 (IL-12), IL-10, and transforming growth factor beta mRNA expression in primary *Mycobacterium bovis* BCG infection in guinea pigs measured by a real-time fluorogenic reverse transcription-PCR assay. *Infect. Immun.* **70(12)** (2002) 6614–6620.

The guinea pig has been utilized as a model for studying infectious diseases because its reactions closely resemble those of humans biologically and immunologically.

However, the cytokine responses in this animal remain to be studied. Initially, we established a quantitative assay using a real-time reverse transcription-PCR (RT-PCR), to measure guinea pig gamma interferon (IFN-gamma), interleukin-12 (IL-12), IL-10, and transforming growth factor beta (TGF-beta) mRNA. By preparing primer-fluorogenic probe sets for these cytokines and standard RNA templates corresponding to the target sequence of each cytokine, we obtained linear standard curves essential for quantitative determination. In guinea pigs immunized by intradermal (i.d.) vaccination with the Tokyo strain of *Mycobacterium bovis* BCG (0.1 mg) or else hyperimmunized with the same vaccine (10 mg) given intravenously (i.v.), peripheral blood mononuclear cells (PBMCs) at 4 weeks showed an increase in IFN-gamma mRNA expression in the latter but not the former animals. However, at week 10, IFN-gamma mRNA expression was markedly elevated in PBMCs, spleen cells, and cells in bronchoalveolar lavage fluid in both the i.d.- and the i.v.-immunized animals, the level of expression being 10 times higher in the latter. In contrast, the expression levels of IL-12 mRNA in PBMCs, spleen cells, and BAL cells were not enhanced in either group at 10 weeks postimmunization. The expression of IL-10 and TGF-beta increased slightly only in PBMCs. Regardless of differences in the levels of cytokine responses, the magnitudes of the purified protein derivative of tuberculin-specific delayed-type hypersensitivity (DTH) skin reactions for the two groups did not differ significantly at 8 weeks postvaccination. In this study, we quantitatively measured IL-10, IL-12, TGF-beta, and IFN-gamma mRNA in BCG-immunized guinea pigs and showed that the level of IFN-gamma mRNA expression does not necessarily reflect the magnitude of the DTH response, suggesting that there may be an intricate relationship between protective immunity, the level of IFN-gamma, and the DTH response. Thus, our quantitative assay would be of use for the development of vaccines using guinea pig models. — Authors' Abstract

**Li, Y. J., Petrofsky, M., and Bermudez, L. E.** *Mycobacterium tuberculosis* uptake by recipient host macrophages is influ-

enced by environmental conditions in the granuloma of the infectious individual and is associated with impaired production of interleukin-12 and tumor necrosis factor alpha. *Infect. Immun.* **70**(11) (2002) 6223–6230.

Transmission of *Mycobacterium tuberculosis* from one individual to another usually is associated with episodes of coughing. The bacteria leave the environment of the lung cavity of the infected person and travel in droplets to reach the recipient's respiratory tract. Therefore, at the time that the bacteria encounter alveolar cells (macrophages and epithelial cells) in the new host, they express virulence determinants that are regulated by the environmental conditions in the infected person. To determine if those environmental conditions encountered in the lung cavity (hyperosmolarity, acidic pH, and low oxygen tension, among others) would influence the uptake of *M. tuberculosis* by the recipient's alveolar macrophages, *M. tuberculosis* H37Rv was incubated under several conditions for different periods of time, washed at 4 degrees C, and used to infect human monocyte-derived macrophages. While increased osmolarity had no effect on *M. tuberculosis* uptake compared to the uptake of bacteria grown on 7H10 Middlebrook medium, both acidic pH and anaerobiosis increased the uptake of the H37Rv strain four- to sixfold. Using anti-CD11b receptor blocking antibodies or mannoside to inhibit the uptake of *M. tuberculosis* by macrophages, we determined that while uptake of *M. tuberculosis* cultured on 7H10 medium was inhibited 77%  $\pm$  6% in the presence of anti-CD11b antibody, the antibody had no effect on the uptake of *M. tuberculosis* incubated at pH 6.0 and was associated with 27% inhibition of *M. tuberculosis* previously exposed to anaerobic conditions. The mannose receptor was also not involved with invasion after exposure to acidic conditions, and mannoside resulted in only 32% inhibition of uptake by macrophages of *M. tuberculosis* exposed to anaerobiosis. Uptake by macrophages also resulted in the secretion of significantly lower amounts of interleukin-12 and tumor necrosis factor alpha than that by macrophages infected with a strain cultured under laboratory conditions. *M. tuberculo-*

*sis* cultured under the pH and oxygen concentration found in the granuloma expresses a large number of proteins that are different from the proteins expressed by bacteria grown under laboratory conditions. The results suggest that *M. tuberculosis in vivo* may be adapted to gain access to the intracellular environment in a very efficient fashion and may do so by using different receptors from the complement and mannose receptors.—Authors' Abstract

**Maeda, N., Nigou, J., Herrmann, J. L., Jackson, M., Amara, A., Langrange, P. H., Puzo, G., Gicquel, B., and Neyrolles, O. J.** The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *J. Biol. Chem.* **278**(8) (2003) 5513–5516.

Interactions between dendritic cells (DCs) and *Mycobacterium tuberculosis*, the etiological agent of tuberculosis, are most likely to play a key role in anti-mycobacterial immunity. We have recently shown that *M. tuberculosis* binds to, and infects DCs through ligation of the DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN), and that *M. tuberculosis* mannose-capped lipoarabinomannan (ManLAM) inhibits binding of the bacilli to the lectin, suggesting that ManLAM might be a key DC-SIGN ligand. In the present study, we investigated the molecular basis of DC-SIGN ligation by LAM. Contrarily to what found for slow-growing mycobacteria, such as *M. tuberculosis* and the vaccine strain *Mycobacterium bovis* bacillus Calmette-Gurin (BCG), our data demonstrate that the fast-growing saprophytic species *Mycobacterium smegmatis* hardly binds to DC-SIGN. Consistent with the former finding, we show that *M. smegmatis*-derived lipoarabinomannan, that is capped by phosphoinositide residues (PILAM), exhibits a limited ability to inhibit *M. tuberculosis* binding to DC-SIGN. Moreover, using enzymatically demannosylated and chemically deacylated ManLAM molecules, we demonstrate that both the acyl chains on the ManLAM mannosyl-phosphatidylinositol anchor and the manno oligosaccharide caps play a critical

role in DC-SIGN-ManLAM interaction. Finally, we report that DC-SIGN binds poorly to the PILAM-containing species *Mycobacterium fortuitum* and *Mycobacterium chelonae*. Interestingly, smooth colony-forming *Mycobacterium avium*, in which ManLAM is capped with single mannose residues, was also poorly recognized by the lectin. Altogether, our results provide molecular insight into the mechanisms of mycobacteria-DC-SIGN interaction, and suggest that DC-SIGN may act as a pattern recognition receptor (PRR) and discriminate between *Mycobacterium* species through selective recognition of the mannose caps on LAM molecules.—Authors' Abstract

**Mariotti, S., Teloni, R., Iona, E., Fat-torini, L., Giannoni, F., Romagnoli, G., Orefici, G., and Nisini, R.** *Mycobacterium tuberculosis* subverts the differentiation of human monocytes into dendritic cells. *Eur. J. Immunol.* **32(11)** (2002) 3050–3058.

Intracellular pathogens have developed strategies for evading elimination by the defenses of the host immune system. Here we describe an escape mechanism utilized by *Mycobacterium tuberculosis* that involves the interference with the generation of fully competent DC from monocytes. We show that monocytes infected with live *M. tuberculosis* differentiated into mature, CD83+ and CCR7+ DC (Mt-MoDC), but were characterized by a selective failure in the expression of the family of CD1 molecules. These cells also showed levels of MHC class II and CD80 (B7.1) that were reduced in comparison with LPS-matured DC. In addition, Mt-MoDC produced TNF-alpha and IL-10, but were unable to secrete IL-12. The generation of Mt-MoDC required the infection of monocytes with live *M. tuberculosis*, since infection with heat-killed bacteria partially abrogated the effects on monocyte differentiation. Interestingly, Mt-MoDC revealed an impaired antigen-presentation function as assessed by the reduced capability to induce proliferation of cord blood T lymphocytes. Further, naive T lymphocytes expanded by Mt-MoDC were unable to secrete cytokines, in particular

IL-4 and IFN-gamma, suggesting that they could be ineffective in helping the macrophage-mediated killing of intracellular mycobacteria. Our results suggest that the interference with monocyte differentiation into fully competent DC is an evasion mechanism of *M. tuberculosis* that could contribute to its intracellular persistence by avoiding immune recognition.—Authors' Abstract

**Mueller-Ortiz, S. L., Sepulveda, E., Olsen, M. R., Jagannath, C., Wanger, A. R., and Norris, S. J.** Decreased infectivity despite unaltered C3 binding by a DeltahbA mutant of *Mycobacterium tuberculosis*. *Infect. Immun.* **70(12)** (2002) 6751–6760.

HbhA of *Mycobacterium tuberculosis* is a multifunctional binding protein, binding to both sulfated sugars such as heparin and to human complement component C3. HbhA may therefore interact with host molecules and/or host cells during *M. tuberculosis* infection and play a role in the pathogenesis of this bacterium. The purpose of this study was to use allelic exchange to create an *M. tuberculosis* strain deficient in expression of HbhA to determine whether this protein's C3-binding activity plays a role in the pathogenesis of *M. tuberculosis*. An in-frame, 576-bp unmarked deletion in the hbhA gene was created using sacB as a counterselectable marker. Southern blotting and PCR analyses confirmed deletion of hbhA in the DeltahbA mutant. The DeltahbA mutant strain grew at a rate similar to that of the parent in broth culture and in J774.A1 murine macrophage-like cells but was deficient in growth compared to the parent strain in the lungs, liver, and spleen of infected mice. In addition, the DeltahbA mutation did not reduce binding of *M. tuberculosis* to human C3 or to J774.A1 cells in the presence or absence of serum, suggesting that in the absence of HbhA, other molecules serve as C3-binding molecules on the *M. tuberculosis* surface. Taken together, these data indicate that HbhA is important in the infectivity of *M. tuberculosis*, but its ability to bind C3 is not required for mycobacterial adherence to macrophage-like cells. Using the DeltahbA mutant

strain, a second *M. tuberculosis* C3-binding protein similar in size to HbhA was identified as HupB, but the role of HupB as a C3-binding protein in intact organisms remains to be determined.—Authors' Abstract

**Riendeau, C. J. and Kornfeld, H.** THP-1 cell apoptosis in response to Mycobacterial infection. *Infect. Immun.* **71(1)** (2003) 254–259.

We previously reported that *Mycobacterium tuberculosis* infection primes human alveolar macrophages (HAM) for tumor necrosis factor alpha (TNF-alpha)-mediated apoptosis and that macrophage apoptosis is associated with killing internalized bacilli. Virulent mycobacterial strains elicit much less apoptosis than attenuated strains, implying that apoptosis is a defense against intracellular infection. The present study evaluated the potential for phorbol myristate acetate-differentiated THP-1 cells to mimic this response of primary macrophages. Consistent with the behavior of alveolar macrophages, attenuated *M. tuberculosis* H37Ra and *Mycobacterium bovis* BCG strongly induce THP-1 apoptosis, which requires endogenous TNF. THP-1 apoptosis is associated with reduced viability of infecting BCG. In contrast, virulent wild-type *M. tuberculosis* H37Rv and *M. bovis* do not increase THP-1 apoptosis over baseline. BCG induced early activation of caspase 10 and 9, followed by caspase 3. In contrast, wild-type *M. bovis* infection failed to activate any caspases in THP-1 cells. BCG-induced THP-1 apoptosis is blocked by retroviral transduction with vectors expressing crmA but not bcl-2. We conclude that differentiated THP-1 cells faithfully model the apoptosis response of HAM. Analysis of the THP-1 cell response to infection with virulent mycobacteria suggests that TNF death signals are blocked proximal to initiator caspase activation, at the level of TNF receptor 1 or its associated intracytoplasmic adaptor complex. Interference with TNF death signaling may be a virulence mechanism that allows *M. tuberculosis* to circumvent innate defenses leading to apoptosis of infected host cells.—Authors' Abstract

**Saunders, B. M., Frank, A. A., Orme, I. M., and Cooper, A. M.** CD4 is required for the development of a protective granulomatous response to pulmonary tuberculosis. *Cell. Immunol.* **216(1–2)** (2002) 65–72.

To confirm the primary role of CD4 T cells in pulmonary tuberculosis, mice with a disruption of their CD4 gene (CD4 KO) were exposed to an aerosol of *Mycobacterium tuberculosis* and survival, cellular responses in the lung and granuloma development followed. CD8 and NK cells from the lungs of infected CD4 KO mice expressed IFN-gamma and were recruited in numbers similar to those seen in the C57BL/6 mice; recruitment correlated with initial control of bacteria. The major defect in mice lacking CD4 was the significant reduction in total cellular recruitment into the lungs. CD4 KO mice did not generate the typical mononuclear granulomatous lesions, instead the cellular influx was macrophage in character and was localized as perivascular cuffing. Early control of *M. tuberculosis* growth is therefore independent of CD4+ cells but such cells are required to ensure recruitment of mononuclear cells to the lung and thus ensure long-term survival.—Authors' Abstract

**Schaible, U. E., Collins, H. L., Priem, F., and Kaufmann, S. H.** Correction of the iron overload defect in beta-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis. *J. Exp. Med.* **196(11)** (2002) 1507–1513.

As a resident of early endosomal phagosomes, *Mycobacterium tuberculosis* is connected to the iron uptake system of the host macrophage. beta-2-microglobulin (beta2m) knockout (KO) mice are more susceptible to tuberculosis than wild-type mice, which is generally taken as a proof for the role of major histocompatibility complex class I (MHC-I)-restricted CD8 T cells in protection against *M. tuberculosis*. However, beta2m associates with a number of MHC-I-like proteins, including HFE. This protein regulates transferrin receptor mediated iron

uptake and mutations in its gene cause hereditary iron overload (hemosiderosis). Accordingly, beta2m-deficient mice suffer from tissue iron overload. Here, we show that modulating the extracellular iron pool in beta2m-KO mice by lactoferrin treatment significantly reduces the burden of *M. tuberculosis* to numbers comparable to those observed in MHC class I-KO mice. In parallel, the generation of nitric oxide impaired in beta2m-KO mice was rescued. Conversely, iron overload in the immunocompetent host exacerbated disease. Consistent with this, iron deprivation in infected resting macrophages was detrimental for intracellular mycobacteria. Our data establish: (a) defective iron metabolism explains the increased susceptibility of beta2m-KO mice over MHC-I-KO mice, and (b) iron overload represents an exacerbating cofactor for tuberculosis. — Authors' Abstract

**Scott, H. M. and Flynn, J. L.** *Mycobacterium tuberculosis* in chemokine receptor 2-deficient mice: influence of dose on disease progression. *Infect. Immun.* **70(11)** (2002) 5946–5954.

Within a *Mycobacterium tuberculosis*-induced granuloma, lymphocytes and macrophages work together to control bacterial growth and limit the spread of infection. Chemokines and chemokine receptors are involved in cell migration and are logical candidates for a role in granuloma formation. In the present study we addressed the role of CC chemokine receptor 2 (CCR2) in *M. tuberculosis* infection. In previous studies (W. Peters, *et al.*, *Proc. Natl. Acad. Sci. USA* 98:7958–7963, 2001), CCR2(–/–) mice were found to be highly susceptible to a moderate or high dose of H37Rv administered intravenously (i.v.). We have expanded those studies to demonstrate that the susceptibility of CCR2(–/–) mice is dose dependent. After low-dose aerosol or i.v. infection of CCR2(–/–) mice with *M. tuberculosis*, there was a substantial delay in cell migration to the lungs and delayed expression of gamma interferon and inducible nitric oxide synthase. The CCR2(–/–) mice had a severe and prolonged deficiency in

the number of macrophages in the lungs and an early increase in the number of neutrophils. Despite these deficiencies in cell migration, the CCR2(–/–) mice did not have increased bacterial loads in the lungs compared to wild-type (C57BL/6) mice and successfully formed granulomas. This finding is in contrast to CCR2(–/–) mice infected with a high dose of *M. tuberculosis* administered i.v. These results indicate that with low-dose infection, a delay in immune response in the lungs does not necessarily have detrimental long-term effects on the progression of the disease. The fact that CCR2(–/–) mice survive with substantially fewer macrophages in the low-dose models implies that the immune response to low-dose *M. tuberculosis* infection in mice is more robust than necessary to control the infection. Finally, these data demonstrate that, in cases of infectious disease in knockout models, clear phenotypes may not be evident when one is solely evaluating bacterial numbers and survival. Functional assays may be necessary to reveal roles for components of the multifactorial immune system. — Authors' Abstract

**Trajkovic, V., Singh, G., Singh, B., Singh, S., and Sharma, P.** Effect of *Mycobacterium tuberculosis*-specific 10-kilodalton antigen on macrophage release of tumor necrosis factor alpha and nitric oxide. *Infect. Immun.* **70(12)** (2002) 6558–6566.

Secreted proteins of *Mycobacterium tuberculosis* are major targets of the specific immunity in tuberculosis and constitute promising candidates for the development of more efficient vaccines and diagnostic tests. We show here that *M. tuberculosis*-specific antigen 10 (MTSA-10, originally designated CFP-10) can bind to the surface of mouse J774 macrophage-like cells and stimulate the secretion of the proinflammatory cytokine tumor necrosis factor alpha (TNF-alpha). MTSA-10 also synergized with gamma interferon (IFN-gamma) for the induction of the microbicidal free radical nitric oxide (NO) in J774 cells, as well as in bone marrow-derived and peritoneal macrophages. On the other hand, pretreatment of J774 cells with

MTSA-10 markedly reduced NO but not TNF-alpha or interleukin 10 (IL-10) release upon subsequent stimulation with lipopolysaccharide or the cell lysate of *M. tuberculosis*. The presence of IFN-gamma during stimulation with *M. tuberculosis* lysate antagonized the desensitizing effect of MTS-10 pretreatment on macrophage NO production. The activation of protein tyrosine kinases (PTK) and the serine/threonine kinases p38 MAPK and ERK was apparently required for MTS-10 induction of TNF-alpha and NO release, as revealed by specific kinase inhibitors. However, only p38 MAPK activity, not PTK or ERK activity, was partly responsible for MTS-10-mediated macrophage desensitization. The modulation of macrophage function by MTS-10 suggests a novel mechanism for its involvement in immunopathogenesis of tuberculosis and might have implications for the prevention, diagnosis, and therapy of this disease.—Authors' Abstract

**Turner, J., Gonzalez-Juarrero, M., Ellis, D. L., Basaraba, R. J., Kipnis, A., Orme, I. M., and Cooper, A. M.** *In vivo* IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. *J. Immunol.* **169(11)** (2002) 6343–6351.

The production of immunosuppressive cytokines, such as IL-10 and TGF-beta, has been documented in individuals diagnosed with active tuberculosis. In addition, IL-10 production is increased within the lungs of mice that have chronic mycobacterial infection. Therefore, we hypothesized that the down-regulatory properties of IL-10 might contribute to the reactivation of chronic *Mycobacterium tuberculosis* infection in mice. To determine the influence of IL-10 on the course of infection, transgenic mice producing increased amounts of IL-10 under the control of the IL-2 promoter were infected with *M. tuberculosis* via the respiratory route. Mice that overexpressed IL-10 showed no increase in susceptibility during the early stages of infection, but during the chronic phase of the infection showed evidence of reactivation tuberculosis with a highly significant increase in bacterial numbers

within the lungs. Reactivation was associated with the formation of macrophage-dominated lesions, decreased mRNA production for TNF and IL-12p40, and a decrease in Ag-specific IFN-gamma secretion. These data support the hypothesis that IL-10 plays a pivotal role during the chronic/latent stage of pulmonary tuberculosis, with increased production playing a potentially central role in promoting reactivation tuberculosis.—Authors' Abstract

**Vankayalapati, R., Wizel, B., Weis, S. E., Klucar, P., Shams, H., Samten, B., and Barnes, P. F.** Serum cytokine concentrations do not parallel *Mycobacterium tuberculosis*-induced cytokine production in patients with tuberculosis. *Clin. Infect. Dis.* **36(1)** (2003) 24–28.

We measured serum cytokine concentrations and *Mycobacterium tuberculosis*-stimulated cytokine production by peripheral blood mononuclear cells (PBMCs) obtained from persons infected with *M. tuberculosis*. Serum interferon-gamma (IFN-gamma) and interleukin-10 (IL-10) concentrations were elevated in patients with tuberculosis compared with healthy persons who had reactions to tuberculin skin tests, but IL-18 concentrations were not. In contrast, *M. tuberculosis*-stimulated PBMCs from patients with tuberculosis produced less IFN-gamma and IL-18 but similar amounts of IL-10, compared with PBMCs from healthy subjects who had reactions to tuberculin skin tests. Pretreatment of PBMCs from healthy subjects with reaction to tuberculin with serum from patients with tuberculosis inhibited IFN-gamma production in response to *M. tuberculosis*, and inhibition was blocked by anti-IL-10. Thus, serum concentrations of IFN-gamma, IL-18, and IL-10 do not parallel *M. tuberculosis*-induced cytokine levels, and increased IL-10 serum levels in patients with tuberculosis inhibit IFN-gamma production in response to mycobacterial antigens.—Authors' Abstract

**Weatherby, K. E., Zwilling, B. S., and Lafuse, W. P.** Resistance of macro-

phages to *Mycobacterium avium* is induced by alpha(2)-adrenergic stimulation. *Infect. Immun.* **71(1)** (2003) 22–29.

The ability of macrophages to control the growth of microorganisms is increased by macrophage activation. Previously, it was shown that epinephrine activated mouse macrophages to resist the growth of *Mycobacterium avium* via alpha(2)-adrenergic stimulation. In the present study, we show that the alpha(2)-adrenergic agonist (alpha(2)-agonist) clonidine induced resistance to *M. avium* growth in the RAW264.7 mouse macrophage cell line. The ability of catecholamines to induce resistance to mycobacteria was specific to alpha(2)-adrenergic stimulation, as alpha(1)-, beta(1)-, and beta(2)-agonists had no effect. Receptor signaling through Gi proteins was required. A G-protein antagonist specific for the alpha subunits of the Go/Gi family blocked the increased resistance induced by clonidine, while a Gs-protein antagonist was

without effect. Both nitric oxide (NO) production and superoxide (O(2)(-)) production were required for the increased resistance to *M. avium* growth induced by clonidine. Although NO production was required, clonidine did not increase the level of NO in *M. avium*-infected cells. Since NO and O(2)(-) interact to produce peroxynitrite (ONOO(-)), we examined whether ONOO(-) mediates the increased resistance to *M. avium* induced by clonidine. 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrinato iron (III) chloride (FeTPPS), a specific scavenger of ONOO(-), inhibited the effect of clonidine on *M. avium* growth. Clonidine also increased the production of ONOO(-) in *M. avium*-infected RAW264.7 cells, as measured by the oxidation of 123-dihydrorhodamine and the production of nitrated tyrosine residues. We therefore conclude that alpha(2)-adrenergic stimulation activates macrophages to resist the growth of *M. avium* by enhancing the production of ONOO(-).—Authors' Abstract

## Microbiology

**Goulding, C. W., Parseghian, A., Sawaya, M. R., Cascio, D., Apostol, M. I., Genaro, M. L., and Eisenberg, D.** Crystal structure of a major secreted protein of *Mycobacterium tuberculosis*-MPT63 at 1.5-Å resolution. *Protein Sci.* **11(12)** (2002) 2887–2893.

MPT63 is a small, major secreted protein of unknown function from *Mycobacterium tuberculosis* that has been shown to have immunogenic properties and has been implicated in virulence. A BLAST search identified that MPT63 has homologs only in other mycobacteria, and is therefore mycobacteria specific. As MPT63 is a secreted protein, mycobacteria specific, and implicated in virulence, MPT63 is an attractive drug target against the deadliest infectious disease, tuberculosis (TB). As part of the TB Structural Genomics Consortium, the X-ray crystal structure of MPT63 was determined to 1.5-Å resolution with the hope of yielding functional information about MPT63. The structure of MPT63 is an antiparallel beta-sandwich immunoglob-

ulin-like fold, with the unusual feature of the first beta-strand of the protein forming a parallel addition to the small antiparallel beta-sheet. MPT63 has weak structural similarity to many proteins with immunoglobulin folds, in particular, Homo sapiens beta2-adaptin, bovine arrestin, and Yersinia pseudotuberculosis invasin. Although the structure of MPT63 gives no conclusive evidence to its function, structural similarity suggests that MPT63 could be involved in cell-host interactions to facilitate endocytosis/phagocytosis.—Authors' Abstract

**Le Dantec, C., Duguet, J. P., Montiel, A., Dumoutier, N., Dubrou, S., and Vincent, V.** Occurrence of mycobacteria in water treatment lines and in water distribution systems. *Appl. Environ. Microbiol.* **68(11)** (2002) 5318–5325.

The frequency of recovery of atypical mycobacteria was estimated in two treatment plants providing drinking water to Paris, France, at some intermediate stages

of treatment. The two plants use two different filtration processes, rapid and slow sand filtration. Our results suggest that slow sand filtration is more efficient for removing mycobacteria than rapid sand filtration. In addition, our results show that mycobacteria can colonize and grow on granular activated carbon and are able to enter distribution systems. We also investigated the frequency of recovery of mycobacteria in the water distribution system of Paris (outside buildings). The mycobacterial species isolated from the Paris drinking water distribution system are different from those isolated from the water leaving the treatment plants. Saprophytic mycobacteria (present in 41.3% of positive samples), potentially pathogenic mycobacteria (16.3%), and unidentifiable mycobacteria (54.8%) were isolated from 12 sites within the Paris water distribution system. *Mycobacterium gordonae* was preferentially recovered from treated surface water, whereas *Mycobacterium nonchromogenicum* was preferentially recovered from groundwater. No significant correlations were found among the presence of mycobacteria, the origin of water, and water temperature.— Authors' Abstract

**Mijs, W., De Vreese, K., Devos, A., Pottel, H., Valgaeren, A., Evans, C., Norton, J., Parker, D., Rigouts, L., Portaels, F., Reischl, U., Watterson, S., Pfyffer, G., and Rossau, R.** Evaluation of a commercial line probe assay for identification of *Mycobacterium* species from liquid and solid culture. *Eur. J. Clin. Microbiol. Infect. Dis.* **21(11)** (2002) 794–802.

The performance of a commercial line probe assay (LiPA) (Inno-LiPA Mycobacteria; Innogenetics, Belgium) for the detection and identification of *Mycobacterium* species from liquid and solid culture was evaluated at five routine clinical laboratories. The LiPA method is based on the reverse hybridization principle, in which the mycobacterial 16S-23S ribosomal RNA (rRNA) spacer region is amplified by polymerase chain reaction (PCR). Amplicons are subsequently hybridized with oligonucleotide probes arranged on a membrane strip and detected by a colorimetric system. The test detects the presence of *Mycobacte-*

*rium* species and specifically identifies *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, *Mycobacterium xenopi*, *Mycobacterium gordonae*, *Mycobacterium avium* complex, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum*, and *Mycobacterium chelonae-Mycobacterium abscessus* complex. The results of LiPA were compared with the results obtained using traditional biochemical and molecular tests (DNA probe-based techniques, PCR restriction enzyme analysis of the 65 kDa heat-shock protein gene, and sequencing of the 16S rDNA). A total of 669 isolates, 642 of which were identified as *Mycobacterium* species and 27 as non-*Mycobacterium* species, were tested by LiPA. After analysis of 14 initially discordant results and exclusion of one isolate, concordant results were obtained for 636 of 641 *Mycobacterium* isolates (99.2% accuracy). All *Mycobacterium* species reacted with the MYC (*Mycobacterium* species) probe (100% sensitivity), and all non-*Mycobacterium* species were identified as such (100% specificity).— Authors' Abstract

**Mougous, J. D., Leavell, M. D., Senaratne, R. H., Leigh, C. D., Williams, S. J., Riley, L. W., Leary, J. A., and Bertozzi, C. R.** Discovery of sulfated metabolites in mycobacteria with a genetic and mass spectrometric approach. *Proc. Natl. Acad. Sci. U. S. A.* **99(26)** (2002) 17037–17042.

The study of the metabolome presents numerous challenges, first among them being the cataloging of its constituents. A step in this direction will be the development of tools to identify metabolites that share common structural features. The importance of sulfated molecules in cell-cell communication motivated us to develop a rapid two-step method for identifying these metabolites in microorganisms, particularly in pathogenic mycobacteria. Sulfur-containing molecules were initially identified by mass spectral analysis of cell extracts from bacteria labeled metabolically with a stable sulfur isotope ( $^{34}\text{S}$  4 2–). To differentiate sulfated from reduced-sulfur-containing molecules, we employed a mutant lacking

the reductive branch of the sulfate assimilation pathway. In these sulfur auxotrophs, heavy sulfate is channeled exclusively into sulfated metabolites. The method was applied to the discovery of several new sulfated molecules in *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. Be-

cause a sulfur auxotrophic strain is the only requirement of the approach, many microorganisms can be studied in this manner. Such genetic engineering in combination with stable isotopic labeling can be applied to various metabolic pathways and their products.—Authors' Abstract

## Microbiology (Leprosy)

**Guerrero, M., Arias, M. T., Garces, M. T., and Leon, C. I.** Developing and using a PCR test to detect subclinical *Mycobacterium leprae* infection. *J. Public Health* **11(4)** (2002) 228–234.

**Objective:** The objective of this study was to investigate the use of a polymerase chain reaction (PCR) test to detect *M. leprae* in samples of nasal mucus from asymptomatic household contacts of patients with leprosy. **Methods:** We standardized and optimized a PCR technique to amplify a 321-base pair DNA fragment using a pair of primers complementary to a segment of an LSR/A15 gene that codes for the 15 kDa *M. leprae* antigen. We investigated the optimal concentrations of all the test components. We used dimethyl sulfoxide (DMSO) to achieve a more specific amplification. We applied the PCR test to 70 healthy household contacts of leprosy patients from 8 municipalities in Colombia where there was a high prevalence of the disease. **Results:** The test's detection limit was 100 fg of DNA. With the optimized technique, bacilli were detected in the nasal mucus samples of 9 (12.8%) of the 70 household contacts. The 3 PCR-positive household contacts of paucibacillary cases were from municipalities with very high prevalence levels. In comparison to contacts who were PCR-negative, the contacts who were PCR-positive had spent significantly less time, as a proportion of their age, living with a patient ( $p = 0.028$ ). This finding demonstrates the test's capacity for early detection. **Conclusions:** The PCR test that we developed is useful as a tool for detection and early follow-up of possible leprosy cases. It can be used to monitor high-risk populations and also to maintain the achievements of leprosy elim-

ination programmes in countries where the disease's prevalence has been significantly reduced.—Tropical Diseases Bulletin

**Hagge, D. A., Oby Robinson, S., Scollard, D., McCormick, G., and Williams, D. L.** A new model for studying the effects of *Mycobacterium leprae* on Schwann cell and neuron interactions. *J. Infect. Dis.* **186(9)** (2002) 1283–1296.

Millions of patients with leprosy suffer from nerve damage resulting in disabilities as a consequence of *Mycobacterium leprae* infection. However, mechanisms of nerve damage have not been elucidated because of the lack of a model that maintains *M. leprae* viability and mimics disease conditions. A model was developed using viable *M. leprae*, rat Schwann cells, and Schwann cell-neuron cocultures incubated at 33 degrees C. *M. leprae* retained 56% viability in Schwann cells for 3 weeks after infection at 33 degrees C, compared with 3.6% viability at 37 degrees C. Infected Schwann cells had altered morphology and expression of genes encoding cellular adhesion molecules at 33 degrees C but were capable of interacting with and myelinating neurons. Cocultures, infected after myelination occurred, showed no morphological changes in myelin architecture after 1 month of incubation at 33 degrees C, and *M. leprae* retained 53% viability. This article describes a new model for studying the effects of *M. leprae* on Schwann cells.—Authors' Abstract

**Paige, C. F., Scholl, D. T., and Truman, R. W.** Prevalence and incidence density of *Mycobacterium leprae* and *Trypanosoma*

*cruzi* infections within a population of wild nine-banded armadillos. Am. J. Trop. Med. Hyg. **67**(5) (2002) 528–532.

A total of 415 wild 9-banded armadillos from the East Atchafalaya River Levee (Point Coupee, LA) were collected over 4 yrs to estimate the incidence and prevalence of *Mycobacterium leprae* and *Trypanosoma cruzi* and to discern any relationship between the 2 agents. *M. leprae* infections were maintained at a high steady prevalence rate year to year averaging 19%. *T. cruzi* antibody prevalence remained relatively low, averaging 3.9%, and varied markedly between years. Prevalence rates were independent, with only 3 armadillos coinfecting with both agents. *M. leprae* incidence density ranged from 0.47 to 3.5 cases per 1,000 animal-days, depending on case definition, confirming active intense transmission of *M. leprae* among armadillos. No incident *T. cruzi* cases were found. These infections seem to occur independently and may be used in comparisons to understand better factors that may influence transmission of these agents.—Authors' Abstract

**Visca, P., Fabozzi, G., Milani, M., Bolognesi, M., and Ascenzi, P.** Nitric oxide and *Mycobacterium leprae* pathogenicity. IUBMB Life **54**(3) (2002) 95–99.

Leprosy is an old, still dreaded infectious disease caused by the obligate intracellular bacterium *Mycobacterium leprae*. During the infectious process, *M. leprae* is faced with the host macrophagic environment, where the oxidative stress and NO release, combined with low pH, low pO<sub>2</sub>, and high pCO<sub>2</sub>, contribute to limit the growth of the bacilli. Comparative genomics has unraveled massive gene decay in *M. leprae*, linking the strictly parasitic lifestyle with the reductive genome evolution. Compared with *Mycobacterium tuberculosis* and *Mycobacterium bovis*, the leprosy bacillus has lost most of the genes involved in the detoxification of reactive oxygen and nitrogen species. The very low reactivity of the unique truncated hemoglobin retained by *M. leprae* could account for the susceptibility of this exceptionally slow-growing microbe to NO.—Authors' Abstract

## Microbiology (Tuberculosis)

**Ahmad, S., Mokaddas, E., and Fares, E.**

Characterization of rpoB mutations in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates from Kuwait and Dubai. Diagn. Microbiol. Infect. Dis. **44**(3) (2002) 245–252.

Mutations conferring resistance to rifampin in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates occur mostly in the 81 bp rifampin-resistance-determining region (RRDR) of the rpoB gene. In this study, 29 rifampin-resistant and 12 susceptible clinical *M. tuberculosis* isolates were tested for characterization of mutations in the rpoB gene by line probe (INNO-LiPA Rif. TB) assay and the results were confirmed and extended by DNA sequencing of the PCR amplified target DNA. The line probe assay identified all 12 susceptible strains as rifampin-sensitive and

the DNA sequence of RRDR in the amplified rpoB gene from two isolates matched perfectly with the wild-type sequence. The line probe assay identified 28 resistant isolates as rifampin-resistant with specific detection of mutation in 22 isolates including one isolate that exhibited hetro-resistance containing both the wild-type pattern as well as a specific mutation within RRDR while one of the rifampin-resistant strain was identified as rifampin-susceptible. DNA sequencing confirmed these results and, in addition, led to the specific detection of mutations in 5 rifampin-resistant isolates in which specific base changes within RRDR could not be determined by the line probe assay. These analyses identified 8 different mutations within RRDR of the rpoB gene including one novel mutation (S522W) that has not been reported so far. The genotyping performed on the isolates

carrying similar mutations showed that majority of these isolates were unique as they exhibited varying DNA banding patterns. Correlating the ethnic origin of the infected TB patients with the occurrence of specific mutations at three main codon positions (516, 526 and 531) in the *rpoB* gene showed that most patients (11 of 15) from South Asian region contained mutations at codon 526 while majority of isolates from patients (6 of 11) of Middle Eastern origin contained mutations at codon 531.—Authors' Abstract

**Boon, C. and Dick, T.** *Mycobacterium bovis* BCG response regulator essential for hypoxic dormancy. *J. Bacteriol.* **184(24)** (2002) 6760–6767.

Obligately aerobic tubercle bacilli are capable of adapting to survive hypoxia by developing into a nonreplicating or dormant form. Dormant bacilli maintain viability for extended periods. Furthermore, they are resistant to antimycobacterials, and hence, dormancy might play a role in the persistence of tuberculosis infection despite prolonged chemotherapy. Previously, we have grown dormant *Mycobacterium bovis* BCG in an oxygen-limited Wayne culture system and subjected the bacilli to proteome analysis. This work revealed the upregulation of the response regulator Rv3133c and three other polypeptides (alpha-crystallin and two “conserved hypothetical” proteins) upon entry into dormancy. Here, we replaced the coding sequence of the response regulator with a kanamycin resistance cassette and demonstrated that the loss-of-function mutant died after oxygen starvation-induced termination of growth. Thus, the disruption of this dormancy-induced transcription factor resulted in loss of the ability of BCG to adapt to survival of hypoxia. Two-dimensional gel electrophoresis of protein extracts from the gene-disrupted strain showed that the genetic loss of the response regulator caused loss of the induction of the other three dormancy proteins. Thus, the upregulation of these dormancy proteins requires the response regulator. Based on these two functions, dormancy survival and regulation, we named the Rv3133c gene *dosR* for dormancy survival

regulator. Our results provide conclusive evidence that *DosR* is a key regulator in the oxygen starvation-induced mycobacterial dormancy response.—Authors' Abstract

**Garcia-Quintanilla, A., Gonzalez-Martin, J., Tudo, G., Espasa, M., and Jimenez de Anta, M. T.** Simultaneous identification of *Mycobacterium genus* and *Mycobacterium tuberculosis* complex in clinical samples by 5'-exonuclease fluorogenic PCR. *J. Clin. Microbiol.* **40(12)** (2002) 4646–4651.

Early diagnosis of tuberculosis and screening of other mycobacteria is required for the appropriate management of patients. We have therefore developed a 5'-exonuclease fluorogenic PCR assay in a single-tube balanced heminested format that simultaneously detects *Mycobacterium tuberculosis* complex (MTC) and members of the *Mycobacterium genus* (MYC) using the 16S ribosomal DNA target directly on clinical samples. One hundred twenty-seven clinical samples (65 smear negative and 62 smear positive) with a positive culture result from 127 patients were tested, including 40 negative control specimens. The finding of both a positive MTC and probe value and a positive MYC probe value confirmed the presence of MTC or mycobacteria with a 100% positive predictive value. However, a negative value for MTC or MYC did not discount the presence of mycobacteria in the specimen. Interestingly, the addition of the MYC probe allowed the diagnosis of an additional 7% of patients with tuberculosis and rapid screening of nontuberculous mycobacteria (NTM). Thus, over 75% of the patients were diagnosed with mycobacterial disease by PCR. The sensitivity was much higher on smear-positive samples (90.3%) than smear-negative samples (49.2%) and was slightly higher for MTC than NTM samples. With regard to the origin of the sample, MTC pulmonary samples gave better results than others. In conclusion, we believe this test may be useful for the rapid detection of mycobacteria in clinical samples and may be a valuable tool when used together with conventional methods and the clinical data available.—Authors' Abstract

**Han, X. Y., Pham, A. S., Tarrand, J. J., Sood, P. K., and Luthra, R.** Rapid and accurate identification of mycobacteria by sequencing hypervariable regions of the 16S ribosomal RNA gene. *Am. J. Clin. Pathol.* **118**(5) (2002) 796–801.

We developed a method to identify mycobacteria by sequencing hypervariable regions of the polymerase chain reaction-amplified 16S ribosomal RNA gene. This method is nearly specific for mycobacteria and uses positive culture from liquid or solid medium without the need for lengthy subculture. It shortens identification time to 3 days, which is much faster than the conventional biochemical method (mean, 8 weeks). It applies to all mycobacteria (approximately 100 species), unlike current nucleic acid hybridization methods, which probe only 4 species. The identifications are the same or are species specific for the well-characterized mycobacteria (59/68 [87%]) or more accurate for recently proposed species (9/68 [13%]). The method requires a single sequencing reaction, which is efficient and cost-effective. Therefore, this method is clinically and academically useful.—Authors' Abstract

**Larsen, M. H., Vilcheze, C., Kremer, L., Besra, G. S., Parsons, L., Salfinger, M., Heifets, L., Hazbon M. H., Alland, D., Sacchetti, J. C., and Jacobs, W. R. Jr.** Overexpression of *inhA*, but not *kasA*, confers resistance to isoniazid and ethionamide in *Mycobacterium smegmatis*, *M. bovis* BCG and *M. tuberculosis*. *Mol. Microbiol.* **46**(2) (2002) 453–466.

The *inhA* and *kasA* genes of *Mycobacterium tuberculosis* have each been proposed to encode the primary target of the antibiotic isoniazid (INH). Previous studies investigating whether overexpressed *inhA* or *kasA* could confer resistance to INH yielded disparate results. In this work, multicopy plasmids expressing either *inhA* or *kasA* genes were transformed into *M. smegmatis*, *M. bovis* BCG and three different *M. tuberculosis* strains. The resulting transformants, as well as previously published *M. tuberculosis* strains with multicopy *inhA* or *kasA* plasmids, were tested

for their resistance to INH, ethionamide (ETH) or thiolactomyacin (TLM). Mycobacteria containing *inhA* plasmids uniformly exhibited 20-fold or greater increased resistance to INH and 10-fold or greater increased resistance to ETH. In contrast, the *kasA* plasmid conferred no increased resistance to INH or ETH in any of the five strains, but it did confer resistance to thiolactomyacin, a known KasA inhibitor. INH is known to increase the expression of *kasA* in INH-susceptible *M. tuberculosis* strains. Using molecular beacons, quantified *inhA* and *kasA* mRNA levels showed that increased *inhA* mRNA levels correlated with INH resistance, whereas *kasA* mRNA levels did not. In summary, analysis of strains harbouring *inhA* or *kasA* plasmids yielded the same conclusion: overexpressed *inhA*, but not *kasA*, confers INH and ETH resistance to *M. smegmatis*, *M. bovis* BCG and *M. tuberculosis*. Therefore, *InhA* is the primary target of action of INH and ETH in all three species.—Authors' Abstract

**Li, Q., Whalen, C. C., Albert, J. M., Larkin, R., Zukowski, L., Cave, M. D., and Silver, R. F.** Differences in rate and variability of intracellular growth of a panel of *Mycobacterium tuberculosis* clinical isolates within a human monocyte model. *Infect. Immun.* **70**(11) (2002) 6489–6493.

Significant differences were observed in the capacities of *Mycobacterium tuberculosis* clinical isolates to grow within human monocytes. Genotyping indicated that the four most rapidly growing isolates were members of the Beijing strain family. *M. tuberculosis* strain H37Rv provided more reproducible infection than the clinical isolates or *M. tuberculosis* Erdman.—Authors' Abstract

**Mukamolova, G. V., Turapov, O. A., Young, D. I., Kaprelyants, A. S., Kell, D. B., and Young, M.** A family of autocrine growth factors in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **46**(3) (2002) 623–635.

*Mycobacterium tuberculosis* and its close relative, *Mycobacterium bovis* (BCG) contain five genes whose predicted products resemble Rpf from *Micrococcus luteus*. Rpf is a secreted growth factor, active at picomolar concentrations, which is required for the growth of vegetative cells in minimal media at very low inoculum densities, as well as the resuscitation of dormant cells. We show here that the five cognate proteins from *M. tuberculosis* have very similar characteristics and properties to those of Rpf. They too stimulate bacterial growth at picomolar (and in some cases, subpicomolar) concentrations. Several lines of evidence indicate that they exert their activity from an extra-cytoplasmic location, suggesting that they are also involved in intercellular signalling. The five *M. tuberculosis* proteins show cross-species activity against *M. luteus*, *Mycobacterium smegmatis* and *M. bovis* (BCG). Actively growing cells of *M. bovis* (BCG) do not respond to these proteins, whereas bacteria exposed to a prolonged stationary phase do. Affinity-purified antibodies inhibit bacterial growth *in vitro*, suggesting that sequestration of these proteins at the cell surface might provide a means to limit or even prevent bacterial multiplication *in vivo*. The Rpf family of bacterial growth factors may therefore provide novel opportunities for preventing and controlling mycobacterial infections. — Authors' Abstract

**Olananmi, O., Schlesinger, L. S., Ahmed, A., and Britigan, B. E.** Intraphagosomal *Mycobacterium tuberculosis* acquires iron from both extracellular transferrin and intracellular iron pools. Impact of interferon-gamma and hemochromatosis. *J. Biol. Chem.* **277(51)** (2002) 49727–49734.

*Mycobacterium tuberculosis* multiplies within the macrophage phagosome and requires iron for growth. We examined the route(s) by which intracellular *M. tuberculosis* acquires iron. During intracellular growth of the virulent Erdman *M. tuberculosis* strain in human monocyte-derived macrophages (MDM), *M. tuberculosis* acquisition of (59)Fe from transferrin (TF) provided extracellularly (exogenous source) was compared

with acquisition when MDM were loaded with (59)Fe from TF prior to *M. tuberculosis* infection (endogenous sources). *M. tuberculosis* (59)Fe acquisition required viable bacteria and was similar from exogenous and endogenous sources at 24 hr and greater from exogenous iron at 48 hr. Interferon-gamma treatment of MDM reduced (59)Fe uptake from TF 51% and TF receptor expression by 34%. Despite this, intraphagosomal *M. tuberculosis* iron acquisition in IFN-gamma-treated cells was decreased by only 30%. Macrophages from hereditary hemochromatosis patients have altered iron metabolism. Intracellular *M. tuberculosis* acquired markedly less iron in MDM from these individuals than in MDM from healthy donors, regardless of the iron source (exogenous and endogenous):  $36 \pm 3.8\%$  and  $17 \pm 9.6\%$  of control, respectively. Thus, intraphagosomal *M. tuberculosis* can acquire iron from both extracellular TF and endogenous macrophage sources. Acquisition of iron from macrophage cytoplasmic iron pools may be critical for the intracellular growth of *M. tuberculosis*. This acquisition is altered by IFN-gamma treatment to a small extent, but is markedly reduced in macrophages from hemochromatosis patients. — Authors' Abstract

**Park, S. W., Hwang, E. H., Park, H., Kim, J. A., Heo, J., Lee, K. H., Song, T. Kim, E., Ro, Y. T., Kim, S. W., and Kim, Y. M.** Growth of mycobacteria on carbon monoxide and methanol. *J. Bacteriol.* **185(1)** (2003) 142–147.

Several mycobacterial strains, such as *Mycobacterium flavescens*, *Mycobacterium gastri*, *Mycobacterium neoaurum*, *Mycobacterium parafortuitum*, *Mycobacterium peregrinum*, *Mycobacterium phlei*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, and *Mycobacterium vaccae*, were found to grow on carbon monoxide (CO) as the sole source of carbon and energy. These bacteria, except for *M. tuberculosis*, also utilized methanol as the sole carbon and energy source. A CO dehydrogenase (CO-DH) assay, staining by activity of CO-DH, and Western blot analysis using an antibody raised against CO-DH of *Mycobacterium* sp. strain JC1 (formerly *Acinetobacter* sp. strain JC1 [J. W. Cho, H. S. Yim, and Y. M. Kim,

Kor. J. Microbiol. 23:1–8, 1985]) revealed that CO-DH is present in extracts of the bacteria prepared from cells grown on CO. Ribulose biphosphate carboxylase/oxygenase (RubisCO) activity was also detected in extracts prepared from all cells, except *M. tuberculosis*, grown on CO. The mycobacteria grown on methanol, except for *M. gastri*, which showed hexulose phosphate synthase activity, did not exhibit activities of classic methanol dehydrogenase, hydroxypyruvate reductase, or hexulose phosphate synthase but exhibited N,N-dimethyl-4-nitrosoaniline-dependent methanol dehydrogenase and RuBisCO activities. Cells grown on methanol were also found to have dihydroxyacetone synthase. Double immunodiffusion revealed that the antigenic sites of CO-DHs, RuBisCOs, and dihydroxyacetone synthases in all mycobacteria tested are identical with those of the *Mycobacterium* sp. strain JC1 enzymes. — Authors' Abstract

**Pheiffer C, Betts J, Lukey P., and van Helden P.** Protein expression in *Mycobacterium tuberculosis* differs with growth stage and strain type. Clin. Chem. Lab. Med. **40(9)** (2002) 869–875.

Different phenotypes are displayed by *Mycobacterium tuberculosis* (*M. tuberculosis*) strains, fuelling speculation that certain strains are “hypervirulent” and able to evade host defenses better than others. Furthermore, differential antigen expression by *M. tuberculosis* strains may explain why certain patients are susceptible to a repeat episode of tuberculosis. The objective of this study was to compare protein expression by *M. tuberculosis* H37Rv and clinical isolates in order to determine whether differential protein expression contributes to the different phenotypes expressed by these strains. Expression of alpha-crystallin, the antigen 85 complex, PstS-1, L-alanine dehydrogenase and the 65 kDa antigen was analysed by Western blotting and enzyme-linked immunosorbent assays, using mouse monoclonal antibodies. We found no significant difference in the growth rate of the *M. tuberculosis* strains *in vitro*, and although *M. tuberculosis* protein expression showed phase variation during growth, expression seemed to be qualitatively, but not quantitatively, conserved in the strains investigated. These results have potentially important implications for vaccine development and serodiagnosis. — Authors' Abstract

## Experimental Infections

**Aldwell, F. E., Tucker, I. G., De Lisle, G. W., and Buddle, B. M.** Oral delivery of *Mycobacterium bovis* BCG in a lipid formulation induces resistance to pulmonary tuberculosis in mice. Infect. Immun. **71(1)** (2003) 101–18.

A lipid-based formulation has been developed for oral delivery of *Mycobacterium bovis* bacille Calmette-Guerin (BCG) vaccine. The formulated *M. bovis* BCG was fed to BALB/c mice to test for immune responses and protection against *M. bovis* infection. The immune responses included antigen-specific cytokine responses, spleen cell proliferation, and lymphocyte-mediated macrophage inhibition of *M. bovis*. Oral delivery of formulated *M. bovis* BCG to mice induced strong splenic gamma interferon

levels and macrophage inhibition of virulent *M. bovis* compared with results with nonformulated *M. bovis* BCG. Formulated oral *M. bovis* BCG significantly reduced the bacterial burden in the spleen and lungs of mice following aerosol challenge with virulent *M. bovis*. Our data suggest that oral delivery of formulated *M. bovis* BCG is an effective means of inducing protective immune responses against tuberculosis. Lipid-based, orally delivered mycobacterial vaccines may be a safe and practical method of controlling tuberculosis in humans and animals. — Authors' Abstract

**Botha, T. and Ryffel, B.** Reactivation of latent tuberculosis by an inhibitor of inducible nitric oxide synthase in an aerosol

murine model. *Immunology* **107**(3) (2002) 350–357.

Exposure to *Mycobacterium tuberculosis* results in clinical tuberculosis only in a small percentage of healthy individuals. In most instances the bacilli are controlled by the immune system and survive in a latent state within granuloma. Immunosuppression, however, may result in reactivation of infection, resulting in clinical disease. Using a low-dose aerosol infection (30 colony-forming units) in mice, we describe a short-duration model for studying spontaneous and drug-induced reactivation of anti-tuberculous drug-treated, latent tuberculosis infection. Although a 4-week treatment with rifampicin and isoniazid reduced the number of bacilli to undetectable levels, the infection spontaneously reactivated following therapy. By contrast, an 8-week treatment period induced a state of latent infection, requiring immunosuppression to reactivate infection. Finally, a 12-week treatment period eliminated the bacilli completely and aminoguanidine did not induce reactivation of infection. In view of the fact that therapy in the selected protocol reduces the mycobacterial load to undetectable levels, the data suggest that an 8-week treatment period is necessary and sufficient to mount protective immunity in mice.—Authors' Abstract

**Fattorini, L., Creti, R., Nisini, R., Pietrobono, R., Fan, Y., Stringaro, A., Arancia, G., Serlupi-Crescenzi, O., Iona, E., and Orefici, G.** Recombinant GroES combination with CpG oligodeoxynucleotides protects mice against *Mycobacterium avium* infection. *J. Med. Microbiol.* **51**(12) (2002) 1071–1079.

The groES gene of *Mycobacterium avium* strain 485 was cloned and expressed in *Escherichia coli* and the recombinant GroES protein was purified by affinity chromatography. The GroES preparation showed high purity by electrophoresis and immunoblotting. Immuno-electron microscopy showed that GroES was located both in the cytoplasm and on the surface of the mycobacterial cells and thus is readily available to interact with the host immune

system. BALB/c mice were immunised intranasally with recombinant GroES, alone or in combination with a synthetic oligodeoxynucleotide containing unmethylated CpG motifs, and tested for protection against infection with *M. avium*. Neither GroES nor CpG alone provided any protection against subsequent challenge with *M. avium*, whereas a combination of the two significantly protected the lungs and spleen against colonisation by *M. avium* after intranasal challenge with a low dose of the organism. This indicates that intranasal administration of GroES and CpG oligodeoxynucleotides increases the resistance of BALB/c mice to *M. avium* infection.—Authors' Abstract

**Gormus, B. J., Baskin, G. B., Xu, K., Ratterree, M. S., Mack, P. A., Bohm, R. P. Jr, Meyers, W. M., and Walsh, G. P.** Anti-leprosy protective vaccination of rhesus monkeys with BCG or BCG plus heat-killed *Mycobacterium leprae*: lepromin skin test results. *Lepr. Rev.* **73**(3) (2002) 254–261.

Groups of rhesus monkeys (RM) were vaccinated and boosted with living *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) or BCG + low dose (LD) heat-killed *Mycobacterium leprae* (HKML) or high dose (HD) HKML or were unvaccinated. Animals vaccinated with BCG + LD and HD HKML were lepromin skin tested 2 weeks after boosting. All groups were lepromin tested 37 and 46 months after challenge with live *M. leprae*. Fernandez (72 hr) and Mitsuda (28 day) responses were recorded. Ten of 10 rhesus monkeys in each of the two BCG + HKML-vaccinated groups significantly converted to strong positive Fernandez status within 2 weeks of boosting, compared to one of six positives in the unvaccinated unchallenged normal control group. Both BCG + HKML groups were significantly protected from clinical leprosy. Six of 10 in each of the two BCG + HKML groups significantly converted to Mitsuda positivity within 2 weeks of boosting compared to zero of six in the normal control group. The sizes of the Mitsuda responses were larger in the LD group than the HD HKML vaccinated/boosted group,

suggesting suppression by vaccination with higher doses of HKML in combination with BCG. Fernandez responses were negative in normal RM as well as in the unvaccinated, ML-challenged group and the BCG-vaccinated, ML-challenged group at 37 or 46 months after ML inoculation, although the BCG-vaccinated group was significantly protected from leprosy and the unvaccinated group was not. In contrast, at 37 months the Fernandez reaction was positive in the BCG plus LD and the BCG plus HD HKML-vaccinated groups, both of which were significantly protected from clinical leprosy. By 46 months, the Fernandez responses were below significance in all groups. Thus, Fernandez reactivity is not a reliable correlate to protection from experimental leprosy in RM. Mitsuda responses became strongly positive in all four ML-challenged groups by 37 months and re-

mained strongly positive at 46 months after ML inoculation, suggesting that strong Mitsuda reactivity reflects responses to living ML. BCG or BCG + LD or HD HKML vaccination/boosting of RM produced significant clinical protection from leprosy and there was a good correlation between protection from LL forms of leprosy and positive Mitsuda skin test responses after challenge with live ML. Positive Mitsuda responses were generated in essentially all individuals after challenge with live ML, and this response was primed by prior vaccination/boosting with BCG + HKML as shown by conversion to positivity 2 weeks after boosting. The data show that resistance to clinical leprosy is reflected by Mitsuda responses in ML-exposed RM, similar to results from human studies, and confirm the suitability of RM as a model for leprosy vaccine studies.—Authors' Abstract

## Epidemiology and Prevention

**Meima, A., Irgens, L. M., van Oortmarsen, G. J., Richardus, J. H., and Habbema, J. D.** Disappearance of leprosy from Norway: an exploration of critical factors using an epidemiological modelling approach. *Int. J. Epidemiol.* **31(5)** (2002) 991–1000.

**BACKGROUND:** By the middle of the 19th century, leprosy was a serious public health problem in Norway. By 1920, new cases only rarely occurred. This study aims to explain the disappearance of leprosy from Norway. **METHODS:** Data from the National Leprosy Registry of Norway and population censuses were used. The patient data include year of birth, onset of disease, registration, hospital admission, death, and emigration. The Norwegian data were analysed using epidemiological models of disease transmission and control. **RESULTS:** The time trend in leprosy new case detection in Norway can be reproduced ad-

equately. The shift in new case detection towards older ages which occurred over time is accounted for by assuming that infected individuals may have a very long incubation period. The decline cannot be explained fully by the Norwegian policy of isolation of patients: an autonomous decrease in transmission, reflecting improvements in for instance living conditions, must also be assumed. The estimated contribution of the isolation policy to the decline in new case detection very much depends on assumptions made on build-up of contagiousness during the incubation period and waning of transmission opportunities due to rapid transmission to close contacts. **CONCLUSION:** The impact of isolation on interruption of transmission remains uncertain. This uncertainty also applies to contemporary leprosy control that mainly relies on chemotherapy treatment. Further research is needed to establish the impact of leprosy interventions on transmission.—Authors' Abstract

## Other Microbacterial Diseases

**Brown-Elliott, B. A., Griffith, D. E., and Wallace, R. J. Jr.** Diagnosis of nontuberculous mycobacterial infections. *Clin. Lab. Med.* **22(4)** (2002) 911–925.

This section discusses the methods of laboratory diagnosis of nontuberculous mycobacteria (NTM) using conventional biochemical and nutritional requirements, acid-fast smear microscopy, high performance liquid chromatography (HPLC), antibiotic susceptibility testing, and newer genetic methods such as molecular probes, polymerase chain reaction restriction fragment length polymorphism analysis (PRA), and 16S rDNA sequence analysis. This article discusses how laboratory results are applied by clinicians, and some of the difficulties and controversies regarding the diagnosis of NTM disease after the laboratory work is complete.—Authors' Abstract

**Enzensberger, R., Hunfeld, K. P., Elshorst-Schmidt, T., Boer, A., and Brade, V.** Disseminated cutaneous *Mycobacterium marinum* infection in a patient with Non-Hodgkin's lymphoma. *Infection* **30(6)** (2002) 393–395.

A 60-year-old woman with non-Hodgkin's lymphoma was admitted to the hospital because of extensive subcutaneous abscesses developing on all limbs. The patient had an aquarium and kept tropical fish as pets. After repeated investigations, the diagnosis of *Mycobacterium marinum* was established from skin biopsy by PCR and culture. Long-term therapy with several drugs regimens had only a limited efficacy and was accompanied by severe adverse reactions. This report highlights the therapeutic problems posed by disseminated cutaneous *M. marinum* infection in the immunosuppressed host.—Authors' Abstract

**Hsu, P. Y., Yang, Y. H., Hsiao, C. H., Lee, P. I., and Chiang, B. L.** *Mycobacterium kansasii* infection presenting as cellulitis

in a patient with systemic lupus erythematosus. *J. Formos. Med. Assoc.* **101(8)** (2002) 581–584.

The prevalence of mycobacterial infection has increased in recent years, especially in patients immunocompromised due to autoimmune disease, malignancy and AIDS. *Mycobacterium kansasii* infection most commonly presents as tuberculosis-like pulmonary disease. We report the case of a 38-year-old woman with systemic lupus erythematosus (SLE) who developed cellulitis over the left lower leg and had poor response to antibiotics. Two months before this admission, she had sustained a small wound over the right pretibial area and had noticed erythematous swelling after swimming at the beach. Pathologic examination of biopsied tissue showed acid-fast bacilli, and culture yielded *M. kansasii*. The cellulitis improved gradually during treatment with antimycobacterial agents for 1 year. This case emphasizes the possibility that cutaneous *M. kansasii* infection may occur in an immunocompromised patient and that exposure to contaminated water is a possible source. With early diagnosis, the response to an antimycobacterial multidrug regimen is usually satisfactory.—Authors' Abstract

**John, G. T. and Shankar, V.** Mycobacterial infections in organ transplant recipients. *Semin. Respir. Infect.* **17(4)** (2002) 274–283.

Tuberculosis has a major adverse impact on solid organ transplant recipients; this article attempts to define this fact. The prevalence of posttransplant tuberculosis is increasing globally and currently is 13.7% at our center. The transplant surgery divides the continuum of pretransplant tuberculosis and posttransplant tuberculosis; immunosuppression accounts for a greater severity of the latter. Cyclosporin and tacrolimus are associated with an earlier onset of tuberculosis when compared with prednisolone and azathioprine immunosuppression. Dissemi-

nated disease is more common in nonrenal transplants. The risk for developing post-transplant tuberculosis in renal transplant recipients increased 2.25 times independently with cytomegalovirus (CMV) and twice with chronic liver disease; OKT3 treatment enhances the risk 1.8-fold. Tuberculosis occurring after 2 yrs of transplantation, diabetes mellitus, posttransplant diabetes mellitus, chronic liver disease, CMV, and deep mycoses each independently confer a risk, 1.5-times or higher, for death. Disseminated disease entails a 2-fold risk. Treatment with or without rifampicin is possible; the former is associated with a higher risk for allograft rejection. Isoniazid prophylaxis is recommended for high-risk patients with apparent clinical efficacy. However, in endemic areas, attendant liver disease makes it a difficult goal.—Authors' Abstract

**Kayal, J. D. and McCall, C. O.** Sporotrichoid cutaneous *Mycobacterium avium* complex infection. *J. Am. Acad. Dermatol.* **47(5 Suppl.)** (2002) S249–250.

*Mycobacterium avium* complex, a common opportunistic pathogen among patients with AIDS, usually manifests as disseminated disease involving the lung, lymph nodes, and gastrointestinal tract. Primary cutaneous infections with *M. avium* complex are extremely rare, and most cutaneous lesions are caused by dissemination. Cutaneous manifestations thus far reported include scaling plaques, crusted ulcers, ecthyma-like lesions, verrucous ulcers, inflammatory nodules, panniculitis, pustular lesions, and draining sinuses. Localized skin involvement resembling sporotrichosis is unusual and to our knowledge has been reported only once in the English-language literature. We describe an additional case of primary cutaneous *M. avium* complex infection manifesting as sporotrichosis-like lesions on a patient with AIDS.—Authors' Abstract

**Malecha, M. A. and Doughman, D. J.** *Mycobacterium chelonae* keratitis associated with soft contact lens wear. *CLAO J.* **28(4)** (2002) 228–230.

**PURPOSE:** To report a case of *Mycobacterium chelonae* keratitis associated with soft contact lens wear. **METHODS:** A 17-year-old boy who wore frequent replacement soft contact lenses developed keratitis in the right eye. There was no history of trauma to the right eye. The patient was treated initially with topical ciprofloxacin but without improvement. On presentation, visual acuity in his right eye was 20/40. A Gram-stained scraping of the corneal infiltrate revealed beaded filamentous rods, and the organisms were acid-fast positive. The patient's right eye was treated with intensive topical amikacin, 20 mg/mL, and 10% sulfacetamide. Eventually, *Mycobacterium chelonae* was cultured on Sabourard's agar, topical sulfacetamide was stopped, and amikacin was continued. **RESULTS:** The patient's keratitis responded well to amikacin and resolved over a period of 4 weeks. Visual acuity in the right eye improved to 20/25. **CONCLUSIONS:** *Mycobacterium chelonae* is a rare cause of keratitis in soft contact lens wearers. We have identified fewer than five cases of *Mycobacterium chelonae* keratitis associated with soft contact lenses in the literature. Prompt and accurate diagnosis of the organism using corneal scraping can lead to appropriate therapy and resolution of the keratitis.—Authors' Abstract

**Palca, A., Aebi, C., Weimann, R., and Bodmer, T.** *Mycobacterium bohemicum* cervical lymphadenitis. *Pediatr. Infect. Dis. J.* **21(10)** (2002) 982–984.

A lymph node excision was performed on a 2-year-old child with right submandibular swelling not responding to oral cefaclor therapy. Histology revealed granulomatous, partly necrotizing lymphadenitis and a large number of acid-fast bacilli subsequently identified by molecular techniques as *Mycobacterium bohemicum*.—Authors' Abstract

**Portaels, F., Aguiar, J., Debacker, M., Steunou, C., Zinsou, C., Guedenon, A., and Meyers, W. M.** Prophylactic effect of *Mycobacterium bovis* BCG vaccina-

tion against osteomyelitis in children with *Mycobacterium ulcerans* disease (Buruli Ulcer). Clin. Diagn. Lab. Immunol. **9**(6) (2002) 1389–1391.

*Mycobacterium ulcerans* disease, or Buruli ulcer (BU), causes significant morbidity in West Africa. In 233 consecutive, laboratory-confirmed samples from BU patients in Benin whose *Mycobacterium bovis* BCG scar status was known, 130 children (<15 yrs old) and 75 adults had a neonatal BCG vaccination scar. Of 130 children with BCG scars, 10 (7.7%) had osteomyelitis, while 3 of 9 children without BCG scars (33.3%) had osteomyelitis. Our observations support the conclusion that having a BCG vaccination scar provides significant protection against *M. ulcerans osteomyelitis* in children with BU disease.—Authors' Abstract

**Ryan, P., Bennett, M. W., Aarons, S., Lee, G., Collins, J. K., O'Sullivan, G. C., O'Connell, J., and Shanahan, F.** PCR detection of *Mycobacterium paratuberculosis* in Crohn's disease granulomas isolated by laser capture microdissection. Gut **51**(5) (2002) 665–670.

**BACKGROUND AND AIMS:** The uncertainty surrounding the role of *Mycobacterium avium* subsp paratuberculosis (Map) in Crohn's disease has been compounded by possible contamination from Map present in the lumen microflora. This study used laser capture microdissection (LCM) and polymerase chain reaction (PCR) to detect Map DNA in subepithelial granulomas, isolated from 15 surgically resected, formalin fixed specimens of granulomatous Crohn's disease and from 12 granulomatous disease controls (10 bowel, 2 non-bowel). **METHODS:** The effect of amplicon size on reliability of PCR from formalin fixed samples was examined by amplifying 435 bp and 133 bp sequences of the human APC gene. After this, nested primers were designed to detect a small fragment (155 bp) of the Map specific IS900 gene in Crohn's granulomas. LCM isolated granulomas from Map culture positive bovine intestine was used as positive control. PCR product specificity

was confirmed by direct DNA sequencing. **RESULTS:** The smaller, but not the larger, fragment of the APC gene amplified reliably in all samples. Amplification of the 155 bp fragment of the IS900 gene detected Map DNA in microdissected Crohn's granulomas in 6 of 15 cases, and in 0 of 12 disease control granulomas. **CONCLUSIONS:** LCM can be used to detect Map DNA in granulomas in a proportion of patients with Crohn's disease. However, formalin fixation requires that comparatively short DNA fragments of the Map specific IS900 gene be targeted, to permit consistent detection. Detection of Map DNA within granulomas might suggest an infectious etiology in a subset of patients; alternatively, a transmissible agent may not be involved but mycobacterial DNA may influence pathogenesis by modifying the local cytokine responses.—Authors' Abstract

**Schmekal, B., Janko, O., Zazgornik, J., Schinko, H., Bogner, S., Syre, G., and Biesenbach, G.** Skin tuberculosis with atypical mycobacteria 8 years after combined pancreas-kidney transplantation. Am. J. Nephrol. **22**(5–6) (2002) 566–568.

We report on a *Mycobacterium marinum* infection in a diabetic woman 8 yrs after undergoing a combined pancreas-kidney transplantation. This is, to our knowledge, the first case report on an isolated skin infection with atypical mycobacteria after simultaneous pancreas-kidney transplantation. A genetic probe categorization revealed an infection with *M. marinum*. Skin tuberculosis caused by *M. marinum* is an uncommon complication in kidney or pancreas-kidney transplant recipients, hence the diagnosis can be delayed.—Authors' Abstract

**Shafran, S. D., Mashinter, L. D., Phillips, P., Lalonde, R. G., Gill, M. J., Walmsley, S. L., Toma, E., Conway, B., Fong, I. W., Rachlis, A. R., Williams, K. E., Garber, G. E., Schlech, W. F., Smaill, F., and Pradier, C.** Successful discontinuation of therapy for disseminated *Mycobacterium avium* complex infec-

tion after effective antiretroviral therapy. *Ann. Intern. Med.* **137(9)** (2002) 734–737.

**BACKGROUND:** Highly active antiretroviral therapy (HAART) is associated with improvement or resolution of several HIV-associated opportunistic infections. Although prophylaxis against disseminated *Mycobacterium avium* complex infection may be successfully discontinued after a favorable response to HAART, the 1999 guidelines from the U.S. Public Health Service/Infectious Diseases Society of America recommend continuing therapy for disseminated *M. avium* complex infection, regardless of the response to HAART. **OBJECTIVE:** To examine the outcome among patients with disseminated *M. avium* complex infection whose antimycobacterial therapy was discontinued after a favorable response to HAART. **DESIGN:** Retrospective chart review between May 2000 and May 2001. **SETTING:** 13 Canadian HIV clinics. **PATIENTS:** 52 HIV-infected adults (43 men; mean age, 37.3 yrs) in whom successful antimycobacterial therapy for disseminated *M. avium* complex infection was discontinued after a favorable virologic response to HAART. **MEASUREMENTS:** Survival, survival free of disseminated *M. avium* complex infection, and CD4(+) cell count responses. **RESULTS:** At the time

of diagnosis of disseminated *M. avium* complex infection, the median CD4(+) cell count was  $0.016 \times 10^9$  cells/L, and the median plasma HIV RNA level was 90,000 copies/mL (plasma HIV RNA levels were available for only 21 patients). The patients received a median of 32 months of antimycobacterial therapy that included ethambutol plus either clarithromycin or azithromycin. When antimycobacterial therapy was discontinued, the median CD4(+) cell count was  $0.23 \times 10^9$  cells/L and the median plasma HIV RNA level was less than 50 copies/mL. A median of 20 months after discontinuation of antimycobacterial therapy, only 1 patient had developed recurrent *M. avium* complex disease (37 months after stopping antimycobacterial therapy). This patient had stopped HAART 2 months earlier because of uncontrolled HIV viremia. Twenty months after stopping antimycobacterial therapy, the other 51 patients had a median CD4(+) cell count of  $0.288 \times 10^9$  cells/L; 34 (67%) had undetectable plasma HIV RNA levels, and 8 (15%) had plasma HIV RNA levels of 50 to 1000 copies/mL. **CONCLUSIONS:** Discontinuation of successful disseminated *M. avium* complex therapy after a successful response to HAART is safe and reduces patients' pill burdens, potential drug adverse effects, drug interactions, and costs of therapy.—Authors' Abstract

## Molecular and Genetic Studies

**DeVito, J. A. and Morris, S.** Exploring the structure and function of the Mycobacterial KatG protein using trans-dominant mutants. *Antimicrob. Agents Chemother.* **47(1)** (2003) 188–195.

In order to probe the structure and function of the mycobacterial catalase-peroxidase enzyme (KatG), we employed a genetic approach using dominant-negative analysis of katG merodiploids. Transformation of *Mycobacterium bovis* BCG with various katG point mutants (expressed from low-copy-number plasmids) resulted in reductions in peroxidase and catalase activities as measured in cell extracts. These

reductions in enzymatic activity usually correlated with increased resistance to the antituberculosis drug isoniazid (INH). However, for the NI38S trans-dominant mutant, the catalase-peroxidase activity was significantly decreased while the sensitivity to INH was retained. Trans-dominance required katG expression from multicopy plasmids and could not be demonstrated with katG mutants integrated elsewhere on the wild-type *M. bovis* BCG chromosome. Reversal of the mutant phenotype through plasmid exchange suggested the catalase-peroxidase deficiency occurred at the protein level and that INH resistance was not due to a second site mu-

tation(s). Electrophoretic analysis of KatG proteins from the trans-dominant mutants showed a reduction in KatG dimers compared to WT and formation of heterodimers with reduced activity. The mutants responsible for these defects cluster around proposed active site residues: N138S, T275P, S315T, and D381G. In an attempt to identify mutants that might delimit the region(s) of KatG involved in subunit interactions, C-terminal truncations were constructed (with and without the D381G dominant-negative mutation). None of the C-terminal deletions were able to complement a DeltakatG strain, nor could they cause a dominant-negative effect on the WT. Taken together, these results suggest an intricate association between the amino- and carboxy-terminal regions of KatG and may be consistent with a domain-swapping mechanism for KatG dimer formation.— Authors' Abstract

**Fenhalls, G., Stevens, L., Moses, L., Bezuidenhout, J., Betts, J. C., Helden, P. V., Lukey, P. T., and Duncan, K.** In situ detection of *Mycobacterium tuberculosis* transcripts in human lung granulomas reveals differential gene expression in necrotic lesions. *Infect. Immun.* **70(11)** (2002) 6330–6338.

We have used RNA-RNA *in situ* hybridization to detect the expression of several *Mycobacterium tuberculosis* genes in tuberculous granulomas in lung tissue sections from tuberculosis patients. The *M. tuberculosis* genes chosen fall into two classes. Four genes (*icl*, *narX*, and *Rv2557* and *Rv2558*) have been implicated in the persistence of the bacterium in the host, and two genes (*iniB* and *kasA*) are upregulated in response to isoniazid exposure. Both necrotic and nonnecrotic granulomas were identified in all of the patients. Necrotic granulomas were divided into three zones: an outer lymphocyte cuff containing lymphocytes and macrophages, a transition zone consisting of necrotic material interspersed with macrophages, and a central acellular necrotic region. Transcripts of all of the genes studied were found in nonnecrotic granulomas and in the lymphocyte cuff of necrotic granulomas. Mycobacterial gene expression was as-

sociated with CD68-positive myeloid cells. *Rv2557* and/or its homolog *Rv2558*, *kasA*, and *iniB* were expressed within the transition zone of necrotic granulomas, whereas *icl* and *narX* transcripts were absent from this area. There was no evidence of transcription of any of the genes examined in the central necrotic region, although mycobacterial DNA was present. The differential expression of genes within granulomas demonstrates that *M. tuberculosis* exists in a variety of metabolic states and may be indicative of the response to different microenvironments. These observations confirm that genes identified in models of persistence or in response to drug treatment *in vitro* are expressed in the human host.— Authors' Abstract

**Fietta, A. M., Morosini, M., Meloni, F., Bianco, A. M., and Pozzi, E.** Pharmacological analysis of signal transduction pathways required for *Mycobacterium tuberculosis*-induced IL-8 and MCP-1 production in human peripheral monocytes. *Cytokine* **19(5)** (2002) 242–249.

Signalling cascades involved in chemokine production by human phagocytes following infection with *Mycobacterium tuberculosis* are still not defined. We used specific pharmacologic inhibitors to identify the signalling molecules which lead to interleukin (IL)-8 and MCP-1 production in human monocytes in response to *M. tuberculosis* infection. Inhibition of extracellular signal-regulated (ERK) or p38 mitogen-activated protein kinase by PD98059 and SB203580 respectively, significantly affected chemokine production. However, only the presence of both inhibitors completely blocked the release. A down-regulation of chemokine secretion was found in presence of inhibitors of protein kinase (PK)C and phospholipase C. Moreover, production depended on transcription activation via the nuclear factor-kappa B (NF-kappaB), as demonstrated by treatment with actinomycin D and caffeic acid phenethyl ester. In addition, activation of PKA and the phosphoinositide 3-kinase (PI-3k)/p70 ribosomal S6 kinase cascade was required to have maximal MCP-1 but not IL-8 production. In conclusion, this study provides evidence that multiple signal

transduction pathways are involved in *M. tuberculosis*-induced chemokine secretion by human monocytes. Moreover, for the first time this report indicates that inhibitors of some signalling molecules are able to dissociate IL-8 from MCP-1 secretion. Differences in the regulatory pathways of chemokine production can potentially be exploited therapeutically.—Authors' Abstract

**Fitness, J., Tosh, K., and Hill, A. V.** Genetics of susceptibility to leprosy. *Genes Immun.* **3(8)** (2002) 441–453.

The ancient disease of leprosy can cause severe disability and disfigurement and is still a major health concern in many parts of the world. Only a subset of those individuals exposed to the pathogen will go on to develop clinical disease and there is a broad clinical spectrum among leprosy sufferers. The outcome of infection is in part due to host genes that influence control of the initial infection and the host's immune response to that infection. Identification of the host genes that influence host susceptibility/resistance will enable a greater understanding of disease pathogenesis. In turn, this should facilitate development of more effective therapeutics and vaccines. So far at least a dozen genes have been implicated in leprosy susceptibility and a genome-wide linkage study has led to the identification of at least one positional candidate. These findings are reviewed here.—Authors' Abstract

**Haile, Y., Bjune, G., and Wiker, H. G.** Expression of the *mceA*, *esat-6*, and *hspX* genes in *Mycobacterium tuberculosis* and their responses to aerobic conditions and to restricted oxygen supply. *Microbiology* **148** (2002) 3881–3886.

The expression of six of the mammalian cell-entry (*mce1a*–*mce1f*) genes of the *mce1* operon of *Mycobacterium tuberculosis* has been described previously. In this study, data are presented for the expression of other mammalian cell-entry homologs (*mce-2a*, *mce-3a* and *mce-4a*) at the RNA level, as determined by RT-PCR. The stress

responses of these genes and of other immunologically important antigens are also characterized with respect to the introduction of oxygen depletion. Analysis of the expression of the *mceA* genes in relation to oxygen depletion revealed that they were expressed differentially. The RT-PCR results showed that *mce-1a*, *mce-2a*, *hspX* (encoding the alpha-crystallin antigen *Acr*) and *esat-6* (encoding the early secretory antigenic target-6) were expressed throughout the cultivation period, whereas the expression of *mce-3a* and *mce-4a* was down-regulated in the later stages of cultivation. This study gives new insights into the expression profiles of the different *mce* operons and the *hspX* and *esat-6* genes in an *in vitro* model of dormant-like bacilli. Identification of the genes that are differentially expressed under aerobic conditions and under oxygen-limited conditions contributes to our understanding of the bacilli involved in latent tuberculosis.—Authors' Abstract

**Harth G., Horwitz, M. A., Tabatadze, D., and Zamecnik, P. C.** Targeting the *Mycobacterium tuberculosis* 30/32-kDa mycolyl transferase complex as a therapeutic strategy against tuberculosis: Proof of principle by using antisense technology. *Proc. Natl. Acad. Sci. USA* **99(24)** (2002) 15614–15619.

We have investigated the effect of sequence-specific antisense phosphorothionate-modified oligodeoxyribonucleotides (PS-ODNs) targeting different regions of each of the 30/32-kDa protein complex (antigen 85 complex) encoding genes on the multiplication of *Mycobacterium tuberculosis*. Single PS-ODNs to one of the three mycolyl transferase transcripts, added either once or weekly over the 6-wk observation period, inhibited bacterial growth by up to 1 log unit. A combination of three PS-ODNs specifically targeting all three transcripts inhibited bacterial growth by approximately 2 logs; the addition of these PS-ODNs weekly for 6 wk was somewhat more effective than a one-time addition. Targeting the 5' end of the transcripts was more inhibitory than targeting internal sites; the most effective PS-ODNs and target sites had minimal or no secondary structure. The

effect of the PS-ODNs was specific, as mismatched PS-ODNs had little or no inhibitory activity. The antisense PS-ODNs, which were highly stable in *M. tuberculosis* cultures, specifically blocked protein expression by their gene target. PS-ODNs targeting the transcript of a related 24-kDa protein (mpt51) had little inhibitory effect by themselves and did not increase the effect of PS-ODNs against the three members of the 3032-kDa protein complex. The addition of PS-ODNs against the transcripts of glutamine synthetase I (glnA1) and alanine racemase (alr) modestly increased the inhibitory efficacy of the 3032-kDa protein complex-specific PS-ODNs to approximately 2.5 logs. This study shows that the three mycolyl transferases are highly promising targets for antituberculous therapy by using antisense or other antimicrobial technologies.—Authors' Abstract

**Le Fleche, P., Fabre, M., Denoed, F., Koeck, J. L., and Vergnaud, G.** High resolution, on-line identification of strains from the *Mycobacterium tuberculosis* complex based on tandem repeat typing. *BMC Microbiol.* **2(1)** (2002) 37.

**BACKGROUND:** Currently available reference methods for the molecular epidemiology of the *Mycobacterium tuberculosis* complex either lack sensitivity or are still too tedious and slow for routine application. Recently, tandem repeat typing has emerged as a potential alternative. This report contributes to the development of tandem repeat typing for *M. tuberculosis* by summarizing the existing data, developing additional markers, and setting up a freely accessible, fast, and easy to use, internet-based service for strain identification. **RESULTS:** A collection of 21 VNTRs incorporating 13 previously described loci and 8 newly evaluated markers was used to genotype 90 strains from the *M. tuberculosis* complex (*M. tuberculosis* (64 strains), *M. bovis* (9 strains including 4 BCG representatives), *M. africanum* (17 strains)). Eighty-four different genotypes are defined. Clustering analysis shows that the *M. africanum* strains fall into three main groups, one of which is closer to the *M. tuberculosis* strains, and another one is closer to the *M.*

*bovis* strains. The resulting data has been made freely accessible over the internet <http://bacterial-genotyping.igmors.u-psud.fr/bnserver> to allow direct strain identification queries. **CONCLUSIONS:** Tandem-repeat typing is a PCR-based assay which may prove to be a powerful complement to the existing epidemiological tools for the *M. tuberculosis* complex. The number of markers to type depends on the identification precision which is required, so that identification can be achieved quickly at low cost in terms of consumables, technical expertise and equipment.—Authors' Abstract

**Mostowy, S. and Behr, M. A.** Comparative genomics in the fight against tuberculosis: diagnostics, epidemiology, and BCG vaccination. *Am. J. Pharmacogenomics* **2(3)** (2002) 189–196.

Although the causative agent of tuberculosis, *Mycobacterium tuberculosis*, has been known for some 120 yrs, the disease continues to plague humanity. In 1998, the sequencing of *M. tuberculosis* H37Rv enabled tuberculosis researchers to draw comparisons between it and other species of the closely-related *M. tuberculosis* complex, including bacillus Calmette-Guerin (BCG), the vaccine administered to prevent human tuberculosis. These efforts have uncovered genomic variability that potentially encodes the discrepant phenotypes displayed by species. Due to the infrequency of single nucleotide polymorphisms (SNPs) and other modes of genomic change, large sequence polymorphisms (LSPs) have presented themselves as the most obvious form of genomic variability among species. This review discusses genomic polymorphism among species of the *M. tuberculosis* complex as revealed through comparative genomics. Attention is drawn towards the impact of comparative genomics in generating several exciting hypotheses towards diagnosis, epidemiology, and prevention of tuberculosis disease.—Authors' Abstract

**Pym, A. S., Brodin, P., Brosch, R., Huerre, M., and Cole, S. T.** Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines *Mycobacte-*

*rium bovis* BCG and *Mycobacterium microti*. Mol. Microbiol. **46**(3) (2002) 709–717.

Although large human populations have been safely immunized against tuberculosis with two live vaccines, *Mycobacterium bovis* BCG or *Mycobacterium microti*, the vole bacillus, the molecular basis for the avirulence of these vaccine strains remains unknown. Comparative genomics has identified a series of chromosomal deletions common to both virulent and avirulent species but only a single locus, RD1, that has been deleted from *M. bovis* BCG and *M. microti*. Restoration of RD1, by gene knock-in, resulted in a marked change in colonial morphology towards that of virulent tubercle bacilli. Three RD1-encoded proteins were localized in the cell wall, and two of them, the immunodominant T-cell antigens ESAT-6 and CFP-10, were also found in culture supernatants. The BCG::RD1 and *M. microti*::RD1 knock-ins grew more vigorously than controls in immunodeficient mice, inducing extensive splenomegaly and granuloma formation. Increased persistence and partial reversal of attenuation were observed when immunocompetent mice were infected with the BCG::RD1 knock-in, whereas BCG controls were cleared. Knocking-in five other RD loci did not affect the virulence of BCG. This study describes a genetic lesion that contributes to safety and opens new avenues for vaccine development. — Authors' Abstract

**Rojas, M., Olivier, M., and Garcia, L. F.** Activation of JAK2/STAT1-alpha-dependent signaling events during *Mycobacterium tuberculosis*-induced macrophage apoptosis. Cell. Immunol. **217**(1–2) (2002) 58–66.

Induction of apoptosis by *Mycobacterium tuberculosis* in murine macrophage involves TNF-alpha and nitric oxide (NO) production and caspase cascade activation; however, the intracellular signaling pathways implicated remain to be established. Our results indicate that infection of the B10R murine macrophage line with *M. tuberculosis* induces apop-

toxis independent of mycobacterial phagocytosis and that *M. tuberculosis* induces protein tyrosine kinase (PTK) activity, JAK2/STAT1-alpha phosphorylation, and STAT1-alpha nuclear translocation. Inhibitors of PTK (AG-126), or JAK2 (AG-490) inhibited TNF-alpha and NO production, caspase 1 activation and apoptosis, suggesting that *M. tuberculosis*-induction of these events depends on JAK2/STAT1-alpha activation. In addition, we have obtained evidence that ManLAM capacity to inhibit *M. tuberculosis*-induced apoptosis involves the activation of the PTP SHP-1. The finding that *M. tuberculosis* infection activate JAK2/STAT1-alpha pathway suggests that *M. tuberculosis* might mimic macrophage-activating stimuli. — Authors' Abstract

**Sanchez, F., Radaeva, T. V., Nikonenko, B. V., Persson, A. S., Sengel, S., Schalling, M., Schurr, E., Apt, A. S., and Lavebratt, C.** Multigenic control of disease severity after virulent *Mycobacterium tuberculosis* infection in mice. Infect. Immun. **71**(1) (2003) 126–131.

Following challenge with virulent *Mycobacterium tuberculosis*, mice of the I/St inbred strain exhibit shorter survival time, more rapid body weight loss, higher mycobacterial loads in organs, and more severe lung histopathology than mice of the A/Sn strain. We previously performed a genome-wide scan for quantitative trait loci (QTLs) that control the severity of *M. tuberculosis*-triggered disease in [(A/Sn × I/St) F1 × I/St] backcross-1 (BC1) mice and described several QTLs that are significantly or suggestively linked to body weight loss. In the present study we expanded our analysis by including the survival time phenotype and by genotyping 406 (A/Sn × I/St) F2 mice for the previously identified chromosomal regions of interest. The previously identified 12-cM-wide QTL on distal mouse chromosome 3 was designated tbs1 (tuberculosis severity 1); the location of the QTL on proximal chromosome 9 was narrowed to a 9-cM interval, and this QTL was designated tbs2. Allelic variants of the tbs2 locus appeared to be involved in control of both body weight loss and sur-

vival time. Also, the data strongly suggested that a QTL located in the vicinity of the H-2 complex on chromosome 17 is involved in control of tuberculosis in mice of both genders, whereas the *tbs1* locus seemed to have an effect on postinfection body weight loss in female mice. Interestingly, these loci appeared to interact with each other, which suggests that there might be a basic genetic network for the control of intracellular parasites. Overall, linkage data reported here for F2 mice are in agreement with, and add to, our previous findings concerning the control of *M. tuberculosis*-triggered disease in the BC1 segregation—Authors' Abstract

**Sirakova, T. D., Fitzmaurice, A. M., and Kolattukudy, P.** Regulation of expression of *mas* and *fadD28*, two genes involved in production of dimycocerosyl phthiocerol, a virulence factor of *Mycobacterium tuberculosis*. *J. Bacteriol.* **184(24)** (2002) 6796–6802.

Transcriptional regulation of genes involved in the biosynthesis of cell wall lipids of *Mycobacterium tuberculosis* is poorly understood. The gene encoding mycocerosic acid synthase (*mas*) and *fadD28*, an adjoining acyl coenzyme A synthase gene, involved in the production of a virulence factor, dimycocerosyl phthiocerol, were cloned from *Mycobacterium bovis* BCG, and their promoters were analyzed. The putative promoters were fused to the *xylE* reporter gene, and its expression was measured in *Escherichia coli*, *Mycobacterium smegmatis*, and *M. bovis* BCG. In *E. coli*, the *fadD28* promoter was not functional but the *mas* promoter was functional. Both *fadD28* and *mas* promoters were functional in *M. smegmatis*, at approximately two- and sixfold-higher levels, respectively, than the BCG *hsp60* promoter. In *M. bovis* BCG, the *fadD28* and *mas* promoters were functional at three- and fivefold-higher levels, respectively, than the *hsp60* promoter. Primer extension analyses identified transcriptional start points 60 and 182 bp upstream of the translational start codons of *fadD28* and *mas*, respectively. Both promoters contain sequences similar to the canonical –10 and –35 hexamers recognized by the sigma(70)

subunit of RNA polymerase. Deletions of the upstream regions of both genes indicated that 324 bp of the *fadD28* and 228 bp of the *mas* were essential for promoter activity. Further analysis of the *mas* promoter showed that a 213-bp region 581 bp upstream of the *mas* promoter acted as a putative transcriptional enhancer, promoting high-level expression of the *mas* gene when present in either direction. This represents the identification of a rare example of an enhancer-like element in mycobacteria.—Authors' Abstract

**Slayden, R A. and Barry, C. E. 3rd.** The role of *KasA* and *KasB* in the biosynthesis of meromycolic acids and isoniazid resistance in *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)* **82(4–5)** (2002) 149–160.

*Mycobacterium tuberculosis* has two discrete beta-ketoacyl synthases encoded by *kasA* and *kasB* that are located in tandem within a five-gene operon that has been implicated in isoniazid-sensitivity and mycolic acid synthesis. We have developed an *in vitro* meromycolic acid synthase assay to elucidate the anabolic role of these enzymes. Overproduction of *KasA* and *KasB* individually and together in *M. smegmatis* enabled cell-free incorporation of [(14)C]malonyl-CoA into lipids whose chain length was dependent upon the *M. tuberculosis* elongating enzyme used. *KasA* specifically elongated palmitoyl-CoA to monounsaturated fatty acids that averaged 40 carbons in length. *KasB* hyperproduction in the presence of *KasA* produced longer chain multiunsaturated hydrocarbons averaging 54 carbons in length. These products comigrated with a synthetic standard of meromycolic acid and their production was sensitive to isoniazid, thiolactomycin, and triclosan. *KasA* mutations associated with isoniazid resistance produced an enzyme that had a diminished overall catalytic activity but conferred enhanced resistance to isoniazid. *In vivo* analysis confirmed that overexpression of each of the four mutant *KasAs* enhanced isoniazid resistance when compared to overexpression of wild-type *KasA*. These results suggest discrete anabolic roles for both *KasA* and *KasB* in my-

colic acid synthesis and substantiate the involvement of KasA mutations in isoniazid resistance.— Authors' Abstract

**Sugawara, I., Yamada, H., and Mizuno, S.** Relative importance of STAT4 in murine tuberculosis. *J. Med. Microbio.* **52(1)** (2003) 29–34.

This study was designed to determine the roles of STAT proteins in defense against mycobacterial infection. Airborne infection of STAT4 knockout (KO) mice with a *Mycobacterium tuberculosis* strain induced large

granulomas with massive neutrophil infiltration over time, while that in STAT6 KO mice did not. The STAT4 KO mice succumbed to mycobacterial infection by the 80th day after infection. Compared with the levels in wild-type (WT) and STAT6 KO mice, pulmonary inducible nitric oxide synthase, interferon-alpha, -beta and -gamma mRNA levels were significantly lower in STAT4 KO mice, but expression of interleukin-2, -6, -12 and -18 mRNAs was slightly higher up to the fifth week after aerial infection. Therefore, STAT4, but not STAT6, appears to be a critical transcription factor in mycobacterial regulations.— Authors' Abstract