

## CURRENT LITERATURE

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

General & Historical	132
Chemotherapy	134
Clinical Sciences	139
Immuno-Pathology	145
Leprosy	152
Tuberculosis	153
Experimental Infections	162
Microbiology	167
Leprosy	170
Tuberculosis	171
Epidemiology	172
Rehabilitation	174
Other Mycobacterial Diseases	174
Molecular and Genetic Studies	180

## General and Historical

**Daemmrich, A.** A tale of two experts: thalidomide and political engagement in the United States and West Germany. *Soc. Hist. Med.* **15**(1) (2002) 137–158.

The physicians, Widukind Lenz and Frances Kelsey, played crucial roles in the thalidomide drama of the early 1960s. Widespread use of the drug in West Germany was only halted when the pediatrician, Lenz, publicized its association with the birth of nearly 4000 children exhibiting abnormal limb growth. Few cases were reported in the United States because Kelsey, a medical officer at the US Food and Drug Administration, repeatedly delayed thalidomide's marketing approval. Experts in both countries were expected to demonstrate publicly the professional 'objectivity' of medicine and the institutional 'disinterestedness' of regulatory bodies. These norms were invoked both by industry representatives seeking to undermine the two experts and by critics desiring stronger regulatory controls. Comparing Lenz and Kelsey demonstrates how institutional structures shape an expert's social and scientific roles. While the United States provided important

protection from external pressure for Kelsey through her regulatory position at the FDA, Lenz was open to sharp criticism, especially when giving expert testimony during a lengthy court trial. The degree of exposure to politically motivated attacks differed for these two experts; they nevertheless faced similar threats to their professional credibility and personal integrity when they publicized links between thalidomide and birth defects. — Author's Abstract

**Fletcher, H. A., Donoghue H. D., Taylor, G. M., van der Zanden, A. G., and Spigelman, M.** Molecular analysis of *Mycobacterium tuberculosis* DNA from a family of 18th century Hungarians. *Microbiology* **149**(Pt 1) (2003) 143–151.

The naturally mummified remains of a mother and two daughters found in an 18th century Hungarian crypt were analyzed, using multiple molecular genetic techniques to examine the epidemiology and evolution of tuberculosis. DNA was amplified from a number of targets on the *Mycobacterium tuberculosis* genome, including DNA from

IS6110, *gyrA*, *katG* codon 463, *oxyR*, *dnaA*-*dnaN*, *mtp40*, *plcD* and the direct repeat (DR) region. The strains present in the mummified remains were identified as *M. tuberculosis* and not *Mycobacterium bovis*, from *katG* and *gyrA* genotyping, PCR from the *oxyR* and *mtp40* loci, and spoligotyping. Spoligotyping divided the samples into two strain types, and screening for a deletion in the MT1801-*plcD* region initially divided the strains into three types. Further investigation showed, however, that an apparent deletion was due to poor DNA preservation. By comparing the effect of PCR target size on the yield of amplicon, a clear difference was shown between 18th century and modern *M. tuberculosis* DNA. A two-center system was used to confirm the findings of this study, which clearly demonstrate the value of using molecular genetic techniques to study historical cases of tuberculosis and the care required in drawing conclusions. The genotyping and spoligotyping results are consistent with the most recent theory of the evolution and spread of the modern tuberculosis epidemic. — Author's Abstract

**Obregon, D.** Building national medicine: leprosy and power in Colombia, 1870–1910. *Soc. Hist. Med.* **15**(1) (2002) 89–108.

As imperialist nations rediscovered leprosy in their colonial world in the late nineteenth century, Colombian physicians found endemic leprosy in their own country. The medical community was interested in constructing a national medicine to conform to 'universal' science. To medicalize leprosy, doctors provoked fears through exaggerating the number of leprosy sufferers to demonstrate that charity was incapable of dealing with the problem. The government approved laws of compulsory segregation of leprosy patients in the 1890s, while the 1897 international conference on leprosy held in Berlin gave international sanction to isolation. Lepers actively resisted segregation as a violation of their individual rights. Dr. Juan de Dios Carrasquilla studied the disease, experimented with sero-therapy to cure it, and claimed that the flea was its agent of transmission. He combatted segregation and proposed instead a hygienic program to improve environmental living conditions, but his approach was defeated.

When the early twentieth century saw the consolidation of the Colombian state, modernization of the country became a national priority. The government started to take control of lazarettos, enforcing segregation of lepers, who were confined within an area circumscribed by a sanitary cordon. This strategy was a failure, since patients resisted segregation. — Author's Abstract

**Pai, H. H., Chen, W. C., and Peng, C. F.** Isolation of non-tuberculous mycobacteria from hospital cockroaches (*Periplaneta americana*). *J. Hosp. Infect.* **53**(3) (2003) 224–248.

This study attempted to isolate mycobacteria from hospital and household cockroaches from 90 hospitals and 40 households in Kaohsiung City and Kaohsiung County, South Taiwan. Among 203 cockroaches (139 *Periplaneta americana* and 64 *Blattella germanica*) collected from the hospitals, six *Mycobacterium* spp. were isolated and identified by polymerase chain reaction-restriction fragment length polymorphism analysis. In 12 cockroaches (*P. americana*): four *Mycobacterium kansasii*, three *Mycobacterium xenopi*, two *Mycobacterium goodii*, one *Mycobacterium hemophilium*, one *Mycobacterium fortuitum*, and one *Mycobacterium avium*. However, no mycobacteria were obtained from the hospital *B. germanica* or 226 household cockroaches (123 *P. americana* and 103 *B. germanica*). As cockroach infestation occurs commonly in the hospital environment, they may potentially be implicated as a cause of hospital-acquired infections due to non-tuberculous mycobacteria. — Authors' Abstract

**Zink, A. R., Sola, C., Reischl, U., Grabner, W., Rastogi, N., Wolf, H., and Nerlich, A. G.** Characterization of *Mycobacterium tuberculosis* complex DNAs from Egyptian mummies by spoligotyping. *J. Clin. Microbiol.* **41**(1) (2003) 359–367.

Bone and soft tissue samples from 85 ancient Egyptian mummies were analyzed for the presence of ancient *Mycobacterium tuberculosis* complex DNA (aDNA) and further characterized by spoligotyping. The specimens were obtained from individuals

from different tomb complexes in Thebes West, Upper Egypt, which were used for upper social class burials between the Middle Kingdom (since ca. 2050 BC) and the Late Period (until ca. 500 BC). A total of 25 samples provided a specific positive signal for the amplification of a 123-bp fragment of the repetitive element IS6110, indicating the presence of *M. tuberculosis* DNA. Further PCR-based tests for the identification of subspecies failed due to lack of specific amplification products in the historic tissue samples. Of these 25 positive specimens, 12 could be successfully characterized by spoligotyping. The spoligotyping signatures were compared to those in an international database. They all show either an *M. tuber-*

*culosis* or an *M. africanum* pattern, but none revealed an *M. bovis*-specific pattern. The results from a Middle Kingdom tomb (used exclusively between ca. 2050 and 1650 BC) suggest that these samples bear an *M. africanum*-type specific spoligotyping signature. The samples from later periods provided patterns typical for *M. tuberculosis*. This study clearly demonstrates that spoligotyping can be applied to historic tissue samples. In addition, our results do not support the theory that *M. tuberculosis* originated from the *M. bovis* type but, rather, suggest that human *M. tuberculosis* may have originated from a precursor complex probably related to *M. africanum*.—Authors' Abstract

## Chemotherapy

**Qurrat-Ul-Ain, Sharma, S., Khuller, G. K., and Garg, S. K.** Alginate-based oral drug delivery system for tuberculosis: pharmacokinetics and therapeutic effects. *J. Antimicrob. Chemother.* **51**(4) (2003) 931–938.

Alginate microparticles were developed as oral sustained delivery carriers for antitubercular drugs in order to improve patient compliance. In the present study, pharmacokinetics and therapeutic effects of alginate microparticle encapsulated antitubercular drugs, i.e., isoniazid, rifampicin and pyrazinamide were examined in guinea pigs. Alginate microparticles containing antitubercular drugs were evaluated for *in vitro* and *in vivo* release profiles. These microparticles exhibited sustained release of isoniazid, rifampicin and pyrazinamide for 3–5 days in plasma and up to 9 days in organs. Peak plasma concentration (C<sub>max</sub>), T<sub>max</sub>, elimination half-life (t<sub>1/2e</sub>) and AUC<sub>0-infinity</sub> of alginate drugs were significantly higher than those of free drugs. The encapsulation of drug in alginate microparticles resulted in up to a nine-fold increase in relative bioavailability compared with free drugs. Chemotherapeutic efficacy of alginate drug microspheres against experimental tuberculosis showed no detectable cfu values at 1:100 and 1:1000 dilutions of spleen and lung homogenates. Histopathological studies further substanti-

ated these observations, thus suggesting that application of alginate-encapsulated drugs could be useful in the effective treatment of tuberculosis.—Authors' Abstract

**Buchmeier, N. A., Newton, G. L., Koledin, T., and Fahey, R. C.** Association of mycothiol with protection of *Mycobacterium tuberculosis* from toxic oxidants and antibiotics. *Mol. Microbiol.* **47**(6) (2003) 1723–1732.

Mycothiol, MSH or 1d-myo-inosityl 2-(N-acetyl-l-cysteinyl)amido-2-deoxy-alpha-d-glucopyranoside, is an unusual conjugate of N-acetylcysteine (AcCys) with 1d-myo-inosityl 2-acetamido-2-deoxy-alpha-d-glucopyranoside (GlcN-Ins), and is the major low-molecular-mass thiol in mycobacteria. Mycothiol has antioxidant activity as well as the ability to detoxify a variety of toxic compounds. Because of these activities, MSH is a candidate for protecting *Mycobacterium tuberculosis* from inactivation by the host during infections as well as for resisting antituberculosis drugs. In order to define the protective role of MSH for *M. tuberculosis*, we have constructed an *M. tuberculosis* mutant in Rv1170, one of the candidate MSH biosynthetic genes. During exponential growth, the Rv1170 mutant bacteria produced approximately 20% of wild-type levels of MSH. Levels of the

Rv1170 substrate, GlcNAc-Ins, were elevated, whereas those of the product, GlcN-Ins, were reduced. This establishes that the Rv1170 gene encodes for the major GlcNAc-Ins deacetylase activity (termed MshB) in the MSH biosynthetic pathway of *M. tuberculosis*. The Rv1170 mutant grew poorly on agar media lacking catalase and oleic acid, and had heightened sensitivities to the toxic oxidant cumene hydroperoxide and to the antibiotic rifampin. In addition, the mutant was more resistant to isoniazid, suggesting a role for MSH in activation of this prodrug. These data indicate that MSH contributes to the protection of *M. tuberculosis* from oxidants and influences resistance to two first-line antituberculosis drugs.—Authors' Abstract

**Crevel, R. van, Alisjahbana, B., de Lange, W. C., Borst, F., Danusantoso, H., van der Meer, J. W., Burger, D., and Nelwan, R. H.** Low plasma concentrations of rifampicin in 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/L. No toxic plasma concentrations of rifampicin (>20 mg/L) 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 found to be normal. The clinical significance of these findings remains to be determined.—Authors' Abstract

**Dhople, A. M. and Namba, K.** In vivo susceptibility of *Mycobacterium leprae* to sitafloxacin (DU-6859a), either singly or in combination with rifampicin analogues. *Int. J. Antimicrob. Agents* **21(3)** (2003) 251–255.

The antimicrobial effects of sitafloxacin (DU-6859a) against *Mycobacterium leprae*, either singly or in combination with either rifampicin, rifabutin or KRM-1648, were studied using a mouse footpad assay technique and the results were compared with those obtained with ofloxacin. When used singly, the minimum concentrations of sitafloxacin and ofloxacin needed to inhibit completely the growth of *M. leprae* were 25 and 100 mg per kg body weight per day, respectively, and the effects were bactericidal. Both sitafloxacin and ofloxacin exhibited excellent synergistic effects when combined with either rifabutin or KRM-1648, but not with rifampicin. Thus, incorporation of sitafloxacin and rifabutin (or KRM-1648) in the multidrug regimen for treating leprosy patients is suggested.—Authors' Abstract

**Doucet-Populaire, F., Buriankova, K., Weiser, J., and Pernodet, J. L.** Natural and acquired macrolide resistance in mycobacteria. *Curr. Drug Targets Infect. Disord.* **2(4)** (2002) 355–370.

The genus *Mycobacterium* contains two of the most important human pathogens, *Mycobacterium tuberculosis* and *Mycobacterium leprae*, the etiologic agents of tuberculosis and leprosy, respectively. Other mycobacteria are mostly saprophytic organisms, living in soil and water, but some of them can cause opportunistic infections. The increasing incidence of tuberculosis as well as infections with non-tuberculous mycobacteria (NTM) in AIDS patients has renewed interest in molecular mechanisms of drug resistance in these pathogens. Mycobacteria show a high

degree of intrinsic resistance to most common antibiotics. For instance, species from the *M. tuberculosis* complex (MTC) are intrinsically resistant to macrolides. Nevertheless, some semi-synthetic macrolides as the erythromycin derivatives clarithromycin, azithromycin and most recently the ketolides, are active against NTM, particularly *Mycobacterium avium*, and some of them are widely used for infection treatment. However, shortly after the introduction of these new drugs, resistant strains appeared due to mutations in the macrolide target, the ribosome. The mycobacterial cell wall with its specific composition and structure is considered to be a major factor in promoting the natural resistance of mycobacteria to various antibiotics. However, to explain the difference in macrolide sensitivity between the MTC and NTM, the synergistic contribution of a specific resistance mechanism might be required, in addition to possible differences in cell wall permeability. This mini-review summarizes the current knowledge on the natural and acquired macrolide resistance in mycobacteria, gives an overview of potential mechanisms implicated in the intrinsic resistance and brings recent data concerning a macrolide resistance determinant in the MTC.—Authors' Abstract

**Dutt, M. and Khuller, G. K.** Poly (DL-lactide-co-glycolide) microparticles as carriers for antimycobacterial drug rifampicin. *Indian J. Exp. Biol.* **38(9)** (2000) 887–894.

Poly (DL-lactide-co-glycolide) polymers were investigated as carriers for the first line antitubercular drug rifampicin. Different formulations of PLG microparticles viz. porous, non porous and hardened exhibited sustained release of rifampicin up to 7 weeks *in vitro*. However, hardened PLG microparticles exhibited the most sustained release *in vivo* in different organs up to 6 weeks. In case of free rifampicin, release was detected *in vivo* only up to 48 hr. In addition, no hepatotoxicity was observed on a biochemical basis (levels of SGPT, ALP and total bilirubin) in comparison to control animals. Taken together, these results suggest that polymer encapsulated antitubercular drug rifampicin may serve as an ideal therapeutic approach for treatment of tuberculous infections.—Authors' Abstract

**Gardam, M. A., Keystone, E. C., Menzies, R., Manners, S., Skamene, E., Long, R., and Vinh, D. C.** Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and Clinical management. *Lancet Infect. Dis.* **3(3)** (2003) 148–155.

Cases of active tuberculosis have been reported worldwide with the use of therapeutic agents that inhibit tumor necrosis factor (TNF) alpha. TNFalpha has a central role in mycobacterial infection and disease. Accordingly, progression of recently acquired tuberculosis infection or reactivation of remotely acquired infection should be expected with the use of anti-TNF agents. The available *in vitro* and epidemiological evidence for the two currently approved agents, infliximab and etanercept, shows that the risk of development of active tuberculosis is greater with infliximab. Tuberculin skin testing (TST) should be undertaken before any significant immunosuppressive therapy including these agents, though the possibility of false-negative reactions in immunocompromised populations must be borne in mind. A positive TST should be followed by medical assessment and chest radiography, as well as by other tests judged appropriate by the physician to identify active disease. Active tuberculosis must be treated appropriately before initiation of treatment with an anti-TNF agent. Treatment of latent tuberculosis can be considered on an individual basis for TST-negative patients receiving anti-TNF agents when significant risk factors for infection are present.—Authors' Abstract

**Ishii, N.** Recent advances in the treatment of leprosy. *Dermatol. Online J.* **9(2)** (2003) 5.

Leprosy, a chronic infectious disease caused by *Mycobacterium leprae*, was identified by G. H. A. Hansen in 1873. The different clinical presentations of the disease are determined by the quality of the host immune response. The bacteria have affinity for the peripheral nerves and are likely the cause of neuropathy, a cardinal manifestation of the disease. WHO recommends a protocol of multidrug therapy (MDT), which effectively controls the disease, hence contributing to the global elimination program. Early detection of leprosy and treatment by MDT are

the most important steps in preventing deformity and disability.— Author's Abstract

**Jain, R., Vaitilingam, B., Nayyar, A., and Palde, P. B.** Substituted 4-methylquinolines as a new class of anti-tuberculosis agents. *Bioorg. Med. Chem. Lett.* **13(6)** (2003) 1051–1054.

We report synthesis and anti-tuberculosis activities of a series of novel ring-substituted quinolines. The most effective compound of the series 3d (MIC = 6.25 microg/mL, *Mycobacterium tuberculosis* H37Rv strain) was synthesized in one step; thus is an attractive lead molecule for anti-tuberculosis drug development. The results of this study represent the discovery of ring-substituted 4-methylquinolines as new class of potential anti-tuberculosis agents.— Authors' Abstract

**Lall, N., Das Sarma, M., Hazra, B., and Meyer, J. J.** Antimycobacterial activity of diospyrin derivatives and a structural analogue of diospyrin against *Mycobacterium tuberculosis in vitro*. *J. Antimicrob. Chemother.* **51(2)** (2003) 435–438.

Three derivatives and one structural analogue of diospyrin were synthesized and investigated for their inhibitory activity against *Mycobacterium tuberculosis* employing the rapid radiometric method *in vitro*. A novel aminoacetate derivative was found to be more active than the parent compound, the MICs being 50 and 100 mg/L, respectively, for a drug-susceptible strain, H37Rv, of *M. tuberculosis*. This derivative also exhibited an MIC of 50 mg/L for a few multidrug-resistant strains of *M. tuberculosis*. The other two derivatives and the analogue did not show any significant antimycobacterial activity at the highest concentration (100 mg/L) tested.— Authors' Abstract

**Luna-Herrera, J., Martinez-Cabrera, G., Parra-Maldonado, R., Enciso-Moreno, J. A., Torres-Lopez, J., Quesada-Pascual, F., Delgadillo-Polanco, R., and Franzblau, S. G.** Use of Receiver Operating Characteristic Curves to Assess the Performance of a Microdilution Assay for Determination of Drug Susceptibility

of Clinical Isolates of *Mycobacterium tuberculosis*. *Eur. J. Clin. Microbiol. Infect. Dis.* **22(1)** (2003) 21–27.

The aim of this study was to apply receiver operating characteristic (ROC) analysis to the microplate Alamar blue assay, a recently developed alternative for drug susceptibility testing of mycobacteria. As this is a quantitative assay, its performance can be determined by ROC analysis, in which the area under the ROC curve represents a summary of test performance (the higher the area, the better the test's performance). Sixty isolates of *Mycobacterium tuberculosis* were tested by the microcolorimetric assay against six twofold dilutions of streptomycin, isoniazid, rifampin, and ethambutol. For each isolate, the susceptibility pattern was simultaneously established by the agar proportion method, the result of which represented the gold standard value for the ROC analysis. The critical concentration, area under the curve, and p value for each drug were determined by ROC curve analysis. The results of the assay were obtained in an average of 8 days of incubation. The performance of the assay was excellent for all four drugs: the area under the curves was >0.97, the p values were 0.000, and sensitivity was 94%, specificity 97%, predictive value for resistance ≥92%, predictive value for susceptibility 97%, and test efficiency 97%. According to ROC analysis, the microplate Alamar blue assay is a reliable method for determination of drug-susceptibility. Rapidity and cost efficiency are two additional qualities that make this test an excellent alternative for the drug susceptibility testing of *Mycobacterium tuberculosis*. The ROC curve analysis is a robust statistical approach for evaluating the performance of new quantitative methods for determination of drug sensitivity of *Mycobacterium tuberculosis* isolates.— Authors' Abstract

**Meierhofer, C. and Wiedermann, C. J.** New insights into the pharmacological and toxicological effects of thalidomide. *Curr. Opin. Drug Discov. Devel.* **6(1)** (2003) 92–99.

The molecular activity of thalidomide comprises a wide range of mechanisms. Al-

teration of cytokine synthesis and release may be as important as changes in lymphocyte trafficking and leukocyte migration. Since endothelial cells play an important role in leukocyte extravasation and maintenance of inflammatory processes in the affected tissue, thalidomide-induced alterations of cellular adhesion molecules, and consequently changes of interaction of leukocytes with the endothelial cell layer, will result in modulation of the response in inflammation and immunity. Thalidomide mainly reduces tumor necrosis factor (TNF)-alpha production by macrophages, and its TNF alpha antagonist properties explain the beneficial effects in several TNF alpha-associated complications of severe diseases. Pathophysiologically relevant alterations most likely include gene regulatory effects, with interference in growth factor-dependent pathways known to be involved in teratogenesis, and effects on the transcriptional control of the inflammatory response via nuclear factor (NF)-kappa B. The effects of thalidomide, its enantiomers and analogs, on a broad range of diseases, and their differential pharmacokinetic and pharmacodynamic properties, give the scope for ongoing investigations in the search for analogs with better selectivity but without thalidomide-related side effects and teratogenicity.—Authors' Abstract

**Murray, M. F.** Nicotinamide: an oral antimicrobial agent with activity against both *Mycobacterium tuberculosis* and human immunodeficiency virus. *Clin. Infect. Dis.* **36(4)** (2002) 453–460.

Coinfection with *Mycobacterium tuberculosis* and human immunodeficiency virus (HIV) is responsible for one-third of all deaths due to acquired immunodeficiency syndrome. More than 99% of cases of HIV-*M. tuberculosis* coinfection occur in the developing world, where limited resources add urgency to the search for effective and affordable therapies. Although antimicrobial agents against each of these infections are available, single agents that have activity against both *M. tuberculosis* and HIV are uncommon. The activity of nicotinamide has been evaluated in 2 different areas: in anti-*M. tuberculosis* studies per-

formed during 1945–1961 and in anti-HIV studies performed from 1991 to the present. This review brings together these 2 bodies of inquiry and raises the possibility that, with more study, this small molecule could emerge at the beginning of the 21st century either as a therapeutic agent in itself or as the lead compound for a new class of agents with activity against both *M. tuberculosis* and HIV.—Author's Abstract

**Ordway, D., Viveiros, M., Leandro, C., Bettencourt, R., Almeida, J., Martins, M., Kristiansen, J. E., Molnar, J., and Amaral, L.** Clinical concentrations of thioridazine kill intracellular multidrug-resistant *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **47(3)** (2003) 917–922.

The phenothiazines chlorpromazine (CPZ) and thioridazine (TZ) have equal *in vitro* activities against antibiotic-sensitive and -resistant *Mycobacterium tuberculosis*. These compounds have not been used as anti-*M. tuberculosis* agents because their *in vitro* activities take place at concentrations which are beyond those that are clinically achievable. In addition, chronic administration of CPZ produces frequent severe side effects. Because CPZ has been shown to enhance the killing of intracellular *M. tuberculosis* at concentrations in the medium that are clinically relevant, we have investigated whether TZ, a phenothiazine whose negative side effects are less frequent and serious than those associated with CPZ, kills *M. tuberculosis* organisms that have been phagocytosed by human macrophages, which have nominal killing activities against these bacteria. Both CPZ and TZ killed intracellular antibiotic-sensitive and -resistant *M. tuberculosis* organisms when they were used at concentrations in the medium well below those present in the plasma of patients treated with these agents. These concentrations *in vitro* were not toxic to the macrophage, nor did they affect *in vitro* cellular immune processes. TZ thus appears to be a serious candidate for the management of a freshly diagnosed infection of pulmonary tuberculosis or as an adjunct to conventional antituberculosis therapy if the patient originates from an area

known to have a high prevalence of multidrug-resistant *M. tuberculosis* isolates. Nevertheless, we must await the outcomes of clinical trials to determine whether TZ itself may be safely and effectively used as an antituberculosis agent.—Authors' Abstract

**Salem, I. I. and Duzgunes, N.** Efficacies of cyclodextrin-complexed and liposome-encapsulated clarithromycin against *Mycobacterium avium* complex infection in human macrophages. *Int. J. Pharm.* **250(2)** (2003) 403–414.

Cyclodextrins and liposomes have been used in recent years as drug delivery vehicles, improving the bioavailability and therapeutic efficacy of many poorly water-soluble drugs. In this study, we used two approaches to enhance the availability of the poorly water-soluble antibiotic, clarithromycin, by inclusion complex formation and by liposome-encapsulation. We examined the efficacies of these formulations against *Mycobacterium avium* complex (MAC) in human peripheral blood monocyte-derived macrophages. The water solubility of clarithromycin was enhanced by about 700-fold by complexation with cyclodextrin. The use of a rapid radiometric (BACTEC) method for the detection of MAC growth and susceptibility showed identical MICs against

MAC for both the free and complexed drug. The anti-MAC efficacy of the cyclodextrin complex of clarithromycin in macrophages was slightly lower than the free drug, probably due to the high stability of the inclusion complex. At higher drug concentrations, Liposome-encapsulated clarithromycin was slightly more effective against intracellular MAC growth than the free drug.—Authors' Abstract

**Salmon-Ceron, D, Groupe Recherche Anti-TNF et Infections Opportunistes, and Agence Francaise de Securite Sanitaire des Produits de Sante.** Recommendations for the prevention and management of tuberculosis in patients taking infliximab. *Ann. Med. Interne.* (Paris) **153(7)** (2002) 429–431.

An unusually large number of cases of tuberculosis, often with miliary or widespread dissemination, has been reported in patients taking infliximab for rheumatoid arthritis or Crohn's disease. Recommendations have been issued in France regarding the definition of high-risk patients, the screening methods to be used in these patients, and possible prophylactic treatments. The present update is also intended to help physicians manage tuberculosis occurring before or during infliximab therapy.—Authors' Abstract

## Clinical Sciences

**Biswas, S. and Mondal, K. K.** Multidrug therapy in leprosy can prevent relapse—a retrospective study. *Indian J. Lepr.* **74(4)** (2002) 313–318.

A retrospective study was done at the Leprosy Control Unit (LCU) in Durgapur of Burdwan district, West Bengal, to determine the relapse rate following multidrug therapy (MDT). A total of 1581 patients (1276 PB and 305 MB) completed MDT regimens during a period of 5 yrs as per WHO recommendations and National Leprosy Eradication Programme (NLEP) guidelines. The treated patients were kept under surveillance as per NLEP guidelines and searched for relapses. The results of

MDT were compared with those of pre-MDT (monotherapy) era at the same center (total: 405 patients; PB-373, MB-32) and also with those of the Leprosy Clinic in Gopalpur (only dapsone was given to a total of 189 patients, PB-167, MB-22). Following monotherapy, the relapse rate was 10.06% at the Gopalpur Leprosy Clinic and 12.4% at the Durgapur LCU during the 2 yrs (PB) and 5 yrs (MB) of surveillance, whereas following MDT no relapse case was encountered both in PB and MB cases during the surveillance periods recommended by WHO. The results of this study are comparable with those of other studies. Though a few studies showed relapses during long-term surveillance beyond the peri-

ods recommended by WHO, it is once again established that MDT can prevent relapse in leprosy.—Authors' Abstract

**Chatterjee, M. and Jaiswal, A. K.** Does pentoxifylline find a place in the armamentarium of leprologists in type II reaction? *Indian J. Lepr.* **74(4)** (2002) 329–334.

Alternative therapeutic interventions in Type II lepra reaction are being considered following serious problems associated with the use of steroids and thalidomide. Pentoxifylline (PTX) has been used in Type II reaction with varying degrees of success. The results of a study on the use of this drug in a dose of 1200 mg per day for a period of 2 months in patients with ENL reaction are discussed. Five patients, one of whom was HIV positive—all with severe Type II reaction, were regularly evaluated for regression of inflammatory symptoms and clinical involution of ENL lesions while on PTX therapy and thereafter. It was found that PTX led to a total elimination of systemic symptoms within a week. ENL lesions regressed in two weeks. However, in one patient, lesions recurred after one month of therapy. It appears that PTX is well tolerated and could be used as an additional drug in the armamentarium of leprologists in the management of Type II reaction, especially in HIV co-infection, where long-term steroids are contraindicated. However, further studies to compare the effects of PTX with currently, widely used drugs for the treatment of ENL reaction are necessary.—Authors' Abstract

**Croft, R. P., Nicholls, P. G., Steyerberg, E. W., Richardus, J. H., Withington, S. G., and Smith, W. C. S.** A clinical prediction rule for nerve function impairment in leprosy patients—revisited after 5 yrs of follow-up. *Lepr. Rev.* **74** (2003) 35–41.

Nerve function impairment (NFI) commonly occurs during or after chemotherapy in leprosy. We previously described a clinical prediction rule to estimate the risk of NFI occurring within 2 years of diagnosis, based on 2510 patients who are followed up in the Bangladesh Acute Nerve Damage

Study (BANDS). This prediction rule assigns new leprosy patients to one of three risk groups based on leprosy group and the presence or absence of NFI at registration. Updated data with up to 5 years of follow-up showed that 95% of all NFI occurred within 2 years. This study confirms the validity of the rule and supports the conclusion that there is little value for the detection of NFI in extending follow-up beyond 2 years.—Leprosy Review

**De Carsalade, G. Y., Achirafi, A., and Flageul, B.** Pentoxifylline in the treatment of erythema nodosum leprosum. *J. Dermatol.* **30(1)** (2003) 64–68.

Erythema nodosum leprosum (ENL) is a well-known serious complication affecting 10% of lepromatous multibacillary leprosy patients. In the chronic form, its morbidity may be considerable. Thalidomide and systemic steroids are the two current effective drugs for the management of ENL. However, their use in endemic countries is often difficult and hazardous, and a search for new therapies is needed. We report our experience on the effects of pentoxifylline, a methylxanthine derivative, which has recently been suggested as a possible effective treatment for ENL attacks.—Authors' Abstract

**Ebenezer, G. J., Norman, G., Joseph, G. A., Daniel, S., and Job, C. K.** Drug resistant-*Mycobacterium leprae*—results of mouse footpad studies from a laboratory in south India. *Indian J. Lepr.* **74(4)** (2002) 301–312.

Out of 265 biopsies of leprosy patients received at the Experimental Pathology Laboratory of Schieffelin Leprosy Research and Training Centre from 1987 to 1997 for evaluating resistant strains of *M. leprae*, using the mouse footpad technique, 49 showed resistant strains of *M. leprae* to varying concentrations of dapsone, rifampicin and clofazimine. 23 (47%) of these were from a control area. With 369 skin-smear positive multibacillary (MB) patients as the risk group (denominator), 23 (6.23%) were resistant to one or more

drugs. 18 (4.88%) had dapsone resistance, 5 (1.36%) were resistant to rifampicin and 9 (2.44%) had resistance to low concentrations of clofazimine (0.0001%). Out of the 23 biopsies with drug resistance from the control area, primary dapsone resistance was seen in 7 (30%) biopsies and secondary dapsone resistance in 11 (48%). Primary rifampicin resistance was seen in 4 (17.4%) patients, secondary rifampicin resistance in 1 (4.35%) and primary clofazimine resistance in 7 (30%). 3 (13%) of the strains showed secondary clofazimine resistance. One biopsy had resistant strains to all the three drugs. In a control area where properly supervised effective multidrug therapy (MDT) was regularly administered over the years, the emergence of drug resistance is negligible. It may not be the case if the content, duration and regularity of the drug regimen were not satisfactory. Aware of the possible shortcomings in mass administration of MDT, it is emphasized that mouse footpad studies on drug resistance should be made available at least in endemic areas where the incidence of the disease has not changed despite good MDT coverage in order to monitor the emergence of drug resistance. Research into molecular biological identification of drug resistant-*M. leprae* should be intensified. These steps would help to institute timely measures to check the spread of any drug-resistant organisms in the community.—Authors' Abstract

**Joshi, R. S.** Follicular mucinosis in suspected leprosy. *Indian J. Lepr.* **74(4)** (2002) 341–347.

Four cases of suspected leprosy showed, on biopsy, follicular mucinosis without any granulomatous inflammation. All the patients were adolescents (12–17 yrs) with a single lesion on the face. Three patients showed complete clearing after anti-leprosy treatment, and the fourth patient is currently taking anti-leprosy treatment and showed good clinical response.—Author's Abstract

**Kheir, M. M., Ahmed, A. M., and Elsar-rag, A. A.** Thyroid functional status in leprosy patients in Sudan. *East Mediterr. Health J.* **7(1–2)** (2001) 79–83.

We studied 45 adult patients with untreated lepromatous leprosy and borderline leprosy, presenting at clinics in Khartoum and Omdurman, to assess clinical and biochemical effects of the disease on thyroid function. A matching control group of 30 subjects, without symptoms or signs of thyroid disease, were included for comparison. Thyroxine, triiodothyronine and thyrotrophin levels were within normal range. Mean serum thyroxine was low in both groups (significant in lepromatous leprosy patients only). Mean serum triiodothyronine was high in both groups (significant in neither group). Mean thyrotrophin was significantly higher in both groups compared with controls.—Authors' Abstract

**Koelewijn, L. F., Meima, A., Broekhuis, S. M., Rishardus, J. H., Mitchell, P. D., Benbow, C., and Saunderson, P. R.** Sensory testing in leprosy: comparison of ballpoint pen and monofilaments. *Lepr. Rev.* **74** (2003) 42–52.

The 10 g monofilament has been replaced by the ballpoint pen in routine sensory testing of nerves in leprosy control in Ethiopia. Results of sensory testing between the ballpoint pen and different monofilaments on hands and feet were compared. Ballpoint pen underdiagnosis of loss of sensation was defined to occur when the pen was felt and the monofilament was not. Differences were evaluated both for individual test points (test point level) and for the test points of extremities collectively (extremity level). An extremity (either a hand or a foot) was defined as having sensory nerve function impairment (SNFI) if a supplying nerve had SNFI, which was the case when sensation was absent in two or more test points in the area supplied by that nerve. At test point level, the percentages with ballpoint pen underdiagnosis relative to the 2, 10, 20, and 50 g monofilaments were 40, 21, 9 and 7%, respectively, in the hands, and 47, 30, 15 and 7% in the feet. Ballpoint pen underdiagnosis percentages of SNFI at extremity level were 32, 18, 8 and 9% in the hands, and 37, 26, 14 and 6% in the feet. The risk of ballpoint pen underdiagnosis appears to be higher in extremities without visible damage. In conclusion, substan-

tial levels of underdiagnosis of sensory loss with the ballpoint pen were observed. However, the consequences for the prognosis of treatment with corticosteroids in patients with the more subtle sensation loss noted here need to be established. Development and testing of guidelines is a prerequisite for the use of the ballpoint pen.—Leprosy Review

**McEvitt, E. and Schwarz, R.** Tendon transfer for triple nerve paralysis of the hand in leprosy. *Lepr. Rev.* **73(4)** (2002) 319–325.

Paralysis of ulnar, median and radial nerves is seen in less than 1% of those affected with leprosy. This condition is a particular challenge for the surgeon, physiotherapist, and patient. A retrospective chart review was conducted at the Green Pastures Hospital and Rehabilitation Centre (GPHRC) and Anandaban Leprosy Hospital (ALH) in Nepal, and results were graded by the system outlined by Sundararaj in 1984. Thirty-one patients were identified, and 21 charts were available for review. Excellent or good results were obtained in 93% of patients for wrist extension, 85% of patients for finger extension, 90% of patients for thumb extension, 71% of patients for intrinsic reconstruction, and 63% of patients for thumb opposition reconstruction. These results are reasonable but inferior to those obtained by Sundararaj in his study. Surgical intervention offers a very significant improvement in function in these very difficult hands. Intensive physiotherapy is required both pre- and postoperatively.—Authors' Abstract

**Oliveira, M. L., Pierro, A. P., Silveira, P. A., Campos, M. M., and Vilela, M. F.** Relapse of lepromatous leprosy after WHO/MDT with rapid bacterial growth. *Lepr. Rev.* **73(4)** (2002) 386–388.

The authors report a case of relapse in a lepromatous patient 6 years after he had been cured by MDT/WHO/24 doses. The atypical aspect emphasized in this case is the bacterial load increase in a short period of time of 1 year after the smear count was negative, and the case reinforces the importance of patient education on release. No leprosy cases were

identified in the patient's close contacts. It seems that relapse was a result of bacillary persistence, since a significant improvement was noted in relapsed lesions after two doses of MDT/WHO.—Authors' Abstract

**Oommen, A. T., Mannam, E., Partheenarajan, S., and Samuel, S.** Excision arthroplasty: an effective method in the management of plantar ulcers with metatarso-phalangeal joint infection in anaesthetic feet. *Lepr. Rev.* **74** (2003) 63–67.

The cases of 30 patients with septic arthritis of the metatarsophalangeal (MTP) joints as a complication of plantar ulceration in leprosy who underwent excision arthroplasty and primary closure of the plantar ulcer were reviewed. Twenty-two of these patients were male. The commonest site of MTP joint involvement was the first MTP joint. The average longitudinal diameter of ulcers was 2 cm, and most ulcers were oval in shape. Diagnosis was made on the basis of signs of infection over the MTP joint, discharge from the ulcer and examination with a probe. Infection in the joint ranged from simple synovial discharge to seropurulent or purulent discharge. Treatment involved excision arthroplasty of the MTP joint, excision of the ulcer with primary closure of the plantar incision and dorsal or lateral drainage depending upon the direction in which the infection extended. In two patients, the plantar wound could not be closed as it was too large. Healing of the plantar incision took 2 weeks in 12 patients and 3 weeks in 14 patients. In four patients, healing did not occur by primary intention. In a follow up of 1–2 years, there was no recurrence in 24 patients, while four patients had recurrent simple ulceration. Two patients were lost to follow up. Review of the results of this procedure dealing with septic arthritis of MTP joints secondary to plantar ulceration shows that primary healing of the plantar incision could be achieved in 3 weeks. With regard to recurrence, even though only four out of 28 ulcers treated by this procedure recurred, other contributing factors should be considered in a prospective control study to support the view that this procedure has contributed to non-recurrence.—Leprosy Review

**Pan Shuq, 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*

**Rajesh, M., Sulochana, K. N., Sundaram, A. L., Krishnakumar, S., Biswas, J., and Ramakrishnan, S.** Presence of a 88 kDa Eales protein in uveitis, tuberculosis, leprosy and rheumatoid arthritis. *Med. Sci. Monit.* **9(2)** (2003) CR95–99.

**BACKGROUND:** Eales disease (ED) is an idiopathic retinal vasculitis affecting young adult males. We have earlier reported the identification, purification and partial characterization of a novel 88 kDa protein found in the serum of patients with ED. The aim of the present study was to look for the 88 kDa protein in serum samples obtained from cases of retinal vasculitis mimicking ED and in other systemic inflammatory diseases. **MATERIAL/METHODS:** Serum samples from healthy volunteers and from patients with ED, uveitis, parsplanitis ocular sarcoidosis, toxoplasmosis, leprosy, diabetic retinopathy, viral hepatitis, and rheumatoid arthritis were analyzed for the presence of the 88 kDa protein by polyacrylamide gel electrophoresis (PAGE). The immunological identity of the 88 kDa protein found in ED and in other diseases was investigated by Western blot. Immunohistochemistry was performed on epiretinal membranes (ERM) obtained from ED patients to localize the 88

kDa protein. **RESULTS:** 88 kDa protein were detected in serum samples obtained from patients with posterior uveitis, tuberculosis, leprosy and rheumatoid arthritis. The 88 kDa protein found in serum from patients with ED is immunologically identical to that found in other systemic inflammatory conditions. 88 kDa protein was localized in inflammatory cells and in nonvascular endothelium in ERMs obtained from patients with ED. **CONCLUSIONS:** We have identified a novel acute phase reactant, which is elaborated in ocular and systemic inflammatory conditions other than Eales disease. Further work is necessary to decipher the precise role of the 88 kDa protein in the pathophysiology of these inflammatory diseases. — *Authors' Abstract*

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

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

cantly affected the functional outcome. ZLP was found to be a more effective procedure in restoring grip strength, whereas FDS-4T was more effective in correcting claw hand deformity.—Leprosy Review

**Turkof, E., Richard, B., Assadian, O., Khatri, B., Knolle, E., and Lucas, S.** Leprosy affects facial nerves in a scattered distribution from the main trunk to all peripheral branches and neurolysis improves muscle function of the face. *Am. J. Trop. Med. Hyg.* **68(1)** (2003) 81–88.

Current literature rejects nerve release in leprosy facial neuropathy and states that lesions are restricted to the peripheral zygomatic branches. Since there are approximately 500,000 patients with this disease throughout the world, we wanted to clarify the precise location of facial nerve's affection and the benefit of neurolysis. Our study showed that in patients with leprosy, the facial nerve's main trunk, the peripheral zygomatic branches, and all other branches were affected. Follow-up showed improvement in lagophthalmos and in misreinnervation, with no improvement in the control cohort. Nerve release improves muscle function in leprosy facial neuropathy, provided surgery is performed on all affected segments. Intraoperative electroneurodiagnostics is an effective tool for detecting the most proximal site of lesion and ensuring effective surgery.—Authors' Abstract

**Ulvi, H., Yoldas, T., Yigiter, R., and Mungen, B.** R-R interval variation and the sympathetic skin response in the assessment of the autonomic nervous system in leprosy patients. *Acta. Neurol. Scand.* **107(1)** (2003) 42–49.

**OBJECTIVES:** The aim of this study was to evaluate possible autonomic nervous system (ANS) dysfunction in leprosy patients with the sympathetic skin response (SSR) and the heart rate (R-R) interval variation (RRIV) measurements which are easy and reliable methods for evaluation of autonomic functions. **MATERIAL AND METHODS:** We studied 37 lepromatous leprosy patients (mean age:  $38 \pm 17$  years, range 23–62 years,

20 females and 17 males) and 35 age-matched healthy subjects (mean age:  $34.19 \pm 12.74$  years, range 24–48 years, 20 females and 15 males). Non-invasive bedside tests (orthostatic test, Valsalva ratio), R-R interval variation (RRIV) during at rest and deep breathing, the SSR latency and amplitude from both palms, and nerve conduction parameters were studied in all the subjects. **RESULTS:** The mean values of RRIV in leprosy patients during at rest [mean RRIV in patients,  $17.42 \pm 8.64\%$  vs controls,  $22.71 \pm 3.77\%$  ( $p < 0.05$ )] and during deep breathing [mean RRIV in patients,  $21.64 \pm 9.08\%$  vs controls,  $30.70 \pm 5.99\%$  ( $p < 0.005$ )] was significantly lower compared with the controls. The mean latency of SSR in leprosy patients [mean SSR latency in patients,  $1.72 \pm 1.13$  ms vs controls,  $1.30 \pm 0.41$  ms ( $p < 0.05$ )] was significantly prolonged compared with the controls. The mean amplitude of SSR in leprosy patients [mean SSR amplitude in patients,  $0.54 \pm 0.57$  microV vs controls,  $1.02 \pm 0.56$  microV ( $p > 0.05$ )] was smaller compared with the controls, but this difference was not significant. The mean Valsalva ratio in leprosy patients [mean in patients,  $1.11 \pm 0.13$  vs controls,  $1.16 \pm 0.07$  ( $p > 0.05$ )] was smaller compared with the controls, but not statistically significant. The mean difference of systolic and diastolic blood pressure between supine rest and during standing in leprosy patients were higher compared with the controls [mean systolic pressure in patients,  $7 \pm 6$  mmHg vs controls,  $6 \pm 8$  mmHg ( $p > 0.05$ ) and mean diastolic pressure in patients,  $3 \pm 3$  mmHg vs controls,  $3 \pm 2$  mmHg ( $p > 0.05$ )], but they did not reach statistical significance. Furthermore, lower RRIV and the prolonged SSR latencies in leprosy patients were closely correlated to some parameters of sensorimotor nerve conduction and each other [median nerve distal latency and RRIV,  $r = -0.67$  ( $p < 0.05$ ), ulnar nerve distal latency and RRIV,  $r = -0.59$  ( $p < 0.05$ ), RRIV and SSR latency,  $r = -0.33$  ( $p < 0.02$ )]. These data indicate that leprosy patients have the functional abnormalities of ANS. **CONCLUSION:** We conclude that combined use of these two tests, both of which can be easily and rapidly performed in the electromyogram (EMG) laboratory using standard equipment, allows separate testing of parasympathetic and sympathetic function, and are very sensitive methods in assessing of ANS function in

peripheral neuropathy in leprosy patients.—  
Authors' Abstract

**Winthrop, K. L., Steinberg, E. B., Holmes, G., Kainer, M. A., Werner, S. B., Winquist, A., and Vugia, D. J.** Epidemic and sporadic cases of nontuberculous mycobacterial keratitis associated with laser *in situ* keratomileusis. *Am. J. Ophthalmol.* **135**(2) (2003) 223–224.

**PURPOSE:** To report national case-finding results for nontuberculous mycobacterial keratitis and describe its association with laser *in situ* keratomileusis (LASIK). **DESIGN:** Enhanced passive disease reporting. **METHODS:** In April 2001, we investigated a California cluster of *Mycobacterium chelonae* keratitis associated with hyperopic

LASIK using a contact lens mask. To identify other possibly related cases, the American Academy of Ophthalmology e-mailed its members asking them to report recent cases of nontuberculous mycobacterial keratitis to the Centers for Disease Control and Prevention. **RESULTS:** Forty-three additional cases of keratitis were reported (onsets between August 2000 and June 2001). Of these, 31 occurred as part of two unrelated LASIK-associated outbreaks. The 12 other reported cases occurred in sporadic fashion. Of the latter cases, 4 were associated with LASIK surgery. None of the reported cases were related to the *M. chelonae* cluster in California. **CONCLUSIONS:** Laser *in situ* keratomileusis-associated keratitis with nontuberculous mycobacteria may be more common than previously known.—Authors' Abstract

## Immuno-Pathology

**Biswas, P., Ferrarini, M., Mantelli, B., Fortis, C., Poli, G., Lazzarin, A., and Manfredi, A. A.** Double-edged effect of Vgamma9/Vdelta2 T lymphocytes on viral expression in an *in vitro* model of HIV-1/mycobacteria co-infection. *Eur. J. Immunol.* **33**(1) (2003) 252–263.

A reciprocal influence exists between mycobacteria and HIV: HIV-infected individuals are more susceptible to mycobacterial infections and, on the other hand, mycobacterial infection results in acceleration of HIV disease progression. Vgamma9/ Vdelta2 T lymphocytes are known to participate in the defense against intracellular pathogens, including *Mycobacterium tuberculosis*. Indeed, they kill mycobacteria-infected macrophages and, upon recognition of mycobacterial Ag, release TNF-alpha and IFN-gamma, which are also up-regulators of HIV expression. To assess whether mycobacteria-activated gamma delta T lymphocytes contribute to the enhancement of HIV replication, we established an *in vitro* model mimicking HIV and mycobacteria co-infection with the latently HIV-infected promonocytic U1 cell line and Vgamma9/Vdelta2 peripheral lymphocytes stimulated with mycobacterial Ag. gamma

delta T cell activation determined two distinct, but connected effects, namely U1 cell death and HIV expression. Both effects were mainly mediated by release of TNF-alpha and IFN-gamma from activated gamma delta lymphocytes, although Fas-FasL interaction also contributed to U1 apoptosis. The final outcome on U1 survival, and thus, on HIV expression, highly depended on mycobacterial Ag concentration coupled to the differential secretory potency of gamma delta cells. In particular, the induction of viral expression prevailed at low Ag concentration and with lower cytokine production by mycobacteria-activated gamma delta cells. Notably, during the course of HIV infection, Vgamma9/ Vdelta2 lymphocytes are reported to be functionally impaired and may thus indirectly influence the progression of HIV disease. In addition, a predominant inhibition of viral replication was encountered when mycobacteria-activated gamma delta T cells were co-cultured with primary HIV-infected macrophages. Thus, we suggest that specific recognition of mycobacterial Ag by gamma delta T lymphocytes in co-infected individuals may modulate viral replication through the complex array of soluble factors released.—Authors' Abstract

**Cheadle, E. J., Selby, P.J., and Jackson, A. M.** *Mycobacterium bovis* bacillus Calmette-Guerin-infected dendritic cells potently activate autologous T cells via a B7 and interleukin-12-dependent mechanism. *Immunology* **108(1)** (2003) 79–88.

Mycobacteria are potent adjuvants, can survive intracellularly and have been safely used for many years as vaccines against tuberculosis and leprosy. They are thus important potential vectors for recombinant vaccines. Many of their adjuvant properties are mediated following phagocytosis by dendritic cells (DC), which are in turn critical for priming naive T cells. Although the maturation of DC in response to mycobacteria, such as *Mycobacterium bovis* bacillus Calmette-Guerin (BCG), is well described the subsequent responses of autologous T cells to mycobacterium-infected DC remains uncharacterized. In our experiments DC infected with BCG expressed more costimulatory molecules than tumor-necrosis factor-alpha (TNF-alpha)-treated DC and stimulated more potent mixed leucocyte reactions. When autologous T cells were cocultured with BCG-exposed DC they became highly activated, as determined by display of CD25, CD54 and CD71 on both CD4+ and CD8+ cells. In contrast, the response of T cells to TNF-alpha-matured DC was significantly less. Cytokine production from T cells cultured with BCG-exposed DC was enhanced with elevated secretion of interleukin-2 (IL-2), IL-10 and interferon-gamma (IFN-gamma) and was produced by both CD4+ and CD8+ lymphocytes as determined by intracellular staining. In particular, IFN-gamma secretion was increased from 50 pg/ml to 25 000 pg/ml and IL-10 secretion increased from 20 pg/ml to 300 pg/ml in BCG-exposed DC co-cultures. Blocking antibodies to B7.1 and B7.2 or IL-12 significantly reduced the secretion of IFN-gamma and reductions were also seen in the expression of CD25 and CD71 by CD4+ cells. These data demonstrate that mycobacterially infected DC are particularly potent activators of autologous T cells compared to TNF-alpha-exposed DC and that the resultant T cells are functionally superior.—Authors' Abstract

**Danelishvili, L. and Bermudez, L. E.** Role of Type I cytokines in host defense against *Mycobacterium avium* infection. *Curr. Pharm. Des.* **9(1)** (2003) 61–65.

*Mycobacterium avium* is a human pathogen that causes infection in immunocompetent as well as immunocompromised patients. Infection is acquired both by the respiratory and gastrointestinal routes, and bacterial invasion of mucosal epithelial cells is characteristic. *M. avium* crosses the mucosal barrier without triggering substantial inflammatory response. Once in the intestinal submucosa or in the alveolar space *M. avium* infects macrophages. Intracellular bacteria block the production of cytokines involved in the host response against the infection, such as TNF-alpha and IL-12, and suppress antigen presentation by the macrophage. Innate response against the infection is effective to certain extent but the ability of the bacterium to remain "silent" for a period of time prevents neutrophil and NK cells from effectively controlling the establishing of the infection. CD4+ T cells as well as CD8+ T cells are activated, although only CD4+ T cells appear to be effective in inducing anti-*M. avium* activity in macrophages. *M. avium*-specific CD8+ T cells undergo apoptosis early in the infection. Therefore, the immune mechanisms of the host and bacterial strategies for survival are complex and fascinating.—Authors' Abstract

**Girvan, A., Aldwell, F. E., Buchan, G. S., Faulkner, L., and Baird, M. A.** Transfer of macrophage-derived mycobacterial antigens to dendritic cells can induce naive T-cell activation. *Scand. J. Immunol.* **57(2)** (2003) 107–114.

Mycobacteria are capable of surviving and replicating in host macrophages, where they can release antigenic material into the environment. However, unlike dendritic cells (DCs), macrophages do not appear to be capable of activating naive T cells. Therefore, this work investigated antigen transfer between macrophages and DCs. We generated culture supernatants from bacille Calmette-Guerin (BCG)-infected and uninfected macrophages and then determined whether

DCs could present these extracellular mycobacterial antigens to T cells. Here, we show that DCs pulsed with antigens released from BCG-infected macrophages can stimulate primed T cells *in vitro* and initiate naive T-cell responses *in vivo*. These results suggest that antigen transfer can occur between macrophages and DCs.— Authors' Abstract

**Gollapudi, S., Jaidka, S., and Gupta, S.** Molecular basis of rifampicin-induced inhibition of anti-CD95-induced apoptosis of peripheral blood T lymphocytes: the role of CD95 ligand and FLIPs. *J. Clin. Immunol.* **23(1)** (2003) 11–22.

Rifampicin modulates immune response; however, mechanisms by which it exerts these effects are incompletely understood. Recently, rifampicin has been shown to bind to and activate glucocorticoid receptors. Because of the evidence for a role of glucocorticoids in lymphocyte apoptosis, we hypothesized that rifampicin may exert its influence on the immune system by regulating apoptosis. Therefore, we examined the effect of rifampicin on signaling pathway of anti-CD95-induced apoptosis in peripheral blood lymphocytes. Rifampicin, in a concentration-dependent manner, inhibited anti-CD95-induced apoptosis in both CD4+ and CD8+ T cells, which was associated with the inhibition of activation of both caspase-3 and caspase-8. In addition, rifampicin down-regulated the expression of CD95L and Bax. The inhibitory effects of rifampicin on apoptosis and caspase activation as well as its effect on the expression of CD95L and FLIPs were reversed by RU486, an antagonist of glucocorticoid receptor. These data suggest that rifampicin inhibits anti-CD95-mediated apoptosis in lymphocytes by modulating the expression of certain proteins that regulate apoptosis, at least in part, via glucocorticoid receptors.— Authors' Abstract

**Hernandez-Garay, M. and Mendez-Samperio, P.** Transforming Growth Factor-beta decreases survival of *Mycobacterium bovis*-activated T cells. *Arch. Med. Res.* **34(1)** (2003) 20–25.

A comprehensive understanding of the immune response induced by *Mycobacterium bovis* Bacille Calmette-Guerin in activation of protective T cells against tuberculosis is important to develop effective therapies to combat this disease. In this study, our experiments were designed to determine effects of transforming growth factor (TGF)-beta on *M. bovis*-induced T-cell activation and survival. Fluorescence-activated cell sorter (FACS) analysis was used for detection of apoptotic cells by three different methods: 1) scattered light change during early phase of apoptosis; 2) detection of hypodiploid DNA, or 3) terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) technique. Quantification of positively stained population was based on samples stained with isotype control antibodies analyzed on a FACScan. TGF-beta added at initiation of culture did not alter percentage of viable cells. By contrast, TGF-beta added 72 hr post-activation decreased percentage of viable cells. This effect was statistically significant ( $p < 0.05$ ). Furthermore, addition of anti-TGF-beta MoAb together with TGF-beta abolished the ability of this cytokine to decrease survival in post-activated human T cells. Role of TGF-beta on post-activated human T cells was further confirmed by staining apoptotic nuclei with propidium iodide, which detects late events of apoptosis, and by DNA fragmentation determined using TUNEL assay. Interestingly, TGF-beta did not promote Fas-mediated killing. Finally, TGF-beta increased apoptosis of CD4(+) T cells after mycobacterial stimulation. This study indicated an important role for TGF-beta in suppression of protective immune response against *M. bovis* by promoting elimination of post-activated T cells. Furthermore, results showed that TGF-beta had no direct effect on *M. bovis*-induced up-regulation of Fas (CD95).— Authors' Abstract

**Kumar, P., Amara, R. R., Challu, V. K., Chadda, V. K., and Satchidanandam, V.** The Apa protein of *Mycobacterium tuberculosis* stimulates gamma interferon-secreting CD4(+) and CD8(+) T cells from purified protein derivative-positive individuals and affords protec-

tion in a guinea pig model. *Infect. Immun.* **71**(4) (2003) 1929–1937.

The search to identify *Mycobacterium tuberculosis* antigens capable of conferring protective immunity against tuberculosis has received a boost owing to the resurgence of tuberculosis over the past two decades. It has long been recognized that lymphoid cells are required for protection against *M. tuberculosis*. While traditionally the CD4(+) populations of T cells were believed to predominantly serve this protective function, a pivotal role for CD8(+) T cells in this task has been increasingly appreciated. We show that the 50- to 55-kDa Apa protein, specified by the Rv1860 gene of *M. tuberculosis*, can elicit both lymphoproliferative response and gamma interferon (IFN-gamma) production from peripheral blood mononuclear cells (PBMC) of purified protein derivative (PPD)-positive individuals, with significant differences recorded in the levels of responsiveness between PPD-positive healthy controls and pulmonary tuberculosis patients. Flow cytometric analysis of whole blood stimulated with the recombinant Apa protein revealed a sizeable proportion of CD8(+) T cells in addition to CD4(+) T cells contributing to IFN-gamma secretion. PBMC responding to the Apa protein produced no interleukin-4, revealing a Th1 phenotype. A DNA vaccine and a poxvirus recombinant expressing the Apa protein were constructed and tested for their ability to protect immunized guinea pigs against a challenge dose of virulent *M. tuberculosis*. Although the DNA vaccine afforded little protection, the poxvirus recombinant boost after DNA vaccine priming conferred a significant level of protective immunity, bringing about a considerable reduction in mycobacterial counts from the challenge bacilli in spleens of immunized guinea pigs, a result comparable to that achieved by BCG vaccination.—Authors' Abstract

**Lichtenauer-Kaligis, E. G., de Boer, T., Verreck, F. A., van Voorden, S., Hoeve, M. A., van de Vosse, E., Ersoy, F., Tezcan, I., van Dissel, J. T., Sanal, O., and Ottenhoff, T. H.** Severe *Mycobacterium bovis* BCG infections in a large series of novel IL-12 receptor beta1 deficient pa-

tients and evidence for the existence of partial IL-12 receptor beta1 deficiency. *Eur. J. Immunol.* **33**(1) (2003) 59–69.

Cell mediated immunity plays a critical role in human host defence against intracellular bacteria. In patients with unusual, severe infections caused by poorly pathogenic species of mycobacteria and salmonellae, genetic deficiencies have been identified in key genes in the type-1 cytokine pathway, especially in IFNGR1 and IL12RB1. Here, we analyzed 11 patients originating from Turkey and suffering from unusual *Mycobacterium bovis* Bacille Calmette-Guerin infections following vaccination, and found that most patients (n = 8) are deficient in IL-12Rbeta1 expression and function. No defects were found in patients' IFN-gammaR or IL-18R. In addition, a first patient suffering from partial IL-12Rbeta1 deficiency is described. This patient presented with an intermediate cellular and immunological phenotype: a consistent, low response to IL-12 was found, which could be further augmented by IL-18. Despite a lack of cell surface IL-12Rbeta1 expression, normal levels of intracellular IL-12Rbeta1 protein were detectable, which was not seen in the other, completely IL-12Rbeta1 deficient patients examined. Moreover, this patient had a relatively mild clinical phenotype and was the only individual with a single homozygous amino acid substitution in IL-12Rbeta1 (C198R). Collectively, our findings indicate that idiopathic, unusually severe infections due to *M. bovis* BCG can be caused by complete as well as partial IL-12Rbeta1 deficiency.—Authors' Abstract

**Morris, K. R., Lutz, R. D., Choi, H. S., Kamitani, T., Chmura, K., and Chan, E. D.** Role of the NF-kappaB signaling pathway and kappaB cis-regulatory elements on the IRF-1 and iNOS promoter regions in mycobacterial lipoarabinomannan induction of nitric oxide. *Infect. Immun.* **71**(3) (2003) 1442–1452.

Nitric oxide (NO(.)) produced by inducible nitric oxide synthase (iNOS) is an important host defense molecule against *Mycobacterium tuberculosis* in mononuclear phagocytes. The objective of this study was to determine the role of the Ikap-

paB $\alpha$  kinase-nuclear factor  $\kappa$ B (IKK-NF- $\kappa$ B) signaling pathway in the induction of iNOS and NO(•) by a mycobacterial cell wall lipoglycan known as mannose-capped lipoarabinomannan (ManLAM) in mouse macrophages costimulated with gamma interferon (IFN- $\gamma$ ). NF- $\kappa$ B was activated by ManLAM as shown by electrophoretic mobility shift assay, by immunofluorescence of translocated NF- $\kappa$ B in intact cells, and by a reporter gene driven by four NF- $\kappa$ B-binding elements. Transduction of an IkappaB $\alpha$  mutant (Ser32/36Ala) significantly inhibited NO(•) expression induced by IFN- $\gamma$  plus ManLAM. An activated SCF complex, a heterotetramer (Skp1, Cul-1, beta-TrCP [F-box protein], and ROC1) involved with ubiquitination, is also required for iNOS-NO(•) induction. Two NF- $\kappa$ B-binding sites ( $\kappa$ BI and  $\kappa$ BII) present on the 5'-flanking region of the iNOS promoter bound ManLAM-induced NF- $\kappa$ B similarly. By use of reporter constructs in which one or both sites are mutated, both NF- $\kappa$ B-binding positions were essential in iNOS induction by IFN- $\gamma$  plus ManLAM. IFN- $\gamma$ -induced activation of the IRF-1 transcriptional complex is a necessary component in host defense against tuberculosis. Although the 5'-flanking region of the IRF-1 promoter contains an NF- $\kappa$ B-binding site and ManLAM-induced NF- $\kappa$ B also binds to this site, ManLAM was unable to induce IRF-1 expression. The influence of mitogen-activated protein kinases on IFN- $\gamma$  plus ManLAM induction of iNOS-NO(•) is not due to any effects on ManLAM induction of NF- $\kappa$ B. — Authors' Abstract

**Pittis, M. G., Muzzolin, L., Giulianini, P. G., and Garcia, R. C.** Mycobacteria-containing phagosomes associate less annexins I, VI, VII and XI, but not II, concomitantly with a diminished phagolysosomal fusion. *Eur. J. Cell. Biol.* **82(1)** (2003) 9–17.

We have studied the intracellular localization of annexins I, II, VI, VII, and XI in cells containing latex beads or *Mycobacterium avium* at different times after ingestion in order to establish whether a correlation

existed between the association of annexins to phagosomes and phagolysosomal fusion, since the intracellular survival of mycobacteria is linked to an impairment of phagosome maturation. We demonstrate an important decrease in the levels of association of annexins I, VI, VII and XI, but not II to phagosomes containing either live or killed mycobacteria compared with phagosomes containing inert latex particles. The reduced association of annexins observed was detected only on *M. avium*-containing phagosomes and not in other cell membrane nor in cytosolic fractions from infected cells, and was apparent from 8 hours through to 4 days after phagocytosis. These findings add elements to the present knowledge of the phagosomal modifications that accompany the survival of intracellular pathogens, suggesting that annexins I, VI, VII, and XI play a secondary role in phagosomal fusion events while annexin II does not seem to be related to the mechanism of regulation of endolysosomal fusion. — Authors' Abstract

**Rodriguez, A., Troye-Blomberg, M., Lindroth, K., Ivanyi, J., Singh, M., and Fernandez, C.** B- and T-cell responses to the mycobacterium surface antigen PstS-1 in the respiratory tract and adjacent tissues. Role of adjuvants and routes of immunization. *Vaccine* **21(5–6)** (2003) 458–467.

Induction of mucosal immunity in the respiratory tract is crucial for protection against respiratory infections. Here, we have investigated the effects of the routes of immunization as well as of three different adjuvants on the induction of mucosal immune responses. Mice were immunized using intranasal (i.n.) or intraperitoneal (i.p.) routes with the mycobacterium PstS-1 antigen. Cholera toxin (CT), detoxified pertussis toxin (detPT) and RU 41.740 from *Klebsiella pneumoniae* were compared as mucosal adjuvants. Our data showed that i.n. route of immunization induced the most favorable stimulation of mucosal antigen-specific IgA responses supported by mixed Th cells producing IL-4, IL-5, IFN- $\gamma$ . In contrast, i.p. immunizations elicited only enhancement of systemic responses, predominantly of the Th2 type. Furthermore, the use of CT as mucosal adjuvant resulted

in the stimulation of a mixed Th cell response whereas detPT evoked mainly Th2 type of responses. Likewise CT, the RU 41.740 adjuvant elicited a mixed Th cell response, albeit supported by much lower numbers of CD4(+) T-cells. Thus, i.n. route of immunization favors the induction of mucosal and systemic immune responses, while the Th cell development at mucosal inductive site is influenced by the adjuvant used for immunizations.—Authors' Abstract

**Schorey, J. S. and Cooper, A. M.** Macrophage signalling upon mycobacterial infection: the MAP kinases lead the way. *Cell. Microbiol.* **5(3)** (2003) 133–142.

Mycobacteria activate a series of macrophage signalling pathways upon engaging host cell receptors and during the invasion process. These signals initiate a cascade of events leading to the production of immune effector molecules including cytokines, chemokines and reactive nitrogen intermediates. This response by the macrophage is critical for the control of the mycobacterial infection and, not surprisingly, pathogenic mycobacteria have evolved mechanisms to limit this macrophage activation. Recent data has suggested that macrophages infected with pathogenic compared to non-pathogenic mycobacteria are restricted in their activation of the mitogen activated protein kinase (MAPK) pathways. Mitogen activated protein kinase activation in macrophages appears to play an important role in promoting antimycobacterial activity and in the production of various effector molecules following a mycobacterial infection. Therefore, the ability of pathogenic mycobacteria to limit MAPK activity is likely an important virulence mechanism and may be a potential therapeutic target.—Authors' Abstract

**Tollefsen, S., Pollock, J. M., Lea, T., Harboe, M., and Wiker, H. G.** T- and B-cell epitopes in the secreted *Mycobacterium bovis* antigen MPB70 in mice. *Scand. J. Immunol.* **57(2)** (2003) 151–161.

MPB70 is a soluble secreted protein highly expressed in *Mycobacterium bovis* and strains of bacille Calmette-Guerin (BCG); as

such, it is a candidate for subunit and DNA vaccines against tuberculosis. MPB70 was screened for T-cell epitopes in four different inbred mouse strains. Major histocompatibility complex (MHC) H-2b-expressing mice (C57BL/6) secreted interferon-gamma (IFN-gamma) after stimulation with peptides from the regions 1–20, 41–50, 81–110, 121–150 and 161–193 of the MPB70 sequence. H-2db mouse (B6D2) splenocytes secreted IFN-gamma after stimulation with some of the same peptides, whereas H-2d mice (BALB/c and DBA/2) did not secrete IFN-gamma upon stimulation with the peptides. Sera from H-2db mice immunized with native MPB70 in incomplete Freund's adjuvant (IFA), mpb70 DNA or live BCG Moreau were found to contain antibodies against the native MPB70 antigen. H-2db mice immunized with native MPB70 in IFA exhibited high titres of peptide-reactive immunoglobulin G1 (IgG1) antibodies, whereas DNA-immunized mice reacted with IgG2a antibodies against some of the same peptides. As some of the epitopes recognized by mouse T and B cells have previously been found to stimulate immune responses in humans, cattle and rabbits, we conclude that these epitopes may be good general epitopes for the stimulation of T- and B-cell responses and candidates for a DNA vaccine with a broad applicability.—Authors' Abstract

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

Reactive oxygen and nitrogen intermediates are important antimicrobial defense mechanisms of macrophages and other phagocytic cells. While reactive nitrogen intermediates have been shown to play an important role in tuberculosis control in the murine system, their role in human disease is not clearly established. Glutathione, a tripeptide and antioxidant, is synthesized at high levels by cells during reactive oxygen intermediate and nitrogen intermediate production. Glutathione has been recently shown to play an important role in apoptosis and to

regulate antigen-presenting-cell functions. Glutathione also serves as a carrier molecule for nitric oxide, in the form of S-nitrosoglutathione. Previous work from this laboratory has shown that glutathione and S-nitrosoglutathione are directly toxic to mycobacteria. A mutant strain of *Mycobacterium bovis* BCG, defective in the transport of small peptides such as glutathione, is resistant to the toxic effect of glutathione and S-nitrosoglutathione. Using the peptide transport mutant as a tool, we investigated the role of glutathione and S-nitrosoglutathione in animal and human macrophages in controlling intracellular mycobacterial growth.— Authors' Abstract

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

We investigated the effects of peripheral blood mononuclear cells expanded with irrelevant control and mycobacterial antigens on the intracellular growth of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) in human macrophages. More than 90% of the cells present after 1 week of *in vitro* expansion were CD3(+). T cells were expanded from purified protein derivative-negative controls, persons with latent tuberculosis, and BCG-vaccinated individuals. T cells expanded with nonmycobacterial antigens enhanced the intracellular growth of BCG in suboptimal cultures of macrophages. T cells expanded with live BCG or lysates of *Mycobacterium tuberculosis* directly inhibited intracellular BCG. Recent intradermal BCG vaccination significantly enhanced the inhibitory activity of T cells expanded with mycobacterial antigens ( $p < 0.02$ ), consistent with the induction of memory-immune inhibitory T-cell responses. Selected mycobacterial antigens (Mtb41 > lipoarabinomannan > 38kd > Ag85B > Mtb39) expanded inhibitory T cells, demonstrating the involvement of antigen-specific T cells in intracellular BCG inhibition. We studied the T-cell subsets and molecular mechanisms involved in the memory-immune inhibition of intracellular BCG. Mycobacteria-specific gammadelta T

cells were the most potent inhibitors of intracellular BCG growth. Direct contact between T cells and macrophages was necessary for the BCG growth-enhancing and inhibitory activities mediated by control and mycobacteria-specific T cells, respectively. Increases in tumor necrosis factor alpha, interleukin-6, transforming growth factor beta, and vascular endothelial growth factor mRNA expression were associated with the enhancement of intracellular BCG growth. Increases in gamma interferon, FAS, FAS ligand, perforin, granzyme, and granulysin mRNA expression were associated with intracellular BCG inhibition. These culture systems provide *in vitro* models for studying the opposing T-cell mechanisms involved in mycobacterial survival and protective host immunity.— Authors' Abstract

**Zhou, D., Lai, X., Shen, Y., Sehgal, P., Shen, L., Simon, M., Qiu, L., Huang, D., Du, G. Z., Wang, Q., Letvin, N. L., and Chen, Z. W.** Inhibition of adaptive Vgamma2Vdelta2+ T-cell responses during active mycobacterial coinfection of simian immunodeficiency virus SIVmac-infected monkeys. *J. Virol.* **77(5)** (2003) 2998–3006.

Adaptive immune responses of gammadelta T cells during active mycobacterial coinfection of human immunodeficiency virus-infected humans have not been studied. Macaques infected with the simian immunodeficiency virus (SIV) SIVmac were employed to determine the extent to which a coincident AIDS virus infection might compromise immune responses of mycobacterium-specific Vgamma2Vdelta2(+) T cells during active mycobacterial infection. Control SIVmac-negative macaques developed primary and recall expansions of phosphoantigen-specific Vgamma2 Vdelta2(+) T cells after *Mycobacterium bovis* BCG infection and BCG reinfection, respectively. In contrast, SIVmac-infected macaques did not exhibit sound primary and recall expansions of Vgamma2 Vdelta2(+) T cells in the blood and pulmonary alveoli following BCG infection and reinfection. The absence of adaptive Vgamma2Vdelta2(+) T-cell responses was associated with profound CD4(+) T-cell

deficiency and subsequent development of SIVmac-related tuberculosis-like disease in the coinfecting monkeys. Consistently, Vgamma2Vdelta2(+) T cells from coinfecting monkeys displayed a reduced capacity to expand *in vitro* following stimulation with phosphoantigen. The reduced ability of Vgamma2Vdelta2(+) peripheral blood lymphocytes (PBL) to expand could be restored to some extent by coculture of these cells with CD4(+) T cells purified from PBL of SIV-negative monkeys. Furthermore, naive monkeys inoculated simultaneously with SIVmac and BCG were unable to sustain

expansion of Vgamma2Vdelta2(+) T cells at the time that the coinfecting monkeys developed lymphoid depletion and a fatal tuberculosis-like disease. Nevertheless, no deletion in Vdelta2 T-cell receptor repertoire was identified in SIVmac-BCG-coinfecting macaques, implicating an SIVmac-induced down-regulation rather than a clonal exhaustion of these cells. Thus, an SIVmac-induced compromise of the adaptive Vgamma2 Vdelta2(+) T-cell responses may contribute to the immunopathogenesis of the SIV-related tuberculosis-like disease in macaques.—Authors' Abstract

## Immuno-Pathology (Leprosy)

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

In recent years there has been increased interest in diseases showing degeneration of dermal elastic tissue, in spite of the fact that the skin elastic system is not well understood. Some inflammatory and non-inflammatory conditions associated with dermal elastolysis have been described.

The coexistence of leprosy and elastolytic giant cell granuloma, for which we use the term "LEGG," so far as we are aware, has not hitherto been recognized nor described.

The present study documents two unusual cases of leprosy combined with elastolytic giant cell granuloma on exposed areas of skin and discusses their possible relation.—*Anais Brasileiros de Dermatologia*

**Kiszewski, C. A., Becerril, E., Baquera, J., Aguilar, L. D., and Hernandez-Pando, R.** Expression of Transforming Growth Factor-beta isoforms and their receptors in lepromatous and tuberculoid leprosy. *Scand. J. Immunol.* **57(3)** (2003) 279–285.

Leprosy is an infectious disease with two polar forms, tuberculoid leprosy (TT) and

lepromatous leprosy (LL), that are characterized by strong cell-mediated immunity (CMI) and CMI anergy, respectively. Transforming growth factor-beta (TGF-beta) belongs to a family of pleiotropic cytokines (TGF-beta1, TGF-beta2 and TGF-beta3) that participate in the control of cell differentiation and proliferation, as well as tissue repair. This cytokine family is unique because it suppresses CMI. In this study, we compared the expression of the three TGF-beta isoforms and their receptors in skin biopsies from LL and TT patients (LL = 20; TT = 20) using immunohistochemistry and automated morphometry. The percentage of cells immunostained for the three TGF-beta isoforms and cells positive for the three TGF-beta receptors in the inflammatory infiltrate located in the papillary dermis, reticular dermis and periadnexal tissue were significantly higher in LL than that in TT, with macrophages being the most common and strongest immunoreactive cells. Some lymphocytes, fibroblasts, keratinocytes and epithelial cells from sweat glands and hair roots were also positive. In situ reverse-transcription polymerase chain reaction corroborated the capacity of these cells to synthesize TGF-beta1 and TGF-beta receptor 2. This high expression of TGF-beta isoforms and their receptors could contribute to CMI anergy and other clinical characteristic features of leprosy, like skin atrophy.—Authors' Abstract

**Oliveira, R. B., Ochoa, M. T., Sieling, P. A., Rea, T. H., Rambukkana, A., Sarno, E. N., and Modlin, R. L.** Expression of Toll-like receptor 2 on human Schwann cells: a mechanism of nerve damage in leprosy. *Infect. Immun.* **71(3)** (2003) 1427–1433.

Nerve damage is a clinical hallmark of leprosy and a major source of patient morbidity. We investigated the possibility that human Schwann cells are susceptible to cell death through the activation of Toll-like receptor 2 (TLR2), a pattern recognition receptor of the innate immune system. TLR2 was detected on the surface of human Schwann cell line ST88-14 and on cultured primary human Schwann cells. Activation

of the human Schwann cell line and primary human Schwann cell cultures with a TLR2 agonist, a synthetic lipopeptide comprising the N-terminal portion of the putative *Mycobacterium leprae* 19-kDa lipoprotein, triggered an increase in the number of apoptotic cells. The lipopeptide-induced apoptosis of Schwann cells could be blocked by an anti-TLR2 monoclonal antibody. Schwann cells in skin lesions from leprosy patients were found to express TLR2. It was possible to identify in the lesions Schwann cells that had undergone apoptosis *in vivo*. The ability of *M. leprae* ligands to induce the apoptosis of Schwann cells through TLR2 provides a mechanism by which activation of the innate immune response contributes to nerve injury in leprosy.—Authors' Abstract

## Immuno-Pathology (Tuberculosis)

**Al-Attayah, R., Shaban, F. A., Wiker, H. G., Oftung, F., and Mustafa, A. S.** Synthetic peptides identify promiscuous human Th1 cell epitopes of the secreted mycobacterial antigen MPB70. *Infect. Immun.* **71(4)** (2003) 1953–1960.

MPB70 is a secreted protein of *Mycobacterium bovis* and *Mycobacterium tuberculosis*, which stimulates both cellular and humoral immune responses during infection with bovine and human tubercle bacilli. In addition, vaccination with MPB70 has been shown to induce Th1 cell responses and protection in animal models of tuberculosis. The present study was carried out to map the dominant human Th1 cell epitopes of MPB70 in relation to major histocompatibility complex (MHC) class II restriction in healthy subjects showing strong T-cell responses to complex mycobacterial antigens. Peripheral blood mononuclear cells (PBMC) from HLA-DR-typed donors were tested with complex mycobacterial antigens (whole-cell *M. tuberculosis* and *M. tuberculosis* culture filtrates), with MPB70 purified from the culture filtrate of *M. bovis* BCG Tokyo, and with 13 synthetic peptides (25-mers overlapping by 10 residues) covering the sequence of MPB70. The donors that

responded to the complex antigens and MPB70 also responded to the cocktail of synthetic MPB70 peptides. Testing of PBMC with individual peptides showed that peptides p5 (amino acids [aa] 61 to 85), p6 (aa 76 to 100), p8 (aa 106 to 130), p12 (aa 166 to 190), and p13 (aa 181 to 193) were most frequently recognized in proliferation and gamma interferon (IFN-gamma) assays. Testing of antigen-specific CD4(+) T-cell lines with the individual peptides of MPB70 confirmed that peptides p8, p12, and p13 contain immunodominant Th1 cell epitopes of MPB70. MHC restriction analysis with HLA-typed donors showed that MPB70 and its immunodominant peptides were presented to T cells promiscuously. The T-cell lines responding to MPB70 and peptides p8, p12, and p13 in IFN-gamma assays mediated antigen-peptide-specific cytotoxic activity against monocytes/macrophages pulsed with the whole-protein antigen or the peptides. In conclusion, the promiscuous recognition of MPB70 and its immunodominant peptide defined epitopes (aa 106 to 130 and 166 to 193) by IFN-gamma-producing Th1 cells supports possible application of this secreted antigen to subunit vaccine design.—Authors' Abstract

**Antas, P. R., Oliveira, E. B., Milagres, A. S., Franken, K. C., Ottenhoff, T. H., Klatser, P., Sarno, E. N., and Sampaio, E. P.** Kinetics of T cell-activation molecules in response to *Mycobacterium tuberculosis* antigens. Mem. Inst. Oswaldo Cruz **97**(8) (2002) 1097–1099.

The phenotypic features acquired subsequent to antigen-specific stimulation *in vitro* were evaluated by means of the kinetic expressions of CD69 and CD25 activation molecules on T lymphocytes and assayed by flow cytometry in response to PPD, Ag85B, and ferritin in PPD-positive healthy control individuals. In response to PHA, CD69 staining on both CD4+ and CD8+ T cells became initially marked after 4 hr, peaked at 24 hr, and quickly decreased after 120 hr. For CD25, a latter expression was detected around 8 hr, having increased after 96 hr. As expected, the response rate to the mycobacterial antigens was much lower than that to the mitogen. Positive staining was high after 96 hr for CD25 and after 24 hr for CD69. CD69 expression was significantly enhanced ( $p < 0.05$ ) on CD8+ as compared to CD4+ T cells. High levels were also found between 96–120 hr. Regarding Ag85B, CD25+ cells were mostly CD4+ instead of CD8+ T cells. Moreover, in response to ferritin, a lower CD25 expression was noted. The present data will allow further characterization of the immune response to new mycobacterial-specific antigens and their evaluation for possible inclusion in developing new diagnostic techniques for tuberculosis as well in a new vaccine to prevent the disease.—Authors' Abstract

**Bao, L., Chen, W., Zhang, H., and Wang, X.** Virulence, Immunogenicity, and protective efficacy of two recombinant *Mycobacterium bovis* bacillus Calmette-Guerin strains expressing the antigen ESAT-6 from *Mycobacterium tuberculosis*. Infect. Immun. **71**(4) (2003) 1656–1661.

We constructed two recombinant *Mycobacterium bovis* BCG (rBCG) strains expressing ESAT-6 of *Mycobacterium tuberculosis*, named rBCG-1 and rBCG-2. rBCG-1 contained the ESAT-6 gene linked

to BCG hsp60 and expressed a fusion protein, while rBCG-2, with a secretory sequence, could secret ESAT-6 into the culture medium. There was no evidence for increased virulence of the two rBCG strains when we made a comparison between them and BCG with regard to organ bacterial loads, lung histology, and survival time. rBCG-1 induced significantly higher specific antibody titers and stronger cellular immune response than BCG, whereas rBCG-2 had immunogenicity similar to that of the parental BCG strain. Both rBCG-1 and rBCG-2 conferred marked protection against *M. tuberculosis* infection, yet in terms of protective efficacy, they showed no significant improvements upon conventional BCG vaccine.—Authors' Abstract

**Barthel, R., Tsytsykova, A. V., Barczak, A. K., Tsai, E. Y., Dascher, C. C., Brenner, M. B., and Goldfeld, A. E.** Regulation of tumor necrosis factor alpha gene expression by mycobacteria involves the assembly of a unique enhanceosome dependent on the coactivator proteins CBP/p300. Mol. Cell. Biol. **23**(2) (2003) 526–533.

Tumor necrosis factor alpha (TNF-alpha) plays an important role in host containment of infection by *Mycobacterium tuberculosis*, one of the leading causes of death by an infectious agent globally. Using the pathogenic *M. tuberculosis* strain H37Rv, we present evidence that upon stimulation of monocytic cells by *M. tuberculosis* a unique TNF-alpha enhanceosome is formed, and it is distinct from the TNF-alpha enhanceosome that forms in T cells stimulated by antigen engagement or virus infection. A distinct set of activators including ATF-2, c-jun, Ets, Sp1, Egr-1 and the coactivator proteins CBP/p300 are recruited to the TNF-alpha promoter after stimulation with *M. tuberculosis*. Furthermore, the formation of this enhanceosome is dependent on inducer-specific helical phasing relationships between transcription factor binding sites. We also show that the transcriptional activity of CBP/p300 is potentiated by mycobacterial stimulation of monocytes. The identification of TNF-alpha regulatory elements and coactivators involved in *M. tuberculosis*-stimulated gene expression thus

provides potential selective molecular targets in the modulation of TNF-alpha gene expression in the setting of mycobacterial infection.—Authors' Abstract

**Britton, W. J. and Palendira, U.** Improving vaccines against tuberculosis. *Immunol. Cell. Biol.* **81(1)** (2003) 34–45.

Tuberculosis remains a major cause of mortality and physical and economic deprivation worldwide. There have been significant recent advances in our understanding of the *Mycobacterium tuberculosis* genome, mycobacterial genetics and the host determinants of protective immunity. Nevertheless, the challenge is to harness this information to develop a more effective vaccine than BCG, the attenuated strain of *Mycobacterium bovis* derived by Calmette and Guerin nearly 90 years ago. Some of the limitations of BCG include the waning of the protective immunity with time, reduced effectiveness against pulmonary tuberculosis compared to disseminated disease, and the problems of a live vaccine in immuno-compromised subjects. Two broad approaches to vaccine development are being pursued. New live vaccines include either attenuated strains of *Mycobacterium tuberculosis* produced by random mutagenesis or targeted deletion of putative virulence factors, or by genetic manipulation of BCG to express new antigens or cytokines. The second approach utilizes non-viable subunit vaccines to deliver immunodominant mycobacterial antigens. Both protein and DNA vaccines induce partial protection against experimental tuberculosis infection in mice, however, their efficacy has generally been equivalent to or less than that of BCG. The comparative effects of cytokine adjuvants and vaccines targeting antigen presenting cells on enhancing protection will be discussed. Coimmunization with plasmid interleukin-12 and a DNA vaccine expressing Antigen 85B, a major secreted protein, was as protective as BCG. The combination of priming with DNA-85B and boosting with BCG was superior to BCG alone. Therefore it is possible to achieve a greater level of protection against tuberculosis than with BCG, and this highlights the potential for new tuberculosis vaccines in humans.—Authors' Abstract

**Chopra, P., Singh, A., Koul, A., Ramachandran, S., Drlica, K., Tyagi, A. K., and Singh, Y.** Cytotoxic activity of nucleoside diphosphate kinase secreted from *Mycobacterium tuberculosis*. *Eur. J. Biochem.* **270(4)** (2003) 625–634.

Pathogenicity of *Mycobacterium tuberculosis* is closely related to its ability to survive and replicate in the hostile environment of macrophages. For some pathogenic bacteria, secretion of ATP-utilizing enzymes into the extracellular environment aids in pathogen survival via P2Z receptor-mediated, ATP-induced death of infected macrophages. A component of these enzymes is nucleoside diphosphate kinase (Ndk). The ndk gene was cloned from *M. tuberculosis* H37Rv and expressed in *Escherichia coli*. Ndk was secreted into the culture medium by *M. tuberculosis*, as determined by enzymatic activity and Western blotting. Purified Ndk enhanced ATP-induced macrophage cell death, as assayed by the release of [<sup>14</sup>C]adenine. A catalytic mutant of Ndk failed to enhance ATP-induced macrophage cell death, and periodate-oxidized ATP (oATP), an irreversible inhibitor of P2Z receptor, blocked ATP/Ndk-induced cell death. Purified Ndk was also found to be autophosphorylated with broad specificity for all nucleotides. Conversion of His117→Gln, which is part of the nucleotide-binding site, abolished autophosphorylation. Purified Ndk also showed GTPase activity. Collectively, these results indicate that secreted Ndk of *M. tuberculosis* acts as a cytotoxic factor for macrophages, which may help in dissemination of the bacilli and evasion of the immune system.—Authors' Abstract

**Condos, R., Raju, B., Canova, A., Zhao, B. Y., Weiden, M., Rom, W. N., and Pine, R.** Recombinant gamma interferon stimulates signal transduction and gene expression in alveolar macrophages in vitro and in tuberculosis patients. *Infect. Immun.* **71(4)** (2003) 2058–2064.

Tuberculosis is the seventh leading cause of morbidity and mortality in the world, with eight million cases per year. Animal and human studies demonstrate an enrichment of CD4 cells at sites of disease, with a

more favorable clinical course when there is a Th1 response with the presence of gamma interferon (IFN-gamma). We previously treated patients who had multidrug-resistant tuberculosis with recombinant IFN-gamma (rIFN-gamma) in aerosol form and were able to convert smear-positive cases to smear negative with 12 treatments over 1 month. We hypothesized that rIFN-gamma would induce signal transducer and activator of transcription (STAT) and interferon regulatory factor (IRF) binding activity in alveolar macrophages (AM). AM treated *in vitro* showed clear upregulation of STAT-1 and IRF-1 by rIFN-gamma. STAT-1 was not activated and IRF-1 was only weakly induced after 1 day of infection by *Mycobacterium tuberculosis* TN913. In bronchoalveolar lavage (BAL) cells obtained from 10 of 10 tuberculosis patients 10 ± 2 days post-antituberculosis treatment, there was no detectable STAT-1 or IRF-1 DNA-binding activity. After 4 weeks of treatment with rIFN-gamma aerosol in addition to the antituberculosis drugs, 10 of 10 patients had increased STAT-1, IRF-1, and/or IRF-9 DNA-binding activity in BAL cells from lung segments shown radiographically to be involved and in those shown to be uninvolved. Symptoms and chest radiographs improved, and amounts of macrophage inflammatory cytokines and human immunodeficiency virus type 1 (HIV-1) viral loads (in five of five HIV-1-coinfected patients) declined in the second BAL specimens. rIFN-gamma aerosol induces signal transduction and gene expression in BAL cells and should be evaluated for efficacy in a randomized, controlled clinical trial.—Authors' Abstract

**Cubillas-Tejeda, A. C., Ruiz-Arguelles, A., Bernal-Fernandez, G., Quiroz-Compean, L., Lopez-Davila, A., Reynaga-Hernandez, E., and Gonzalez-Amaro, R.** Cytokine production and expression of leucocyte-differentiation antigens by human mononuclear cells in response to *Mycobacterium tuberculosis* antigens. *Scand. J. Immunol.* **57(2)** (2003) 115–124.

The aim of this work was to characterize a leucocyte-differentiation antigen or chemo-

kine receptor that allows the identification of type 1 (T helper 1 (Th1), Tc1) and type 2 (Th2, Tc2) lymphocytes in short-term-cultured human peripheral blood mononuclear cells. In addition, we assessed the type of response induced by mycobacterial antigens in tuberculosis patients and healthy contacts. Cells were stimulated with an unfractionated culture filtrate or 30 kDa antigen from *Mycobacterium tuberculosis*. Then, CD4 and CD8 cell labelling was combined with CD30, CD27, CD28, CD45RA or CD45R0 staining, detection of intracellular interferon-gamma (IFN-gamma) or interleukin-4 (IL-4) and analysis by three-color flow cytometry. In separate experiments, the expression of different chemokine receptors (CCR1, CCR3, CCR5, CXCR3 and CXCR4) was also studied. We found that none of the cell-surface molecules studied was preferentially expressed by Th1 or Th2 cells. Thus, our results indicate that these lymphocyte subsets cannot be identified in short-term-cultured mononuclear cells on the basis of preferential expression of the cell markers studied, and that it is necessary to look for additional molecules that allow the discrimination of Th1 and Th2 cells.—Authors' Abstract

**Ferrero, E., Biswas, P., Vettoretto, K., Ferrarini, M., Uguccioni, M., Piali, L., Leone, B. E., Moser, B., Rugarli, C., and Pardi, R.** Macrophages exposed to *Mycobacterium tuberculosis* release chemokines able to recruit selected leucocyte subpopulations: focus on gamma-delta cells. *Immunology* **108(3)** (2003) 365–374.

Granuloma is a typical feature of tuberculosis. We evaluated the chemotaxis of selected human leucocyte subsets induced by macrophages incubated with *Mycobacterium tuberculosis* (MT)-derived products *in vitro*. The release of monocyte chemotactic protein 1 (MCP-1) and interleukin-8 (IL-8) correlated with the specific induction of strong chemotaxis towards monocytes and polymorphonuclear leucocytes (PMNs). gammadelta and T helper type 1 (Th1) alphabeta lymphocytes were chemoattracted, while T-resting, IL-2-activated and Th2 lymphocytes were unaffected. Activation

with mycobacterium-derived, phosphate-containing components, modulated the chemokine receptor profile of gammadelta T lymphocytes as well as their pattern of cyto-chemokine production, disclosing a potential for their active participation in granuloma formation. In particular, CXCR3 and IP-10, which we found to be released by MT-pulsed alveolar macrophages, seem to represent the receptor-counter-receptor pair implicated in the chemotaxis of gammadelta lymphocytes. Immunohistochemical analysis and *in situ* hybridization revealed the *in vivo* presence of IL-8, MCP-1 and IL-10 in lymph node and lung tuberculous granulomas. Our results underscore the role of MT extracts in the induction of macrophage-derived chemokines responsible for the orchestrated recruitment of PMNs, monocytes, and Th1 and gammadelta T cells, as well as in the regulation of gammadelta function.— Authors' Abstract

**Geijtenbeek, T. B., Van Vliet, S. J., Koppel, E. A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C. M., Appelmelk, B., and Van Kooyk, Y.** Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.* **197(1)** (2003) 7–17.

*Mycobacterium tuberculosis* represents a world-wide health risk and immunosuppression is a particular problem in *M. tuberculosis* infections. Although macrophages are primarily infected, dendritic cells (DCs) are important in inducing cellular immune responses against *M. tuberculosis*. We hypothesized that DCs represent a target for *M. tuberculosis* and that the observed immunosuppression results from modulation of DC functions. We demonstrate that the DC-specific C-type lectin DC-SIGN is an important receptor on DCs that captures and internalizes intact *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) through the mycobacterial cell wall component ManLAM. Antibodies against DC-SIGN block *M. bovis* BCG infection of DCs. ManLAM is also secreted by *M. tuberculosis*-infected macrophages and has been implicated as a virulence factor. Strikingly, ManLAM binding to DC-SIGN prevents mycobacteria- or LPS-induced DC

maturation. Both mycobacteria and LPS induce DC maturation through Toll-like receptor (TLR) signaling, suggesting that DC-SIGN, upon binding of ManLAM, interferes with TLR-mediated signals. Blocking antibodies against DC-SIGN reverse the ManLAM-mediated immunosuppressive effects. Our results suggest that *M. tuberculosis* targets DC-SIGN both to infect DCs and to down-regulate DC-mediated immune responses. Moreover, we demonstrate that DC-SIGN has a broader pathogen recognition profile than previously shown, suggesting that DC-SIGN may represent a molecular target for clinical intervention in infections other than HIV-1.— Authors' Abstract

**Lande, R., Giacomini, E., Grassi, T., Remoli, M. E., Iona, E., Miettinen, M., Julkunen, I., and Coccia, E. M.** IFN-alpha beta released by *Mycobacterium tuberculosis*-infected human dendritic cells induces the expression of CXCL10: selective recruitment of NK and activated T cells. *J. Immunol.* **170(3)** (2003) 1174–1182.

We recently reported that dendritic cells (DC) infected with *Mycobacterium tuberculosis* (Mtb) produce Th1/IFN-gamma-inducing cytokines, IFN-alpha beta and IL-12. In the present article, we show that maturing Mtb-infected DC express high levels of CCR7 and they become responsive to its ligand CCL21. Conversely, CCR5 expression was rapidly lost from the cell surface following Mtb infection. High levels of CCL3 and CCL4 were produced within 8 hr after infection, which is likely to account for the observed CCR5 down-modulation on Mtb-infected DC. In addition, Mtb infection stimulated the secretion of CXCL9 and CXCL10. Interestingly, the synthesis of CXCL10 was mainly dependent on the Mtb-induced production of IFN-alpha beta. Indeed, IFN-alpha beta neutralization down-regulated CXCL10 expression, whereas the expression of CXCL9 appeared to be unaffected. The chemotactic activity of the Mtb-infected DC supernatants was evaluated by migration assays using activated NK, CD4(+), and CD8(+) cells that expressed both CCR5 and CXCR3. Mtb-induced expression of CCL3,

CCL4, CXCL9, and CXCL10 was involved in the stimulation of NK and T cell migration. In accordance with the data on the IFN- $\alpha$  beta-induced expression of CXCL10, neutralization of IFN- $\alpha$  beta significantly reduced the chemotactic activity of the supernatant from Mtb-infected DC. This indicates that IFN- $\alpha$  beta may modulate the immune response through the expression of CXCL10, which along with CXCL9, CCL3, and CCL4 participates in the recruitment and selective homing of activated/effector cells, which are known to accumulate at the site of Mtb infection and take part in the formation of the granulomas.—Authors' Abstract

**Leemans, J. C., Florquin, S., Heikens, M., Pals, S. T., van der Neut, R., and Van Der Poll, T.** CD44 is a macrophage binding site for *Mycobacterium tuberculosis* that mediates macrophage recruitment and protective immunity against tuberculosis. *J. Clin. Invest.* **111**(5) (2003) 681–689.

Cell migration and phagocytosis are both important for controlling *Mycobacterium tuberculosis* infection and are critically dependent on the reorganization of the cytoskeleton. Since CD44 is an adhesion molecule involved in inflammatory responses and is connected to the actin cytoskeleton, we investigated the role of CD44 in both these processes. Macrophage (Mphi) recruitment into *M. tuberculosis*-infected lungs and delayed-type hypersensitivity sites was impaired in CD44-deficient (CD44 $^{-/-}$ ) mice. In addition, the number of T lymphocytes and the concentration of the protective key cytokine IFN- $\gamma$  were reduced in the lungs of infected CD44 $^{-/-}$  mice. The production of IFN- $\gamma$  by splenocytes of CD44 $^{-/-}$  mice was profoundly increased upon antigen-specific stimulation. Flow cytometry analysis revealed that soluble CD44 can directly bind to virulent *M. tuberculosis*. Mycobacteria also interacted with Mphi-associated CD44, as reflected by reduced binding and internalization of bacilli by CD44 $^{-/-}$  Mphis. This suggests that CD44 is a receptor on Mphis for binding of *M. tuberculosis*. CD44 $^{-/-}$  mice displayed a decreased sur-

vival and an enhanced mycobacterial outgrowth in lungs and liver during pulmonary tuberculosis. In summary, we have identified CD44 as a new Mphi binding site for *M. tuberculosis* that mediates mycobacterial phagocytosis, Mphi recruitment, and protective immunity against pulmonary tuberculosis.—Authors' Abstract

**Lopez, M., Sly, L. M., Luu, Y., Young, D., Cooper, H., and Reiner, N. E.** The 19-kDa *Mycobacterium tuberculosis* protein induces macrophage apoptosis through Toll-like receptor-2. *J. Immunol.* **170**(5) (2003) 2409–2416.

Macrophages infected with *Mycobacterium tuberculosis* undergo increased rates of apoptosis. Important objectives are to define the microbial factors that cause apoptosis, the mechanisms involved and the impact on infection. The 19-kDa *M. tuberculosis* glycolipoprotein (p19) is both cell wall-associated and secreted and is a candidate virulence factor. We investigated the potential of recombinant, His-tagged p19 lacking the secretion/acylation signal to induce macrophage apoptosis. The TUNEL assay and annexin V binding to membrane phosphatidylserine were used to measure apoptosis. The results show that p19 does act to induce apoptosis in differentiated THP-1 cells and monocyte-derived macrophages and that this effect is both dose- and time-dependent. Furthermore, this effect of p19 is Toll-like receptor (TLR)-2-mediated because preincubation of either THP-1 cells or TLR-2-expressing CHO cells with anti-TLR-2 mAb inhibited apoptosis induced by p19. Apoptosis of macrophages in response to p19 was found to be caspase-8 dependent and caspase-9 independent consistent with a transmembrane pathway signaling cell death through TLR-2. The viability of *M. tuberculosis* in cells undergoing apoptosis induced by p19 was significantly reduced suggesting the possibility that this may favor containment of infection. Although native p19 is a mycobacterial glycolipoprotein, based upon the use of recombinant p19 where the acylation signal had been removed, we conclude that it is the polypeptide component of p19 that is responsible for signaling through TLR-2 and that the

lipid moiety is not required.—Authors' Abstract

**Malik, Z. A., Thompson, C. R., Hashimi, S., Porter, B., Iyer, S. S., and Kusner, D. J.** Cutting edge: *Mycobacterium tuberculosis* blocks Ca(2+) signaling and phagosome maturation in human macrophages via specific inhibition of sphingosine kinase. *J. Immunol.* **170(6)** (2003) 2811–2815.

One-third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb), and three million people die of tuberculosis each year. Following its ingestion by macrophages (MPs), Mtb inhibits the maturation of its phagosome, preventing progression to a bactericidal phagolysosome. Phagocytosis of Mtb is uncoupled from the elevation in MP cytosolic Ca(2+) that normally accompanies microbial ingestion, resulting in inhibition of phagosomal-lysosome fusion and increased intracellular viability. This study demonstrates that the mechanism responsible for this failure of Ca(2+)-dependent phagosome maturation involves mycobacterial inhibition of MP sphingosine kinase. Thus, inhibition of sphingosine kinase directly contributes to survival of Mtb within human MPs and represents a novel molecular mechanism of pathogenesis.—Authors' Abstract

**Mustafa, A. S., Shaban, F. A., Al-Attayah, R., Abal, A. T., El-Shamy, A. M., Andersen, P., and Oftung, F.** Human Th1 cell lines recognize the *Mycobacterium tuberculosis* ESAT-6 antigen and its peptides in association with frequently expressed HLA class II molecules. *Scand. J. Immunol.* **57(2)** (2003) 125–134.

We have used a synthetic-peptide approach to map epitope regions of the *Mycobacterium tuberculosis* ESAT-6 antigen recognized by human T cells in relation to major histocompatibility complex (MHC) restriction. ESAT-6-specific CD4+ T-cell lines were established by stimulating peripheral blood mononuclear cells from 25 HLA-DR-typed tuberculosis patients with complete antigen *in vitro*. The established

T-cell lines were then screened for proliferation and interferon-gamma (IFN-gamma) secretion in response to eight overlapping 20-mer peptides covering the ESAT-6 sequence. The response of the T-cell lines to ESAT-6 and peptides from a human leucocyte antigen (HLA)-heterogeneous group of donors suggested the presence of multiple epitopes and promiscuous recognition of the antigen. Analysis of antigen and peptide recognition in the presence of anti-HLA class I and class II antibodies suggested that the T-cell lines recognized ESAT-6 in association with HLA-DR and -DQ molecules. Furthermore, testing of selected T-cell lines with ESAT-6 and the peptides in the presence of autologous and allogeneic HLA-DR- and -DQ-typed antigen-presenting cells identified HLA-DR2, -DR52 and -DQ2 amongst the HLA molecules involved in the presentation of ESAT-6 and its peptides to human Th1 cells. In addition, the T-cell lines were cytotoxic for monocytes and macrophages pulsed with ESAT-6 and peptides. In conclusion, the recognition of ESAT-6 by IFN-gamma-secreting and cytotoxic CD4+ T cells in association with frequently expressed HLA class II molecules supports the application of this antigen to either specific diagnosis or subunit vaccine design.—Authors' Abstract

**Peters, W. and Ernst, J. D.** Mechanisms of cell recruitment in the immune response to *Mycobacterium tuberculosis*. *Microbes Infect.* **5(2)** (2003) 151–158.

Recent advances in understanding cell traffic, especially the roles of adhesion proteins, chemokines, and chemokine receptors, provide the opportunity for understanding mechanisms involved in the immune response to tuberculosis. This review concentrates on the roles of these molecules and the immune response in tuberculosis, based on studies of humans and mice infected with *Mycobacterium tuberculosis*.—Authors' Abstract

**Samten, B., Wizel, B., Shams, H., Weis, S. E., Klucar, P., Wu, S., Vankayalapati, R., Thomas, E. K., Okada, S., Krensky, A. M., and Barnes, P. F.** CD40 ligand

trimer enhances the response of CD8+ T cells to *Mycobacterium tuberculosis*. J. Immunol. **170**(6) (2003) 3180–3186.

We investigated the effect of recombinant CD40 ligand trimer (CD40LT) on the functional capacity of peripheral blood CD8(+) T cells from healthy tuberculin reactors that were cultured with *Mycobacterium tuberculosis*-infected autologous monocytes. CD40LT enhanced the capacity of *M. tuberculosis*-responsive CD8(+) T cells to produce IFN-gamma by increasing the number of IFN-gamma-producing CD8(+) T cells and the amount of IFN-gamma produced per cell. CD40LT-induced IFN-gamma production was dependent on production of IL-12 and IL-18, but did not require IL-15. CD40LT up-regulated expression of the transcription factors phosphorylated CREB and c-Jun, both of which have been previously shown to stimulate IFN-gamma mRNA transcription by binding to the IFN-gamma promoter. CD40LT also enhanced the capacity of CD8(+) T cells to lyse *M. tuberculosis*-infected monocytes, and increased CTL activity was associated with higher expression of perforin and granulysin, but not of Fas ligand. We conclude that CD40LT can enhance CD8(+) T cell effector function in response to *M. tuberculosis*.—Authors' Abstract

**Szere day, L., Baliko, Z., and Szekeres-Bartho, J.** Gamma/delta T cell subsets in patients with active *Mycobacterium tuberculosis* infection and tuberculin anergy. Clin. Exp. Immunol. **131**(2) (2003) 287–291.

Earlier data suggest that gamma/delta T cells may play an important role in the immune response to *Mycobacterium tuberculosis*. The aim of this study was to determine the percentage of different gamma/delta subsets in peripheral blood of active tuberculosis patients with a positive or negative tuberculin reaction. Thirty-eight patients infected with *M. tuberculosis* and 22 healthy controls were included in the study. Venous blood was taken before starting antimycobacterial treatment. Lymphocytes were reacted with monoclonal antibodies specific for different gamma/delta V chains (Vdelta1,

Vdelta2, Vgamma9 and Vgamma4). The results were analysed in the context of tuberculin reactivity and X-ray findings. Our results revealed a selective loss of Vgamma9/Vdelta2 T cells in the peripheral blood of tuberculin-negative patients with active tuberculosis compared to healthy controls, while the ratio of Vgamma9/Vdelta2 T cells in the peripheral blood of patients with a positive skin test did not differ from that of healthy controls. These findings demonstrate a relationship between the loss of the major *M. tuberculosis*-reactive subset of gammadelta T cells and the absence of tuberculin reactivity. The data are consistent with the hypothesis that gammadelta T cells play a role in the protective immune response to *M. tuberculosis* infection.—Authors' Abstract

**Tailleux, L., Schwartz, O., Herrmann, J. L., Pivert, E., Jackson, M., Amara, A., Legres, L., Dreher, D., Nicod, L. P., Gluckman, J. C., Lagrange, P. H., Gicquel, B., and Neyrolles, O.** DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. J. Exp. Med. **197**(1) (2003) 121–127.

Early interactions between lung dendritic cells (LDCs) and *Mycobacterium tuberculosis*, the etiological agent of tuberculosis, are thought to be critical for mounting a protective anti-mycobacterial immune response and for determining the outcome of infection. However, these interactions are poorly understood, at least at the molecular level. Here we show that *M. tuberculosis* enters human monocyte-derived DCs after binding to the recently identified lectin DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN). By contrast, complement receptor (CR)3 and mannose receptor (MR), which are the main *M. tuberculosis* receptors on macrophages (Mphis), appeared to play a minor role, if any, in mycobacterial binding to DCs. The mycobacteria-specific lipoglycan lipoarabinomannan (LAM) was identified as a key ligand of DC-SIGN. Freshly isolated human LDCs were found to express DC-SIGN, and *M. tuberculosis*-derived material was detected in CD14(-)HLA-DR(+)DC-SIGN(+) cells in lymph nodes (LNs) from

patients with tuberculosis. Thus, as for human immunodeficiency virus (HIV), which is captured by the same receptor, DC-SIGN-mediated entry of *M. tuberculosis* in DCs *in vivo* is likely to influence bacterial persistence and host immunity.—Authors' Abstract

**Talreja, J., Bhatnagar, A., Jindal, S. K., and Ganguly, N. K.** Influence of *Mycobacterium tuberculosis* on differential activation of helper T-cells. Clin. Exp. Immunol. **131(2)** (2003) 292–298.

Host defence against tuberculosis infection involves T-lymphocyte mediated cellular immune responses. In this study we assessed T-cell activation by studying the early signal transduction events and production of cytokines by human CD4+ T-cells. The study constituted of five groups of subjects: (a) untreated acid fast bacilli (AFB)+ve TB patients who have not started anti-tuberculosis therapy (ATT) [New]; (b) patients who have taken ATT for two months [2T]; (c) patients who have taken ATT for six months [6T]; (d) mantoux positive healthy controls [T + ve]; (e) mantoux negative healthy controls [T - ve]. We found that mantoux positive healthy controls produced significantly higher levels of IP3, intracellular Ca<sup>2+</sup> and presented increased PKC activity when CD4+ T-cells were stimulated with *M. tuberculosis* H37Rv cell lysate as compared to mantoux negative controls. Furthermore, decreased expression of CD54 (ICAM-1) and reduced [Ca<sup>2+</sup>]<sub>i</sub> were seen in TB patients as compared to T+ve healthy controls. TB patients showed significantly lower levels of IL-2 and IFN $\gamma$  and higher levels of IL-4 as compared to normal healthy controls, suggesting a diminished Th1 response. Thus, the reciprocal changes in cytokines, reduced [Ca<sup>2+</sup>]<sub>i</sub> levels, and CD54 expression in patients imply phenotype shifting of Th precursors to Th2 type in TB patients.—Authors' Abstract

**Urdahl, K. B., Liggitt, D., and Bevan, M. J.** CD8(+) T cells accumulate in the lungs of *Mycobacterium tuberculosis*-infected K(b-/-)D(b-/-) mice, but pro-

vide minimal protection. J. Immunol. **170(4)** (2003) 1987–1994.

Recent studies have shown that MHC class I molecules play an important role in the protective immune response to *Mycobacterium tuberculosis* infection. Here we showed that mice deficient in MHC class Ia, but possessing MHC class Ib (K(b-/-)D(b-/-) mice), were more susceptible to aerosol infection with *M. tuberculosis* than control mice, but less susceptible than mice that lack both MHC class Ia and Ib (beta(2)m(-/-) mice). The susceptibility of K(b-/-)D(b-/-) mice cannot be explained by the failure of CD8(+) T cells (presumably MHC class Ib-restricted) to respond to the infection. Although CD8(+) T cells were a relatively small population in uninfected K(b-/-)D(b-/-) mice, most already expressed an activated phenotype. During infection, a large percentage of these cells further changed their cell surface phenotype, accumulated in the lungs at the site of infection, and were capable of rapidly producing IFN- $\gamma$  following TCR stimulation. Histopathologic analysis showed widespread inflammation in the lungs of K(b-/-)D(b-/-) mice, with a paucity of lymphocytic aggregates within poorly organized areas of granulomatous inflammation. A similar pattern of granuloma formation has previously been observed in other types of MHC class I-deficient mice, but not CD8 $\alpha$ (-/-) mice. Thus, neither the presence of MHC class Ib molecules themselves, nor the activity of a population of nonclassical CD8(+) effector cells, fully restored the deficit caused by the absence of MHC class Ia molecules, suggesting a unique role for MHC class Ia molecules in protective immunity against *M. tuberculosis*.—Authors' Abstract

**Yamada, H., Mizuno, S., and Sugawara, I.** Interferon regulatory factor 1 in mycobacterial infection. Microbiol. Immunol. **46(11)** (2002) 751–760.

In order to understand the role of IRF-1 in the development of murine tuberculosis *in vivo*, IRF-1 knockout mice were infected with *Mycobacterium tuberculosis* by placing them in the exposure chamber of an air-

borne infection apparatus. These knockout mice developed multifocal necrotic lesions in the lung, liver and spleen tissues and died of disseminated tuberculosis within 43 days of infection. Compared with the levels in wild-type mice, the pulmonary inducible NO synthase (iNOS) mRNA expression level was significantly lower, but IL-18 and IL-6 mRNA levels were higher. There was no statistically significant difference in the expression of IFN-gamma and TNF-alpha mRNA between the IRF-1 knockout and wild-type mice. IRF-1 is indirectly responsible for iNOS mRNA expression and plays an important role in the pathogenesis of murine tuberculosis.—Authors' Abstract

**Zahrt, T. C.** Molecular mechanisms regulating persistent *Mycobacterium tuberculosis* infection. *Microbes. Infect.* **5(2)** (2003) 159–167.

Establishing persistent infection and resisting elimination by the host's immune system are key factors contributing to latent infection by *Mycobacterium tuberculosis*. Recently, bacterial determinants regulating these processes have been identified. Here, we review molecular mechanisms regulating persistent infection and discuss the highly dynamic interaction of *M. tuberculosis* with the host.—Author's Abstract

## Experimental Infections

**Chen, J. M., Alexander, D. C., Behr, M. A., and Liu, J.** *Mycobacterium bovis* BCG vaccines exhibit defects in alanine and serine catabolism. *Infect. Immun.* **71(2)** (2003) 708–716.

*Mycobacterium bovis* BCG is the only accepted vaccine for the prevention of tuberculosis (TB) in humans. BCG is a live vaccine, and induction of immunity to TB requires productive infection of the host by BCG. However, BCG is not a satisfactory vaccine, because it fails to protect against pulmonary TB in adults. In this study, we found that BCG strains cannot utilize many naturally occurring amino acids as the sole nitrogen source for growth. This defect is caused, at least partially, by the lack of functional metabolic enzymes. All BCG strains are unable to catabolize L-alanine or D-alanine due to a frameshift mutation in the L-alanine dehydrogenase gene (*ald*). Some BCG strains, such as BCG-Pasteur and BCG-Frappier, cannot catabolize L-serine, apparently due to inadequate expression of L-serine deaminase (*sdaA*). We also found that undegraded alanine and serine inhibit the growth of BCG through blockage of glutamine synthetase. These results suggest that BCG strains are limited in nitrogen metabolic capacity and predict defects that may restrict multiplication and persistence of the live vaccine within the host.—Authors' Abstract

**de Bruyn, G. and Garner, P.** *Mycobacterium vaccae* immunotherapy for treating tuberculosis. *Cochrane Database Syst. Rev.* 2003; **(1)**: CD001166.

**BACKGROUND:** Some authorities have advocated *Mycobacterium vaccae* immunotherapy for treating tuberculosis and other infections caused by mycobacteria. **OBJECTIVES:** To assess the effects of *Mycobacterium vaccae* as an adjunct to chemotherapy for treating tuberculosis. **SEARCH STRATEGY:** We searched the Cochrane Infectious Diseases Group trials register (September 2002), the Cochrane Controlled Trials register (Issue 3, 2002), MEDLINE (1966 to October 2002), EMBASE (1980 to September 2002), and reference lists of articles. We also contacted organizations and individuals working in the field. **SELECTION CRITERIA:** Randomized and quasi-randomized trials using whole, killed *Mycobacterium vaccae* for patients with tuberculosis. **DATA COLLECTION AND ANALYSIS:** One reviewer assessed trial quality and extracted data. **MAIN RESULTS:** Seven trials met the inclusion criteria. There was no effect on mortality (4 trials, OR 1.09, 95% CI 0.79 to 1.49). No consistent effect on sputum negativity or sputum culture was shown. Most immunotherapy recipients experienced local adverse reactions (2 trials, OR 18.2, 95% CI 9 to 37), some of which progressed to ul-

ceration and scarring. REVIEWER'S CONCLUSIONS: *Mycobacterium vaccae* does not benefit patients with tuberculosis.— Authors' Abstract

**Dietrich, G., Viret, J. F., and Hess, J.** *Mycobacterium bovis* BCG-based vaccines against tuberculosis: novel developments. *Vaccine* **21**(7–8) (2003) 667–670.

*Mycobacterium bovis* Bacille Calmette-Guerin (BCG) is one of the most widely used vaccines. Modern techniques in genome manipulation allow the construction of recombinant (r)-BCG strains that can be employed as highly immunogenic vaccines against tuberculosis (TB) with an enhanced safety profile. In addition, the development of novel procedures to cultivate BCG will allow the large-scale production of future BCG-based vaccines.— Authors' Abstract

**Dietrich, G., Viret, J. F., and Hess, J.** Novel vaccination strategies based on recombinant *Mycobacterium bovis* BCG. *Int. J. Med. Microbiol.* **292**(7–8) (2003) 441–451.

In this manuscript, we will review the utilization of *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) as a vaccine against tuberculosis (TB) and as a carrier system for heterologous antigens. BCG is one of the most widely used vaccines. Novel techniques in genome manipulation allow the construction of virulence-attenuated recombinant (r)-BCG strains that can be employed as homologous vaccines, or as heterologous antigen delivery systems, for priming pathogen-specific immunity against infectious diseases, including TB. Several approaches are available for heterologous antigen expression and compartmentalization in BCG and recent findings show the potential to modulate and direct the immune responses induced by r-BCG strains as desired. Recent achievements in complete genome analysis of various target pathogens, combined with a better understanding of protective pathogen-specific immune responses, form the basis for the rational design of a new generation of recombinant mycobacterial vaccines against a multitude of infectious diseases.— Authors' Abstract

**Gruppo, V. and Orme, I. M.** Dose of BCG does not influence the efficient generation of protective immunity in mice challenged with *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)* **82**(6) (2002) 267–273.

It is generally agreed that BCG vaccination is relatively ineffective in adults exposed to tuberculosis infection. The reasons for this may well be multiple, and may include the possibility that higher doses of BCG may induce a mixed TH1 and TH2 response, which may lessen the protective effect of the vaccine. To test this hypothesis, mice were vaccinated with a range of doses of BCG and then challenged by the intravenous or aerogenic routes with virulent *Mycobacterium tuberculosis*. While the data support the hypothesis that a TH2 response is induced by higher doses of BCG, this was found to have no influence whatsoever on the capacity of the vaccinated mouse to express acquired specific resistance to the challenge infection.— Authors' Abstract

**Horwitz, M. A. and Harth, G.** A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the guinea pig model of pulmonary tuberculosis. *Infect. Immun.* **71**(4) (2003) 1672–1679.

Tuberculosis (TB) remains an enormous global health problem, and a new vaccine against TB more potent than the current inadequate vaccine, *Mycobacterium bovis* BCG, is urgently needed. We describe a recombinant BCG vaccine (rBCG30) expressing and secreting the 30-kDa major secretory protein of *Mycobacterium tuberculosis*, the primary causative agent of TB, that affords greater survival after challenge than parental BCG in the highly demanding guinea pig model of pulmonary TB. Animals immunized with rBCG30 and then challenged by aerosol with a highly virulent strain of *M. tuberculosis* survived significantly longer than animals immunized with conventional BCG. The parental and recombinant vaccine strains are comparably avirulent in guinea pigs, as they display a similar pattern of growth and clearance in

the lung, spleen, and regional lymph nodes. The pMTB30 plasmid encoding the 30-kDa protein is neither self-transmissible nor mobilizable to other bacteria, including mycobacteria. The pMTB30 plasmid can be stably maintained in *Escherichia coli* but is expressed only in mycobacteria. The recombinant and parental strains are sensitive to the same antimycobacterial antibiotics. rBCG30, the first vaccine against TB more potent than nearly century-old BCG, is being readied for human clinical trials.—Authors' Abstract

**Jiao, X., Lo-Man, R., Winter, N., Deriaud, E., Gicquel, B., and Leclerc, C.** The shift of Th1 to Th2 immunodominance associated with the chronicity of *Mycobacterium bovis* bacille Calmette-Guerin infection does not affect the memory response. *J. Immunol.* **170(3)** (2003) 1392–1398.

In the present study we investigated the shaping and evolution of the immunodominance of the T cell response during a chronic mycobacterial infection. Using a recombinant bacille Calmette-Guerin expressing a reporter Ag, the *Escherichia coli* MalE protein, we analyzed the peptide specificity and the cytokine profile of the T cell response to the reporter Ag by ELISPOT. During the early steps of infection, the T cell response was focused on two dominant MalE epitopes and was characterized by a pure IFN- $\gamma$  response. Then, in the course of infection the initial IFN- $\gamma$  response to these two epitopes shifted to a mixed IFN- $\gamma$ /IL-4 response. At the same time, the peptide specificity of the T cell response was broadened to two additional MalE epitopes characterized by a unique IL-4 response resulting in the establishment of a dominant IL-4 response to the MalE protein at 16 wk postinfection. However, this phenomenon did not impair the outcome of a predominant IFN- $\gamma$  response upon subsequent MalE recall *in vivo* performed in the presence of CFA, a Th1-driving adjuvant. These results indicate that the Th2 nature of the immune response established during a chronic infection, which most likely reflects regulatory mechanisms to allow the return to T cell homeostasis, does not shape the Th1/Th2 nature of the memory response.—Authors' Abstract

**Marsollier, L., Honore, N., Legras, P., Manceau, A. L., Kouakou, H., Carbonnelle, B., and Cole, S. T.** Isolation of three *Mycobacterium ulcerans* strains resistant to rifampin after experimental chemotherapy of mice. *Antimicrob. Agents Chemother.* **47(4)** (2003) 1228–1232.

By use of a murine model for Buruli ulcer, *Mycobacterium ulcerans* was found to be susceptible to rifampin, with the MIC being 0.5 to 1 micro g/ml. Three mutants were isolated after rifampin monotherapy. Two were resistant to rifampin at 8 micro g/ml, and one was resistant to rifampin at 32 micro g/ml. The mutants harbored Ser416Phe mutations and His420Tyr mutations in the rpoB gene, and these mutations have also been found to be responsible for rifampin resistance in the leprosy and tubercle bacilli. The results indicate that while rifampin may be active against *M. ulcerans*, it should never be used as monotherapy in humans.—Authors' Abstract

**Minion, F. C., Menon, S. A., Mahairas, G. G., and Wannemuehler, M. J.** Enhanced murine antigen-specific gamma Interferon and immunoglobulin G2a responses by using mycobacterial ESAT-6 sequences in DNA vaccines. *Infect. Immun.* **71(4)** (2003) 2239–2243.

The *Mycobacterium tuberculosis* protein ESAT-6 has unusual immune stimulating activities, has been implicated in the recall of long-lived immunity, and induces protection against tuberculosis in mice. For many diseases caused by bacterial or viral pathogens, a strong cell-mediated immune (i.e., type 1) response is often required for recovery or protection. Therefore, it is important to design immunization regimens that induce agent-specific type 1 immunity. We have shown in previous studies that ESAT-6 could enhance antigen-specific type 1 immune responses in BALB/c mice against a second antigen when presented as a purified fusion protein. It was also of interest to determine if ESAT-6 could enhance the type 1 response against a second antigen beyond that afforded by DNA vaccination through CpG motifs. This was tested by using gene fusions of ESAT-6 and

the *Mycoplasma hyopneumoniae* surface antigen P71. Modified P71 gene sequences were cloned with or without ESAT-6 sequences into a DNA vaccine vector and were used to immunize mice. Splenic lymphocytes from vaccinated mice were tested for gamma interferon (IFN-gamma) and interleukin-10 (IL-10) secretion. Serum antibodies were examined for P71 antigen-specific isotype responses. When stimulated *in vitro* with purified P71 antigen, splenocytes from the ESAT-6:P71 vaccinates secreted higher levels of IFN-gamma and lower levels of IL-10 compared to those of vaccinates receiving the P71 construct alone. Furthermore, the immunoglobulin G2a serum antibody levels were significantly higher in the ESAT-6:P71 vaccinates compared to those of the vaccinates receiving P71 alone. In conclusion, ESAT-6 was shown to enhance antigen-specific type 1 immune responses in BALB/c mice when used in DNA vaccines. — Authors' Abstract

**Ngamying, M., Sawanpanyalert, P., Butraporn, R., Nikasri, J., Cho, S. N., Levy, L., and Brennan, P. J.** Effect of vaccination with refined components of the organism on infection of mice with *Mycobacterium leprae*. *Infect. Immun.* **71**(3) (2003) 1596–1598.

Only native products of *Mycobacterium leprae*, whether cell wall, cytosol, or membrane derived, can confer protective immunity against challenge in the mouse footpad. Previously, recombinant proteins were shown to be ineffective. The cell wall skeleton—the mycolyl-arabinogalactan-peptidoglycan complex—devoid of proteins is not protective. — Authors' Abstract

**Shi, L., Jung, Y. J., Tyagi, S., Gennaro, M. L., and North, R. J.** Expression of Th1-mediated immunity in mouse lungs induces a *Mycobacterium tuberculosis* transcription pattern characteristic of nonreplicating persistence. *Proc. Natl. Acad. Sci. U. S. A.* **100**(1) (2003) 241–246.

The lung is the primary target of infection with *Mycobacterium tuberculosis*. It is

well established that, in mouse lung, expression of adaptive, Th1-mediated host immunity inhibits further multiplication of *M. tuberculosis*. Here, real-time RT-PCR was used to define the pattern of expression against time of lung infection of key genes involved in Th1-mediated immunity and of selected genes of *M. tuberculosis*. Inhibition of bacterial multiplication was preceded by increased mRNA synthesis for IFN-gamma and inducible NO synthase (NOS2) and by NOS2 protein synthesis in infected macrophages. Concurrently, the pattern of transcription of bacterial genes underwent dramatic changes. mRNA synthesis increased for alpha-crystallin (acr), rv2626c, and rv2623 and decreased for superoxide dismutase C (sodC), sodA, and fibronectin-binding protein B (fbpB). This pattern of *M. tuberculosis* transcription is characteristic of the nonreplicating persistence [Wayne, L. G. & Sohaskey, C. D. (2001) *Annu. Rev. Microbiol.* **55**, 139–163] associated with adaptation of tubercle bacilli to hypoxia *in vitro*. Based on this similarity, we infer that host immunity induces bacterial growth arrest. In IFN-gamma gene-deleted mice, bacterial growth was not controlled; NOS2 protein was not detected in macrophages; sodC, sodA, and fbpB transcription showed no decrease; and acr, rv2626c, and rv2623 transcription increased only at the terminal stages of lung pathology. These findings define the transcription signature of *M. tuberculosis* as it transitions from growth to persistence in the mouse lung. The bacterial transcription changes measured at onset of Th1-mediated immunity are likely induced, directly or indirectly, by nitric oxide generated by infected macrophages. — Authors' Abstract

**Taylor, J. L., Turner, O. C., Basaraba, R. J., Belisle, J. T., Huygen, K., and Orme, I. M.** Pulmonary necrosis resulting from DNA vaccination against tuberculosis. *Infect. Immun.* **71**(4) (2003) 2192–2198.

The use of DNA constructs encoding mycobacterial proteins is a promising new approach to vaccination against tuberculosis. A DNA vaccine encoding the hsp60 molecule of *Mycobacterium leprae* has previously

been shown to protect against intravenous infection of mice with *Mycobacterium tuberculosis* in both the prophylactic and immunotherapeutic modes. It is shown here, however, that this vaccine was not effective in a more realistic aerosol infection model or in a model of latent tuberculosis in the lungs. Moreover, when given in an immunotherapeutic model the immunized mice developed classical Koch reactions characterized by multifocal discrete regions of cellular necrosis throughout the lung granulomas. Similar and equally severe reactions were seen in mice given a vaccine with DNA coding for the Ag85 antigen of *M. tuberculosis*. This previously unanticipated safety problem indicates that DNA vaccines should be used with caution in individuals who may have already been exposed to tuberculosis.—Authors' Abstract

**Turner, O. C., Basaraba, R. J., and Orme, I. M.** Immunopathogenesis of pulmonary granulomas in the guinea pig after infection with *Mycobacterium tuberculosis*. *Infect. Immun.* **71(2)** (2003) 864–871.

Pulmonary tuberculosis in guinea pigs is similar to the disease in humans and is accordingly widely used as a model to test tuberculosis vaccines. The primary site of expression of acquired immunity and the hallmark of tuberculosis is the granuloma. Granuloma morphology is well described, but there is limited information regarding T-cell subset influx. We monitored the course of pulmonary tuberculosis in guinea pigs and observed four distinct immunohistopathological stages. In all stages there were similar numbers and arrangement of CD4 and CD8 T cells. There were only small numbers of apoptotic lymphocytes, scattered around and within the necrotic core, and acid-fast bacilli were visible both within macrophages and free within airway debris. A key finding of the study was the observation that the development of the necrotic core was an early event and almost certainly preceded the emergence of the acquired immune response. This in turn suggests that innate mechanisms are the basis of the early lesions and that subsequent acquired responses are unable to moderate

them. This hypothesis differs from the current dogma that excessive activity of T cells mediates delayed-type hypersensitivity and that cellular cytolysis is the root cause of the necrosis.—Authors' Abstract

**Winslow, G. M., Roberts, A. D., Blackman, M. A., and Woodland, D. L.** Persistence and turnover of antigen-specific CD4 T cells during chronic tuberculosis infection in the mouse. *J. Immunol.* **170(4)** (2003) 2046–2052.

CD4 T cells are critical for resistance to *Mycobacterium tuberculosis* infection, but how effective T cell responses are maintained during chronic infection is not well understood. To address this question we examined the CD4 T cell response to a peptide from ESAT-6 during tuberculosis infection in the mouse. The ESAT-6(1–20)/IA(b)-specific CD4 T cell response in the lungs, mediastinal lymph nodes, and spleen reached maxima 3–4 wk postinfection, when the bacteria came under the control of the immune response. Once chronic infection was established, the relative frequencies of Ag-specific CD4 T cells were maintained at nearly constant levels for at least 160 days. ESAT-6(1–20)/IA(b)-specific CD4 T cells that responded *in vitro* expressed activation markers characteristic of chronically activated effector cells and used a limited Vbeta repertoire that was clonally stable *in vivo* for at least 12 wk. 5-Bromo-2-deoxyuridine incorporation studies indicated a relatively high rate of cell division among both total CD4 and ESAT-6(1–20)/IA(b)-specific CD4 T cells during acute infection, but the degree of 5-bromo-2-deoxyuridine incorporation by both the CD4 T cells and the Ag-specific cells declined at least 3-fold during chronic infection. The data indicate that the peripheral ESAT-6(1–20)/IA(b)-specific CD4 T cell response to *M. tuberculosis* is characterized during the acute phase of infection by a period of extensive proliferation, but once bacterial control is achieved, this is followed during chronic infection by an extended containment phase that is associated with a persistent response of activated, yet more slowly proliferating, T cells.—Authors' Abstract

## Microbiology

**Bhattacharya, B., Karak, K., Ghosal, A. G., Roy, A., Das, S., Dandapat, P., Khetawat, D., Mondal, D. K., Bhattacharya, S., and Chakrabarti, S.** Development of a new sensitive and efficient multiplex polymerase chain reaction (PCR) for identification and differentiation of different mycobacterial species. *Trop. Med. Int. Health* **8(2)** (2003) 150–157.

For early detection and species differentiation of mycobacteria, polymerase chain reaction (PCR) techniques are currently in wide use. However, individual techniques using amplification of different targets with appropriate primers still have some limitations, which have to be overcome. The ideal technique would use DNA sequences which should be present in all mycobacteria and absent in others and would be able to discriminate one species from the other, as non-tuberculous mycobacteria (NTM) are on rise in terms of frequency of detection. We developed a multiplex PCR based on amplification of 16S, 365 and 541 bp target fragments of unrelated genes, hsp 65 coding for 65 kDa antigen, dnaJ gene of mycobacteria and insertion element IS 6110 of *Mycobacterium tuberculosis*, respectively. This multiplex PCR was tested over 5 years from 1996 to 2001 with 411 clinical specimens from suspected cases of tuberculosis and mycobacterioses and compared with standard laboratory techniques. The multiplex PCR was positive for 379 cases compared with 280 cases by standard techniques ( $p < 0.0001$ ). It could distinguish between strains of the *M. tuberculosis* complex and NTM; the results are comparable with standard techniques. Thus the multiplex PCR can be useful in early detection, species differentiation and epidemiology. — Authors' Abstract

The clinical profile of nontuberculous mycobacteria (NTM) has been raised by the human immunodeficiency virus and AIDS pandemic. Different laboratory techniques, often molecular based, are available to facilitate the rapid and accurate identification of NTM. The expense of these advanced techniques has been questioned. At the National Reference Center for Mycobacteriology and the Health Sciences Center, University of Manitoba, in Winnipeg, Canada, we performed a direct cost analysis of laboratory techniques for commercial DNA probe-negative (Gen-Probe, Inc., San Diego, Calif.), difficult-to-identify NTM. We compared the costs associated with conventional phenotypic methodology (biochemical testing, pigment production, growth, and colony characteristics) and genotypic methodology (16S ribosomal DNA [rDNA] sequence-based identification). We revealed a higher cost per sample with conventional methods, and this cost varied with organism characteristics: \$80.93 for slowly growing, biochemically active NTM; \$173.23 for slowly growing, biochemically inert NTM; and \$129.40 for rapidly growing NTM. The cost per sample using 16S rDNA sequencing was \$47.91 irrespective of organism characteristics, less than one-third of the expense associated with phenotypic identification of biochemically inert, slow growers. Starting with a pure culture, the turnaround time to species identification is 1 to 2 days for 16S rDNA sequencing compared to 2 to 6 weeks for biochemical testing. The accuracy of results comparing both methodologies is briefly discussed. 16S rDNA sequencing provides a cost-effective alternative in the identification of clinically relevant forms of probe-negative NTM. This concept is not only useful in mycobacteriology but also is highly applicable in other areas of clinical microbiology. — Authors' Abstract

**Cook, V. J., Turenne, C. Y., Wolfe, J., Pauls, R., and Kabani, A.** Conventional methods versus 16S ribosomal DNA sequencing for identification of nontuberculous mycobacteria: cost analysis. *J. Clin. Microbiol.* **41(3)** (2003) 1010–1015.

**Dauendorffer, J. N., Guillemin, I., Aubry, A., Truffot-Pernot, C., Sougakoff, W., Jarlier, V., and Cambau, E.** Identification of mycobacterial species by PCR sequencing of quinolone resistance-determining regions of DNA gyrase

genes. *J. Clin. Microbiol.* **41(3)** (2003) 1311–1315.

The determination of the amino acid sequence of quinolone resistance-determining regions (QRDRs) in the A and B subunits of DNA gyrase is the molecular test for the detection of fluoroquinolone resistance in mycobacteria. We looked to see if the assignment of mycobacterial species could be obtained simultaneously by analysis of the corresponding nucleotide sequences. PCR sequencing of *gyrA* and *gyrB* QRDRs was performed for 133 reference and clinical strains of 21 mycobacterial species commonly isolated in clinical laboratories. Nucleotide sequences of *gyrA* and *gyrB* QRDRs were species specific, regardless of fluoroquinolone susceptibility. — Authors' Abstract

**Li, Z., Qin, W., Yue, Q., Meng, K., and Lin, Q.** [Study on detection of the Mycobacteria DNA in formalin-fixed, paraffin-embedded tissue samples by triplex polymerase chain reaction] *Wei Sheng Wu Xue Bao* **42(1)** (2002) 69–75. [Article in Chinese]

To supply an additional differential diagnostic method for pathological diagnosis of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria infections in formalin-fixed, paraffin-embedded tissue samples by triplex-PCR. Three pairs of oligonucleotide primer were used in triplex-PCR. A 383 bp DNA fragment encoding part of the 65 kD mycobacterial surface antigen, a 123 bp fragment corresponding to a specific *Mycobacterium tuberculosis* complex sequence which was the insertion sequence 6110 (IS6110) and a 268 bp fragment for human beta-globin were amplified by triplex-PCR respectively. The sensitivity of the triplex-PCR-electrophoresis for the mycobacteria DNA was 0.6 picogram. The specific bands of 383 bp and 123 bp among the amplified DNA from *Mycobacterium tuberculosis*, *M. bovis*, *M. bovis* BCG and *M. simiae* were present in the agarose gel. By contrast, only a band of 383 bp was found among the nontuberculosis mycobacteria which contained *M. avium*, *M. cheiloneae*, *M. scrofulaceum*, *M. xenopi*, *M.*

*kansasii*, *M. intracellulare* and *M. smegmatis*. Compared with the standard strains, there was an additional 268 bp band in simulated clinic samples infected by mycobacteria, 209 formalin-fixed, paraffin-embedded tissue samples of the patients diagnosed as scrofula by clinic doctor at first visit were examined by triplex polymerase chain reaction. Among them, 193 tissue samples of the patients pathologically diagnosed as scrofula, tuberculous granulomatous tissue or tuberculous granulomatous inflammation were positive: the specific bands of 383 bp, 123 bp and 268 bp were present in the agarose gel and this tallied with *Mycobacterium tuberculosis* complex infection. Of 16 tissue samples of the patients pathologically diagnosed as suspicious scrofula, 15 samples were same positive results and this tallied with *Mycobacterium tuberculosis* complex infection, too; 1 sample could find the specific bands of 383 bp and 268 bp which were present in the agarose gel and this tallied with nontuberculous mycobacteria infection. The results showed that the triplex-PCR could detect and identify the DNA of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria except *M. simiae*. It is a valuable detecting method which has high sensitivity and specificity. — Authors' Abstract

**O'Toole, R., Smeulders, M. J., Blokpoel, M. C., Kay, E. J., Lougheed, K., and Williams, H. D.** A two-component regulator of universal stress protein expression and adaptation to oxygen starvation in *Mycobacterium smegmatis*. *J. Bacteriol.* **185(5)** (2003) 1543–1554.

We identified a response regulator in *Mycobacterium smegmatis*, which plays an important role in adaptation to oxygen-starved stationary phase. The regulator exhibits strong sequence similarity to DevR/Rv3133c of *M. tuberculosis*. The structural gene is present on a multigene locus, which also encodes a sensor kinase. A *devR* mutant of *M. smegmatis* was adept at surviving growth arrest initiated by either carbon or nitrogen starvation. However, its culturability decreased several orders of magnitude below that of the wild type under oxygen-starved stationary-phase conditions. Two-

dimensional gel analysis revealed that a number of oxygen starvation-inducible proteins were not expressed in the *devR* mutant. Three of these proteins are universal stress proteins, one of which is encoded directly upstream of *devR*. Another protein closely resembles a proposed nitroreductase, while a fifth protein corresponds to the alpha-crystallin (HspX) orthologue of *M. smegmatis*. None of the three universal stress proteins or nitroreductase, and a considerably lower amount of HspX was detected in carbon-starved wild-type cultures. A fusion of the *hspX* promoter to *gfp* demonstrated that DevR directs gene expression when *M. smegmatis* enters stationary phase brought about, in particular, by oxygen starvation. To our knowledge, this is the first time a role for a two-component response regulator in the control of universal stress protein expression has been shown. Notably, the *devR* mutant was 10(4)-fold more sensitive than wild type to heat stress. We conclude that DevR is a stationary-phase regulator required for adaptation to oxygen starvation and resistance to heat stress in *M. smegmatis*. — Authors' Abstract

**Patterson, J. H., Waller, R. F., Jeevarajah, D., Billman-Jacobe, H., and McConville, M. J.** Mannose metabolism is required for mycobacterial growth. *Biochem. J.* **372(Pt 1)** (2003) 77–86.

Mycobacteria are the causative agents of tuberculosis and several other significant diseases in humans. All species of mycobacteria synthesize abundant cell wall mannolipids (phosphatidylinositolmannosides, lipoarabinomannan), a cytoplasmic methylmannose polysaccharide and O-mannosylated glycoproteins. To investigate whether these molecules are essential for mycobacterial growth, we have generated a *M. smegmatis* mannose auxotroph by targeted deletion of the gene encoding phosphomannose isomerase (PMI). The PMI deletion mutant displayed a mild hyperseptation phenotype, but grew normally in media containing an exogenous source of mannose. When this mutant was suspended in media without mannose, ongoing synthesis of both the mannolipids and methylmannose polysaccharides was halted and the hyperseptation

phenotype became more pronounced. These changes preceded a dramatic loss of viability after 10 hr in mannose-free media. Mannose starvation did not lead to detectable changes in cell wall ultrastructure or permeability to hydrophobic drugs, or to changes in the rate of biosynthesis of other plasma membrane or wall-associated phospholipids. These results show that mannose metabolism is required for growth of *M. smegmatis* and that one or more mannose-containing molecules may play a role in regulating septation and cell division in these bacteria. — Authors' Abstract

**Rodriguez, G. M. and Smith, I.** Mechanisms of iron regulation in mycobacteria: role in physiology and virulence. *Mol. Microbiol.* **47(6)** (2003) 1485–1494.

The role of iron in mycobacteria as in other bacteria goes beyond the need for this essential cofactor. Limitation of this metal triggers an extensive response aimed at increasing iron acquisition while coping with iron deficiency. In contrast, iron-rich environments prompt these prokaryotes to induce synthesis of iron storage molecules and to increase mechanisms of protection against iron-mediated oxidative damage. The response to changes in iron availability is strictly regulated in order to maintain sufficient but not excessive and potentially toxic levels of iron in the cell. This response is also linked to other important processes such as protection against oxidative stress and virulence. In bacteria, iron metabolism is regulated by controlling transcription of genes involved in iron uptake, transport and storage. In mycobacteria, this role is fulfilled by the iron-dependent regulator IdeR. IdeR is an essential protein in *Mycobacterium tuberculosis*, the causative agent of human tuberculosis. It functions as a repressor of iron acquisition genes, but is also an activator of iron storage genes and a positive regulator of oxidative stress responses. — Authors' Abstract

**Snyder, D. S. and Small, P. L.** Uptake and cellular actions of mycolactone, a virulence determinant for *Mycobacterium ulcerans*. *Microb. Pathog.* **34(2)** (2003) 91–101.

Mycolactone is a macrolide secreted by *Mycobacterium ulcerans*. Experimental evidence suggests that mycolactone plays a prominent role in the pathogenesis of Buruli ulcer by causing both tissue destruction and immunosuppression. To understand the cell biology of mycolactone activity, we have synthesized the fluorescent mycolactone derivative bodipy mycolactone. Although derivatization resulted in a modest decrease in cytopathic activity, the derivatized and native molecules produce identical phenotypes in cultured cells. Confocal microscopy of bodipy mycolactone added to cultured fibroblasts, shows that it is localized to the cytosol. Bodipy mycolactone fails to bind to the cell membrane and is excluded from the nucleus. Uptake is both nonsaturable and noncompetitive with excess mycolactone, consistent with passive diffusion of this toxin through the cell membrane. These facts, combined with the inability of signal transduction inhibitors to inhibit mycolactone cytopathicity point towards the presence of an cytosolic target for mycolactone. A dose dependent increase in intracellular calcium levels at occurs upon mycolactone exposure, but chelation of intracellular calcium alters neither the cytopathicity nor the caspase induction profile of treated cells. Mitochondrial polarization is maintained in treated cells for up to 3 days arguing that the rise in intracellular calcium levels may be a result of cytoskeletal remodeling.— Authors' Abstract

**Torrelles, J. B., Ellis, D., Osborne, T., Hoefler, A., Orme, I. M., Chatterjee, D., Brennan, P. J., and Cooper, A. M.**

Characterization of virulence, colony morphotype and the glycopeptidolipid of *Mycobacterium avium* strain 104. Tuberculosis (Edinb) **82(6)** (2002) 293–300.

**SETTING:** Members of the *Mycobacterium avium* complex (MAC) are responsible for mycobacterial disease in children, the aged and in immunocompromised individuals. The complex consists of different species, serovars and morphologic forms that vary in virulence. One isolate of the MAC is currently being sequenced (MAC 104) and was chosen based on its derivation from an AIDS patient and the fact that it could be genetically manipulated. **OBJECTIVE:** MAC 104 was therefore analyzed for virulence, colony morphotype and expression of the glycopeptidolipid (GPL) responsible for serotyping differences and the rough to smooth morphological switch. **RESULTS:** The isolate was found to be virulent in the murine model of low-dose aerosol infection in that it could colonize the lung, proliferate within the tissue and disseminate to other organs. MAC 104 expressed a variety of colony morphotypes, the most prevalent of which were smooth opaque, smooth transparent and rough. All three morphotypes could persist in the lung; however, the transparent and rough morphotypes grew more rapidly *in vivo*. The rough morphotype was unusual in that it expressed an atypical form of the GPL usually absent from rough morphotypes. **CONCLUSION:** This characterization complements the genome data and confirms that MAC 104 behaves similarly to other MAC isolates.— Authors' Abstract

## Microbiology (Leprosy)

**Guerrero, M. I., Arias, M. T., Garcés, M. T., and León, C. I.** Developing and using a PCR test to detect subclinical *Mycobacterium leprae* infection. Revista Panamericana de Salud Pública/Pan Amer. J. Pub. Health **11(4)** (2002) 228–234.

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

**Torres, P., Camarena, J. J., Gomez, J. R., Nogueira, J. M., Gimeno, V., Navarro, J. C., and Olmos, A.** Comparison of PCR mediated amplification of DNA and the classical methods for detection of *Mycobacterium leprae* in different types of clinical samples in leprosy patients and contacts. *Lepr. Rev.* **74** (2003) 18–30.

Traditional staining and microscopic examination techniques for the detection of *Mycobacterium leprae*, DNA amplification by polymerase chain reaction (PCR) of a 531-bp fragment of the *M. leprae* specific gene encoding the 36-kDa antigen, and serodiagnosis with *M. leprae* specific antigens (PGL-1 and D-BSA) were compared on different clinical specimens (serum samples, slit-skin smears, biopsies and swabs) from 60 leprosy patients attending the Sanatorium of Fontilles. Patients were divided into groups; (i) 20 multibacillary patients (MB) with positive bacteriological index (BI) by conventional methods and on WHO multidrug therapy (MDT); (ii) 30 MB patients with negative BI and completed minimum 2 years treatment MDT; (iii) 10 paucibacillary (PB) patients who had completed 6 months MDT at least 8 years ago. Control groups included four non-leprosy patients for PCR methods and 40 health control patients and 10 tuberculosis patients for serological methods. In the multibacillary BI positive group, there was a good correlation between all methods. All tests were negative in the paucibacillary group, although only a few patients were tested and all had been treated many years ago. One must be cautious concerning the diagnostic potential of these techniques in this type of leprosy. We also studied different combinations of leprosy diagnosis methods to determine the potential risk in a leprosy contact individuals group. The prevalence of antibodies to *M. leprae* antigens in serum was measured, together with the presence of *M. leprae* DNA in the nose and lepromin status in a group of 43 contacts of leprosy patients (12 household and 31 occupational) to evaluate the maintenance of infection reservoirs and transmission of the disease. Only two individuals were found to form a potential high risk group.—Leprosy Review

## Microbiology (Tuberculosis)

**Parish, T., Smith, D. A., Kendall, S., Casali, N., Bancroft, G. J., and Stoker, N. G.** Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis*. *Infect. Immun.* **71**(3) (2003) 1134–1140.

Two-component regulatory signal transduction systems are widely distributed among bacteria and enable the organisms to make coordinated changes in gene expression in response to a variety of environmental stimuli. The genome sequence of *Mycobacterium tuberculosis* H37Rv has revealed the presence of a large number of two-component regulatory systems. Two-component regulatory systems are widely distributed among bacteria and enable the organisms to make coordinated changes in gene expression in response to a variety of environmental stimuli. The genome sequence of *Mycobacterium tuberculosis* H37Rv has revealed the presence of a large number of two-component regulatory systems.

*bacterium tuberculosis* contains 11 complete two-component systems, four isolated homologous regulators, and three isolated homologous sensors. We have constructed defined mutations in six of these genes and measured virulence in a SCID mouse model. Mice infected with four of the mutants (deletions of *devR*, *trcXY*, *trcS*, and *kdpDE*) died more rapidly than those infected with wild-type bacteria. The other two mutants (*narL* and *Rv3220c*) showed no change compared to the wild-type H37Rv strain. The most hypervirulent mutant (*devRdelta*) also grew more rapidly in the acute stage of infection in immunocompetent mice and in gamma interferon-activated macrophages. These results define a novel class of genes in this pathogen whose presence slows down its multiplication *in vivo* or increases its susceptibility to host killing mechanisms. Thus, *M. tuberculosis* actively maintains a balance between its own survival and that of the host.—Authors' Abstract

**Sharma, V., Arockiasamy, A., Ronning, D. R., Savva, C. G., Holzenburg, A.,**

**Braunstein, M., Jacobs, W. R. Jr., and Sacchetti, J. C.** Crystal structure of *Mycobacterium tuberculosis* SecA, a preprotein translocating ATPase. Proc. Natl. Acad. Sci. U.S.A. **100(5)** (2003) 2243–2248.

In bacteria, the majority of exported proteins are translocated by the Sec system, which recognizes the signal sequence of a preprotein and uses ATP and the proton motive force to mediate protein translocation across the cytoplasmic membrane. SecA is an essential protein component of this system, containing the molecular motor that facilitates translocation. Here we report the three-dimensional structure of the SecA protein of *Mycobacterium tuberculosis*. Each subunit of the homodimer contains a “motor” domain and a translocation domain. The structure predicts that SecA can interact with the SecYEG pore and function as a molecular ratchet that uses ATP hydrolysis for physical movement of the preprotein. Knowledge of this structure provides a framework for further elucidation of the translocation process.—Authors' Abstract

## Epidemiology

**Byamungu, D. C. and Osahon, O. I.** Integrating leprosy control in general health service in a war situation: the level after 5 years in Eastern Congo. *Lepr. Rev.* **74** (2003) 68–78.

South Kivu Province of the Democratic Republic of Congo, plagued by a turbulent civil war, started a process of integrating leprosy into general health services in 1995. A questionnaire survey was carried out in September 2000 to assess the level of structural and functional integration, after 5 years of the integration process, in nine of its 14 health districts. The survey revealed that a total of 76 clinic nurses remained of those trained in leprosy since 1993. In all, 33.6% of the total 226 health facilities had a trained nurse, but according to the district supervisors who filled the questionnaires, nurses in only 28.3% of health facilities could diagnose leprosy. Less than 40% of the total 226 health facilities were struc-

turally integrated with MDT and other leprosy services. Functionally, the clinic nurses were involved in dispensing MDT drugs and keeping leprosy records in 90.8 and 81.6%, respectively, of the integrated facilities, and diagnostic activities in 43.7%. The degree of involvement put health facilities into four grades of functional integration: 1) fully-functional integrated, 2) semi-functional integrated, 3) semi-integrated (structural but not functional), 4) not integrated (vertical). On this scale, 80% of 107 health facilities reported by the supervisors had some form of integration and 20% were not integrated. Treatment activities were significantly more functionally integrated than the diagnostic and POD activities, which require more skills. The presence of a trained nurse in a health facility made no significant difference to the involvement of clinic nurses in dispensing MDT drugs and performing POD activities, but significantly affected

their performance of diagnostic activities and records keeping. The endemic districts had higher levels of *structural* integration, were not more likely to be *functionally* integrated. The levels of structural integration after 5 years are considered low in South Kivu Province, and reflect the significant negative effect of civil conflicts on integration leprosy programs in Africa.—Leprosy Review

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

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

**Vynnycky, E., Borgdorff, M. W., Van Soolingen, D., and Fine, P. E.** Annual *Mycobacterium tuberculosis* infection risk

and interpretation of clustering statistics. *Emerg. Infect. Dis.* **9(2)** (2003) 176–183.

Several recent studies have used proportions of tuberculosis cases sharing identical DNA fingerprint patterns (i.e., isolate clustering) to estimate the extent of disease attributable to recent transmission. Using a model of introduction and transmission of strains with different DNA fingerprint patterns, we show that the properties and interpretation of clustering statistics may differ substantially between settings. For some unindustrialized countries, where the annual risk for infection has changed little over time, 70% to 80% of all age groups may be clustered during a 3-year period, which underestimates the proportion of disease attributable to recent transmission. In contrast, for a typical industrialized setting (the Netherlands), clustering declines with increasing age (from 75% to 15% among young and old patients, respectively) and underestimates the extent of recent transmission only for young patients. We conclude that, in some settings, clustering is an unreliable indicator of the extent of recent transmission.—Authors' Abstract

**Young, D.** Prospects for molecular epidemiology of leprosy. *Lepr. Rev.* **74** (2003) 11–17.

The identification of markers of genetic diversity among clinical isolates of *Mycobacterium tuberculosis* has had an important impact on tuberculosis research over the last decade, promoting development of an active field of molecular epidemiology. Molecular typing methods have been used to analyze reactivation disease, to distinguish relapse from reinfection, and to track local chains of tuberculosis transmission. The use of molecular methods has demonstrated differences in the extent of recent transmission as compared to reactivation disease in different geographic settings, with important implications for appropriate targeting of tuberculosis control strategies. Information about genetic diversity has also stimulated fundamental research into the evolutionary origins of the *M. tuberculosis* complex. Is it possible to take an analogous molecular epidemiology approach to help in our understanding of leprosy?—Leprosy Review

## Rehabilitation

**Kaur, H. and Van Brakel, W.** Is beggary a chosen profession among people living in a 'leprosy colony'? *Lepr. Rev.* **73(4)** (2003) 334–345.

Leprosy is a highly stigmatized disease that apart from the physical ailments and the deformities causes psycho-socio-economic problems to the people affected. As a result of social rejection, leprosy colonies were formed inhabited by the leprosy-affected families. With inadequate socioeconomic support and help, these people often have resorted to beggary as a way to earn their living. This study is an attempt to look into the lives of the leprosy-affected people living in the leprosy colony in Ambala City, Haryana, north-west State of India and who have accepted beggary as their source of income. The psychosocial impact of leprosy and the subjects' attitude towards beggary has been studied. The study comprised 21 families, including, 22 men, 21 women and 40 children. Seventy-one percent of the families came from Southern

India. All the men and nine of the women were leprosy-affected. The proportion of people with deformity was 89%. Prior to contracting leprosy, all of the men were employed, mainly in agriculture and physical labor. At present, all are beggars. Of the 20 who were interviewed, 65% of those who beg and 83% of other adults were illiterate. Fifty percent of the children were in need of education. Due to leprosy, the social interaction of 85% of the interviewees was limited to within the colony and of 88% to only other leprosy-affected people. Through their own organized efforts, they raised welfare services and housing for themselves. None of them liked begging to start with but have accepted it as a source of income. If given a chance and support, 80% said they were ready to quit begging. They were concerned about the education of their children. The study highlighted the need to develop alternate avenues of income generation utilizing the existing desires and potential of the inhabitants. — Authors' Abstract

## Other Mycobacterial Diseases

**Barr, K. L., Lowe, L., and Su, L. D.** *Mycobacterium marinum* infection simulating interstitial granuloma annulare: a report of two cases. *Am. J. Dermatopathol.* **25(2)** (2003) 148–151.

We report two cases of *Mycobacterium marinum* infection that histologically simulated interstitial granuloma annulare (GA). In one case, an infectious etiology was not suspected in histologic sections, but a tissue culture performed during the patient's clinic visit identified *M. marinum*, and a subsequent Fite stain revealed mycobacteria. Interstitial granulomatous dermatitis is a rare presentation for cutaneous nontuberculous mycobacteria and has yet to be attributed specifically to *M. marinum*. In both immunocompetent and immunosuppressed patients, infection with *M. marinum* should be considered in lesions histologically resembling interstitial GA, particularly when there is clinical suspicion for an infectious process. — Authors' Abstract

**Busin, M., Ponzin, D., and Arffa, R. C.** *Mycobacterium chelonae* interface infection after endokeratoplasty. *Am. J. Ophthalmol.* **135(3)** (2003) 393–395.

**PURPOSE:** To report a case of interface infection by *Mycobacterium chelonae* in a patient who underwent endokeratoplasty. **DESIGN:** Interventional case report. **SETTING:** Clinical practice. **METHODS:** Two weeks after endokeratoplasty, a 74-year-old woman developed multiple enlarging interface infiltrates in her right eye. Cultures performed on the preservation medium grew *Mycobacterium chelonae*. Penetrating keratoplasty (PK) surgery was performed after failure of conservative antibiotic therapy, including topical and systemic clarithromycin. **RESULTS:** Five months after PK surgery, the graft was clear and no signs of extraocular or intraocular inflammation were present. Cultures taken from the corneal interface at the time of PK surgery confirmed the presence of *M. chelonae*.

Acid-fast bacilli were seen in the excised corneal button. **CONCLUSIONS:** *M. chelonae* should be ruled out as a possible etiologic agent when postoperative infection of the corneal interface occurs. Surgical intervention can lead to eradication of the infection when conservative treatment fails.— Authors' Abstract

**Dauendorffer, J. N., Laurain, C., Weber, M., and Dailloux, M.** *In vitro* sensitivity of *Mycobacterium xenopi* to five antibiotics. *Pathol. Biol. (Paris)* **50(10)** (2003) 591–594.

We tested 20 strains of *Mycobacterium xenopi* (*M. xenopi*) in order to evaluate their *in vitro* sensitivity to amikacin, clarithromycin, ethambutol, ofloxacin and rifampicin, by establishing minimal inhibitory concentration (MIC) on agar medium. MICs of amikacin, clarithromycin and ofloxacin are low, so that these antibiotics can be used in the treatment of *M. xenopi* infections. MICs of ethambutol are higher than seric concentrations. Though, its therapeutic use is due to its *in vivo* ability to enhance penetration of other antibiotics in mycobacteria. Strain sensitivity to rifampicin seems heterogeneous but the small number of tested strains does not entitle the exclusion of rifampicin from the treatment of *M. xenopi* infections.— Authors' Abstract

**Debacker, M., Zinsou, C., Aguiar, J., Meyers, W. M., and Portaels, F.** First case of *Mycobacterium ulcerans* disease (Buruli ulcer) following a human bite. *Clin. Infect. Dis.* **36(5)** (2003) e67–68.

*Mycobacterium ulcerans* disease (MUD) is rapidly reemerging in many countries, especially in West African countries. Antecedent trauma has often been related to the lesions that characterize this frequently crippling disease. We report here the first case of MUD that followed a human bite at the site where the lesion later occurred.— Authors' Abstract

**Freitas, D., Alvarenga, L., Sampaio, J., Mannis, M., Sato, E., Sousa, L., Vieira, L., Yu, M. C., Martins, M. C., Hoffling-**

**Lima, A., and Belfort, R. Jr.** An outbreak of *Mycobacterium chelonae* infection after LASIK. *Ophthalmology* **110(2)** (2003) 276–285.

**OBJECTIVE:** To describe an outbreak of mycobacterial keratitis after laser in situ keratomileusis (LASIK), including the microbiologic investigation, clinical findings, treatment response, and outcome. **DESIGN:** Retrospective, noncomparative, interventional case series. **PARTICIPANTS:** Patients (n = 10) who underwent LASIK surgery between August 22 and September 4, 2000, and developed mycobacterial infection. **METHODS:** Patients were prospectively followed in relation to microbiologic investigation, clinical findings, treatment response, and outcome. **MAIN OUTCOME MEASURES:** Most patients underwent bilateral simultaneous LASIK. Postoperative infection was signaled by the appearance of corneal infiltrates in the third postoperative week. The microbiologic workup was performed on cultures obtained either by direct scraping of the cornea or by lifting the flap. Medical therapy was instituted based on drug susceptibility testing. Surgical interventions such as corneal debridement and flap removal were performed during recurrences or when there was no satisfactory clinical response. **RESULTS:** Cultures revealed *Mycobacterium* subspecies *chelonae*. Patients were treated with topical clarithromycin (1%), tobramycin (1.4%), and ofloxacin (0.3%). Oral clarithromycin (500 mg twice a day) was prescribed for those patients who did not respond clinically to topical treatment. Four eyes healed on this regimen. Flap removal was necessary in seven eyes. **CONCLUSIONS:** This report highlights mycobacteria as an etiologic infectious agent after LASIK. Diagnosis can be difficult and is often delayed. The treatment mainstay is prolonged antibiotic therapy. Surgical debridement and flap removal may shorten the disease course.— Authors' Abstract

**Garcia-Rio, I., Fernández-Peñas, P., Fernández-Herrera, J., and García-Díez, A.** Cutaneous *Mycobacterium chelonae* infection. A review of 6 cases. *ACTAS Dermo-Sifiliográficas* **93(10)** (2002) 584–587. [Article in Spanish].

*Mycobacterium chelonae* (*M. chelonae*) is a rapid-growth atypical mycobacteria, which belongs to Runyon group IV. Infection often develops either in patients with immunosuppression or in immunocompetent hosts associated with penetrating trauma or injury.

We present six female immunocompetent patients, who presented nodules, plaques or ulcers, with abscess formation and clear fluid drainage that followed a protracted course in spite of cloxaciline therapy. Although one patient referred a previous history of liposuction, the others did not recall an inoculation site. Treatment of *M. chelonae* infection required oral clarithromycin for at least 3 months, in addition to other drugs. The histopathologic analysis showed an inflammatory infiltrate, and abscess and granuloma formation. *M. chelonae* was cultured from skin biopsy specimens.

Although *M. chelonae* infection usually occurs in immunologically compromised patients or may follow puncture wound or trauma, our series shows that chronic suppurative lesions in otherwise healthy women with no previous trauma might be due to *M. chelonae*.—ACTAS Dermo-Sifiliográficas

**Hayek, S., Issakov, J., Ezra, E., Wientroub, S., and Yaniv, M.** Atypical mycobacterial granulomatous epiphyseal osteomyelitis in an immunocompetent child. *J. Pediatr. Orthop. B.* **12(2)** (2003) 151–154.

Osteomyelitis caused by atypical mycobacteria is rare in children. The majority of affected patients have had some kind of predisposing factor, namely a penetrating injury or surgery, or were immune-compromised. Our experience shows that this diagnosis should be considered in apparently healthy children as well. The use of polymerase chain reaction has now made it possible to identify the pathogen in cases that were previously diagnosed as granulomatous osteomyelitis. We present a case of atypical mycobacterial osteomyelitis affecting the distal femoral epiphysis in an immunocompetent 10-year-old child. The diagnosis in this case was made by the use of the polymerase chain reaction assay.—Authors' Abstract

**Hisamichi, K., Hiruma, M., Yamazaki, M., Matsushita, A., and Ogawa, H.** Efficacy of oral minocycline and hyperthermic treatment in a case of atypical mycobacterial skin infection by *Mycobacterium marinum*. *J. Dermatol.* **29(12)** (2002) 810–811.

We report a case of atypical mycobacterial dermal infection caused by *M. marinum*, which was effectively treated with oral administration of minocycline and local hyperthermic treatment using chemical pocket warmers. A daily oral dose of 200 mg of minocycline was given, and local hyperthermic treatment was applied every evening for 5–6 hours with a disposable chemical pocket warmer. After 2.5 months of therapy, the lesion healed completely with scar formation. At 24 months after the completion of treatments, there is no sign of recurrence.—Authors' Abstract

**Khosrovaneh, A., Briski, L. E., Janowski, E., and Khatib, R.** Nostril infection due to *Mycobacterium marinum* in an immunocompetent host. *Scand. J. Infect. Dis.* **34(12)** (2002) 929–931.

*Mycobacterium marinum* infection developed in the nostril of an immunocompetent host whose only risk factors were infrequent swimming and cleaning of a small fish bowl on a single occasion. The lesion relapsed after 2 surgical excisions but resolved slowly with a 9-month course of trimethoprim/sulfa therapy.—Authors' Abstract

**Mushtaq, I. and Martin, O.** Atypical mycobacterial disease in children: a personal series. *Pediatr. Surg. Int.* **18(8)** (2002) 707–711.

Atypical mycobacterial disease is common in children in Australia. Over 22 years, records were kept prospectively by the senior author. The diagnosis was confirmed in 118 patients, either by culture or by the combination of a positive skin test plus typical histology. There were 46 boys and 72 girls with a median age at diagnosis of 28 months. Most children (n = 56) presented with chronic lymphadenitis or abscess for-

mation (n = 55). The duration of illness varied from 4 days to 18 months. The most common sites affected were the head and neck (n = 112), with the pre-auricular region and anterior end of the submandibular triangle being characteristic. Nine patients had multifocal disease. The aim of treatment is to excise as much of the infected tissue as possible: 47 children had node excision through a planned incision that was closed primarily, with only 4 needing a second operation; 42 had excision of a node through the base of the superficial part of a collar-stud abscess with 6 recurrences. However, of the 33 children who had only drainage/curettage of the cavity or node 10 had recurrences requiring re-operation. Only 1 patient required a third operation. Morbidity was extremely low, with 1 staphylococcal wound infection. No child suffered permanent paresis of the mandibular division of the facial nerve. It is our belief that surgical excision of both the macroscopically affected and adjacent macroscopically unaffected nodes is necessary to achieve cure in the majority of cases.—Authors' Abstract

**Mve-Obiang, A., Lee, R. E., Portaels, F., and Small, P. L.** Heterogeneity of mycolactones produced by clinical isolates of *Mycobacterium ulcerans*: implications for virulence. *Infect. Immun.* **71(2)** (2003) 774–783.

*Mycobacterium ulcerans* is the causative agent of Buruli ulcer, a severe necrotizing skin disease endemic in tropical countries. Clinical evidence suggests that *M. ulcerans* isolates from Asia, Mexico, and Australia may be less virulent than isolates from Africa. *In vivo* studies suggest that mycolactone, a polyketide-derived macrolide toxin, plays a major role in the tissue destruction and immune suppression which occur in cases of Buruli ulcer. Mycolactones were extracted from 34 isolates of *M. ulcerans* representing strains from Africa, Malaysia, Asia, Australia, and Mexico. Thin-layer chromatography, mass spectroscopic analysis, and cytopathic assays of partially purified mycolactones from these isolates revealed that *M. ulcerans* produces a heterogeneous mixture of mycolactone variants. Mycolactone A/B, the most bio-

logically active mycolactone species, was identified by mass spectroscopy as [M(+Na)](+) at m/z 765.5 in all cytotoxic isolates except for those from Mexico. Mycolactone C [M+Na](+) at m/z 726.3 was the dominant mycolactone species in eight Australian isolates, and mycolactone D [M+Na](+) m/z 781.2 was characteristic of two Asian strains. Mycolactone species are conserved within specific geographic areas, suggesting that there may be a correlation between mycolactone profile and virulence. In addition, the core lactone, [M+Na](+) m/z 447.4, was identified as a minor species, supporting the hypothesis that mycolactones are synthesized by two polyketide synthases. A cytopathic assay of the core lactone showed that this molecule is sufficient for cytotoxicity, although it is much less potent than the complete mycolactone.—Authors' Abstract

**Ouattara, D., Meningaud, J. P., and Saliba, F.** Formes plurifocales de l'ulcère de Buruli: aspects cliniques et difficultés de prise en cha à propos de 11 cas. *Bull. Soc. Pathol. Exot.* **95(4)** (2002) 287–291. [Article in French]

Summary: Multifocal forms of Buruli ulcer: clinical aspects and difficulties of care, about 11 cases.

Objective: A certain number of authors have in literature pointed out multifocal forms of Buruli ulcer but no study was ever dedicated to them. The purpose of this study is to be more specific about the clinical aspects and to show how difficult it is for those multifocal forms of Buruli ulcer to be operated on.

Method: The 11 patients who were accepted for the study were subjected to an interrogation, a thorough clinical examination, research of BAAR in ulcers and operative pieces with a direct examination after Ziehl-Neelsen coloring. Each of these patients underwent a surgical treatment under general anesthesia or spinal anesthesia depending on the seat of the lesions under cover of pre and post operative therapy by antibiotics.

Results: Initial lesions preferentially were located at limbs level; new foci appeared within an average period of 3 months, rang-

ing from 1 to 15 months in some cases. All body parts could be the seats of secondary foci. Depending on the patients, the number of foci varied from 3 to 7. Furthermore, amputation has been necessary for the complete healing of four patients. The average operation was 2.4 by patient ranged from 2 to 5. We observed the healing of all the patients within an average hospitalization time of 6.3 months running sometimes from 4 to 13 months. In addition to amputations, 4 patients presented after-effects as articular stiffness, retractions of the hand's dorsal face and knee's retraction.

Conclusion: Those multifocal forms can, with good reason, be considered as malignant form of Buruli ulcer.—Bulletin de la Société de Pathologie Exotique

**Piersimoni, C., Zitti, P. G., Nista, D., and Bornigia, S.** *Mycobacterium celatum* pulmonary infection in the immunocompetent: case report and review. *Emerg. Infect. Dis.* **9(3)** (2003) 399–402.

*Mycobacterium celatum* has been shown to cause disease in immunocompromised patients. We report a case of serious pulmonary infection caused by *M. celatum* in an apparently immunocompetent patient and review the characteristics of two other reported cases. Clinical and radiologic symptoms and signs included cough, malaise, and weight loss associated with cavitory lesions and pulmonary infiltrates. Although *M. celatum* is easy to detect in clinical specimens by liquid and solid media, it may be misidentified as a member of the *M. tuberculosis* complex or as *M. xenopi*. *M. celatum* pulmonary infection appears to respond to antimycobacterial chemotherapy, particularly with clarithromycin.—Authors' Abstract

**Saggese, D., Compadretti, G. C., and Burnelli, R.** Nontuberculous mycobacterial adenitis in children: diagnostic and therapeutic management. *Am. J. Otolaryngol.* **24(2)** (2003) 79–84.

We reviewed a series of 45 patients affected by nontuberculous mycobacterial adenitis of the neck observed in the Ear,

Nose, and Throat Institute of S. Orsola-Malpighi Hospital-Bologna over a 20-year period between 1981 and 2001. The mean age was 5.5 years. Patients were tested by using the differential Mantoux test, which was the principal diagnostic tool in the case of atypical mycobacterial infections. Forty-two patients were surgically treated by total excision of infected nodes, whereas parotidectomy with sparing of facial nerve was performed in those 3 cases with intraparotid nodes involvement. In all cases, the histopathological diagnosis was tubercular granulomatous lymphadenitis. The culture growth of nontuberculous mycobacteria was positive in 13 cases with a marked prevalence of the avium-intracellular germs. The disease was eradicated in all patients. The diagnostic and therapeutic management of nontuberculous mycobacterial adenitis is discussed in this retrospective study.—Authors' Abstract

**Turenne, C., Chedore, P., Wolfe, J., Jamieson, F., Broukhanski, G., May, K., and Kabani, A.** *Mycobacterium lacus* sp. nov., a novel slowly growing, non-chromogenic clinical isolate. *Int. J. Syst. Evol. Microbiol.* **52(Pt 6)** (2002) 2135–2140.

A strain of a novel non-chromogenic mycobacterium was isolated from synovial tissue from a 68-year-old female with bursitis of her right elbow. The slowly growing strain had a unique PCR-restriction enzyme analysis (PRA) profile of the hsp65 gene and 16S rRNA gene sequence in comparison with other mycobacterium species. The most closely related species, as determined by 16S rRNA gene sequence analysis, are *Mycobacterium malmoense*, *Mycobacterium marinum*, *Mycobacterium ulcerans* and members of the *Mycobacterium tuberculosis* complex. The HPLC and biochemical profiles resembled those of *Mycobacterium gastri*, although differences were noted in the peak-height ratio of the HPLC pattern and the nitrate and pyrazinamidase tests. On the basis of PRA, HPLC, biochemical and 16S rRNA gene sequence analyses, the name *Mycobacterium lacus* sp. nov. is proposed for this potential pathogen. The type strain is strain NRCM

00-255(T) (=ATCC BAA-323(T) = DSM 44577(T)).—Authors' Abstract

**Whitehead, S. E., Allen, K. D., Abernethy, V. E., Feldberg, L., and Ridyrd, J. B.** *Mycobacterium malmoeense* septic arthritis. *J. Infect.* **46**(1) (2003) 60–61.

We report a case of septic arthritis of an interphalangeal joint and osteomyelitis of the phalanx due to *Mycobacterium malmoeense* in a 61-year-old man with a 20 year history of rheumatoid arthritis treated with steroids and azathioprine. This was successfully treated with ethambutol, rifampicin and clarithromycin. To our knowledge this is the only reported case of septic arthritis due to this pathogen which is usually associated with respiratory disease or cervical lymphadenitis.—Authors' Abstract

**Wong, A. K. and Lam, D. S.** *Mycobacterium chelonae* keratitis: report of a case and review of the literature. *Yan Ke Xue Bao* **14**(3) (1998) 156–163.

**BACKGROUND:** *Mycobacterium (M.) chelonae* keratitis is a rare opportunistic eye infection that can cause significant morbidity when not being treated properly. The first case was documented by Gangadharam et al in 1978 and since then, a total of 49 cases were reported in the literature. One alarming fact is that more than 50% of cases were found in the Chinese population and mostly reported in recent years. The key to successful management of *M. chelonae* keratitis is early diagnosis by high index of suspicion. In order to alert ophthalmologists of this condition, we report a typical case of *M. chelonae* keratitis and review the literature of all the reported cases with special reference to its risk factors, treatments and outcome. **METHODS:** The cases reported in the literature and a case of our own were reviewed and analyzed. **RE-**

**SULTS:** Our case was a 42-year-old gentleman who developed *M. chelonae* keratitis following pterygium surgery. He had typical clinical features of irregular infiltrates with radiating projections, indistinct fluffy lesion margins, satellite lesions and associated epithelial defect. Penetrating keratoplasty was performed after failed medical treatments. He recovered fully with a best corrected visual acuity (BCVA) of 20/30 at 24 months after the corneal transplant. A total of 49 cases were reported in the literature. The major risk factor was corneal injury, including surgical trauma. Corneal foreign bodies (24 cases, 48%) were found to be highly correlated, especially metallic foreign bodies (16 cases, 32%). Diagnosis was usually delayed for weeks or months and medical treatment alone often failed. Amikacin is usually the treatment of choice but its efficacy is just sub-optimal. Multi-resistance to the commonly used broad spectrum antibiotics is not uncommon. The drug sensitivity test against atypical mycobacterium is technically difficult to perform and *in vitro* results are well known to be poorly related to clinical response. Combined extirpative keratectomy and topical antibiotics had been tried and was shown to be effective. Penetrating keratoplasty appeared to be a good definitive treatment for drug-resistance and advanced cases. **CONCLUSION:** *M. chelonae* keratitis is a rare opportunistic infection. The major risk factor is eye injury, with foreign bodies or surgical trauma. Diagnosis is often missed and delayed as a result of its scarcity and variable presentations. High index of clinical suspicion with early diagnosis and prompt combined medical and surgical intervention seem to be the best measure to decrease ocular morbidity. Good alertness and knowledge of this condition would help our patients in the Far East as the literature review has shown a recent trend of increase in frequency and more than 50% of the reported cases come from the Chinese patients.—Authors' Abstract

## Molecular and Genetic Studies

**Al-Zarouni, M. and Dale, J. W.** Expression of foreign genes in *Mycobacterium bovis* BCG strains using different promoters reveals instability of the hsp60 promoter for expression of foreign genes in *Mycobacterium bovis* BCG strains. *Tuberculosis (Edinb)* **82(6)** (2002) 283–291.

**SETTING:** Optimization of BCG as a vehicle for live recombinant vaccines requires improved strategies for stable antigen expression. **OBJECTIVES:** To investigate the effects of various combinations of post-translational signals and promoters on expression and stability in different BCG strains. **DESIGN:** Plasmids were constructed using mycobacterial promoters (hsp60, 19-kDa antigen, 85A antigen—from the *Mycobacterium tuberculosis* complex—and the 18-kDa antigen from *Mycobacterium leprae*) and post-translation signals (85A antigen secretion and 19-kDa antigen acylation signals), coupled with reporter genes. **RESULTS:** The 19-kDa acylation signal had little effect on expression, while the 85A secretion signal enhanced markedly the levels of cell-associated product. Inclusion of the hsp60 promoter caused plasmid instability; various deletions affecting the promoter region occurred during or soon after transformation, but not during subsequent growth of the transformants, nor with other promoters. BCG Moreau appeared to be more susceptible to deletions than other BCG strains. **CONCLUSIONS:** The 85A signal may prove useful in optimizing gene expression in BCG, irrespective of secretion of the product. Deletions associated with the hsp60 promoter may be due to a transient lethal induction of the hsp60 promoter associated with electroporation. With intact plasmid there was no marked difference in expression between BCG strains.—Authors' Abstract

**Akahoshi, M., Nakashima, H., Miyake, K., Inoue, Y., Shimizu, S., Tanaka, Y., Okada, K., Otsuka, T., and Harada, M.** Influence of interleukin-12 receptor beta1 polymorphisms on tuberculosis. *Hum. Genet.* **112(3)** (2003) 237–243.

Host genetic factors may be important determinants of susceptibility to tuberculosis, and several candidate gene polymorphisms have been shown to date. A series of recent reports concerning rare human deficiencies in the type-1 cytokine pathway suggest that more subtle variants of relevant genes may also contribute to susceptibility to tuberculosis at the general population level. To investigate whether polymorphisms in the interleukin-12 receptor (IL-12R) gene predispose individuals to tuberculosis, we studied these genes by single-strand conformational polymorphism analysis and direct sequencing. Although no common polymorphisms could be identified in the IL-12Rbeta2 gene (IL-12RB2), we confirmed four single nucleotide polymorphisms (SNPs; 641A→G, 684C→T, 1094T→C, and 1132G→C) causing three missense variants (Q214R, M365T, G378R) and one synonymous substitution in the extracellular domain of the IL-12Rbeta1 gene (IL12RB1). All SNPs were in almost perfect linkage disequilibrium ( $D' = 0.98$ ), and two common haplotypes of IL12RB1 (allele 1: Q214-M365-G378; allele 2: R214-T365-R378) were revealed. Polymerase chain reaction/restriction fragment length polymorphism and sequence analyses were used to type IL12RB1 polymorphisms in 98 patients with tuberculosis and 197 healthy controls in Japanese populations. In our case-control association study of tuberculosis, the R214-T365-R378 allele (allele 2) was over-represented in patients with tuberculosis, and homozygosity for R214-T365-R378 (the 2/2 genotype) was significantly associated with tuberculosis (odds ratio: 2.45; 95% CI: 1.20–4.99;  $p = 0.013$ ). In healthy subjects, homozygotes for R214-T365-R378 had lower levels of IL-12-induced signaling, according to differences in cellular responses to IL-12 between two haplotypes. These data suggest that the R214-T365-R378 allele, i.e., variation in IL12RB1, contribute to tuberculosis susceptibility in the Japanese population. This genetic variation may predispose individuals to tuberculosis infection by diminishing receptor responsiveness to IL-12 and to IL-23, leading to partial dysfunction of interferon-gamma-mediated immunity.—Authors' Abstract

**Begum, N. A., Kobayashi, M., Moriwaki, Y., Matsumoto, M., Toyoshima, K., and Seya, T.** *Mycobacterium bovis* BCG cell wall and lipopolysaccharide induce a novel gene, BIGM103, encoding a 7-TM protein: identification of a new protein family having Zn-transporter and Zn-metalloprotease signatures. *Genomics* **80(6)** (2002) 630–645.

To identify novel genes induced during innate immune activation, we screened a cDNA library prepared from monocytes stimulated with *Mycobacterium bovis* BCG cell wall. A novel transcript with three-protein coding potential was identified, and the expressed proteins from individual frames showed distinct intracellular localization. Live and heat-killed *Mycobacterium*, bacterial cell wall, and inflammatory cytokines like TNF $\alpha$  were found to be potent inducers of the transcript. Expression of this gene is very low or undetectable in unstimulated monocytes, while a steady expression level was observed during differentiation of monocytes to dendritic cells and macrophages. The entire gene consisted of eight major exons and was localized on chromosome 4q22–q24, spanning approximately 84 kb. The main open reading frame of the transcript encoded a putative seven-transmembrane (TM) protein that showed homology with a number of functionally unknown proteins in the database. Further analysis revealed that all of these proteins have detectable similarity with the ZIP family of metal transporters. In fact, increased accumulation of intracellular Zn(2+) was observed due to the expression of BIGM103 in CHO cells. However, the identified proteins are structurally unique compared to known ZIP members and they also possess the hallmark of Zn-metalloproteases, suggesting a new class of multi-TM protein with dual features. Here we present a collection of these proteins and discuss the functional aspects of BIGM103, based on our results and current findings on two members of the family, *Drosophila* Catsup and *Arabidopsis* IAR1.—Authors' Abstract

**Bellamy, R.** Susceptibility to mycobacterial infections: the importance of host genetics. *Genes Immun.* **4(1)** (2003) 4–11.

There is substantial evidence that host genetic factors are important in determining susceptibility to mycobacteria. Several different techniques have been used to identify the genes involved. Studies of an inbred strain of mice with increased susceptibility to mycobacteria, salmonella and leishmania infections led to the identification of the natural resistance-associated macrophage protein gene (Nramp1). Case-control studies have confirmed the importance of the human equivalent of this gene, NRAMP1, and have also suggested that the major histocompatibility complex and vitamin-D receptor genes may be involved in determining human susceptibility to mycobacteria. Studies of individuals with the rare condition of increased susceptibility to disseminated bacille Calmette-Guerin and other atypical mycobacterial infections have identified several abnormalities in the genes encoding the interferon gamma receptor (IFN $\gamma$ R) ligand binding chain, IFN $\gamma$ R signal transduction chain, IFN $\gamma$  signal transduction and activation of transcription-1, interleukin 12 receptor beta1 subunit and interleukin 12 p40 subunit. A genome-wide linkage study has been performed to identify genes exerting a major effect on tuberculosis susceptibility in the general population. Linkages were found to markers on chromosomes 15 and X. Studies to identify the genes responsible are in progress.—Author's Abstract

**Bochud, P. Y., Hawn, T. R., and Aderem, A.** Cutting edge: a toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate mycobacterial signaling. *J. Immunol.* **170(7)** (2003) 3451–3454.

Toll-like receptors (TLRs) are key mediators of the innate immune response to microbial pathogens. We investigated the role of TLRs in the recognition of *Mycobacterium leprae* and the significance of TLR2Arg(677) Trp, a recently discovered human polymorphism that is associated with lepromatous leprosy. In mice, TNF- $\alpha$  production in response to *M. leprae* was essentially absent in TLR2-deficient macrophages. Similarly, human TLR2 mediated *M. leprae*-dependent activation of NF- $\kappa$ B in transfected

Chinese hamster ovary and human embryonic kidney 293 cells, with enhancement of this signaling in the presence of CD14. In contrast, activation of NF-kappaB by human TLR2Arg(677)Trp was abolished in response to *M. leprae* and *Mycobacterium tuberculosis*. The impaired function of this TLR2 variant provides a molecular mechanism for the poor cellular immune response associated with lepromatous leprosy and may have important implications for understanding the pathogenesis of other mycobacterial infections.—Authors' Abstract

**Ge, Y., ElNaggar, M., Sze, S. K., Oh, H. B., Begley, T. P., McLafferty, F. W., Boshoffm, H., and Barry, C. E.** Top down characterization of secreted proteins from *Mycobacterium tuberculosis* by electron capture dissociation mass spectrometry. *J. Am. Soc. Mass Spectrom.* **14(3)** (2003) 253–261.

Secreted proteins of *Mycobacterium tuberculosis* are implicated in its disease pathogenesis and so are considered as potential diagnostic and vaccine candidates. The search for these has been slow, even though the entire genome sequence of *M. tuberculosis* is now available; of the 620 protein spots resolved by 2-D gel electrophoresis, 114 secreted proteins have been identified, but for only 13 has the primary structure been partly characterized. For comparison, in this top down mass spectrometry (MS) approach the secreted proteins were precipitated from cell culture filtrate, resuspended, and examined directly by electrospray ionization (ESI) Fourier transform MS. The ESI spectra of three precipitates showed 93, 535, and 369 molecular weight (M(r)) values, for a total of 689 different values. However, only approximately 10% of these values matched ( $\pm 1$  Da) the DNA predicted M(r) values, but these identifications were unreliable. Of nine molecular ions characterized by MS/MS, only one protein match was confirmed, and its isotopic molecular ions were overlapped by those of another protein. MS/MS identified a total of ten proteins by sequence tag search, of which three were unidentified previously. The low success of M(r) matching was due to unusually exten-

sive posttranslational modifications, including loss of a signal sequence, loss of the N-terminal residue, proteolytic degradation, oxidation, and glycosylation. Although in eubacteria the latter is relatively rare, a 9 kDa protein showed 7 hexose attachments and two 20 kDa proteins each had 20 attachments. For MS/MS, electron capture dissociation was especially effective.—Authors' Abstract

**Madan Babu, M.** Did the loss of sigma factors initiate pseudogene accumulation in *M. leprae*? *Trends Microbiol.* **11(2)** (2003) 59–61.

Pseudogenes are non-functional regions in the genome that have arisen as a consequence of accumulating mutations that either result in the premature termination of proteins during protein synthesis or the disruption of transcription. There have been various discussions of the origins of pseudogenes and the models for their formation, but there has been little input on how pseudogenes could have accumulated in an organism. In this brief communication, I propose a two-step model for the accretion of pseudogenes in the *Mycobacterium leprae* genome, triggered by the loss of different sets of sigma factors at different time points during the course of evolution.—Author's Abstract

**Malaga, W., Perez, E., and Guilhot, C.** Production of unmarked mutations in mycobacteria using site-specific recombination. *FEMS Microbiol. Lett.* **219(2)** (2003) 261–268.

Gene disruption experiments play an important role in the functional characterization of genes in mycobacteria and rely mostly on the use of one or two antibiotic resistance markers. We have developed a system for mycobacteria, which features both the advantages of the use of antibiotic resistance markers for gene disruption experiments and the ability to efficiently rescue the marker leaving an unmarked mutation on the chromosome. This new genetic tool relies on the transposon gammadelta site-specific recombination system. A res-

OmegaKm-res cassette was used to generate an insertional mutation by allelic exchange both in *Mycobacterium smegmatis* and *Mycobacterium bovis* BCG. Upon expression in the mutated strains of tnpR, the transposon gammadelta resolvase gene, res-OmegaKm-res, was excised efficiently leaving behind a single res sequence at the mutated locus. A plasmid was engineered allowing expression of tnpR from an easily curable mycobacterial vector. This system will be useful for simple construction of unmarked mutations or repeated use of the same antibiotic marker to generate multiple mutants.—Authors' Abstract

**Mira, M. T., Alcais, A., Di Pietrantonio, T., Thuc, N. V., Phuong, M. C., Abel, L., and Schurr, E.** Segregation of HLA/TNF region is linked to leprosy clinical spectrum in families displaying mixed leprosy subtypes. *Genes Immun.* **4(1)** (2003) 67–73.

Each year an estimated 600,000 new leprosy cases are diagnosed worldwide. The spectrum of the disease varies widely from limited tuberculoid forms to extensive lepromatous forms. A measure of the risk to develop lepromatous forms of leprosy is provided by the extent of skin reactivity to lepromin (Mitsuda reaction). To address a postulated oligogenic control of leprosy pathogenesis, we investigated in the present study linkage of leprosy susceptibility, leprosy clinical subtypes, and extent of the Mitsuda reaction to six chromosomal regions carrying known or suspected leprosy susceptibility loci. The only significant result obtained was linkage of leprosy clinical subtype to the HLA/TNF region on human chromosome 6p21 ( $p(\text{corrected}) = 0.00126$ ). In addition, we established that within the same family different HLA/TNF haplotypes segregate into patients with different leprosy subtypes directly demonstrating the importance of this genome region for the control of clinical leprosy presentation.—Authors' Abstract

**Mira, M. T., Alcais, A., Van Thuc, N., Thai, V. H., Huong, N. T., Ba, N. N., Verner, A., Hudson, T. J., Abel, L., and**

**Schurr, E.** Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. *Nat. Genet.* **33(3)** (2003) 412–415.\*

Leprosy, a chronic infectious disease caused by *Mycobacterium leprae*, affects an estimated 700,000 persons each year. Clinically, leprosy can be categorized as paucibacillary or multibacillary disease. These clinical forms develop in persons that are intrinsically susceptible to leprosy per se, that is, leprosy independent of its specific clinical manifestation. We report here on a genome-wide search for loci controlling susceptibility to leprosy per se in a panel of 86 families including 205 siblings affected with leprosy from Southern Vietnam. Using model-free linkage analysis, we found significant evidence for a susceptibility gene on chromosome region 6q25 (maximum likelihood binomial (MLB) lod score 4.31;  $p = 5 \times 10^{-6}$ ). We confirmed this by family-based association analysis in an independent panel of 208 Vietnamese leprosy simplex families. Of seven microsatellite markers underlying the linkage peak, alleles of two markers (D6S1035 and D6S305) showed strong evidence for association with leprosy ( $p = 6.7 \times 10^{-4}$ ) and  $p = 5.9 \times 10^{-5}$ , respectively).—Authors' Abstract [See commentary on this article by Dr. Buschman & Dr. Skamene on page 115.]

**Oliveira, R. S., Sircili, M. P., Oliveira, E. M., Balian, S. C., Ferreira-Neto, J. S., and Leao, S. C.** Identification of *Mycobacterium avium* genotypes with distinctive traits by combination of IS1245-based restriction fragment length polymorphism and restriction analysis of hsp65. *J. Clin. Microbiol.* **41(1)** (2003) 44–49.

One-hundred eight *Mycobacterium avium* isolates from pigs, humans, birds, and bovines were typed by the IS1245-based restriction fragment length polymorphism (RFLP) method and PCR-restriction enzyme analysis (PRA) of hsp65. Nine clusters of isolates showing more than 80% similarity in their RFLP profiles were detected. The largest cluster (cluster B) included 32 of 79 pig isolates (40.5%), 3 of 25 human isolates (12%), and 1 of 2 bovine isolates, comprising

33% of all isolates. The second largest cluster (cluster A) included 18 pig isolates (22.8%) and 6 human isolates (24%). Six smaller clusters included six pig isolates (clusters C and D), four and two human isolates (clusters E and F, respectively), two pig isolates (cluster I), and two pig isolates plus one bovine isolate and the avian purified protein derivative strain (cluster H). Cluster G represented the "bird-type" profile and included the bird isolate in this series, one pig isolate, plus reference strain R13. PRA revealed four allelic variants. Seventy-seven isolates were identified as *M. avium* PRA variant I, 24 were identified as *M. avium* PRA variant II, 6 were identified as *M. avium* PRA variant III, and 1 was identified as *M. avium* PRA variant IV. Except for three isolates from cluster B, each of the RFLP clusters was associated with a single PRA pattern. Isolates with unique (nonclustered) RFLP profiles were distributed between PRA variants I and II, and there was one unique isolate of PRA variant IV. These observations are consistent with divergent evolution within *M. avium*, resulting in the emergence of distinct lineages with particular competence to infect animals and humans. — Authors' Abstract

**Pashley, C. A., Parish, T., McAdam, R. A., Duncan, K., and Stoker, N. G.** Gene replacement in mycobacteria by using incompatible plasmids. *Appl. Environ. Microbiol.* **69**(1) (2003) 517–523.

A simple and efficient delivery system was developed for making targeted gene knockouts in *Mycobacterium smegmatis*. This delivery system relies on the use of a pair of replicating plasmids, which are incompatible. Incompatible plasmids share elements of the same replication machinery and so compete with each other during both replication and partitioning into daughter cells. Such plasmids can be maintained together in the presence of antibiotics; however, removal of selection leads to the loss of one or both plasmids. For mutagenesis, two replicating plasmids based on pAL5000 are introduced; one of these plasmids carries a mutated allele of the targeted gene. Homologous recombination is allowed to take place, and either one or both of the vectors are lost through the pressure of incompatibility, allowing the

phenotypic effects of the mutant to be studied. Several different plasmid combinations were tested to optimize loss in the absence of antibiotic selection. pAL5000 carries two replication genes (repA and repB), which act in trans, and the use of vectors that each lack one rep gene and complement each other resulted in the loss of both plasmids in *M. smegmatis* and *Mycobacterium bovis* BCG. The rate of loss was increased by the incorporation of an additional incompatibility region in one of the plasmids. To facilitate cloning when the system was used, we constructed plasmid vector pairs that allow simple addition of selection and screening genes on flexible gene cassettes. Using this system, we demonstrated that *M. smegmatis* pyrF mutants could be isolated at high frequency. This method should also be useful in other species in which pAL5000 replicates, including *Mycobacterium tuberculosis*. — Authors' Abstract

**Reynard, M. P., Turner, D., Junqueira-Kipnis, A. P., Ramos De Souza, M., Moreno, C., and Navarrete, C. V.** Allele frequencies for an interferon-gamma microsatellite in a population of Brazilian leprosy patients. *Eur. J. Immunogenet.* **30**(2) (2003) 149–151.

A group of Brazilian leprosy patients and controls were genotyped for a CA-repeat microsatellite polymorphism within the interferon (IFN)-gamma gene. A significantly higher frequency of alleles 5–7 was observed in this patient population, indicating that IFN-gamma gene polymorphism may contribute to the course of leprosy post-infection. — Authors' Abstract

**Salazar, L., Guerrero, E., Casart, Y., Turcios, L., and Bartoli, F.** Transcription analysis of the dnaA gene and oriC region of the chromosome of *Mycobacterium smegmatis* and *Mycobacterium bovis* BCG, and its regulation by the DnaA protein. *Microbiology* **149**(Pt 3) (2003) 773–784.

The regions flanking the *Mycobacterium* dnaA gene have extensive sequence conservation, and comprise various DnaA boxes.

Comparative analysis of the *dnaA* promoter and *oriC* region from several mycobacterial species revealed that the localization, spacing and orientation of the DnaA boxes are conserved. Detailed transcriptional analysis in *M. smegmatis* and *M. bovis* BCG shows that the *dnaN* gene of both species and the *dnaA* gene of *M. bovis* BCG are transcribed from two promoters, whereas the *dnaA* gene of *M. smegmatis* is transcribed from a single promoter. RT-PCR with total RNA showed that *dnaA* and *dnaN* were expressed in both species at all growth stages. Analysis of the promoter activity using *dnaA*-gfp fusion plasmids and DnaA expression plasmids indicates that the *dnaA* gene is autoregulated, although the degree of transcriptional autorepression was moderate. Transcription was also detected in the vicinity of *oriC* of *M. bovis* BCG, but not of *M. smegmatis*. These results suggest that a more complex transcriptional mechanism may be involved in the slow-growing mycobacteria, which regulates the expression of *dnaA* and initiation of chromosomal DNA replication.—Authors' Abstract

**Saviola, B., Woolwine, S. C., and Bishai, W. R.** Isolation of acid-inducible genes of *Mycobacterium tuberculosis* with the use of recombinase-based *in vivo* expression technology. *Infect. Immun.* **71(3)** (2003) 1379–1388.

A better understanding of mycobacterial gene regulation under certain stress conditions (e.g., low pH) may provide insight into mechanisms of adaptation during infection. To identify mycobacterial promoters induced at low pH, we adapted the recombinase-based *in vivo* expression technology (RIVET) promoter trap system for use with mycobacteria. Our results show that the TnpR recombinase of transposon  $\gamma$ -*madelta* is active in *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*. We developed a method to perform sequential double selection with mycobacteria by using RIVET, with a kanamycin preselection and a sucrose postselection. A library of *M. tuberculosis* DNA inserted upstream of *tnpR* was created, and using the double selection, we identified two promoters which are upregulated at low pH. The promoter re-

gions drive the expression of a gene encoding a putative lipase, *lipF* (Rv3487c), as well as a PE-PGRS gene, Rv0834c, in a pH-dependent manner in both *M. smegmatis* and *M. tuberculosis*. The acid inducibility of *lipF* and Rv0834c was independent of the stress response sigma factor, SigF, as acid induction of the two genes in an *M. tuberculosis* sigF mutant strain was similar to that in the wild-type strain. No induction of *lipF* or Rv0834c was observed during infection of J774 murine macrophages, an observation which is in agreement with previous reports on the failure of phagosomes containing *M. tuberculosis* to acidify.—Author's Abstract

**Steyn, A. J., Joseph, J., and Bloom, B. R.** Interaction of the sensor module of *Mycobacterium tuberculosis* H37Rv KdpD with members of the Lpr family. *Mol. Microbiol.* **47(4)** (2003) 1075–1089.

The genetic and biochemical mechanisms by which *Mycobacterium tuberculosis* senses and responds to the complex environment that it encounters during infection and persistence within the host remain unknown. In a number of bacterial species, the Kdp signal transduction pathway appears to be the primary response to environmental osmotic stress, which is primarily mediated by K<sup>+</sup> concentration in bacteria. We show that *kdp* encodes for components of a mycobacterial signalling pathway by demonstrating the K<sup>+</sup> dependence of *kdpFABC* expression in both *M. tuberculosis* H37Rv and *Mycobacterium smegmatis*. To identify proteins of *M. tuberculosis* that participate in this signalling pathway, we used the N-terminal sensing module of the histidine kinase KdpD as bait in a yeast two-hybrid screen. We show that the sensing domain of KdpD interacts specifically with two membrane lipoproteins, LprJ (Rv1690) and LprF (Rv1368). Overexpression of *lprF* and *lprJ* alleles in mycobacterial *kdpF-lacZ* reporter strains enabled us to identify alleles that modulate *kdpFABC* expression. By exploiting the yeast three-hybrid system, we have found that the histidine kinase domain of KdpD forms ternary complexes with LprF and LprJ and the sensing module of KdpD. Our results establish a role for membrane proteins in the Kdp signalling pathway and

suggest that LprF and LprJ function as accessory or ligand-binding proteins that communicate directly with the sensing domain of KdpD to modulate kdp expression.—Authors' Abstract

**Yin, Y., Suzuki, Y., Makino, M., and Wu, Q.** [Gene cloning and expression of *Mycobacterium leprae* alpha 2 antigen]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* **21(1)** (1999) 62–68. [Article in Chinese].

**OBJECTIVES:** The recombinant alpha 2 antigen of *M. leprae* was prepared using the molecular biologic tools and the recombinant DNA expression technology. **METHODS:** Screening of the *M. leprae* expression library was performed by the plaque

hybridization technique. Nucleotide sequences were determined by dideoxy termination method. **RESULTS:** The gene coding for alpha 2 antigen of *M. leprae* was cloned and characterized, and the complete nucleotide sequence data has been assigned in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databank. The over expression system of alpha 2 antigen gene in *E. coli* was constructed, and the recombinant alpha 2 antigen has been purified by amylose column chromatography at the purity of more than 95%. More than 10 mg of recombinant alpha 2 antigen has been obtained from 200 ml of liquid culture. **CONCLUSION:** The recombinant alpha 2 antigen of *M. leprae* could be used as one of the specific antigens for the sero-diagnosis of leprosy.—Authors' Abstract