

## CURRENT LITERATURE

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

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## General and Historical

**Britton, W. J., and Lockwood, D. N.** Leprosy. *Lancet* **363(9416)** (2004) 1209–1219. Comment in: *Lancet* **363(9424)** (2004) 1911.

Leprosy remains an important health problem worldwide. The disease is caused by a chronic granulomatous infection of the skin and peripheral nerves with *Mycobacterium leprae*. The clinical range from tuberculoid to lepromatous leprosy is a result of variation in the cellular immune response to the mycobacterium. The resulting impairment of nerve function causes the disabilities associated with leprosy. This review summarises recent advances in understanding of the biology of leprosy, clinical features of the disease, the current diagnostic criteria, and the new approaches to treatment of the infection and the immune-mediated complications. Supervised multi-drug therapy (MDT) for fixed durations is highly effective for all forms of the disease. The widespread implementation of MDT has been associated with a fall in the prevalence of the leprosy but as yet no reduction in the case-detection rate

globally. Thus, leprosy control activities must be maintained for decades to interrupt transmission of infection.—Authors' Abstract

**Job, C. K.** Nine-banded armadillo and leprosy research. *Indian J. Pathol. Microbiol.* **46(4)** (2003) 541–550.

In this presentation an attempt has been made to describe the nine-banded armadillo as an animal model, probably the only one in which lepromatous leprosy similar to that found in humans can be experimentally produced. Some unique features of the physiology of the animal are mentioned. The pathology and the microbiology of leprosy in the armadillo are described in detail. The discovery of lepromatous leprosy in the wild armadillos in the southern parts of United States, the transmission of disease among them through trauma and thorn pricks and the pathogenesis of the disease are presented. The impact of leprosy in the wild animals may have on human leprosy is discussed.—Author's Abstract

**Kalk, A., and Fleischer, K.** The decentralization of the health system in Colombia and Brazil and its impact on leprosy control. *Lepr. Rev.* **75**(1) (2004) 67–78.

Decentralization policies are an integrated component of health sector reform in an increasing number of countries. The ability of such policies to improve the health system's quality and efficiency is backed up by limited scientific evidence. This study intends to evaluate the impact of decentralization on a specialized field of disease control (leprosy control) in Colombia and Brazil. It analyses the respective juridical base, epidemiological indicators and local publications. Furthermore, 39 semi-structured interviews with key informants were conducted. In both countries, the devolution of technical responsibility and financial resources to the municipalities was the implemented form of decentralization. Access to preventive and curative health care and the community participation in decision-making improved clearly only in Brazil. The decentralization to private providers in Colombia had dubious effects on service quality in general and still more on public health. The flow of finances (including finance collection through state-owned taxes instead of insurance companies) seemed to be better controlled in Brazil. Leprosy control in Brazil took advantage of the decentralization process; in Colombia, it came close to a collapse.—Authors' Abstract

**Mori, S., Kato, S., Yokoyama, H., Tanaka, U., and Kaneda, S.** [The history of Yunosawa village and the policy of leprosy of Japan. IV]. *Nihon Hansenbyo Gakkai Zasshi* **73**(1) (2004) 47–63. [Article in Japanese]

There was a village which was called Yunosawa, lots of leprosy patients lived, existed from 1887 to 1941, Kusatu town, Gunma Prefecture, Japan. It was the only place continued securing self-government to the last as area was free from the isolation policy of State in prewar days there. The aim of this study will make clear the dynamism of "The protection from the tension of the society of leprosy patient currently persecuted" to "The defense of the society from

the leprosy patient who is a source of infection." In this study, explained the factor of confusion to a National Leprosarium Kuryu Rakusen-en during World War II and considered relation between patient movement and residents of Yunosawa village at the postwar period.—Authors' Abstract

**Ostrer, B. S.** Leprosy: medical views of leviticus rabba. *Early Sci. Med.* **7**(2) (2002) 138–154.

This article discusses chapters 15 and 16 of the ancient midrash (allegorical commentary) *Leviticus Rabba* (IV–V AD) and its view of leprosy. The phenomenon of Biblical leprosy is here not investigated from a paleo-pathological point of view. The focus lies on its physiological, aetiological, pathological and therapeutic aspects as represented in *Leviticus Rabba*. It is argued that the medical views of *Leviticus Rabba* show a certain resemblance to some of the view of the Hippocratic School, notably with respect to humoral theory, the belief in the correspondence between the macrocosm and the individual microcosm, and the notion of *paideia* as a way of healing. Finally, it is shown that the ancient myth of the two floods (of water and of fire) is connected to the understanding of leprosy in *Leviticus Rabba*.—Author's Abstract

**Saha, K., Kapoor, L., Arora, V. M., and Chattopadhyay, D.** Malaria, leprosy and dapsone. *Southeast Asian J. Trop. Med. Public Health.* **34**(3) (2003) 501–504.

Although the preventive action of dapsone against *P. falciparum* malaria was known for many years, there was no report about the incidence of *P. falciparum* malaria in leprosy patients treated with dapsone, especially from areas of Southeast Asia where both leprosy and malaria are endemic. Therefore, two clinic-based malaria surveys were undertaken at a gap of 12 years, comprising 506 lepromatous leprosy patients and 499 febrile nonleprosy control subjects. Both the surveys showed that the lepromatous patients treated with MDT had only *P. vivax* malaria (incidence comparable to the febrile nonleprosy controls) with complete

freedom from *P. falciparum*. On the contrary, control subjects not taking any-leprosy drugs and staying with the leprosy patients at the same beggars' home, had both *P. vivax* and *P. falciparum* malaria. It is postulated that dapsone provided protection against *P. falciparum* among leprosy patients.—Authors' Abstract

**Smit, J. J., Folkerts, G., and Nijkamp, F.** *P. Mycobacteria, genes and the 'hygiene hypothesis'.* Curr. Opin. Allergy Clin. Immunol. **4**(1) (2004) 57–62.

**PURPOSE OF REVIEW:** The “hygiene hypothesis” suggests that a relationship exists between improved hygiene and an increase in allergic diseases. As an underlying mechanism for this hypothesis it is proposed that due to the lack of microbial stimulation either a misbalance in T helper type responses or a misbalance in regulatory mechanisms develops. As yet, however, a specific infectious factor responsible for the hygiene hypothesis has not been found. **RECENT FINDINGS:** Animal models have lent support for mycobacteria as important candidates in the hygiene hypothesis. These animal studies have also suggested that mycobacterial treatment generated regulatory mechanisms which restored the immune balance. In contrast, the relationship between mycobacterial infection or treatment and the development of allergy and asthma in humans is unclear and highly controversial. **SUMMARY:** Mycobacteria have been found to unambiguously reduce allergic and asthmatic manifestations, suggesting that mycobacteria perhaps can be used as an “anti-asthma” vaccine. Conflicting results in humans, however, confirm that the complex and multifactorial interactions between the environment and the genetic background of the individual contribute to the development of allergic disease. Therefore, the hygiene hypothesis should involve the genetic and the environmental background of the individual.—Authors' Abstract

**Verma, G., Upshur, R. E., Rea, E., and Benatar, S. R.** Critical reflections on evidence, ethics and effectiveness in the

management of tuberculosis: public health and global perspectives. BMC Med. Ethics **5**(2) (2004) 2.

**BACKGROUND:** Tuberculosis is a major cause of morbidity and mortality globally. Recent scholarly attention to public health ethics provides an opportunity to analyze several ethical issues raised by the global tuberculosis pandemic. **DISCUSSION:** Recently articulated frameworks for public health ethics emphasize the importance of effectiveness in the justification of public health action. This paper critically reviews the relationship between these frameworks and the published evidence of effectiveness of tuberculosis interventions, with a specific focus on the controversies engendered by the endorsement of programs of service delivery that emphasize direct observation of therapy. The role of global economic inequities in perpetuating the tuberculosis pandemic is also discussed. **SUMMARY:** Tuberculosis is a complex but well understood disease that raises important ethical challenges for emerging frameworks in public health ethics. The exact role of effectiveness as a criterion for judging the ethics of interventions needs greater discussion and analysis. Emerging frameworks are silent about the economic conditions contributing to the global burden of illness associated with tuberculosis and this requires remediation—Authors' Abstract

**Visschedijk, J., Engelhard, A., Lever, P., Grossi, M. A., and Feenstra, P.** Leprosy control strategies and the integration of health services: an international perspective. Cad. Saude Publica. **19**(6) (2003) 1567–1581.

Integration of leprosy services into the general health services is regarded as the core strategy to ensure that leprosy control remains cost-effective and equitable, and, thus, sustainable in the coming years. In this article an extensive review is presented of the integration of leprosy services into the general health services. After the rationale of integration is discussed, the article highlights several recent developments within leprosy control and the health sector that are in support of the integration process. An

overview is presented of recent experiences in countries that have already embarked on the integration process. Based on these experiences important lessons can be learned and incorporated into a model for the

process of integration. This model, which is presented at the end of the article, will assist countries to successfully integrate leprosy services into the general health services.—Authors' Abstract

## Chemotherapy

**Abraham, M. H.** Hydrogen bond and other descriptors for thalidomide and its N-alkyl analogs; prediction of physicochemical and biological properties. *Eur. J. Pharm. Sci.* **21(4)** (2004) 465–469.

Descriptors for thalidomide and its N-alkyl derivatives have been obtained from solubilities reported by Goosen, *et al.* It is shown that thalidomide and the three N-alkylthalidomides are reasonably strong hydrogen bond bases, and that thalidomide is a hydrogen bond acid. From the obtained descriptors, a large number of physicochemical and biochemical properties of the four thalidomides were predicted, including several water-solvent partition coefficients. Predictions of skin permeation of the four thalidomides are in excellent agreement with experiment. Thalidomide is predicted to be only moderately to poorly distributed to the brain, but N-pentylthalidomide, is predicted to be very well distributed to the brain. All four thalidomides have very high predicted % human intestinal absorption.—Author's Abstract

**Adeniyi, B. A., Groves, M. J., and Gangadharam, P. R.** *In vitro* anti-mycobacterial activities of three species of Cola plant extracts (Sterculiaceae). *Phytother. Res.* **18(5)** (2004) 414–418.

Extracts obtained from three Nigerian Sterculiaceae plants: *Cola accuminata*, *C. nitida* and *C. milleni* were screened for anti-mycobacterium properties using a slow growing *Mycobacterium bovis* ATCC 35738 (designated BCG Mexican and known to have some virulence in mouse and guinea pig) at 1000 microg/ml using the radiometric (BACTEC) method. The extracts were also tested against six fast growing ATCC

strains of *M. vaccae* using the broth microdilution method. The methanol extracts from both leaves, stem bark and root bark of *Cola accuminata* and from the leaves and stem bark of *C. nitida* and *C. milleni* were not active at the highest concentration of 1000 microg/ml. Only the methanol extract of root bark for both *C. nitida* and *C. milleni* were found to be potent against both *M. bovis* and strains of *M. vaccae*. The minimum inhibitory concentration (MIC) of *C. nitida* against *M. bovis* is 125 microg/ml while the MIC of *C. milleni* against *M. bovis* is 62.5 microg/ml after at least 6 days of inhibition with growth index (GI) units lesser than or equal to the change in GI units inoculated with a 1/100 of the BACTEC inoculum for a control vial. The minimum inhibitory concentration of *C. milleni* against the six ATCC strain of *M. vaccae* ranged from 62.5 microg/ml to 250 microg/ml while for *C. nitida* ranged from 500 microg/ml to above 1000 microg/ml. Evidently, *C. milleni* has the highest inhibitory activity against both *M. bovis* and strains of *M. vaccae* used. Rifampicin, the positive control used has strong activity against *M. bovis* at the tested concentration of 5 microg and 10 microg/ml and 4 to 8 microg/ml against the six strains of *M. vaccae*.—Authors' Abstract

**Bidstrup, T. B., Stilling, N., Damkier, P., Scharling, B., Thomsen, M. S., and Brosen, K.** Rifampicin seems to act as both an inducer and an inhibitor of the metabolism of repaglinide. *Eur. J. Clin. Pharmacol.* **60(2)** (2004) 109–114.

**OBJECTIVE:** To investigate if rifampicin is both an inducer and an inhibitor of repaglinide metabolism, it was determined whether the timing of rifampicin co-

administration influences the pharmacokinetics of repaglinide. **METHODS:** Male volunteers ( $N = 12$ ) participated in a randomised, two-period, crossover trial evaluating the effect of multiple doses of 600 mg rifampicin once daily for 7 days on repaglinide metabolism. Subjects were, after baseline measurements of repaglinide pharmacokinetics, randomized to receive, on either day 7 or day 8 of the rifampicin administration period, a single dose of 4 mg repaglinide and vice versa in the following period. **RESULTS:** When repaglinide was given, together with the last rifampicin dose, on day 7, an almost 50% reduction of the median repaglinide area under the plasma concentration-time curve (AUC) was observed. Neither the peak plasma concentration ( $C(\max)$ ), time to reach  $C(\max)$  ( $t(\max)$ ) nor terminal half-life ( $t(1/2)$ ) was statistically significantly affected. When repaglinide was given on day 8, 24 hr after the last rifampicin dose, an almost 80% reduction of the median repaglinide AUC was observed. The median  $C(\max)$  was now statistically significantly reduced from 35 ng/ml to 7.5 ng/ml. Neither  $t(\max)$  nor  $t(1/2)$  was significantly affected. **CONCLUSION:** When rifampicin and repaglinide are administered concomitantly, rifampicin seems to act as both an inducer and an inhibitor of the metabolism of repaglinide. After discontinuing rifampicin administration, while the inductive effect on CYP3A4 and probably also CYP2C8 is still present, an even more marked reduction in the plasma concentration of repaglinide was observed. Our results suggest that concomitant administration of rifampicin and repaglinide may cause a clinically relevant decrease in the glucose-lowering effect of repaglinide, in particular when rifampicin treatment is discontinued or if the drugs are not administered simultaneously or within a few hours of each other.—Authors' Abstract

**Bopape, M. C., Steel, H. C., Cockeran, R., Matlola, N. M., Fourie, P. B., and Anderson, R.** Antimicrobial activity of clofazimine is not dependent on mycobacterial C-type phospholipases. *J. Antimicrob. Chemother.* **53**(6) (2004) 971–974.

We have used a phospholipase C (PLC)-deletion mutant ( $plcABC$ ) of the H37Rv

strain of *Mycobacterium tuberculosis* (MTB), as well as a  $plcA$ -insertion mutant of *Mycobacterium smegmatis*, to investigate the possible involvement of PLCs in clofazimine-mediated inhibition of mycobacterial  $K(+)$  transport and growth. Inactivation of the PLCs of MTB and insertion of the  $plcA$  gene into *M. smegmatis* resulted in a substantial reduction and increase in hydrolysis of phosphatidylcholine (PC), respectively. However, both the mutant and wild-type strains of MTB and *M. smegmatis* were equally sensitive to the inhibitory effects of clofazimine on  $K(+)$  uptake and growth. These observations demonstrate that the PLCs of MTB are not involved in the antimicrobial activity of clofazimine.—Authors' Abstract

**Flageul, B.** [Current guidelines for leprosy treatment]. *Bull. Soc. Pathol. Exot.* **96**(5) (2003) 357–361. [Article in French]

During the last 20 years, the global leprosy situation has strikingly changed with a decrease of cases from 12 millions estimated cases in 1982 to 600,000 registered cases in the year 2000. However, during the past 15 years, about 700,000 new cases are still detected annually. The systematic use of multidrug therapy (MDT), as recommended by a WHO Study Group in 1982, has proven its efficacy as assessed by the low reported relapse rate (less than 1% per year). The initial PCT schedule has been modified several times, but this PCT remains the recommended chemotherapy for the great majority of patients. New potent antibacillary drugs (ofloxacin, minocycline, clarithromycin) have been discovered; however, their current use is limited and should remain limited until under way trials could confirm their efficacy. With the use of PCT, the frequency of immunologically mediated reactional states have changed. The occurrence of reversal reaction, (type 1 reaction) has significantly increased while that of erythema nodosum leprosum (ENL, type 2) appeared less common. Because of the high risk of neurological permanent damage, reversal reaction needs to be diagnosed and treated as soon as possible. Here in, the current antibacillary and antireactional treatments are being reviewed.—Author's Abstract



**Franks, M. E., Macpherson, G. R., and Figg, W. D.** Thalidomide. *Lancet* **363(9423)** (2004) 1802–1811.

Despite its history as a human teratogen, thalidomide is emerging as a treatment for cancer and inflammatory diseases. Although the evolution of its clinical application could not have been predicted from the tragedy associated with its misuse in the past, its history serves as a lesson in drug development that underscores the need to understand the molecular pharmacology of a compound's activity, including associated toxicities. Here, we summarise the applications for thalidomide with an emphasis on clinical trials published over the past 10 years, and consider our knowledge of the molecular pharmacology of the drug in the context of clinical trial data, attempting to provide a mechanism-guided understanding of its activity.—Authors' Abstract

**Gasymov, O. K., Abduragimov, A. R., Gasimov, E. O., Yusifov, T. N., Dooley, A. N., and Glasgow, B. J.** Tear lipocalin: potential for selective delivery of rifampin. *Biochem. Biophys. Acta.* **1688(2)** (2004) 102–111.

The potential of ligand binding proteins as drug carriers and delivery systems has recently sparked great interest. We investigated the potential of tear lipocalin (TL) to bind the antibiotic, rifampin, and the environmental conditions for controlled release. To determine if TL binds rifampin, gel filtration was used to isolate protein fractions of tears. Rifampin was detected by absorbance spectroscopy in the elution fractions containing TL. The bound complex of rifampin-TL generates optical activity at about 360 nm, indicating a unique conformation at the binding site. Rifampin has a higher affinity for TL ( $K_d = 128$   $\mu$ M) than albumin. Rifampin is released from the TL calyx in acidic conditions and is displaced by palmitic acid. Autooxidation of free rifampin begins in minutes but is delayed by at least 3 hr in the presence of TL. These properties are conducive to stabilization and delivery of rifampin to tubercles that are acidic and rich in fatty acids. These studies show the potential of TL as a carrier

for rifampin with controlled release to a targeted environment.—Authors' Abstract

**Gidoh, M., Namisato, M., Kumano, K., Goto, M., Nogami, R., Ozaki, M.; and the Committee for standards on therapeutic usage of newquinolones.** [Guideline for the treatment of leprosy by new quinolones]. *Nihon Hansenbyo Gakkai Zasshi.* **73(1)** (2004) 65–67. [Article in Japanese]

Ofloxacin(OFLX) is often applied today as a substitution drug of MDT for drug resistance to dapsone, rifampicin or clofazimine. However, OFLX resistance is also becoming a great concern. Low and/or irregular administration are considered to be the major causes of OFLX resistance. OFLX should be used as a combined therapy, and minimal daily dose of 400 mg of OFLX or 200–300 mg of levofloxacin is required. Quinolone resistance should be considered when no improvement of clinical and/or bacterial index is observed after the treatment for 6 months. In such cases, resistance gene detection is necessary.—Authors' Abstract

**Gursoy, A., Kut, E., and Ozkirimli, S.** Co-encapsulation of isoniazid and rifampicin in liposomes and characterization of liposomes by derivative spectroscopy. *Int. J. Pharm.* **271(1–2)** (2004) 115–123.

Taking into consideration the benefits of the combined therapy of isoniazid (INH) and rifampicin (RIF), this study focused on co-encapsulation of INH and RIF in the same liposome formulation. INH was incorporated in the aqueous phase and RIF in the lipid layer. Liposomes containing either INH or RIF were also prepared. All liposome formulations were compared for their loading capacity, encapsulation percentage and release properties. Drug amounts in the liposomes were estimated using peak-to-peak first-order derivative UV spectroscopy. Among the liposome formulations DPPC:chol liposomes showed the highest loading capacity ( $106.70 \pm 0.12$  for INH and  $18.17 \pm 0.06 (\times 10^{-3})$  for RIF) and encapsulation percentage ( $73.84 \pm 0.78$  for INH and  $81.53 \pm 2.06$  for RIF) compared to EPC:chol liposomes

(loading capacity  $93.36 \pm 0.58$  for INH and  $17.87 \pm 0.11$  ( $\times 10^{-3}$ ) for RIF; encapsulation percentage  $64.61 \pm 0.51$  for INH and  $74.45 \pm 0.48$  for RIF). Co-encapsulation of INH and RIF increased their individual encapsulation percentage and extended drug release compared to the formulations containing drug alone (Table 2). Results of this study support the conclusion that lipid and water soluble drugs can be successfully co-encapsulated in the same liposome formulation and also show that derivative UV spectroscopy is a sensitive method for direct and accurate quantification of these co-encapsulated drugs.—Authors' Abstract

**Hummel, M. A., Dickmann, L. J., Rettie, A. E., Haining, R. L., and Tracy, T. S.** Differential activation of CYP2C9 variants by dapsone. *Biochem. Pharmacol.* **67(10)** (2004) 1831–1841.

Studies have shown that CYP2C9.1 mediated metabolism of flurbiprofen or naproxen is activated by co-incubation with dapsone. However, dapsone activation has not been examined in the known variant forms of CYP2C9. Six concentrations of flurbiprofen (2–300 microM) or naproxen (10–1800 microM) were co-incubated with six concentrations of dapsone (0–100 microM) and with reconstituted, purified CYP2C9.1, CYP2C9.2 (R144C), CYP2C9.3 (I359L), or CYP2C9.5 (D360E), in order to assess degrees of activation. Dapsone increased the efficiency ( $V(m)/K(m)$ ) of flurbiprofen 4'-hydroxylation by CYP2C9.1, CYP2C9.2, CYP2C9.3, and CYP2C9.5 by 8-, 31-, 47-, and 22-fold, respectively. In similar experiments using the substrate naproxen, dapsone increased the efficiency of naproxen demethylation 7-, 15-, 13-, and 22-fold, in CYP2C9.1, CYP2C9.2, CYP2C9.3, and CYP2C9.5, respectively. Also, dapsone normalized naproxen's kinetic profile from biphasic (CYP2C9.1 and CYP2C9.2) or linear (CYP2C9.3 and CYP2C9.5) to hyperbolic for all variant forms. Thus, amino acid substitutions of CYP2C9 variants affect the degree of dapsone activation in a genotype-dependent fashion. Furthermore, the degree of effect noted across variants appeared to be dependent on the substrate studied.—Authors' Abstract

**Kantardjieff, K. A., Kim, C. Y., Naranjo, C., Waldo, G. S., Legin, T., Segelke, B.W., Zemla, A., Park, M. S., Terwilliger, T. C., and Rupp, B.** *Mycobacterium tuberculosis* RmlC epimerase (Rv3465): a promising drug-target structure in the rhamnose pathway. *Acta Crystallogr. D. Biol. Crystallogr.* **60(Pt 5)** (2004) 895–902.

The *Mycobacterium tuberculosis* rmlC gene encodes dTDP-4-keto-6-deoxyglucose epimerase, the third enzyme in the *M. tuberculosis* dTDP-L-rhamnose pathway which is essential for mycobacterial cell-wall synthesis. Because it is structurally unique, highly substrate-specific and does not require a cofactor, RmlC is considered to be the most promising drug target in the pathway, and the *M. tuberculosis* rmlC gene was selected in the initial round of TB Structural Genomics Consortium targets for structure determination. The 1.7 Å native structure determined by the consortium facilities is reported and implications for in silico screening of ligands for structure-guided drug design are discussed.—Authors' Abstract

**Lepper, E. R., Ng, S. S., Gutschow, M., Weiss, M., Hauschildt, S., Hecker, T. K., Luzzio, F. A., Eger, K., and Figg, W. D.** Comparative molecular field analysis and comparative molecular similarity indices analysis of thalidomide analogues as angiogenesis inhibitors. *J. Med. Chem.* **47(9)** (2004) 2219–2227.

Thalidomide, 2-(2,6-dioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione, has been shown to inhibit angiogenesis, the formation of new blood vessels from existing vasculature. As a result, there is renewed interest in this drug as a potential therapy for solid tumors. Thalidomide forms a number of metabolites and has been shown to require metabolic activation for antiangiogenic activity. A series of 39 compounds, based upon the structure of some of these metabolites, was synthesized and tested for their ability to inhibit microvessel growth in the rat aortic ring assay. The results of this testing have been used as the basis for a three-dimensional quantitative structure-activity relationship (3D-QSAR) study, utilizing comparative

molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) procedures. The best resulting CoMFA and CoMSIA models have conventional  $r(2)$  values of 0.924 and 0.996, respectively. The cross-validated  $q(2)$  values are 0.666 and 0.635, respectively. These models offer insight into the structural requirements for activity of thalidomide analogues as angiogenesis inhibitors, since there is only speculative knowledge of the target. Additionally, it appears as though there is more than one active site or mechanism of action.—Authors' Abstract

**Nuermberger, E., and Grosset, J.** Pharmacokinetic and pharmacodynamic issues in the treatment of mycobacterial infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **23**(4) (2004) 243–255.

The therapy of mycobacterial infections is challenging for a number of reasons. Because mycobacteria are not susceptible to many classes of antibacterial agents, treatment typically requires the use of antimicrobial drugs that are not commonly used and may have small therapeutic windows. For many species, procedures for drug susceptibility testing and optimal treatment regimens have yet to be defined. Finally, because mycobacteria are generally slow to succumb to antimicrobial agents, therapy must be given with multiple drugs for prolonged periods of time, making it necessary to monitor for drug toxicity, drug interactions, and patient nonadherence. Better understanding of the pharmacokinetics and pharmacodynamics of antimycobacterial agents should improve the therapy of mycobacterial infections. Using current treatment strategies for tuberculosis and *Mycobacterium avium* complex infections as examples, this review highlights basic pharmacokinetic and pharmacodynamic principles and the rationale for combination chemotherapy that should also be applicable to other mycobacterial infections.—Authors' Abstract

**Pandey, R., and Khuller, G. K.** Chemotherapeutic potential of alginate-chitosan microspheres as anti-tubercular drug carriers. *J. Antimicrob. Chemother.* **53**(4) (2004) 635–640.

**OBJECTIVES:** This study was designed to develop alginate-chitosan microspheres as drug carriers to reduce dose/dosing frequency in the management of tuberculosis (TB), which otherwise demands prolonged chemotherapy. **METHODS:** Alginate-chitosan microspheres encapsulating three frontline anti-tuberculous drugs (ATDs), rifampicin, isoniazid and pyrazinamide, were formulated. A therapeutic dose and a half-therapeutic dose of the microsphere-encapsulated ATDs were orally administered to guinea pigs for pharmacokinetic/chemotherapeutic evaluations, respectively. **RESULTS:** The drug encapsulation efficiency ranged from 65% to 85% with a loading of 220–280 mg of drug per gram microspheres. Administration of a single oral dose of the microspheres to guinea pigs resulted in sustained drug levels in the plasma for 7 days and in the organs for 9 days. The half-life and mean residence time of the drugs were increased 13- to 15-fold by microsphere encapsulation, along with an enhanced relative/absolute bioavailability. The sustained release and increase in bioavailability were also observed with a sub-therapeutic dose of the microspheres. In *Mycobacterium tuberculosis* H37Rv-infected guinea pigs, administration of a therapeutic dose of microspheres spaced 10 days apart produced a clearance of bacilli equivalent to conventional treatment for 6 weeks. The most important observation, however, was the documentation of therapeutic benefit with a half-therapeutic dose of the microspheres administered weekly. **CONCLUSION:** Alginate-chitosan microspheres hold promise as a potential natural polymer-based oral ATD carrier for better management of TB.—Authors' Abstract

**Peloquin, C. A., Berning, S. E., Nitta, A. T., Simone, P. M., Goble, M., Huitt, G. A., Iseman, M. D., Cook, J. L., and Curran-Everett, D.** Aminoglycoside toxicity: daily versus thrice-weekly dosing for treatment of mycobacterial diseases. *Clin. Infect. Dis.* **38**(11) (2004) 1538–1544.

Aminoglycoside use is limited by ototoxicity and nephrotoxicity. This study compared the incidences of toxicities associated



with 2 recommended dosing regimens. Eighty-seven patients with tuberculosis or nontuberculous mycobacterial infections were prospectively randomized by drug to receive 15 mg/kg per day or 25 mg/kg 3 times per week of intravenous streptomycin, kanamycin, or amikacin. Doses were adjusted to achieve target serum concentrations. The size of the dosage and the frequency of administration were not associated with the incidences of ototoxicity (hearing loss determined by audiogram), vestibular toxicity (determined by the findings of a physical examination), or nephrotoxicity (determined by elevated serum creatinine levels). Risk of ototoxicity (found in 32 [37%] of the patients) was associated with older age and with a larger cumulative dose received. Vestibular toxicity (found in 8 [9%] of the patients) usually resolved, and nephrotoxicity (found in 13 [15%] of the patients) was mild and reversible in all cases. Subjective changes in hearing or balance did not correlate with objective findings. Streptomycin, kanamycin, and amikacin can be administered either daily or 3 times weekly without affecting the likelihood of toxicity.—Authors' Abstract

**Rossi, L., Brandi, G., Malatesta, M., Serafini, S., Pierige, F., Celeste, A. G., Schiavano, G. F., Gazzanelli, G., and Maggiani, M.** Effect of listeriolysin O-loaded erythrocytes on *Mycobacterium avium* replication within macrophages. *J. Antimicrob. Chemother.* **53**(5) (2004) 863–866.

**OBJECTIVE:** To evaluate the efficacy of erythrocytes loaded with the haemolytic toxin listeriolysin O against *Mycobacterium avium* replication within human macrophages. **METHODS:** Recombinant listeriolysin O was loaded in human erythrocytes by a procedure of hypotonic dialysis and isotonic resealing. Loaded erythrocytes were modified to allow them to be recognized and taken up by human macrophages infected with *M. avium*. The antimycobacterial activity of the erythrocytes loaded with listeriolysin O was evaluated by supernatant and intracellular cfu counts on days 4 and 7 post-erythrocyte administration. **RESULTS:** Recombinant listeriolysin O was encapsulated in human erythrocytes

to reach final concentrations ranging from 1 to 4 ng/mL of erythrocytes. Erythrocytes loaded with increasing quantities of recombinant protein were able to reduce (at most by 50%) *M. avium* replication in a dose-dependent fashion when administered to infected macrophages. **CONCLUSIONS:** Erythrocytes loaded with listeriolysin O are effective against *M. avium* replication within macrophages. We are confident that the strategy presented could be useful against mycobacteria other than *M. avium* (such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*) by itself or as part of an antimycobacterial treatment.—Authors' Abstract

**Teo, S. K., Colburn, W. A., Tracewell, W. G., Kook, K. A., Stirling, D. I., Jaworsky, M. S., Scheffler, M. A., Thomas, S. D., and Laskin, O. L.** Clinical pharmacokinetics of thalidomide. *Clin. Pharmacokinet.* **43**(5) (2004) 311–327.

Thalidomide is a racemic glutamic acid derivative approved in the US for erythema nodosum leprosum, a complication of leprosy. In addition, its use in various inflammatory and oncologic conditions is being investigated. Thalidomide interconverts between the (R)- and (S)-enantiomers in plasma, with protein binding of 55% and 65%, respectively. More than 90% of the absorbed drug is excreted in the urine and faeces within 48 hr. Thalidomide is minimally metabolised by the liver, but is spontaneously hydrolysed into numerous renally excreted products. After a single oral dose of thalidomide 200 mg (as the US-approved capsule formulation) in healthy volunteers, absorption is slow and extensive, resulting in a peak concentration (C(max)) of 1–2 mg/L at 3–4 hours after administration, absorption lag time of 30 minutes, total exposure (AUC (infinity)) of 18 mg hr/L, apparent elimination half-life of 6 hours and apparent systemic clearance of 10 L/hr. Thalidomide pharmacokinetics are best described by a one-compartment model with first-order absorption and elimination. Because of the low solubility of the drug in the gastrointestinal tract, thalidomide exhibits absorption rate-limited pharmacokinetics (the 'flip-flop' phenomenon), with its elim-

ination rate being faster than its absorption rate. The apparent elimination half-life of 6 hours therefore represents absorption, not elimination. The 'true' apparent volume of distribution was estimated to be 16L by use of the faster elimination-rate half-life. Multiple doses of thalidomide 200 mg/day over 21 days cause no change in the pharmacokinetics, with a steady-state  $C(\max)$  ( $C(ss)(\max)$ ) of 1.2 mg/L. Simulation of 400 and 800 mg/day also shows no accumulation, with  $C(ss)(\max)$  of 3.5 and 6.0 mg/L, respectively. Multiple-dose studies in cancer patients show pharmacokinetics comparable with those in healthy populations at similar dosages. Thalidomide exhibits a dose-proportional increase in AUC at doses from 50 to 400 mg. Because of the low solubility of thalidomide,  $C(\max)$  is less than proportional to dose, and  $t(\max)$  is prolonged with increasing dose. Age, sex and smoking have no effect on the pharmacokinetics of thalidomide, and the effect of food is minimal. Thalidomide does not alter the pharmacokinetics of oral contraceptives, and is also unlikely to interact with warfarin and grapefruit juice. Since thalidomide is mainly hydrolysed and passively excreted, its pharmacokinetics are not expected to change in patients with impaired liver or kidney function.—Authors' Abstract

**Villahermosa, L. G., Fajardo, T. T., Jr., Abalos, R. M., Cellona, R. V., Balagon, M. V., Dela Cruz, E. C., Tan, E. V., Walsh, G. P., and Walsh, D. S.** Parallel assessment of 24 monthly doses of rifampin, ofloxacin, and minocycline versus two years of World Health Organization multi-drug therapy for multi-bacillary leprosy. *Am. J. Trop. Med. Hyg.* **70**(2) (2004) 197–200.

Monthly doses of rifampin, ofloxacin, and minocycline (ROM) are expected to be effective treatment for multi-bacillary leprosy. Patients with MB leprosy received ROM (N = 10) or World Health Organization multi-drug therapy (MDT) (N = 11). Treatment with ROM was given as 24 consecutive monthly observed doses of rifampin (600 mg), ofloxacin (400 mg), and minocycline (100 mg). Treatment with MDT was given as 24 consecutive monthly

observed doses of rifampin (600 mg) and clofazimine (300 mg), and unobserved daily dapsone (100 mg) and clofazimine (50 mg). Twenty patients completed the 24-month regimens with >99% compliance. Treatments with ROM and MDT were safe, tolerable, and caused similar improvements in lesions, bacterial indices, and histology. All MDT recipients developed clofazimine-induced pigmentation. Six ROM and nine MDT recipients assessed at five or more years after completion of treatment had no evidence of relapse. Twenty-four months of treatment with ROM is a safe, well-tolerated, and convenient regimen that may provide an alternate therapy to MDT for MB leprosy. Larger trials with sufficient follow-up would better define the role of ROM.—Authors' Abstract

**Yamamoto, F., Harada, S., Mitsuyama, T., Harada, Y., Kitahara, Y., Yoshida, M., and Nakanishi, Y.** Concentration of clarithromycin and 14-R-hydroxy-clarithromycin in plasma of patients with *Mycobacterium avium* complex infection, before and after the addition of rifampicin. *Jpn. J. Antibiot.* **57**(1) (2004) 124–133.

Clarithromycin (CAM) and rifampicin (RFP) have both been recognized to be effective antibiotic agents against *Mycobacterium avium* complex (MAC) infection. Rifamycin derivatives including RFP and rifabutin modulate the CAM metabolism by inducing the hepatic cytochrome p-450 3A4. To clarify the effect of RFP on the CAM metabolism, we measured the plasma concentration of CAM and 14-R-hydroxyclearithromycin (M-5), the major metabolite of CAM, in 9 patients suffering from MAC infection before and after the addition of RFP. After the addition of RFP, the mean plasma concentration of CAM significantly decreased, while that of M-5 did not. In addition, the amount of CAM + M-5 concentration also significantly decreased. As M-5 is less effective against MAC infection than CAM, more attention should thus be paid to the plasma CAM concentration in patients administered CAM and RFP concomitantly.—Authors' Abstract

## Clinical Sciences

**Alioua, Z., Sbai, M., Elhaouri, M., Bouzidi, A., Boudi, O., Ghfir, M., Benomarm S., and Sedrati, O. L.** [Histoid leprosy with erythema nodosum leprosum]. *Acta. Leprol.* **12(3)** (2003) 107–111. [Article in French]

Histoid leprosy is a particular variant of lepromatous leprosy presenting as cutaneous or subcutaneous nodular and/or plaque-like lesions arising from apparently normal skin. It is characterized histologically by spindle-shaped histiocytes in interlacing bundles and whorls, containing numerous intact and rod-shaped *Mycobacterium leprae*. It can occur de novo or secondary in patients treated for a long course by dapsone alone. We describe a case of lepromatous leprosy treated according to the national Moroccan protocol who developed histoid lesions during his treatment by dapsone. The patient responded well to fluoroquinolone, rifampicin and clofazimine, with however, the occurrence of erythema nodosum leprosum.—Authors' Abstract

**Apaydin, R., Bahadir, S., Kakklikkaya, N., Bilen, N., and Bayramgurler, D.** Possible role of *Mycobacterium tuberculosis* complex in Melkersson-Rosenthal syndrome demonstrated with Gen-Probe amplified *Mycobacterium tuberculosis* direct test. *Australas. J. Dermatol.* **45(2)** (2004) 94–99.

Melkersson-Rosenthal (MRS) syndrome is characterized by a classical triad of recurrent or persistent orofacial swelling, peripheral facial nerve paralysis and lingua plicata. Granulomatous cheilitis (GC) is regarded as a monosymptomatic form of MRS. The exact aetiologies of MRS and GC are unknown. In this study we investigated the possible role of mycobacteria in these two conditions. A ribosomal RNA amplification-based Gen-Probe amplified *Mycobacterium tuberculosis* direct test was used to investigate the presence of *M. tuberculosis* complex in paraffin-embedded skin biopsy specimens from five patients with MRS and one patient with GC. Three of the six specimens were shown to be positive using this system;

one of the positive specimens also showed positive Ziehl-Neelsen staining. These results suggest a possible mycobacterial aetiology for MRS and GC.—Authors' Abstract

**Daniel, E., Duriasamy, M., Ebenezer, G. J., Shobhana, and Job, C. K.** Elevated free tear lactoferrin levels in leprosy are associated with Type 2 reactions. *Indian J. Ophthalmol.* **52(1)** (2004) 51–56.

**PURPOSE:** To determine the association of demographics, leprosy and ocular characteristics with altered levels of lactoferrin in the tears of normal subjects and leprosy patients, and to detect the presence of antibodies to lactoferrin in these tear samples. **METHOD:** We collected light-stimulated tears from 298 leprosy patients and an equal number of normal subjects using the glass capillary method. Free lactoferrin levels were estimated using ELISA and the presence of antibodies to lactoferrin was detected using the immuno-blotting method. Significant associations were looked for between tear lactoferrin levels and demographic characteristics, leprosy characteristics such as type of disease, duration of disease, reactions, deformity and bacterial load, and ocular complications, using chi-square and regression analysis. **RESULTS:** Tear lactoferrin levels with a mean (S.D.) of 2.55 (2.83) mg/ml in the control group were significantly different ( $p < 0.000$ ) from leprosy patients with a mean (S.D.) of 5.66 (7.21) mg/ml. Age showed an inverse correlation with tear lactoferrin levels in controls. Increased bacterial load, grade 2 leg deformity and Type 2 reactions were significantly associated ( $p < 0.05$ ) with increased tear lactoferrin levels. Type 2 reactions remained significantly associated ( $p = 0.01$ ) on multiple regression analysis. Tear lactoferrin levels were not associated with gender, serum lactoferrin levels, Type 1 reactions, face patches, treatment status, orbicularis oculi weakness, lagophthalmos, ectropion, entropion, corneal opacity, cataract and iridocyclitis. **CONCLUSION:** Age is inversely related to tear lactoferrin levels in normal subjects. Free lactoferrin levels in tears are

significantly higher in leprosy patients compared with normal controls. Type 2 reactions in leprosy are significantly associated with elevated tear lactoferrin levels.—Authors' Abstract

**Dave, S., Thappa, D. M., Nori, A. V., and Jayanthi, S.** A rare variant of erythema nodosum leprosum: a case report. *Dermatol. Online J.* **9(5)** (2003) 11.

We report a patient with lepromatous leprosy who developed a rare variant of type-2 lepra reaction, characterized by pustular lesions, on switching from WHO multi drug therapy (MDT) to ofloxacin-aided MDT.—Authors' Abstract

**de Carsalade, G. Y., Achirafi, A., and Flageul, B.** [Pentoxifylline in the treatment of erythema nodosum leprosum: results of an open study]. *Acta. Leprol.* **12(3)** (2003) 117–122. [Article in French]

Erythema nodosum leprosum (ENL) is a well-known immunological serious complication affecting lepromatous multibacillary leprosy patients. For a long time, ENL has been regarded as an immune complex-mediated disease or Arthus phenomenon. Recently, it has been reported that ENL was associated with high serum tumor necrosis factor-alpha (TNF $\alpha$ ) levels, suggesting that this cytokine could also play a central role in the manifestations of ENL. Thalidomide (TH) and systemic steroids (S), both TNF $\alpha$  production inhibitors, are the two current effective drugs for the management of ENL. However, TH is rarely available in leprosy endemic countries, and its teratogenicity and neurotoxicity strongly limit its use. Moreover, the morbidity of S and the frequent steroid-dependence of ENL also create real therapeutic problems. Recently, the efficacy of pentoxifylline (PTX), which also inhibits *in vitro* and *in vivo* production of TNF $\alpha$ , has been suggested for ENL treatment. We report our experience on its use for the treatment of 15 leprosy patients suffering from a first ENL attack. (11 cases), a chronic steroid-dependent ENL (3 cases) or chronic steroid- and thalidomide-dependent ENL (1 case). PTX has been given at 800 mg t.i.d.,

(2 cases) or 400 mg t.i.d. (13 cases) doses. The patients received PTX at the initiating dosage until complete clinical cure. At the end of ENL attacks, PTX was either abruptly stopped or tapered down over the next 4 months. In ten of 11 patients who developed ENL for the first time, the systemic symptoms and neuritic pains disappeared within one week; at three weeks, half of the patients were cured and the other half had striking clinical improvement; complete cure was obtained within 7 to 35 days (mean: 27 days). A relapse occurred within 2–3 months in the 5 patients, in which PTX was abruptly stopped. In contrast, no relapse occurred in the patients who benefited from decreasing doses of PTX. Recurrent ENL episodes also responded well to PTX. The 3 patients who had chronic steroid-dependent ENL failed to show any improvement after 3 to 6 weeks of PTX. In contrast, steroid therapy could be stopped in the steroid- and thalidomide-dependent patient. Our results confirm the action of PTX if it is slowly tapered down (4 months seem sufficient) and not abruptly to avoid relapses. As it is safe use, PTX could constitute the first line of ENL attack treatment.—Authors' Abstract

**Ghorpade, A.** Reactional tattoo inoculation borderline tuberculoid leprosy with oedematous tattoos. *Lepr. Rev.* **75(1)** (2004) 91–94.

Tattoo inoculation borderline tuberculoid (BT) leprosy in upgrading reaction with prominent tattoo oedema developing after starting paucibacillary multidrug therapy (PB MDT) is reported. The diagnosis was confirmed by histopathology. An excellent response to oral steroids and PB MDT was seen. There is only one similar report in the literature.—Author's Abstract

**Haanpaa, M., Lockwood, D. N., and Hietaharju, A.** Neuropathic pain in leprosy. *Lepr. Rev.* **75(1)** (2004) 7–18.

Chronic neuropathic pain in treated leprosy has received scant attention. In this article the concept, clinical features and diagnosis of neuropathic pain are reviewed. The



possible pathophysiological mechanisms, treatment challenges and research needs in this area are discussed.—Authors' Abstract

**Kondo, S.** [Ocular impairment in leprosy]. *Nihon Hansenbyo Gakkai Zasshi*. **73**(1) (2004) 23–35. [Article in Japanese]

Leprosy causes several ocular disorders, and it also causes aftereffect with high frequency in various ways. Primary impairment is the ocular disturbance caused with direct invasion of nerve and ocular tissue by *Mycobacterium leprae*. Secondary impairment is the complication of nerve paralysis and residual inflammation due to primary disorder. Main work at Japanese national leprosariums has been the control of primary and secondary impairment in recent years. Clinical ophthalmic study in the leprosarium revealed an increase of age-related ocular disease in addition to aftereffect of leprosy. Severe sequelae due to sensory and functional disturbance will require suitable applications of advanced clinical technologies.—Author's Abstract

**Kumar, A., Girdhar, A., and Girdhar, B. K.** Pattern of bacillary clearance in multibacillary leprosy patients with multidrug therapy. *Acta. Leprol.* **12**(3) (2003) 123–128.

The bacteriological index (BI) of the skin smears is traditionally one of the important parameters of assessment of severity and of progress of leprosy under multidrug therapy. The present study reports on BI clearance among 578 multibacillary treated leprosy patients and the factors that influence this clearance. The patients were treated till smear negativity or for 2 years fixed duration and their skin smears periodically examined every 6 to 12 months till negativity (and even afterwards). We confirm that bacterial clearance is a slow process. The time taken for each log-unit decline in BI is between 13.6 to 24 months probably depending on initial BI level. The rate of smear negativity appears to be dependent on immune competence of the patients as reflected by a rapid BI decline in borderline BT-BB patients vis-a-vis BL-LL lepromatous patients both in the low and high BI group. Patients who had several episodes of ENL, took significantly longer time (63.7

months versus 53.5 months,  $p < 0.0001$ ) to become smear negative than those without ENL.—Authors' Abstract

**Nepal, B. P., and Shrestha, U. D.** Ocular findings in leprosy patients in Nepal in the era of multidrug therapy. *Am. J. Ophthalmol.* **137**(5) (2004) 888–892.

**PURPOSE:** Detailed ophthalmic evaluation was performed to determine the prevalence of ocular complications among leprosy patients on multidrug therapy and those released from multidrug treatment. **DESIGN:** Observational case series. **METHODS:** Leprosy patients at Tribhuvan University Teaching Hospital from April 1, 2001, through September 30, 2002, underwent detailed ophthalmic evaluation including slit-lamp biomicroscopy, dilated funduscopy, and applanation tonometry. **RESULTS:** We evaluated 58 leprosy patients. A majority (72%) was receiving treatment for multibacillary leprosy; 14% belonged to posttreatment multibacillary and paucibacillary groups. Ocular involvement was found in 57% of patients. In the multibacillary group, 55% had ocular involvement, which was more than double that found in the paucibacillary group (25%), although this finding was not statistically significant ( $p = 0.187$ ). Among patients with ocular complications, 48% had visual disability and another 45% had threatened vision; 9% met World Health Organization guidelines for blindness. Uveitis and its complications were the predominant causes of visual disability (88%). **CONCLUSION:** Ocular complications and visual disability are high among leprosy patients in Nepal even after completing multidrug therapy.—Authors' Abstract

**Nunez-Marti, J. M., Bagan, J. V., Scully, C., and Penarrocha, M.** Leprosy: dental and periodontal status of the anterior maxilla in 76 patients. *Oral Dis.* **10**(1) (2004) 19–21.

**OBJECTIVE:** To evaluate the presence of oral disease, as assessed by dental and periodontal indices, in the anterior maxilla of a group of 76 patients with leprosy, compared with a group of matched control subjects. **MATERIALS AND METHODS:** The study included 76 patients with leprosy (age range

40–82 years; 39 males), resident in the sanatorium of San Francisco de Borja de Fontilles (Alicante, Spain). Clinical examination was carried out to evaluate the decayed missing and filled index, and the periodontal status in the anterior maxilla, using the Loe-Silness dental plaque index, mean periodontal probing depth and the average periodontal attachment loss. **RESULTS:** In the leprosy patients, a large proportion of maxillary incisors and canines were missing. The mean plaque index in leprosy was  $2.35 \pm 0.7$ , with a probing depth of  $2.96 \pm 0.8$ , and an average attachment loss of  $4.18 \pm 1.3$ , indices all statistically greater than in controls. There were no differences detected in the oral indices measured according to the presence or absence of facial destruction or the type of leprosy. **CONCLUSIONS:** Patients with leprosy show a tendency to poor dental and periodontal health, unrelated to the presence of facial destruction or the type of leprosy.—Authors' Abstract

**Pignataro, P., Rocha Ada, S., Nery, J. A., Miranda, A., Sales, A. M., Ferreira, H., Valentim, V., and Suffys, P. N.** Leprosy and AIDS: two cases of increasing inflammatory reactions at the start of highly active antiretroviral therapy. *Eur. J. Clin. Microbiol. Infect. Dis.* **23**(5) (2004) 408–411.

Reported here are the cases of two HIV-positive patients with skin lesions suggestive of leprosy, based on clinical and pathological analysis, which worsened during the few weeks following initiation of highly active antiretroviral therapy. The lesions improved after a few weeks of multidrug therapy for leprosy. *Mycobacterium leprae* was confirmed by polymerase chain reaction analysis of blood in case 1 and of a biopsy sample in case 2. Neither *Mycobacterium avium* complex nucleic acid, which is usually associated with immune restoration syndrome, nor mycobacterial cutaneous manifestations were detected in either case.—Authors' Abstract

**Richardus, J. H., Nicholls, P. G., Croft, R. P., Withington, S. G., and Smith, W. C.** Incidence of acute nerve function impairment and reactions in leprosy: a

prospective cohort analysis after 5 years of follow-up. *Int. J. Epidemiol.* **33**(2) (2004) 337–343

**BACKGROUND:** Nerve function impairment (NFI) is the key outcome of the pathological processes of infection with *Mycobacterium leprae*, which can continue after completion of multidrug therapy (MDT) and lead to disability after leprosy patients are released from treatment. The objective of this study was to assess the need for and duration of surveillance of NFI. **METHODS:** Prospective cohort study of 2664 new leprosy patients in Bangladesh, with an observation period of 36 months in paucibacillary (PB) patients, and 60 months in multibacillary (MB) patients. Incidence rates (IR) were calculated with the number of patients developing NFI, type 1 and type 2 reactions, and silent neuritis for the first time after registration as the numerator, and cumulative person-years at risk (PYAR) as the denominator. Survival curves to the first event of NFI were also calculated. **RESULTS:** The IR of first event of NFI amongst MB patients was 16.1 per 100 PYAR, with 121/357 (34%) developing NFI during the observation period. Of the 121 with a first event of NFI, 77 (64%) had this within a year after registration, 35 (29%) in the second year, and the remaining 9 (7%) after 2 years. The IR of first event of NFI amongst PB patients was 0.9 per 100 PYAR, with 54/2153 (2.5%) developing NFI during the observation period. Of the 54 with a first event of NFI, 48 (89%) had this within a year after registration, 3 (5.5%) in the second year, and the remaining 3 (5.5%) cases after 2 years. The percentage of PB patients with no NFI at registration surviving without developing NFI during the observation period was 99% and for PB patients with NFI at registration 92%. In MB patients without NFI at registration, the percentage surviving with no NFI during the observation period was 84% and for MB patients with NFI at registration only 36%. **CONCLUSION:** New episodes of NFI and reactions after registration are common, in particular in MB patients with long-standing NFI at registration. The study highlights the importance of continuing surveillance for NFI of this risk group after registration for 2 years. Active surveillance beyond 2 years is not indicated.—Authors' Abstract

## Immunopathology

**Aoki, N., Zganiacz, A., Margetts, P., and Xing, Z.** Differential regulation of DAP12 and molecules associated with DAP12 during host responses to mycobacterial infection. *Infect. Immun.* **72**(5) (2004) 2477–2483.

DAP12 and its associating molecules MDL-1, TREM-1, and TREM-2 are the recently identified immune regulatory molecules, expressed primarily on myeloid cells including monocytes/macrophages, dendritic cells, NK cells, and neutrophils. However, little is known about the regulation of their expression during host antimicrobial responses. We have investigated the effect of pulmonary mycobacterial infection and type 1 cytokines on the expression of these molecules both *in vivo* and *in vitro*. While DAP12 was constitutively expressed at high levels in the lungs, the MDL-1, TREM-1, and TREM-2 molecules were inducible during mycobacterial infection. Their kinetic expression was correlated with that of the type 1 cytokines tumor necrosis factor alpha (TNF-alpha) and gamma interferon (IFN-gamma). In primary lung macrophage cultures, high constitutive levels of DAP12 and TREM-2 were not modulated by mycobacterial or type 1 cytokine exposure. In contrast, expression of both MDL-1 and TREM-1 was markedly induced by mycobacterial infection and such induction was inhibited by concurrent exposure to IFN-gamma. On mycobacterial infection of TNF-alpha(-/-) and IFN-gamma(-/-) mice *in vivo* or their lung macrophages *in vitro*, TNF-alpha was found to be critical for mycobacterially induced MDL-1, but not TREM-1, expression whereas IFN-gamma negatively regulated mycobacterially induced MDL-1 and TREM-1 expression. Our findings thus suggest that DAP12 and its associating molecules are differentially regulated by mycobacterial infection and type 1 cytokines and that MDL-1- and TREM-1-triggered DAP12 signaling may play an important role in antimicrobial type 1 immunity.—Authors' Abstract

**Clayton, D., Shapovalov, G., Maurer, J. A., Dougherty, D. A., Lester, H. A., and Kochendoerfer, G. G.** Total chemical synthesis and electrophysiological characterization of mechanosensitive channels from *Escherichia coli* and *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U. S. A.* **101**(14) (2004) 4764–4769.

Total chemical protein synthesis was used to generate multimilligram quantities of the mechanosensitive channel of large conductance from *Escherichia coli* (Ec-MscL) and *Mycobacterium tuberculosis* (Tb-MscL). Cysteine residues introduced to allow chemical ligation were masked with cysteine-reactive molecules, resulting in side chain functional groups similar to those of the wild-type protein. Synthetic channel proteins were transferred to 2,2,2-trifluoroethanol and reconstituted into vesicle membranes. Fluorescent imaging of vesicles showed that channel proteins were membrane-localized. Single-channel recordings showed that reconstituted synthetic Ec-MscL has conductance, pressure dependence, and substate distribution similar to those of the recombinant channel. Reconstituted synthetic Tb-MscL also displayed conductance and pressure dependence similar to that of the recombinant protein. Possibilities for the incorporation of unnatural amino acids and biophysical probes, and applications of such synthetic ion channel analogs, are discussed.—Authors' Abstract

**Demangel, C., Brodin, P., Cockle, P. J., Brosch, R., Majlessi, L., Leclerc, C., and Cole, S. T.** Cell envelope protein PPE68 contributes to *Mycobacterium tuberculosis* RD1 immunogenicity independently of a 10-kilodalton culture filtrate protein and ESAT-6. *Infect. Immun.* **72**(4) (2004) 2170–2176.

The protective efficacy of *Mycobacterium bovis* BCG can be markedly augmented by stable integration of *Mycobacterium tuberculosis* genomic region RD1. BCG comple-

mented with RD1 (BCG::RD1) encodes nine additional proteins. Among them, 10-kDa culture filtrate protein (CFP-10) and ESAT-6 (6-kDa early secreted antigenic target) are low-molecular-weight proteins that induce potent Th1 responses. Using pools of synthetic peptides, we have examined the potential immunogenicity of four other RD1 products (PE35, PPE68, Rv3878, and Rv3879c). PPE68, the protein encoded by rv3873, was the only one to elicit gamma interferon (IFN-gamma)-producing cells in C57BL/6 mice infected with *M. tuberculosis*. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of rv3873 in BCG::RD1 did not modify CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.—Authors' Abstract

**Esin, S., Batoni, G., Pardini, M., Favilli, F., Bottai, D., Maisetta, G., Florio, W., Vanacore, R., Wigzell, H., and Campa, M.** Functional characterization of human natural killer cells responding to *Mycobacterium bovis* bacille Calmette-Guerin. *Immunology* **112**(1) (2004) 143–152.

The kinetics of activation and induction of several effector functions of human natural killer (NK) cells in response to *Mycobacterium bovis* bacille Calmette-Guerin (BCG) were investigated. Owing to the central role of monocytes/macrophages (MM) in the initiation and maintenance of the immune response to pathogens, two different experimental culture conditions were analysed. In the first, monocyte-depleted nylon wool non-adherent (NW) cells from healthy donors were stimulated with autologous MM preinfected with BCG (intracellular BCG). In the second, the NW cells were directly incubated with BCG, which was therefore extracellular. In the presence of MM, CD4+ T lymphocytes were the cell subset mainly expressing the activation

marker, CD25, and proliferating with a peak after 7 days of culture. In contrast, in response to extracellular BCG, the peak of the proliferative response was observed after 6 days of stimulation, and CD56+ CD3– cells (NK cells) were the cell subset preferentially involved. Such proliferation of NK cells did not require a prior sensitization to mycobacterial antigens, and appeared to be dependent upon contact between cell populations and bacteria. Following stimulation with extracellular BCG, the majority of interferon-gamma (IFN-gamma)-producing cells were NK cells, with a peak IFN-gamma production at 24–30 hr. Interleukin (IL)-2 and IL-4 were not detectable in NK cells or in CD3+ T lymphocytes at any time tested. IL-12 was not detectable in the culture supernatant of NW cells stimulated with extracellular BCG. Compared to the non-stimulated NW cells, the NW cells incubated for 16–20 hr with BCG induced the highest levels of expression of apoptotic/ death marker on the NK-sensitive K562 cell line. BCG also induced expression of the activation marker, CD25, and proliferation, IFN-gamma production and cytotoxic activity, on negatively selected CD56+ CD3– cells. Altogether, the results of this study demonstrate that extracellular mycobacteria activate several NK-cell functions and suggest a possible alternative mechanism of NK-cell activation as the first line of defence against mycobacterial infections.—Authors' Abstract

**Fairbairn, I. P.** Macrophage apoptosis in host immunity to mycobacterial infections. *Biochem. Soc. Trans.* **32**(Pt 3) (2004) 496–498.

Macrophage apoptosis occurs within the granuloma, which is essential for successful immunity to tuberculosis. *In vitro* macrophage apoptosis is associated with the killing of intracellular *Mycobacterium tuberculosis*. A greater understanding of these observations will lead to new immunotherapies and improved vaccine design. The relevant apoptotic stimuli, the anti-mycobacterial mechanisms that they stimulate and their physiological relevance are reviewed in this paper.—Author's Abstract



**Florido, M., Goncalves, A. S., Gomes, M. S., and Appelberg, R.** CD40 is required for the optimal induction of protective immunity to *Mycobacterium avium*. Immunology **111**(3) (2004) 323–327.

C57Bl/6 mice and mice deficient in the CD40 molecule were infected with three strains of *Mycobacterium avium*. Two of the *M. avium* strains proliferated more extensively in CD40-deficient (CD40<sup>-/-</sup>) mice than in control mice. The increased susceptibility to infection of CD40<sup>-/-</sup> mice was associated with the generation of poorer interleukin-12 (IL-12) p40 and interferon-gamma (IFN-gamma) responses as compared to the controls, suggesting a role for CD40 in the development of protective immunity. In contrast, direct triggering of CD40 on infected macrophages failed to induce any anti-mycobacterial activity in infected macrophages.—Authors' Abstract

**Gockel, H. R., Lugerling, A., Heidemann, J., Schmidt, M., Domschke, W., Kucharzik, T., and Lugerling, N.** Thalidomide induces apoptosis in human monocytes by using a cytochrome c-dependent pathway. J. Immunol. **172**(8) (2004) 5103–5109.

Thalidomide has been shown to be an effective treatment in various immunologic diseases such as Crohn's disease and rheumatoid arthritis. Its major effect is thought to be mediated by the inhibition of TNF-alpha, but the exact mechanism of action is still uncertain. Recent observations could demonstrate that the induction of monocyte apoptosis is a common feature of a variety of anti-inflammatory agents. Therefore, we investigated the role of thalidomide on monocyte apoptosis. Treatment with thalidomide resulted in apoptosis of human peripheral blood monocytes in a time- and dose-dependent manner as demonstrated by annexin V staining. Monocyte apoptosis required the activation of caspases, as combined stimulation by thalidomide together with the broad caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone markedly prevented monocyte cell death. Apoptosis was triggered by a CD95/CD95 ligand, TNF-RI, and

TRAIL-R1 independent pathway with an inhibition of AKT-1 kinase and consecutive mitochondrial release of cytochrome c, followed by the proteolytic activation of initiator caspase-9 and effector caspase-3. Our data suggest that thalidomide-induced monocyte apoptosis is at least partially mediated by a mitochondrial signaling pathway and might contribute to the complex immunomodulatory properties of the drug.—Authors' Abstract

**Gomes, M. S., Florido, M., Cordeiro, J. V., Teixeira, C. M., Takeuchi, O., Akira, S., and Appelberg, R.** Limited role of the Toll-like receptor-2 in resistance to *Mycobacterium avium*. Immunology **111**(2) (2004) 179–185.

The role of the Toll-like receptor (TLR)-2 in the generation of protective immunity to *Mycobacterium avium* was evaluated using gene-disrupted mice. TLR-2<sup>-/-</sup> mice were more susceptible than wild-type C57Bl/6 mice to *M. avium* strains that were able to proliferate *in vivo* before the development of protective immunity and mycobacteriostasis. In contrast, the elimination of non-virulent strains was not affected by the mutation. The generation of interferon-gamma (IFN-gamma)-producing T cells and the expression of the interleukin-12 p40 gene were reduced in TLR-2-deficient mice as compared to C57Bl/6 mice early during infection with *M. avium* strain 2447. The generation of protective CD4<sup>+</sup> T cells was also compromised in the mutated mice as compared with the controls. Our data show that TLR-2 is required for optimal immunity against certain virulent *M. avium* strains.—Authors' Abstract

**Gupta, G., Singh, H. B., Om Parkash, Girdhar, B. K., and Katoch, V. M.** Detection of *Mycobacterium leprae* DNA in blood samples from patients: a preliminary study. J. Immunol. Immunopathol. **6**(S-1) (2004) 144–145.

**Hmama, Z., Sendide, K., Talal, A., Garcia, R., Dobos, K., and Reiner, N. E.** Quantitative analysis of phagolysosome fusion in intact cells: inhibition by mycobacterial lipoarabinomannan and rescue by an 1 $\alpha$ ,25-dihydroxyvitamin D3-phosphoinositide 3-kinase pathway. *J. Cell. Sci.* **117**(Pt 10) (2004) 2131–2140.

Macrophage cell membranes were labeled with PKH26 and subsequently incubated with latex beads to generate phagosomes surrounded by a red-fluorescent membrane suitable for flow cytometry. Following cell disruption and partial purification of phagosomes, these vesicles were readily distinguished from both cell debris and free beads released from disrupted vacuoles. Flow cytometry analysis of phagosomes stained with specific mAbs and FITC-labeled secondary antibodies showed progressive acquisition of both Rab7 and LAMP-1 consistent with movement along the endocytic pathway. Alternatively, macrophages were preloaded with the lysosomal tracer FITC-dextran before membrane labeling with PKH and incubation with latex beads. Phagosome-lysosome fusion was then quantified on the basis of the colocalization of red and green signals. Using these flow cytometry-based systems, we showed that co-internalization of beads with lysates of *Mycobacterium tuberculosis*, but not lysates from the nonpathogenic organism *Mycobacterium smegmatis*, markedly decreased phagosome acquisition of Rab7 and LAMP-1 and vesicle fusion with FITC-dextran-loaded lysosomes. Inhibition of phagolysosome fusion could be attributed, at least in part, to the mycobacterial cell wall glycolipid lipoarabinomannan, and further analysis showed complete rescue of phagosome maturation when cells were pretreated with vitamin D3 before exposure to lipoarabinomannan. Moreover, the ability of vitamin D3 to reverse the phenotype of phagosomes in the presence of the glycolipid was completely abrogated by LY-294002, suggesting that vitamin D3 promotes phagolysosome fusion via a phosphoinositide 3-kinase signaling pathway. These findings establish a robust platform technology based on labeling of phagocyte cell membranes and flow cytometry capable of supporting broad-based screens to identify microbial and other

bioactive compounds that influence phagosome biology.—Authors' Abstract

**Kim, B. J., Lee, K. H., Yun, Y. J., Park, E. M., Park, Y. G., Bai, G. H., Cha, C. Y., and Kook, Y. H.** Simultaneous identification of rifampin-resistant *Mycobacterium tuberculosis* and nontuberculous mycobacteria by polymerase chain reaction-single strand conformation polymorphism and sequence analysis of the RNA polymerase gene (rpoB). *J. Microbiol. Methods* **58**(1) (2004) 111–118.

Interspecies variations and mutations associated with rifampin resistance in rpoB of *Mycobacterium* allow for the simultaneous identification of rifampin-resistant *Mycobacterium tuberculosis* and nontuberculous mycobacteria by PCR-SSCP analysis and PCR-sequencing. One hundred and ten strains of rifampin-susceptible *M. tuberculosis*, 14 strains of rifampin-resistant *M. tuberculosis*, and four strains of the *M. avium* complex were easily identified by PCR-SSCP. Of another seven strains, which showed unique SSCP patterns, three were identified as rifampin-resistant *M. tuberculosis* and four as *M. terrae* complex by subsequent sequence analysis of their rpoB DNAs (306 bp). These results were concordant with those obtained by susceptibility testing, biochemical identification, and 16S rDNA sequencing.—Authors' Abstract

**Kim, B. J., Hong, S. K., Lee, K. H., Yun, Y. J., Kim, E. C., Park, Y. G., Bai, G. H., and Kook, Y. H.** Differential identification of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria by duplex PCR assay using the RNA polymerase gene (rpoB). *J. Clin. Microbiol.* **42**(3) (2004) 1308–1312.

A novel duplex PCR method that can amplify the 235- and 136-bp rpoB DNAs of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria (NTM), respectively, with two different sets of primers was used to differentially identify 44 reference strains and 379 clinical isolates of mycobacteria in a single-step assay. Showing 100% sensitivity and specificity, the duplex

PCR method could clearly differentiate *M. tuberculosis* complex and NTM strains. In addition, restriction fragment length polymorphism analysis and direct sequencing of the amplicon of NTM could be used to supplement species identification.—Authors' Abstract

**Koul, A., Herget, T., Klebl, B., and Ullrich, A.** Interplay between mycobacteria and host signalling pathways. *Nat. Rev. Microbiol.* **2**(3) (2004) 189–202.

Pathogenesis by mycobacteria requires the exploitation of host-cell signalling pathways to enhance the intracellular survival and persistence of the pathogen. The disruption of these pathways by mycobacteria causes impaired maturation of phagosomes into phagolysosomes, modulates host-cell apoptotic pathways and suppresses the host immune response. This review highlights the strategies employed by mycobacteria to subvert host-cell signalling and identifies key molecules involved in these processes that might serve as potential targets for new antimycobacterial therapies.—Authors' Abstract

**Marino, S., and Kirschner, D. E.** The human immune response to *Mycobacterium tuberculosis* in lung and lymph node. *J. Theor. Biol.* **227**(4) (2004) 463–486.

A key issue for the study of tuberculosis is to understand why individuals infected with *Mycobacterium tuberculosis* (Mtb) experience different clinical outcomes. To better understand the dynamics of Mtb infection and immunity, we have previously developed a temporal mathematical model that qualitatively and quantitatively characterizes the cellular and cytokine control network during infection. In this work we extend that model to a two compartmental model to capture the important processes of cellular activation and priming that occur between the lung and the nearest draining lymph node. We are able to reproduce typical disease progression scenarios including primary infection, latency or clearance. Then we use the model to predict key processes determining these different dis-

ease trajectories (i.e., identify bifurcation parameters), suggesting directions for further basic science study and potential new treatment strategies.—Authors' Abstract

**McGarvey, J. A., Wagner, D., and Bermudez, L. E.** Differential gene expression in mononuclear phagocytes infected with pathogenic and non-pathogenic mycobacteria. *Clin. Exp. Immunol.* **136**(3) (2004) 490–500.

The pathogenic mycobacteria are an insidious group of bacterial pathogens that cause the deaths of millions of people every year. One of the reasons these pathogens are so successful is that they are able to invade and replicate within host macrophages, one of the first lines of defence against intruding pathogens. In contrast, non-pathogenic mycobacteria, such as *Mycobacterium smegmatis* are killed rapidly by macrophages. In order to understand better the series of events that allow pathogenic mycobacteria to survive and replicate within macrophages, while the non-pathogenic mycobacteria are killed rapidly, we inoculated the human monocytic cell line U937 with pathogenic (*M. tuberculosis* and *M. avium*) and non-pathogenic (*M. smegmatis*) mycobacteria and monitored the expression of over 3500 genes at 4, 12 and 24 hr post-inoculation using a commercially available gene array system. We observed multiple differences in the gene expression patterns of monocytes infected with pathogenic and non-pathogenic mycobacteria including genes involved in cytokine, lymphokine and chemokine production, adhesion, apoptosis, signal transduction, transcription, protein cleavage, actin polymerization and growth. We also observed differences in gene expression profiles in monocytes infected with *M. tuberculosis* or *M. avium*, indicating that there are differences in the host pathogen interactions of mononuclear phagocytes infected with different pathogenic mycobacterial species. These results increase the understanding of the mechanisms used by pathogenic mycobacteria to cause disease, the host response to these organisms, and provide new insights for antimycobacterial intervention strategies.—Authors' Abstract

**Miller, B. H., Fratti, R. A., Poschet, J. F., Timmins, G. S., Master, S. S., Burgos, M., Marletta, M. A., and Deretic, V.** Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. *Infect. Immun.* **72(5)** (2004) 2872–2878.

Inducible nitric oxide synthase (iNOS) is a cytoplasmic protein responsible for the generation of nitric oxide (NO.) in macrophages. In this work, we hypothesized that the intracellular localization of iNOS is significant for effective delivery of NO. to phagosomes containing ingested microorganisms. Using immunofluorescence microscopy and Western blot analysis, iNOS was shown to localize in the vicinity of phagosomes containing latex beads in stimulated macrophages. iNOS also localized to phagosomes containing *Escherichia coli*. The colocalization of iNOS with ingested latex beads was an actin-dependent process, since treatment with the actin microfilament disrupter cytochalasin D prevented iNOS recruitment to latex bead phagosomes. In contrast to *E. coli* and inert particle phagosomes, mycobacterial phagosomes did not colocalize with iNOS. This study demonstrates that (i). iNOS can be recruited to phagosomes; (ii). this recruitment is dependent on a functional actin cytoskeleton; (iii). certain microorganisms have the ability to prevent or reduce colocalization with iNOS; and (iv). spatial exclusion of iNOS may play a role in *Mycobacterium tuberculosis* pathogenesis.—Authors' Abstract

**Orme, I.** Adaptive immunity to mycobacteria. *Curr. Opin. Microbiol.* **7(1)** (2004) 58–61.

Advances are now being made in terms of understanding both the initiation of the adaptive or acquired response to tuberculosis infection and its interface with elements of the innate response, as well as much later events in terms of the chronic disease state where reactivation can potentially occur. Despite this, there are still several elements of the adaptive response that remain poorly understood.—Author's Abstract

**Pais, T. F., and Appelberg, R.** Induction of *Mycobacterium avium* growth restriction and inhibition of phagosome-endosome interactions during macrophage activation and apoptosis induction by picolinic acid plus IFN $\gamma$ . *Microbiology* **150(Pt 5)** (2004) 1507–1518.

Treatment of mouse macrophages with picolinic acid (PA) and gamma-interferon (IFN $\gamma$ ) led to the restriction of *Mycobacterium avium* proliferation concomitant with the sequential acquisition of metabolic changes typical of apoptosis, mitochondrial depolarization, annexin V staining and caspase activation, over a period of up to 5 days. However, triggering of cell death by ATP, staurosporine or H(2)O(2) failed to affect mycobacterial viability. In contrast to untreated macrophages where extensive interactions between phagosomes and endosomes were observed, phagosomes from treated macrophages lost the ability to acquire endosomal dextran. N-Acetylcysteine was able to revert both the anti-mycobacterial activity of treated macrophages as well as the block in phagosome-endosome interactions. The treatment, however, induced only a minor increase in the acquisition of lysosomal markers, namely Lamp-1, and did not increase to any great extent the acidification of the phagosomes. These data thus suggest that the anti-mycobacterial activity of PA and IFN $\gamma$  depends on the interruption of intracellular vesicular trafficking, namely the blocking of acquisition of endosomal material by the microbe.—Authors' Abstract

**Puissegur, M. P., Botanch, C., Duteyrat, J. L., Delsol, G., Caratero, C., and Altare, F.** An *in vitro* dual model of mycobacterial granulomas to investigate the molecular interactions between mycobacteria and human host cells. *Cell. Microbiol.* **6(5)** (2004) 423–433.

In the majority of individuals infected with *Mycobacterium tuberculosis*, the bacilli cause a long-term asymptomatic infection called latent tuberculosis, a state during which the bacilli reside within granulomas. Latently infected individuals have around 10% risk of progression to clinical disease at a later stage. Determining the state of the



mycobacteria and the host cells during this latent phase, i.e., within the granulomas, would greatly improve our understanding of the physiopathology of tuberculosis, and thus enable the development of new therapeutic means to treat the one-third of the world's population who are latently infected. We have developed an *in vitro* model of human mycobacterial granulomas, enabling the cellular and molecular analysis of the very first steps in the host granulomatous response to either mycobacterial compounds or live mycobacterial species. *In vitro* mycobacterial granulomas mimic natural granulomas very well, with the progressive recruitment of macrophages around live bacilli or mycobacterial antigen-coated beads, their differentiation into multinucleated giant cells and epithelioid cells, and the final recruitment of a ring of activated lymphocytes. Besides morphological similarities, *in vitro* granulomas also functionally resemble natural ones, with the development of intense cellular co-operation and intracellular mycobactericidal activities.—Authors' Abstract

**Rook, G. A., Adams, V., Hunt, J., Palmer, R., Martinelli, R., and Brunet, L. R.** Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin. Immunopathol. **25**(3–4) (2004) 237–255.

In the rich, developed parts of the world there has been a steady and simultaneous increase in at least three groups of disease: (1) allergies, (2) inflammatory bowel diseases (IBD; e.g., Crohn's disease and ulcerative colitis) and (3) autoimmunity (e.g., type 1 diabetes and multiple sclerosis). Because the medical world is so compartmentalised it was some time before the connection between these increases was noticed and understood. There is now evidence that the simultaneous increase in these diseases of immunodysregulation is at least partly attributable to malfunction of regulatory T cells (Treg). This paper provides an overview of relevant work in each of these fields of medicine (though with emphasis on the allergic disorders), and concludes that the increasing failure of Treg is a consequence of diminished exposure to certain

micro-organisms that are "old friends," because of their continuous presence throughout mammalian evolution. These organisms, which include saprophytic mycobacteria, helminths and lactobacilli, are recognised by the innate immune system as harmless, and as adjuvants for Treg induction. Polymorphisms of components of the innate immune system such as TLR2 and NOD2 appear to define subsets of the population that will develop immunoregulatory disorders when living in the modern environment. A further role of the "old friends" and of the Treg that they induce might be to maintain the levels of regulatory IL-10 secreting macrophages and antigen-presenting cells, which are depleted in asthma and Crohn's disease. These concepts are leading to novel therapies based on harmless organisms or their components. Phase I/II clinical trials have yielded some statistically significant results, and phase II trials are in progress.—Authors' Abstract

**Shimizu, T., Sano, C., and Tomioka, H.**

The role of B7 molecules in the cell contact-mediated suppression of T cell mitogenesis by immunosuppressive macrophages induced with mycobacterial infection. Clin. Exp. Immunol. **135**(3) (2004) 373–379.

We found previously that immunosuppressive macrophages (Mphis) induced by *Mycobacterium intracellulare* infection (MI-Mphis) transmitted their suppressor signals to target T cells through cell contact with target T cells. In this study, we examined what kinds of Mphi surface molecules are required for such cell-to-cell interaction. First, it was found that a B7-1-like molecule (B7-1LM) recognizable with one of three test clones of anti-B7-1 monoclonal antibodies (mAbs) was required for expression of the Mphi suppressor activity. Neither anti-B7-2, anti-ICAM-1, nor anti-VCAM-1 mAb blocked the Mphi suppressor activity. Second, MI-Mphis increased the expression of B7-1LM in parallel with the acquisition of the suppressor activity. Moreover, MI-Mphis bound with target T cells in a B7-1LM-dependent fashion. Third, mAb blocking of CTLA-4 on target T cells did not reduce the suppressor activity of MI-Mphis,

suggesting the role of a putative molecule on target T cells other than CTLA-4 as the receptor for B7-1LM of MI-Mphis. Fourth, concanavalin A (Con A) stimulation of MI-Mphis was needed for effective cell contact with target T cells and subsequent expression of the suppressor activity of MI-Mphis. Fifth, the Con A-induced increase in the suppressor activity of MI-Mphis was inhibited by KN-62 but not by herbimycin A, H-7, nor H-88, indicating that Con A-induced up-regulation of MI-Mphi function is mediated by calmodulin-dependent protein kinase II or ATP/P2Z receptors, but independent of protein tyrosine kinase, protein kinase C, and protein kinase A. These findings indicate that a B7/CTLA-4-independent mechanism is needed for the transmission of the suppressor signals from MI-Mphis to target T cells.—Authors' Abstract

**Stermann, M., Sedlacek, L., Maass, S., and Bange, F. C.** A promoter mutation causes differential nitrate reductase activity of *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *J. Bacteriol.* **186(9)** (2004) 2856–2861.

The recent publication of the genome sequence of *Mycobacterium bovis* showed >99.95% identity to *M. tuberculosis*. No genes unique to *M. bovis* were found. Instead numerous single-nucleotide polymorphisms (SNPs) were identified. This has led to the hypothesis that differential gene expression due to SNPs might explain the differences between the human and bovine tubercle bacilli. One phenotypic distinction between *M. tuberculosis* and *M. bovis* is nitrate reduction, which not only is an essential diagnostic tool but also contributes to mycobacterial pathogenesis. We previously showed that narGHJI encodes a nitrate reductase in both *M. tuberculosis* and *M. bovis* and that NarGHJI-mediated nitrate reductase activity was substantially higher in the human tubercle bacillus. In the present study we used a genetic approach to demonstrate that an SNP within the promoter of the nitrate reductase gene cluster narGHJI is responsible for the different nitrate reductase activity of *M. tuberculosis* and *M. bovis*. This is the first example of an SNP that leads to differential gene expression between the

human and bovine tubercle bacilli.—Authors' Abstract

**Talaat, A. M., Lyons, R., Howard, S. T., and Johnston, S. A.** The temporal expression profile of *Mycobacterium tuberculosis* infection in mice. *Proc. Natl. Acad. Sci. U.S.A.* **101(13)** (2004) 4602–4607.

See *Current Literature, Molecular and Genetic Studies*, p. 420.

**Thakurdas, S. M., Hasan, Z., and Husain, R.** IgG1 antimycobacterial antibodies can reverse the inhibitory effect of pentoxifylline on tumor necrosis factor alpha (TNF-alpha) secreted by mycobacterial antigen-stimulated adherent cells. *Clin. Exp. Immunol.* **136(2)** (2004) 320–327.

Chronic inflammation associated with cachexia, weight loss, fever and arthralgia is the hallmark of advanced mycobacterial diseases. These symptoms are attributed to the chronic stimulation of tumour necrosis factor (TNF)-alpha. Mycobacterial components directly stimulate adherent cells to secrete TNF-alpha. We have shown recently that IgG1 antimycobacterial antibodies play a role in augmenting TNF-alpha in purified protein derivative (PPD)-stimulated adherent cells from non-BCG-vaccinated donors. We now show that IgG1 antibodies can also augment TNF-alpha expression in stimulated adherent cells obtained from BCG-vaccinated donors and this augmentation is not linked to interleukin (IL)-10 secretion. In addition IgG1 antimycobacterial antibodies can reverse the effect of TNF-alpha blockers such as pentoxifylline and thalidomide. These studies therefore have clinical implications for anti-inflammatory drug treatments which are used increasingly to alleviate symptoms associated with chronic inflammation.—Authors' Abstract

**Torres, D., Barrier, M., Bihl, F., Quesniaux, V. J., Maillet, I., Akira, S., Ryffel, B., and Erard, F.** Toll-like receptor 2 is required for optimal control of *Listeria*

*monocytogenes* infection. Infect. Immun. **72**(4) (2004) 2131–2139.

The control of *Listeria monocytogenes* infection depends on the rapid activation of the innate immune system, likely through Toll-like receptors (TLR), since mice deficient for the common adapter protein of TLR signaling, myeloid differentiation factor 88 (MyD88), succumb to *Listeria* infection. In order to test whether TLR2 is involved in the control of infections, we compared the host response in TLR2-deficient mice with that in wild-type mice. Here we show that TLR2-deficient mice are more susceptible to systemic infection by *Listeria* than are wild-type mice, with a reduced survival rate, increased bacterial burden in the liver, and abundant and larger hepatic microabscesses containing increased numbers of neutrophils. The production of tumor necrosis factor, interleukin-12, and nitric oxide and the expression of the costimulatory molecules CD40 and CD86, which are necessary for the control of infection, were reduced in TLR2-deficient macrophages and dendritic cells stimulated by *Listeria* and were almost abolished in the absence of MyD88, coincident with the high susceptibility of MyD88-deficient mice to *in vivo* infection. Therefore, the present data demonstrate a role for TLR2 in the control of *Listeria* infection, but other MyD88-dependent signals may contribute to host resistance.—Authors' Abstract

**Toyoda, H., Ido, M., Hayashi, T., Gabazza, E. C., Suzuki, K., Bu, J., Tanaka, S., Nakano, T., Kamiya, H., Chipeta, J., Kisenge, R. R., Kang, J., Hori, H., and Komada, Y.** Impairment of IL-12-dependent STAT4 nuclear translocation in a patient with recurrent *Mycobacterium avium* infection. J. Immunol. **172**(6) (2004) 3905–3912.

We examined the immunological abnormality in a patient with recurrent *Mycobacterium avium* infection. T cells from the patient showed decreased ability both to produce IFN- $\gamma$  and to proliferate in response to IL-12. Despite decreased expression of IL-12R  $\beta$ 1 and  $\beta$ 2 chains in the patient's PHA-activated T cells, there was no differ-

ence in IL-12-induced tyrosine and serine phosphorylation of STAT4 in PHA-activated T cells between the patient and healthy subjects, suggesting that IL-12R signals are transmitted to STAT4 in the patient's PHA-activated T cells. Using EMSA, confocal laser microscopy, and Western blotting, we demonstrated that the nuclear translocation of STAT4 in response to IL-12 is reduced in PHA-activated T cells from the patient when compared with those from healthy subjects. Leptomycin B was used to examine whether nuclear export of STAT4 is increased in the patient's T cells. However, leptomycin B treatment did not reverse impaired IL-12-induced nuclear accumulation of STAT4. Although the exact mechanism responsible for the impaired STAT4 nuclear translocation in this patient remains unclear, the absence of mutation in the IL-12R $\beta$ 1, IL-12R $\beta$ 2, STAT4, and STAT4-binding sequence of the IFN- $\gamma$  gene and preservation of STAT4 tyrosine and serine phosphorylation suggest the existence of a defective STAT4 nuclear translocation. This defect is likely responsible for the impaired STAT4 nuclear translocation in IL-12-stimulated T cells, leading to impairment of both IFN- $\gamma$  production and cell proliferation. To the best of our knowledge, this is the first report of a patient with atypical mycobacterial infection associated with impairment of STAT4 nuclear translocation.—Authors' Abstract

**van Faassen, H., Dudani, R., Krishnan, L., and Sad, S.** Prolonged antigen presentation, APC-, and CD8+ T cell turnover during mycobacterial infection: comparison with *Listeria monocytogenes*. J. Immunol. **172**(6) (2004) 3491–3500.

We expressed the CTL epitope of OVA (OVA(257–264)) in an acute (*Listeria monocytogenes* (LM)-OVA) and a chronic intracellular pathogen (*Mycobacterium bovis* (BCG)-OVA), to evaluate the kinetics of Ag presentation. LM-OVA proliferated rapidly *in vivo*, resulting in profound LM-OVA expansion within the first 24 hr of infection, culminating in the generation of a potent CD8+ T cell response, which peaked on day 7 but underwent a rapid attrition subsequently. In contrast, BCG-OVA exhibited reduced growth *in vivo*, resulting in a de-

layed CD8+ T cell response that increased progressively with time. Relative to LM-OVA, BCG-OVA induced persistently increased numbers of apoptotic (annexin V+) CD8+ T cells. Ag presentation *in vivo* was evaluated by transferring Thy1.2+ carboxy-fluorescein-labeled OT1 transgenic CD8+ T cells into infected Thy1.1+ congenic recipient mice. LM-OVA induced rapid Ag presentation that was profound in magnitude, with most of the transferred cells getting activated within 4 days and resulting in a massive accumulation of activated donor CD8+ T cells. In contrast, Ag presentation induced by BCG-OVA was delayed, weaker in magnitude, which peaked around the second week of infection and declined to a low level subsequently. Increasing the dose of BCG-OVA while enhancing the magnitude of Ag presentation did not change the kinetics. Furthermore, a higher dose of BCG-OVA also accelerated the attrition of OVA(257–264)-specific CD8+ T cells. Relative to LM-OVA, the dendritic cells in BCG-OVA-infected mice were apoptotic for prolonged periods, suggesting that the rapid death of APCs may limit the magnitude of Ag presentation during chronic stages of mycobacterial infection.—Authors' Abstract

**Walburger, A., Koul, A., Ferrari, G., Nguyen, L., Prescianotto-Baschong, C., Huygen, K., Klebl, B., Thompson, C., Bacher, G., and Pieters, J.** Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. *Science* **304**(5678) (2004) 1800–1804.

Pathogenic mycobacteria resist lysosomal delivery after uptake into macrophages, allowing them to survive intracellularly. We found that the eukaryotic-like serine/threonine protein kinase G from pathogenic mycobacteria was secreted within macrophage phagosomes, inhibiting phagosome-lysosome fusion and mediating intracellular survival of mycobacteria. Inactivation of protein kinase G by gene disruption or chemical inhibition resulted in lysosomal localization and mycobacterial cell death in infected macrophages. Besides identifying a target for the control of mycobacterial infections, these findings suggest that pathogenic mycobacteria have evolved eukaryotic-like signal

transduction mechanisms capable of modulating host cell trafficking pathways.—Authors' Abstract

**Wallace, C., Fitness, J., Hennig, B., Sichali, L., Mwaungulu, L., Pon-nighaus, J. M., Warndorff, D. K., Clayton, D., Fine, P. E., and Hill, A. V.** Linkage analysis of susceptibility to leprosy type using an IBD regression method. *Genes Immun.* **5**(3) (2004) 221–225.

Leprosy is a chronic disease caused by infection with *Mycobacterium leprae*, which is manifested across a wide clinical spectrum. There is evidence that susceptibility both to leprosy per se and to the clinical type of leprosy is influenced by host genetic factors. This paper describes the application of an identity by descent regression search for genetic determinants of leprosy type among families from Karonga District, Northern Malawi. Suggestive evidence was found for linkage to leprosy type on chr 21q22 ( $p < 0.001$ ). The methodological implications of the approach and the findings are discussed.—Authors' Abstract

**Wallis, R. S., Kyambadde, P., Johnson, J. L., Horter, L., Kittle, R., Pohle, M., Ducar, C., Millard, M., Mayanja-Kizza, H., Whalen, C., and Okwera, A.** A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. *AIDS.* **18**(2) (2004) 257–264.

**OBJECTIVE:** Tumor necrosis factor (TNF), an important inflammatory mediator in tuberculosis, has been implicated in causing accelerated HIV disease progression in HIV-associated tuberculosis. However, TNF blockade, particularly by monoclonal antibody, has been associated with the reactivation of latent *Mycobacterium tuberculosis* infection by the impairment of mycobacterial immunity. This phase 1 study examined the safety, microbiology, immunology, and virology of TNF blockade using etanercept (soluble TNF receptor, Enbrel) during the initial treatment of HIV-associated tuberculosis. **DESIGN:** A single-arm trial, with key endpoints compared with historical controls,



conducted in Mulago Hospital, Kampala, Uganda. **SUBJECTS::** Sixteen HIV-1-infected patients and 42 CD4-frequency-matched controls with sputum smear-positive tuberculosis and CD4 cell counts >200 cells/microl. **INTERVENTION:** Etanercept 25 mg, eight doses administered subcutaneously twice weekly beginning on day 4 of tuberculosis therapy. **MAIN OUTCOME MEASURES:** Serial examination, radiography, sputum culture, CD4 T-cell counts, plasma log<sub>10</sub> HIV-RNA copy numbers. **RESULTS:** Trends towards superior responses to tuberculosis treatment were evident in etanercept-treated subjects in body mass, performance score, number of involved lung zones, cavitory closure, and time to sputum culture conversion. Etanercept treatment resulted in a 25% increase in CD4 cells by week 4 ( $p = 0.1$  compared with controls). The change in CD4 cell count was inversely related to the change in serum neopterin, a marker of macrophage activation. There was no effect on plasma HIV RNA. **CONCLUSION:** Etanercept can be safely administered during the initial treatment of pulmonary tuberculosis. Further studies are warranted to examine the effects of etanercept on T-cell numbers, activation and apoptosis in AIDS and tuberculosis.—Authors' Abstract

**Wright, C. A., van Zyl, Y., Burgess, S. M., Blumberg, L., and Leiman, G.** Mycobacterial autofluorescence in Papanicolaou-stained lymph node aspirates: a glimmer in the dark? *Diagn. Cytopathol.* **30(4)** (2004) 257–260.

This study was undertaken to determine the value of incorporating fluorescence into cytopathological evaluation of lymph node fine-needle aspiration (FNA) specimens suspected of harboring mycobacterial species. The study population consisted of 1,044 HIV-positive and -negative patients referred for FNA to the cytopathology unit of a South African medical school located in a very high HIV prevalence region. Each aspirate was assessed on routine Papanicolaou-stained slides for morphologic characteristics of mycobacterial infection. The same glass slides were then viewed under fluorescent microscopy to determine the presence

or absence of mycobacterial autofluorescence. Using multivariate analysis, results of both cytology and fluorescence were compared with mycobacterial culture as the final arbiter of the presence of organisms. In this large clinical study, compared with culture, cytomorphology showed sensitivity of 84.9%, but low specificity of only 50.9%. Fluorescence demonstrated lower sensitivity of 65.9%, but improved specificity of 73.0%. Taken together, positivity of both cytology and fluorescence improved specificity to 81.8%. Fluorescent microscopy is rapid, inexpensive, and cost-effective; neither radioactive materials nor further staining are required. It is felt that this methodology would be of diagnostic benefit if used on morphologically suspicious samples in areas with a high prevalence of HIV and mycobacterial infections. Appropriate therapy could be commenced within hours of FNA, with reduction in the current number of patients lost to follow-up while awaiting results of culture. The technique is readily extended to other FNA types such as deep organ aspirates. Autofluorescence of organisms specifically requires usage of Papanicolaou staining; the technique cannot be used in histopathologic specimens stained with hematoxylin-eosin.—Authors' Abstract

**Yadav, M., Roach, S. K., and Schorey, J. S.**

Increased mitogen-activated protein kinase activity and TNF- $\alpha$  production associated with *Mycobacterium smegmatis*-but not *Mycobacterium avium*-infected macrophages requires prolonged stimulation of the calmodulin/calmodulin kinase and cyclic AMP/protein kinase A pathways. *J. Immunol.* **172(9)** (2004) 5588–5597.

Previous studies have shown the mitogen-activated protein kinases (MAPKs) to be activated in macrophages upon infection with *Mycobacterium*, and that expression of TNF- $\alpha$  and inducible NO synthase by infected macrophages was dependent on MAPK activation. Additional analysis demonstrated a diminished activation of p38 and extracellular signal-regulated kinase (ERK)1/2 in macrophages infected with pathogenic strains of *Mycobacterium avium* compared with infections with the fast-

growing, nonpathogenic *Mycobacterium smegmatis* and *Mycobacterium phlei*. However, the upstream signals required for MAPK activation and the mechanisms behind the differential activation of the MAPKs have not been defined. In this study, using bone marrow-derived macrophages from BALB/c mice, we determined that ERK1/2 activation was dependent on the calcium/calmodulin/calmodulin kinase II pathway in both *M. smegmatis*- and *M. avium*-infected macrophages. However, in macrophages infected with *M. smegmatis* but not *M. avium*, we observed a marked increase in cAMP production that remained elevated for 8 hr postinfection. This *M.*

*smegmatis*-induced cAMP production was also dependent on the calmodulin/calmodulin kinase pathway. Furthermore, stimulation of the cAMP/protein kinase A pathway in *M. smegmatis*-infected cells was required for the prolonged ERK1/2 activation and the increased TNF-alpha production observed in these infected macrophages. Our studies are the first to demonstrate an important role for the calmodulin/calmodulin kinase and cAMP/protein kinase A pathways in macrophage signaling upon mycobacterial infection and to show how cAMP production can facilitate macrophage activation and subsequent cytokine production.—Authors' Abstract

## Immunopathology, Leprosy

**Pradhan, V., Badakere, S. S., and Shankar Kumar, U.** Increased incidence of cytoplasmic ANCA (cANCA) and other autoantibodies in leprosy patients from western India. *Lepr. Rev.* **75**(1) (2004) 50–56.

The prevalence of various autoantibodies was studied in 75 leprosy patients comprising eight patients with lepromatous leprosy (LL), 36 patients with borderline lepromatous leprosy (BL) and 31 patients with borderline tuberculoid leprosy (BT), along with 100 normal controls. Certain autoantibodies such as anti-nuclear antibodies (ANA), anti-single stranded DNA (anti-ssDNA) and anti-neutrophil cytoplasmic antibodies (ANCA) were raised among leprosy patients. When ANCA specificities to anti-myeloperoxidase (anti-MPO), anti-proteinase3 (anti-PR3) and anti-lactoferrin (anti-LF) were studied, it was found that the patterns of immunofluorescence such as perinuclear (p-ANCA), cytoplasmic (c-ANCA) and atypical (X-ANCA) and specificity by ELISA to anti-MPO, anti-PR3 and anti-LF varied in the LL, BL and BT groups. However, a higher amount of c-ANCA was observed in 62.5% of leprosy cases, while the incidences of p-ANCA and X-ANCA were lower. The LL group showed a higher incidence of autoantibodies as compared with the BL and BT groups, along with a male preponder-

ance for autoantibody development. Some unusual antibody profiles such as 'X'-ANCA were also observed. The study suggests that autoantibody formation could be quite prevalent and also variable in the spectrum of leprosy cases, and there seems to be a serological overlap among leprosy and autoimmune disease, which could have pathogenetic importance in the leprosy patients developing complications.—Authors' Abstract

**Rojas-Espinosa, O., Arenas, R., Arce-Parades, P., and Miranda-Contreras, G.** Antibodies to diverse lipids in the serum of patients with clinically cured leprosy and tuberculosis. *Acta. Leprol.* **12**(3) (2003) 112–116.

In this study we looked for the presence of antibodies to cardiolipin, cerebroside, and whole lipids extracted from *M. leprae*, *M. tuberculosis* and *M. habana*, in the serum of patients with clinically cured lepromatous leprosy (sixteen) or tuberculosis (sixteen), 8 to 12 months after arresting the corresponding multi-drug therapy (MDT). Compared to healthy controls (sixteen), both leprosy and tuberculosis ex-patients had still significant levels of antibodies to the three mycobacterial lipids but no detectable levels of antibodies to cardiolipin or cerebroside

lipids. Although leprosy and tuberculosis sera recognized the homologous mycobacterial lipids in a preferential fashion, all of them, on the average, reacted more strongly with the lipids of *M. habana*. This observation backs up, in a certain way, the proposition of using *M. habana* as a prospective vaccine for leprosy and tuberculosis.—Authors' Abstract

**Schon, T., Hernandez-Pando, R., Baquera-Heredia, J., Negesse, Y., Becerril-Villanueva, L. E., Eon-Contreras, J. C., Sundqvist, T., and Britton, S.** Nitrotyrosine localization to dermal nerves in borderline leprosy. *Br. J. Dermatol.* **150**(3) (2004) 570–574.

**BACKGROUND:** Nerve damage is a common and disabling feature of leprosy, with unclear aetiology. It has been reported that the peroxidizing agents of myelin lipids-nitric oxide (NO) and peroxynitrite—are produced in leprosy skin lesions. **OBJECTIVES:** To investigate the localization of nitrotyrosine (NT)—a local end-product of peroxynitrite—in leprosy lesions where dermal nerves are affected by a granulomatous reaction. **METHODS:** We investigated by immunohistochemistry and immunoelectron microscopy the localization of the inducible NO synthase (iNOS) and NT in biopsies exhibiting dermal nerves from patients with untreated leprosy. **RESULTS:** There were abundant NT-positive and iNOS-positive macrophages in the borderline leprosy granulomas infiltrating peripheral nerves identified by light microscopy, S-100 and neurofilament immunostaining. Immunoelectron microscopy showed NT reactivity in neurofilament aggregates and in the cell wall of *Mycobacterium leprae*. **CONCLUSIONS:** Our results suggest that NO and peroxynitrite could be involved in the nerve damage following borderline leprosy.—Authors' Abstract

**Singh, N., Manucha, V., Bhattacharya, S. N., Arora, V. K., and Bhatia, A.** Pitfalls in the cytological classification of borderline leprosy in the Ridley-Jopling scale. *Diagn. Cytopathol.* **30**(6) (2004) 386–388.

This is a blinded, retrospective, correlative study of classification of leprosy by cytomorphology, clinical examination, and bacterial density. One hundred consecutive adequate aspirates from skin lesions of leprosy were studied. The Ridley-Jopling (R-J) five-group classification system was used. May-Gruenwald-Giemsa (MGG) and Ziehl-Neelsen (Z-N) stains were employed. Complete clinical, cytological, and bacteriological concordance was found in 88 patients. One-step mismatch in classification was seen in 12 patients with cytomorphological features of borderline-borderline (BB/mid-borderline) leprosy. Cytomorphological features of BB leprosy in aspirates from skin lesions should alert the cytopathologist to the possibility that the bacteriological index (BI) may vary widely. Appropriate steps must be taken to ensure accurate reporting of BI.—Authors' Abstract

**Singhi, M. K., Kachhawa, D., and Ghiya, B. C.** A retrospective study of clinico-histopathological correlation in leprosy. *Indian J. Pathol. Microbiol.* **46**(1) (2003) 47–48.

Leprosy is a chronic infectious disease caused by *Mycobacterium Leprae* and is characterized by well-recognized pathological changes. But there are various disagreement in clinical type and histological finding of leprosy. We observed highest parity in LL and TT group followed by histoid, BT, BL, BB, & indeterminate respectively. There was 10% minor disagreement (difference of one group) and 5% major disagreement (difference of two or more group). Non-specific histological finding was present in 20% cases.—Authors' Abstract

**Smith, W. C., Anderson, A. M., Withington, S. G., van Brakel, W. H., Croft, R. P., Nicholls, P. G., and Richardus, J. H.** Steroid prophylaxis for prevention of nerve function impairment in leprosy: randomised placebo controlled trial (TRIPOD 1). *BMJ.* **328**(7454) (2004) 1459.

**OBJECTIVE:** To determine whether addition of low dose prednisolone to multidrug treatment can prevent reaction and nerve

function impairment in leprosy. **DESIGN:** Multicenter, double blind, randomized, placebo controlled, parallel group trial. **SETTING:** Six centers in Bangladesh and Nepal. **PARTICIPANTS:** 636 people with newly diagnosed multibacillary leprosy. **INTERVENTION:** Prednisolone 20 mg/day for three months, with tapering dose in month 4, plus multidrug treatment, compared with multidrug treatment alone. **MAIN OUTCOME MEASURES:** Signs of reaction, impairment of sensory and motor nerve function, and nerve tenderness needing full dose prednisolone at four months and one year. **RESULTS:** Prednisolone had a significant effect in the prevention of reaction and nerve function impairment at four months (relative risk 3.9, 95% confidence interval 2.1 to 7.3), but this was not maintained at one year (relative risk 1.3, 0.9 to 1.8). Fewer events occurred in the prednisolone group at all time points up to 12 months, but the difference at 12 months was small. Subgroup analysis showed a difference in response between people with and without impairment of nerve function at diagnosis. **CONCLUSIONS:** The use of low dose prophylactic prednisolone during the first four months of multidrug treatment for leprosy reduces the incidence of new reactions and nerve function impairment in the short term, but the effect is not sustained at one year. The presence of nerve function impairment at diagnosis may influence the response to low dose prednisolone.—Authors' Abstract

**Spencer, J. S., Kim, H. J., Marques, A. M., Gonzalez-Juarerro, M., Lima, M. C., Vissa, V. D., Truman, R. W., Genaro, M. L., Cho, S. N., Cole, S. T., and Brennan, P. J.** Comparative analysis of B- and T-cell epitopes of *Mycobacterium leprae* and *Mycobacterium tuberculosis* culture filtrate protein 10. *Infect. Immun.* **72(6)** (2004) 3161–3170.

Culture filtrate protein 10 (CFP-10) from *Mycobacterium tuberculosis* is a well-characterized immunodominant 10-kDa protein antigen known to elicit a very potent early gamma interferon response in T cells from *M. tuberculosis*-infected mice and humans. The sequence of the *Mycobacterium*

*leprae* homologue of CFP-10 shows only 40% identity (60% homology) at the protein level with *M. tuberculosis* CFP-10 and thus has the potential for development as a T- or B-cell reactive antigen for specific diagnosis of leprosy. Antisera raised in mice or rabbits against recombinant *M. leprae* and *M. tuberculosis* CFP-10 proteins reacted only with homologous peptides from arrays of overlapping synthetic peptides, indicating that there was no detectable cross-reactivity at the antibody level. Sera from leprosy and tuberculosis patients were also specific for the homologous protein or peptides and showed distinct patterns of recognition for either *M. leprae* or *M. tuberculosis* CFP-10 peptides. At the cellular level, only 2 of 45 mouse T-cell hybridomas raised against either *M. leprae* or *M. tuberculosis* CFP-10 displayed a cross-reactive response against the N-terminal heterologous CFP-10 peptide, the region that exhibits the highest level of identity in the two proteins; however, the majority of peptide epitopes recognized by mouse T-cell hybridomas specific for each protein did not cross-react with heterologous peptides. Coupled with the human serology data, these results raise the possibility that peptides that could be used to differentiate infections caused by these two related microorganisms could be developed. Immunohistochemical staining of sections of *M. leprae*-infected nude mouse footpads resulted in strongly positive staining in macrophages and dendritic cells, as well as weaker staining in extracellular areas, suggesting that *M. leprae* CFP-10, like its homologue in *M. tuberculosis*, is a secreted protein.—Authors' Abstract

**Sunderkotter, C. H., Tomimori-Yamashita, J., Nix, V., Maeda, S. M., Sindrilaru, A., Mariano, M., Sorg, C., and Roth, J.** High expression of myeloid-related proteins 8 and 14 characterizes an inflammatorily active but ineffective response of macrophages during leprosy. *Immunology* **111(4)** (2004) 472–480.

Macrophages are decisive cells for the course of leprosy as they phagocytose *Mycobacterium leprae* and have the potential to influence the specific immune response. Expression and release of the myeloid-related



protein (MRP) 8 and MRP14 (S100A8 and S100A9) characterize a proinflammatory subtype of macrophage that is prominent in, for example, murine infection with lack of a T helper 1 cell response and in certain highly active chronic inflammations of mice and humans. We investigated cutaneous biopsies of the different forms of leprosy (41 untreated patients) including leprosy reaction type 1 (reversal reaction) and type 2 (erythema nodosum leprosum) (N = 18) for expression of MRP8 and MRP14 by subtypes of macrophages. Concomitantly we determined serum levels of MRP8 and MRP14 by sandwich enzyme-linked immunosorbent assay. Expression of MRP8 and MRP14 by CD68-positive macrophages was low in tuberculoid leprosy and rose significantly in borderline tuberculoid leprosy and especially in multibacillary forms, there being expressed by mycobacteria-loaded foam cells. A significant rise of MRP8 and MRP14 expression also occurred in lepra reactions compared to the corresponding non-reactional forms. In type 2 reactions this additional increase was associated with a significant elevation of serum levels. In type 1 it was associated with expression of MRP8 and MRP14 by epithelioid and giant cells, which so far were considered not to express both proteins. In conclusion, we present evidence that the two prominent proteins MRP8 and MRP14 can be re-expressed *in vivo* by tissue macrophages in chronic infection, that their increased expression is characteristic for a macrophage subtype associated with high inflammatory but low antimycobacterial activity in the absence of a T helper 1 response, and that their significant rise in serum during erythema nodosum leprosum bears diagnostic and pathophysiological relevance.—Authors' Abstract

**Vardhini, D., Suneetha, S., Ahmed, N., Joshi, D. S., Karuna, S., Magee, X., Vijayalakshmi, D. S., Sridhar, V., Karunakar, K. V., Archelos, J. J., and Suneetha, L. M.** Comparative proteomics of the *Mycobacterium leprae* binding protein myelin P0: its implication in leprosy and other neurodegenerative diseases. *Infect. Genet. Evol.* 4(1) (2004) 21–28.

*Mycobacterium leprae*, the causative agent of leprosy invades Schwann cells of the peripheral nerves leading to nerve damage and disfigurement, which is the hallmark of the disease. Wet experiments have shown that *M. leprae* binds to a major peripheral nerve protein, the myelin P zero (P0). This protein is specific to peripheral nerve and may be important in the initial step of *M. leprae* binding and invasion of Schwann cells which is the feature of leprosy. Though the receptors on Schwann cells, cytokines, chemokines and antibodies to *M. leprae* have been identified the molecular mechanism of nerve damage and neurodegeneration is not clearly defined. Recently pathogen and host protein/nucleotide sequence similarities (molecular mimicry) have been implicated in neurodegenerative diseases. The approach of the present study is to utilise bioinformatic tools to understand leprosy nerve damage by carrying out sequence and structural similarity searches of myelin P0 with leproma and other genomic database. Since myelin P0 is unique to peripheral nerve, its sequence and structural similarities in other neuropathogens have also been noted. Comparison of myelin P0 with the *M. leprae* proteins revealed two characterised proteins, Ferredoxin NADP reductase and a conserved membrane protein, which showed similarity to the query sequence. Comparison with the entire genomic database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) by basic local alignment search tool for proteins (BLASTP) and fold classification of structure-structure alignment of proteins (FSSP) searches revealed that myelin P0 had sequence/structural similarities to the poliovirus receptor, coxsackie-adenovirus receptor, anthrax protective antigen, diphtheria toxin, herpes simplex virus, HIV gag-1 peptide, and gp120 among others. These proteins are known to be associated directly or indirectly with neurodegeneration. Sequence and structural similarities to the immunoglobulin regions of myelin P0 could have implications in host-pathogen interactions, as it has homophilic adhesive properties. Although these observed similarities are not highly significant in their percentage identity, they could be functionally important in molecular mimicry, receptor binding and cell signaling events involved in neurodegeneration.—Authors' Abstract

## Immunopathology, Tuberculosis

**Algood, H. M., Lin, P. L., Yankura, D., Jones, A., Chan, J., and Flynn, J. L.** TNF influences chemokine expression of macrophages *in vitro* and that of CD11b+ cells *in vivo* during *Mycobacterium tuberculosis* infection. *J. Immunol.* **172(11)** (2004) 6846–6857.

Granulomas, focal accumulations of immune cells, form in the lung during *Mycobacterium tuberculosis* infection. Chemokines, chemotactic cytokines, are logical candidates for inducing migration of T lymphocytes and monocytes to and within the lung. TNF influences chemokine expression in some models. TNF-deficient mice infected with *M. tuberculosis* are highly susceptible to disease, and granuloma formation is inhibited. Through *in vitro* assays, we demonstrate that neutralization of TNF in *M. tuberculosis*-infected macrophages led to a reduction in many inflammatory chemokines, such as C-C chemokine ligand 5, CXC ligand 9 (CXCL9), and CXCL10. In TNF-deficient mice, immune cells migrated to the lungs early after infection, but did not organize to form granulomas within the lung. Although chemokine expression, as measured in whole lung tissue, was not different, the expression of chemokines in the CD11b(+) subset of cells isolated *ex vivo* from the lungs of TNF-deficient mice had reduced expression of C-C chemokine ligand 5, CXCL9, and CXCL10 at early time points after TNF neutralization. Local expression of CXCR3-binding chemokines within the lungs, as determined by *in situ* hybridization, was also affected by TNF. Therefore, TNF affects the expression of chemokines by macrophages *in vitro* and CD11b(+) cells *in vivo*, which probably influences the local chemokine gradients and granuloma formation.—Authors' Abstract

**Balkhi, M. Y., Sinha, A., and Natarajan, K.** Dominance of CD86, transforming growth factor- $\beta$  1, and interleukin-10 in *Mycobacterium tuberculosis* secretory antigen-activated dendritic cells regulates T helper 1 responses to mycobacterial

antigens. *J. Infect. Dis.* **189(9)** (2004) 1598–1609.

We report that stimulation of *Mycobacterium tuberculosis* (*M. tuberculosis*) secretory antigen (MTSA)-differentiated dendritic cells (DCs) and MTSA-matured DCs with *M. tuberculosis* cell extract (CE) down-regulated proinflammatory responses to CE-primed T (CE-T) cells by increasing surface expression of CD86 after CE stimulation. CE stimulation also decreased interleukin (IL)-12p40 and interferon (IFN)- $\gamma$  levels and increased IL-10 and transforming growth factor- $\beta$  1 (TGF- $\beta$  1) levels from these DCs. Blocking either CD86, IL-10, or TGF- $\beta$  with monoclonal antibodies before CE stimulation restored the attenuated T helper 1 (Th1) responses of CE-T cells. Conversely, treatment of these DCs with IL-12p70 and/or IFN- $\gamma$  completely restored Th1 responses from CE-T cells. These results indicate that *M. tuberculosis* secretory antigens down-regulate proinflammatory Th1 responses to mycobacteria by differentially modulating the cytokine profiles and surface densities of costimulatory molecules on DCs. Of importance, this down-regulation is independent of the maturation status of MTSA-activated DCs and can be rescued after treatment of DCs with IFN- $\gamma$  or IL-12.—Authors' Abstract

**Brock, I., Weldingh, K., Leyten, E. M., Arend, S. M., Ravn, P., and Andersen, P.** Specific T-Cell Epitopes for Immunoassay-Based Diagnosis of *Mycobacterium tuberculosis* Infection. *J. Clin. Microbiol.* **42(6)** (2004) 2379–2387.

The currently used method for immunological detection of tuberculosis infection, the tuberculin skin test, has low specificity. Antigens specific for *Mycobacterium tuberculosis* to replace purified protein derivative are therefore urgently needed. We have performed a rigorous assessment of the diagnostic potential of four recently identified antigens (Rv2653, Rv2654, Rv3873, and

Rv3878) from genomic regions that are lacking from the *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) vaccine strains as well as from the most common nontuberculous mycobacteria. The fine specificity of potential epitopes in these molecules was evaluated by sensitive testing of the T-cell responses of peripheral blood mononuclear cells derived from *M. bovis* BCG-vaccinated healthy individuals to synthesized overlapping peptides. Three of the four molecules contained regions with significant specificity problems (Rv2653, Rv3873, and Rv3878). We selected and combined the specific peptide stretches from the four proteins not recognized by *M. bovis* BCG-vaccinated individuals. These peptide stretches were tested with peripheral blood mononuclear cells obtained from patients with microscopy- or culture-confirmed tuberculosis and from healthy *M. bovis* BCG-vaccinated controls. The combination of the most promising stretches from this analysis showed a sensitivity level (57%) comparable to the level found with the two well-known *M. tuberculosis*-specific proteins ESAT-6 and CFP-10 (75 and 66%, respectively). The combination of ESAT-6, CFP-10, and the novel specific peptide stretches gave an overall sensitivity of 84% at a specificity of 97%. In a validation experiment with new experimental groups, the sensitivities obtained were 57% for the combination of peptides and 90% for the combination of the peptides, ESAT-6, and CFP-10. This combination gave a specificity of 95%.—Authors' Abstract

**Chandra, G., Selvaraj, P., Jawahar, M. S., Banurekha, V. V., and Narayanan, P. R.** Effect of vitamin D(3) on phagocytic potential of macrophages with live *Mycobacterium tuberculosis* and lymphoproliferative response in pulmonary tuberculosis. *J. Clin. Immunol.* **24**(3) (2004) 249–257.

Immune responses are elicited through antigen presentation and recognition by macrophages and T-lymphocytes, respectively. The immunomodulatory effect of vitamin D(3) on macrophage phagocytic potential with live *Mycobacterium tuberculosis*, spontaneous and *M. tuberculosis* culture filtrate antigen induced lymphocyte responses

were studied in pulmonary tuberculosis patients (PTBPs) (N = 31) and normal healthy subjects (NHSs) (N = 43). Vitamin D(3) at a concentration of 10(–7) M significantly enhanced the macrophage phagocytosis of live *M. tuberculosis* in normal subjects with low phagocytic potential (less than 10%) (p = 0.015). No such increase was observed in PTBPs. Vitamin D(3) significantly decreased the spontaneous lymphoproliferative response (p = 0.022) and increased the apoptosis of peripheral blood mononuclear cells in PTBPs (p = 0.024). In normals, vitamin D(3) increased the spontaneous lymphoproliferative response. An inverse correlation between macrophage phagocytosis and spontaneous response was observed in NHSs, whereas a direct correlation was seen between vitamin D(3)-treated cells in normal subjects under *in vitro* condition. Vitamin D(3) decreased the *M. tuberculosis* culture filtrate antigen induced lymphocyte response significantly in normal subjects (p = 0.0003), while it had no influence on the lymphocyte response in PTBPs. The present study suggests that exposure to vitamin D(3) increases the phagocytic potential and spontaneous lymphoproliferative response but brings down the antigen-induced response in normals. In tuberculosis, addition of vitamin D(3) has no significant effect on antigen-induced lymphoproliferative response. This may be due to the unresponsive nature of the cells to the action of vitamin D(3) by virtue of the disease, which renders them inactive.—Authors' Abstract

**Chen, Y., Lu, H. Z., Hu, J. G., and Li, B. Q.** [Characterization of *Mycobacterium tuberculosis* polypeptide antigen capable of stimulating the proliferation of human gammadeltaT cells]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.* **19**(2) (2003) 121–123. [Article in Chinese]

AIM: To analyse biological properties of *Mycobacterium tuberculosis* polypeptide antigen(Mtb-Ag) that could stimulate the proliferation of human gammadeltaT cells. METHODS: Mtb secretory proteins and Mtb-Ag treated via dialysis or with pronase were used to stimulate human PBMCs. After being cultured for 10 days, the phenotype of responsive cells was analyzed by

flow cytometry. **RESULTS:** The proliferation stimulating activity to human gammadeltaT cells of dialysed Mtb-Ag did not decrease remarkably, whereas that of the pronase-treated Mtb-Ag reduced significantly. Moreover, the proliferative stimulating activity to gammadeltaT cells of Mtb-Ag was notably higher than Mtb secretory protein. **CONCLUSION:** The component in Mtb-Ag that can stimulate the proliferation of human gammadeltaT cells is a non-secretory and protease-sensitive polypeptides with M(r) over 10,000.—Authors' Abstract

LM with Toll-like receptor 2 (TLR-2) but not with TLR-4 suggested that these responses were mediated via the TLR-2 signaling pathway. Our experiments revealed an important immunostimulatory activity of the biosynthetic LAM precursor LM. The ratio of LAM to LM in the cell wall of mycobacteria may be an important determinant of virulence, and enzymes that modify LM could provide targets for development of antituberculosis drugs and for derivation of attenuated strains of *M. tuberculosis*.—Authors' Abstract

**Dao, D. N., Kremer, L., Guerardel, Y., Molano, A., Jacobs, W. R., Jr., Porcelli, S. A., and Brien, V.** *Mycobacterium tuberculosis* lipomannan induces apoptosis and interleukin-12 production in macrophages. *Infect. Immun.* **72**(4) (2004) 2067–2074.

The mycobacterial cell wall component lipoarabinomannan (LAM) has been described as a virulence factor of *Mycobacterium tuberculosis*, and modification of the terminal arabinan residues of this compound with mannose caps (producing mannosyl-capped LAM [ManLAM]) in *M. tuberculosis* or with phosphoinositol caps (producing phosphoinositol-capped LAM [PILAM]) in *Mycobacterium smegmatis* has been implicated in various functions associated with these lipoglycans. A structure-function analysis was performed by using LAMs and their biosynthetic precursor lipomannans (LMs) isolated from different mycobacterial species on the basis of their capacity to induce the production of interleukin-12 (IL-12) and/or apoptosis of macrophage cell lines. Independent of the mycobacterial species, ManLAMs did not induce IL-12 gene expression or apoptosis of macrophages, whereas PILAMs induced IL-12 secretion and apoptosis. Interestingly, uncapped LAM purified from *Mycobacterium chelonae* did not induce IL-12 secretion or apoptosis. Furthermore, LMs, independent of their mycobacterial origins, were potent inducers of IL-12 and apoptosis. The precursor of LM, phosphatidyl-myo-inositol dimannoside, had no activity, suggesting that the mannan core of LM was required for the activity of LM. The specific interaction of

**Das, S. D., Subramanian, D., and Prabha, C.** Cell proliferation and apoptosis: dual-signal hypothesis tested in tuberculous pleuritis using mycobacterial antigens. *FEMS Immunol. Med. Microbiol.* **41**(1) (2004) 85–92.

Antigens and mitogens have the innate ability to trigger cell proliferation and apoptosis thus exhibiting a dual-signal phenomenon. This dual-signal hypothesis was tested with mycobacterial antigens (PPD and heat killed *Mycobacterium tuberculosis*—MTB) in tuberculous pleuritis patients where the immune response is protective and compartmentalized. We compared and correlated the cell-cycle analysis and antigen-induced apoptosis in normal and patients' peripheral blood mononuclear cells (PBMCs) and patients' pleural fluid mononuclear cells (PFMCs). In cell-cycle analysis, PFMCs showed good mitotic response with PPD and MTB antigens where 10% and 7% of resting cells entered the S and G2/M phases of cell cycle, respectively. This antigen-induced proliferation of PFMCs correlated well with the lymphocyte transformation test (LTT) results. On the other hand, PFMCs also showed 21% of spontaneous apoptosis, which further increased to 43%, by induction with known apoptotic agent like Dexamethasone (DEX) and the mycobacterial antigens PPD and MTB. Further we demonstrated by anti-CD3 induction experiments that prior activation of cells is prerequisite for them to undergo apoptosis. Our results showed that PPD and MTB antigens induced both cell proliferation and apoptosis in PFMCs, which were pre-sensitized to mycobacterial antigens *in vivo*.



Thus the dual-signal phenomenon was operative against these antigens in tuberculous pleuritis. We also demonstrated that the activated cells are more predisposed to apoptosis.—Authors' Abstract

**Demissie, A., Abebe, M., Aseffa, A., Rook, G., Fletcher, H., Zumla, A., Weldingh, K., Brock, I., Andersen, P., Doherty, T. M.; and VACSEL Study Group.** Healthy individuals that control a latent infection with *Mycobacterium tuberculosis* express high levels of Th1 cytokines and the IL-4 antagonist IL-4delta2. *J. Immunol.* **172(11)** (2004) 6938–6943.

The majority of healthy individuals exposed to *Mycobacterium tuberculosis* will not develop disease and identifying what constitutes “protective immunity” is one of the holy grails of *M. tuberculosis* immunology. It is known that IFN-gamma is essential for protection, but it is also apparent that IFN-gamma levels alone do not explain the immunity/susceptibility dichotomy. The controversy regarding correlates of immunity persists because identifying infected but healthy individuals (those who are immune) has been problematic. We have therefore used recognition of the *M. tuberculosis* virulence factor early secretory antigenic target 6 to identify healthy, but infected individuals from tuberculosis (TB)-endemic and nonendemic regions (Ethiopia and Denmark) and have compared signals for cytokines expressed directly *ex vivo* with the pattern found in TB patients. We find that TB patients are characterized by decreased levels of Th1 cytokines and increased levels of IL-10 compared with the healthy infected and noninfected community controls. Interestingly, the healthy infected subjects exhibited a selective increase of message for the IL-4 antagonist, IL-4delta2, compared with both TB patients or noninfected individuals. These data suggest that long-term control of *M. tuberculosis* infection is associated not just with elevated Th1 responses but also with inhibition of the Th2 response.—Authors' Abstract

**Fortune, S. M., Solache, A., Jaeger, A., Hill, P. J., Belisle, J. T., Bloom, B. R.,**

**Rubin, E. J., and Ernst, J. D.** *Mycobacterium tuberculosis* inhibits macrophage responses to IFN-gamma through myeloid differentiation factor 88-dependent and -independent mechanisms. *J. Immunol.* **172(10)** (2004) 6272–6280.

*Mycobacterium tuberculosis* overcomes macrophage bactericidal activities and persists intracellularly. One mechanism by which *M. tuberculosis* avoids macrophage killing might be through inhibition of IFN-gamma-mediated signaling. In this study we provide evidence that at least two distinct components of *M. tuberculosis*, the 19-kDa lipoprotein and cell wall peptidoglycan (contained in the mycolylarabinogalactan peptidoglycan (mAGP) complex), inhibit macrophage responses to IFN-gamma at a transcriptional level. Moreover, these components engage distinct proximal signaling pathways to inhibit responses to IFN-gamma: the 19-kDa lipoprotein inhibits IFN-gamma signaling in a Toll-like receptor (TLR)2-dependent and myeloid differentiation factor 88-dependent fashion whereas mAGP inhibits independently of TLR2, TLR4, and myeloid differentiation factor 88. In addition to inhibiting the induction of specific IFN-gamma responsive genes, the 19-kDa lipoprotein and mAGP inhibit the ability of IFN-gamma to activate murine macrophages to kill virulent *M. tuberculosis* without inhibiting production of NO. These results imply that inhibition of macrophage responses to IFN-gamma may contribute to the inability of an apparently effective immune response to eradicate *M. tuberculosis*.—Authors' Abstract

**Fulton, S. A., Reba, S. M., Pai, R. K., Pennini, M., Torres, M., Harding, C. V., and Boom, W. H.** Inhibition of major histocompatibility complex II expression and antigen processing in murine alveolar macrophages by *Mycobacterium bovis* BCG and the 19-kilodalton mycobacterial lipoprotein. *Infect. Immun.* **72(4)** (2004) 2101–2110.

Alveolar macrophages constitute a primary defense against *Mycobacterium tuberculosis*, but they are unable to control *M. tuberculosis* without acquired T-cell immu-

nity. This study determined the antigen-presenting cell function of murine alveolar macrophages and the ability of the model mycobacterium, *Mycobacterium bovis* BCG, to modulate it. The majority (80 to 85%) of alveolar macrophages expressed both CD80 (B7.1) and CD11c, and 20 to 30% coexpressed major histocompatibility complex II (MHC-II). Gamma interferon (IFN-gamma) enhanced MHC-II but not B7.1 expression. Naive or IFN-gamma-treated alveolar macrophages did not express CD86 (B7.2), CD11b, Mac-3, CD40, or F4/80. *M. bovis* BCG and the 19-kDa mycobacterial lipoprotein inhibited IFN-gamma-regulated MHC-II expression on alveolar macrophages, and inhibition was dependent on Toll-like receptor 2. The inhibition of MHC-II expression by the 19-kDa lipoprotein was associated with decreased presentation of soluble antigen to T cells. Thus, susceptibility to tuberculosis may result from the ability of mycobacteria to interfere with MHC-II expression and antigen presentation by alveolar macrophages.—Authors' Abstract

**Hoshino, Y., Tse, D. B., Rochford, G., Prabhakar, S., Hoshino, S., Chitkara, N., Kuwabara, K., Ching, E., Raju, B., Gold, J. A., Borkowsky, W., Rom, W. N., Pine, R., and Weiden, M.** *Mycobacterium tuberculosis*-induced CXCR4 and chemokine expression leads to preferential X4 HIV-1 replication in human macrophages. *J. Immunol.* **172**(10) (2004) 6251–6258.

Opportunistic infections such as pulmonary tuberculosis (TB) increase local HIV-1 replication and mutation. As AIDS progresses, alteration of the HIV-1 gp120 V3 sequence is associated with a shift in viral coreceptor use from CCR5 (CD195) to CXCR4 (CD184). To better understand the effect of HIV/TB coinfection, we screened transcripts from bronchoalveolar lavage cells with high density cDNA arrays and found that CXCR4 mRNA is increased in patients with TB. Surprisingly, CXCR4 was predominately expressed on alveolar macrophages (AM). *Mycobacterium tuberculosis* infection of macrophages *in vitro* increased CXCR4 surface expression, whereas amelioration of disease reduced CXCR4 ex-

pression *in vivo*. Bronchoalveolar lavage fluid from TB patients had elevated levels of CCL4 (macrophage inflammatory protein-1beta), CCL5 (RANTES), and CX3CL1 (fractalkine), but not CXCL12 (stromal-derived factor-1alpha). We found that *M. tuberculosis* infection of macrophages *in vitro* increased viral entry and RT of CXCR4, using HIV-1, but not of CCR5, using HIV-1. Lastly, HIV-1 derived from the lung contains CD14, suggesting that they were produced in AM. Our results demonstrate that TB produces a permissive environment for replication of CXCR4-using virus by increasing CXCR4 expression in AM and for suppression of CCR5-using HIV-1 by increasing CC chemokine expression. These changes explain in part why TB accelerates the course of AIDS. CXCR4 inhibitors are a rational therapeutic approach in HIV/TB coinfection.—Authors' Abstract

**Takegawa, H., Matano, Y., Inubushi, T., and Katunuma, N.** Significant accumulations of cathepsin B and prolylendopeptidase in inflammatory focus of delayed-type hypersensitivity induced by *Mycobacterium tuberculosis* in mice. *Biochem. Biophys. Res. Commun.* **316**(1) (2004) 78–84.

To clarify what kinds of proteinases are secreted into the foci of allergic-inflammation involving delayed-type hypersensitivity reaction, we examined the characteristic releases of various proteinases into the foci of *Mycobacterium tuberculosis* (*M. tuber.*)-induced delayed-type allergic-inflammation in mice. The significant activities of cathepsin B and prolylendopeptidase were observed in the washing-fluids of subcutaneous inflammatory foci of *M. tuber.*-induced delayed-type allergic-inflammation, but not *M. tuber.*-induced acute-inflammation. The SDS-resistant complex of cathepsin B and a protein substrate with apparent molecular mass of 74 kDa was observed by Western blot analysis. On the other hand, no significant accumulations of other proteinases, such as matrix metalloproteinases, cathepsin D, and serine proteinases, were determined. CA-074, a specific inhibitor of cathepsin B, suppressed both swelling and cathepsin B activity in the footpad having *M. tuber.*-

induced delayed-type allergic-inflammation *in vivo*. These results suggest that cathepsin B may play an important role in the formation of *M. tuber.*-induced delayed-type allergic-inflammation.—Authors' Abstract

**Kaur, S., and Singh, P. P.** Serum amyloid P-component-mediated inhibition of the uptake of *Mycobacterium tuberculosis* by macrophages, *in vitro*. *Scand. J. Immunol.* **59**(5) (2004) 425–431.

The effect of purified mouse serum amyloid P-component (SAP) treatment of mouse alveolar macrophages (AMs) on their uptake of *Mycobacterium tuberculosis* Erdman was investigated, *in vitro*. SAP (0.5–50.0 micro g/ml), in a concentration-dependent manner, inhibited the *M. tuberculosis* uptake by the AMs; maximum inhibition (33.43%) occurred at 10.0 micro g/ml. The inhibition of uptake could be observed as early as 30 min after the incubation of AMs with 10.0 micro g/ml SAP; however, an incubation of 60 min induced maximum inhibition beyond which the response became static. The SAP-mediated decreased uptake of *M. tuberculosis* also resulted in their reduced intramacrophage growth as determined by colony-forming unit counts. SAP inhibited the uptake of mycobacteria in the presence of Ca(2+), and at pH = 5.6, the inhibition was abrogated. Deglycosylation of purified SAP with N-glycanase, and not with O-glycanase, blocked the SAP-mediated inhibition of the uptake. Heat-inactivated (80 degrees C; 1 hr; pH 7.0) SAP did not inhibit the uptake of *M. tuberculosis* by AMs. These data, apparently for the first time, indicate that purified mouse SAP, in a divalent cation- and N-linked oligosaccharide glycosylation-dependent manner, inhibited the *in vitro* uptake of *M. tuberculosis* Erdman by mouse AMs, which was also associated with their reduced intracellular growth.—Authors' Abstract

**Kawakami, K., Kinjo, Y., Uezu, K., Miyagi, K., Kinjo, T., Yara, S., Koguchi, Y., Miyazato, A., Shibuya, K., Iwakura, Y., Takeda, K., Akira, S., and Saito, A.** Interferon-gamma production and host protective response against *My-*

*cobacterium tuberculosis* in mice lacking both IL-12p40 and IL-18. *Microbes. Infect.* **6**(4) (2004) 339–349.

Interferon (IFN)-gamma plays an essential role in host defense against infection with *Mycobacterium tuberculosis*, and its synthesis is critically regulated by interleukin (IL)-12, IL-18 and the recently identified IL-23. The present study was designed to determine the roles of these cytokines in IFN-gamma-mediated host defenses against *M. tuberculosis*. For this purpose, we compared host protective responses in IL-12p40 and IL-18 double-knockout (DKO) mice (which lacked both IL-12/IL-18 and also IL-23) and IFN-gamma gene-disrupted (GKO) mice. DKO mice were more resistant to the infection than GKO mice, as indicated by their extended survival and reduced live colony numbers in spleen, liver and lung. IFN-gamma was detected by ELISA in liver and lung homogenates, but not in spleen and serum, and in all organs by RT-PCR in DKO mice at comparable or reduced levels to those in wild-type mice. IFN-gamma production was reduced by depletion of CD4+ T cells, but not of natural killer (NK), NKT, gammadeltaT and dendritic cells. Neutralization of IFN-gamma or TNF-alpha by specific monoclonal antibodies (mAbs) significantly shortened the survival time of the infected DKO mice. Furthermore, anti-TNF-alpha mAb partially attenuated IFN-gamma synthesis in the liver of these mice. Finally, the expression level of inducible nitric oxide synthase (iNOS) mRNA in the spleen, liver and lung was considerable in DKO mice but only marginal or undetected in GKO mice. Our results indicate the presence of IL-12-, IL-18- and IL-23-independent host protective responses against mycobacterial infection mediated by IFN-gamma, which was secreted from helper T cells.—Authors' Abstract

**Liu, X. Q., Dosanjh, D., Varia, H., Ewer, K., Cockle, P., Pasvol, G., and Lalvani, A.** Evaluation of T-cell responses to novel RD1- and RD2-encoded *Mycobacterium tuberculosis* gene products for specific detection of human tuberculosis infection. *Infect. Immun.* **72**(5) (2004) 2574–2581.

The tuberculin skin test for diagnosing *Mycobacterium tuberculosis* infection suffers from antigenic cross-reactivity of purified protein derivative with BCG, resulting in poor specificity in BCG-vaccinated populations. Comparative genomics has identified several genetic regions in *M. tuberculosis* and *M. bovis* that are deleted in *M. bovis* BCG. Proteins encoded in these regions will form the basis of new specific T-cell-based blood tests that do not cross-react with BCG, but only two, early secretory antigen target 6 and culture filtrate protein 10, have been studied in detail in humans. We investigated four novel gene products, encoded by RD2 (Rv1989c) and RD1 (Rv3873, Rv3878, and Rv3879c), that are absent from most or all of the vaccine strains of BCG, respectively. Sixty-seven overlapping peptides were tested in *ex vivo* gamma interferon enzyme-linked immunospot assays in 49 patients with culture-confirmed tuberculosis and 38 healthy BCG-vaccinated donors. Forty-five percent (95% confidence interval [CI], 31 to 57%) and 53% (95% CI, 39 to 67%) of the tuberculosis patients responded to Rv3879c and Rv3873, respectively, identifying these proteins as major *M. tuberculosis* T-cell antigens in humans, while 35 and 25% of the patients responded to Rv3878 and Rv1989c, respectively. Of the 38 BCG-vaccinated donors, 1 (2.6%) responded to peptides from Rv3878 and Rv3879c, 3 (7.9%) responded to Rv3873, and none responded to Rv1989c. Exclusion of cross-reactive peptides encoded in conserved motifs of Rv3873, a PPE family member, increased its specificity to 97.4%. The high specificity of Rv3879c peptides and nonconserved Rv3873 sequences, together with their moderate sensitivity in tuberculosis patients, identifies these peptides as candidates for inclusion in new T-cell-based tests for *M. tuberculosis* infection.—Authors' Abstract

**Louie, L. G., Hartogensis, W. E., Jackman, R. P., Schultz, K. A., Zijenah, L. S., Yiu, C. H., Nguyen, V. D., Sohsman, M. Y., Katzenstein, D. K., and Mason, P. R.** *Mycobacterium tuberculosis*/HIV-1 coinfection and disease: role of human leukocyte antigen variation. *J. Infect. Dis.* **189**(6) (2004) 1084–1090.

Genetic variation influences immune responses and may contribute to differential development of tuberculosis (TB), particularly in immunosuppressed individuals. To examine the risk of *Mycobacterium tuberculosis* infection progressing to disease in the context of *M. tuberculosis*/human immunodeficiency virus (HIV) type 1 coinfection, HIV-1 RNA load and human leukocyte antigen (HLA) genotypes were determined among subjects from Harare, Zimbabwe, an area where both TB and HIV-1 are endemic. Patients with TB were compared with control subjects, stratified by HIV-1 infection status and progression of TB disease. Alleles of class I HLA-A and -C were associated with risk of developing active TB, depending on HIV-1 status. Among HIV-positive subjects, HIV-1 load was independently associated with increased risk of developing pulmonary TB. HLA DRB1 homozygosity among HIV-positive subjects was associated with reduced risk of developing pulmonary TB but increased risk of rapid progression to pleural effusion TB. These observations suggest that HLA plays a role in risk of developing symptomatic TB at various stages of disease and that these effects are modified by HIV-1 coinfection.—Authors' Abstract

**Mwandumba, H. C., Russell, D. G., Nyirenda, M. H., Anderson, J., White, S. A., Molyneux, M.E., and Squire, S. B.** *Mycobacterium tuberculosis* resides in nonacidified vacuoles in endocytically competent alveolar macrophages from patients with tuberculosis and HIV infection. *J. Immunol.* **172**(7) (2004) 4592–4598.

Alveolar macrophages (AM) are the first professional phagocytes encountered by aerosols containing infections in the lungs, and their phagocytic capacity may be affected by these infections or environmental particles. The aim of this study was to evaluate the innate endocytic and phagocytic properties of human AM obtained from patients with pulmonary tuberculosis and to characterize the vacuoles in which *Mycobacterium tuberculosis* bacilli reside *in vivo*. AM were obtained by bronchoalveolar lavage from patients with suspected tuberculosis and from asymptomatic volunteers (controls). Clinical case definitions were based on mycobacterial culture



of respiratory specimens and HIV serology. To assess phagocytosis, endocytosis, and acidification of the endosomal system, AM were cultured with IgG-coated polystyrene beads, dextran, and a pH-sensitive reporter (3-(2,4-dinitroanilino)-3-amino-N-methylpropylamine) and were evaluated by light and immunoelectron microscopy. Cells from 89 patients and 10 controls were studied. We found no significant difference between the two groups in the ability of AM either to ingest beads and dextran or to deliver them to acidified lysosomes. In AM from patients with tuberculosis, the bacilli were located in vacuoles that failed to accumulate endocytosed material and were not acidified. We concluded that AM from patients with tuberculosis and HIV infections were competent to endocytose and phagocytose material and to deliver the material to functional, acidified lysosomes. *M. tuberculosis* residing in these AM arrests the progression of their phagosomes, which fail to fuse with acidified lysosomes. This confirms, for the first time in humans with tuberculosis and HIV, the conclusions from previous animal and *in vitro* studies.—Authors' Abstract

**Romano, M., Denis, O., D'Souza, S., Wang, X. M., Ottenhoff, T. H., Brulet, J. M., and Huygen, K.** Induction of *in vivo* functional Db-restricted cytolytic T cell activity against a putative phosphate transport receptor of *Mycobacterium tuberculosis*. *J. Immunol.* **172**(11) (2004) 6913–6921.

Using plasmid vaccination with DNA encoding the putative phosphate transport receptor PstS-3 from *Mycobacterium tuberculosis* and 36 overlapping 20-mer peptides spanning the entire PstS-3 sequence, we determined the immunodominant Th1-type CD4(+) T cell epitopes in C57BL/10 mice, as measured by spleen cell IL-2 and IFN-gamma production. Furthermore, a potent IFN-gamma-inducing, D(b)-restricted CD8(+) epitope was identified using MHC class I mutant B6.C-H-2(bm13) mice and intracellular IFN-gamma and whole blood CD8(+) T cell tetramer staining. Using adoptive transfer of CFSE-labeled, peptide-pulsed syngeneic spleen cells from naive animals into DNA vaccinated or *M. tuberculosis*-infected

recipients, we demonstrated a functional *in vivo* CTL activity against this D(b)-restricted PstS-3 epitope. IFN-gamma ELISPOT responses to this epitope were also detected in tuberculosis-infected mice. The CD4(+) and CD8(+) T cell epitopes defined for PstS-3 were completely specific and not recognized in mice vaccinated with either PstS-1 or PstS-2 DNA. The H-2 haplotype exerted a strong influence on immune reactivity to the PstS-3 Ag, and mice of the H-2(b, p, and f) haplotype produced significant Ab and Th1-type cytokine levels, whereas mice of H-2(d, k, r, s, and q) haplotype were completely unreactive. Low responsiveness against PstS-3 in MHC class II mutant B6.C-H-2(bm12) mice could be overcome by DNA vaccination. IFN-gamma-producing CD8(+) T cells could also be detected against the D(b)-restricted epitope in H-2(p) haplotype mice. These results highlight the potential of DNA vaccination for the induction and characterization of CD4(+) and particularly CD8(+) T cell responses against mycobacterial Ags.—Authors' Abstract

**Safi, H., Barnes, P. F., Lakey, D. L., Shams, H., Samten, B., Vankayalapati, R., and Howard, S. T.** IS6110 functions as a mobile, monocyte-activated promoter in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **52**(4) (2004) 999–1012.

The mobile insertion sequence, IS6110, is an important marker in tracking of *Mycobacterium tuberculosis* strains. Here, we demonstrate that IS6110 can upregulate downstream genes through an outward-directed promoter in its 3' end, thus adding to the significance of this element. Promoter activity was orientation dependent and was localized within a 110 bp fragment adjacent to the right terminal inverted repeat. Transcripts from this promoter, named OP6110, begin approximately 85 bp upstream of the 3' end of IS6110. Use of green fluorescent protein (GFP) expression constructs showed that OP6110 was upregulated in *M. tuberculosis* during growth in human monocytes and in late growth phases in broth. Analysis of natural insertion sites in *M. tuberculosis* showed that IS6110 upregulated expression of several downstream genes during growth

in human monocytes, including Rv2280 in H37Rv and the PE-GRS gene, Rv1468c, in the clinical strain 210, which is a member of the Beijing family. Transcription between IS6110 and downstream genes was confirmed by reverse transcription polymerase chain reaction. The ability to activate genes during infection suggests that IS6110 has the potential to influence growth characteristics of different strains, and indicates another mechanism by which IS6110 can impact *M. tuberculosis* evolution.—Authors' Abstract

**Scanga, C. A., Bafica, A., Feng, C. G., Cheever, A. W., Hieny, S., and Sher, A.** MyD88-deficient mice display a profound loss in resistance to *Mycobacterium tuberculosis* associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. *Infect. Immun.* **72(4)** (2004) 2400–2404.

*Mycobacterium tuberculosis* possesses agonists for several Toll-like receptors (TLRs), yet mice with single TLR deletions are resistant to acute tuberculosis. MyD88(–/–) mice were used to examine whether TLRs play any role in protection against aerogenic *M. tuberculosis* H37Rv infection. MyD88(–/–) mice failed to control mycobacterial replication and rapidly

succumbed. Moreover, expressions of interleukin 12, tumor necrosis factor alpha, gamma interferon, and nitric oxide synthase 2 were markedly decreased in the knockout animals. These results argue that resistance to *M. tuberculosis* must depend on MyD88-dependent signals mediated by an as-yet-undetermined TLR or a combination of TLRs.—Authors' Abstract

**Trajkovic, V., Natarajan, K., and Sharma, P.** Immunomodulatory action of mycobacterial secretory proteins. *Microbes. Infect.* **6(5)** (2004) 513–519.

The recently discovered RD1 locus encodes proteins that are actively secreted by pathogenic mycobacteria, including *Mycobacterium tuberculosis*. Since they are missing in non-tuberculous mycobacteria, these proteins are promising not only as candidates for vaccination and diagnostic tests, but also in understanding mycobacterial evasion of protective immunity in susceptible individuals. Here we analyze the possible role of *M. tuberculosis* secretory proteins in immunity against tuberculosis, with emphasis on their immunomodulatory action and the potential involvement in mycobacterial subversion of the host immune defense.—Authors' Abstract

## Microbiology

**Bhakta, S., Besra, G. S., Upton, A. M., Parish, T., Sholto-Douglas-Vernon, C., Gibson, K. J., Knutton, S., Gordon, S., DaSilva, R. P., Anderton, M. C., and Sim, E.** Arylamine N-Acetyltransferase is required for synthesis of mycolic acids and complex lipids in *Mycobacterium bovis* BCG and represents a novel drug target. *J. Exp. Med.* **199(9)** (2004) 1191–1199.

Mycolic acids represent a major component of the unique cell wall of mycobacteria. Mycolic acid biosynthesis is inhibited by isoniazid, a key frontline antitubercular drug that is inactivated by mycobacterial and human arylamine N-acetyltransferase (NAT). We show that an in-frame deletion

of *Mycobacterium bovis* BCG nat results in delayed entry into log phase, altered morphology, altered cell wall lipid composition, and increased intracellular killing by macrophages. In particular, deletion of nat perturbs biosynthesis of mycolic acids and their derivatives and increases susceptibility of *M. bovis* BCG to antibiotics that permeate the cell wall. Phenotypic traits are fully complemented by introduction of *Mycobacterium tuberculosis* nat. We infer from our findings that NAT is critical to normal mycolic acid synthesis and hence other derivative cell wall components and represents a novel target for antituberculosis therapy. In addition, this is the first report of an endogenous role for NAT in mycobacteria.—Authors' Abstract

**Chua, J., Vergne, I., Master, S., and Deretic, V.** A tale of two lipids: *Mycobacterium tuberculosis* phagosome maturation arrest. *Curr. Opin. Microbiol.* **7**(1) (2004) 71–77.

*Mycobacterium tuberculosis* persistence in human populations relies on its ability to inhibit phagosomal maturation. *M. tuberculosis* resides in a pathogen-friendly phagosome escaping lysosomal bactericidal mechanisms and efficient antigen presentation in the host phagocytic cell. *M. tuberculosis* phagosome maturation arrest includes the action of mycobacterial lipid products, which mimic mammalian phosphatidylinositols, targeting host cell membrane trafficking processes. These products interfere with membrane trafficking and organelle biogenesis processes initiated by Ca(2+) fluxes, and ending with host cell Rab GTP-binding proteins and their effectors. The block includes phosphatidylinositol 3-kinase and membrane tethering molecules that prepare phagosomes for fusion with other organelles. Understanding these processes could provide new targets for pharmacological intervention in tuberculosis.—Authors' Abstract

**Douglas, T., Daniel, D. S., Parida, B. K., Jagannath, C., and Dhandayuthapani, S.** Methionine sulfoxide reductase A (MsrA) deficiency affects the survival of *Mycobacterium smegmatis* within macrophages. *J. Bacteriol.* **186**(11) (2004) 3590–3598.

Methionine sulfoxide reductase A (MsrA) is an antioxidant repair enzyme which reduces oxidized methionine to methionine. Since oxidation of methionine in proteins impairs their function, an absence of MsrA leads to abnormalities in different organisms, including alterations in the adherence patterns and *in vivo* survival of certain pathogenic bacteria. To understand the role of MsrA in intracellular survival of bacteria, we disrupted the gene encoding MsrA in *Mycobacterium smegmatis* through homologous recombination. The *msrA* mutant strain of *M. smegmatis* exhibited significantly reduced intracellular survival in murine J774A.1 macrophages compared to

the survival of its wild-type counterpart. Furthermore, immunofluorescence and immunoblotting of phagosomes containing *M. smegmatis* strains revealed that the phagosomes with the *msrA* mutant strain acquired both p67(phox) of phagocyte NADPH oxidase and inducible nitric oxide synthase much earlier than the phagosomes with the wild-type strain. In addition, the *msrA* mutant strain of *M. smegmatis* was observed to be more sensitive to hydroperoxides than the wild-type strain was *in vitro*. These results suggest that MsrA plays an important role in both extracellular and intracellular survival of *M. smegmatis*.—Authors' Abstract

**Dover, L. G., Cerdeno-Tarraga, A. M., Pallen, M. J., Parkhill, J., and Besra, G. S.** Comparative cell wall core biosynthesis in the mycolated pathogens, *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae*. *FEMS Microbiol. Rev.* **28**(2) (2004) 225–250.

The recent determination of the complete genome sequence of *Corynebacterium diphtheriae*, the aetiological agent of diphtheria, has allowed a detailed comparison of its physiology with that of its closest sequenced pathogenic relative *Mycobacterium tuberculosis*. Of major importance to the pathogenicity and resilience of the latter is its particularly complex cell envelope. The corynebacteria share many of the features of this extraordinary structure although to a lesser level of complexity. The cell envelope of *M. tuberculosis* has provided the molecular targets for several of the major anti-tubercular drugs. Given a backdrop of emerging multi-drug resistant strains of the organism (MDR-TB) and its continuing global threat to human health, the search for novel anti-tubercular agents is of paramount importance. The unique structure of this cell wall and the importance of its integrity to the viability of the organism suggest that the search for novel drug targets within the array of enzymes responsible for its construction may prove fruitful. Although the application of modern bioinformatics techniques to the “mining” of the *M. tuberculosis* genome has already increased our knowledge of the biosynthesis and assembly of the mycobac-

terial cell wall, several issues remain uncertain. Further analysis by comparison with its relatives may bring clarity and aid the early identification of novel cellular targets for new anti-tuberculosis drugs. In order to facilitate this aim, this review intends to illustrate the broad similarities and highlight the structural differences between the two bacterial envelopes and discuss the genetics of their biosynthesis.—Authors' Abstract

**Gerston, K. F., Blumberg, L., Tshabalala, V. A., and Murray, J.** Viability of mycobacteria in formalin-fixed lungs. *Hum. Pathol.* **35**(5) (2004) 571–575.

It is generally accepted that the risk of contracting tuberculosis is relatively high among medical laboratory workers and pathologists. Nevertheless, there is an assumption that once tissue is fixed in formalin, the risk for transmission and subsequent infection of mycobacteria is greatly reduced, if not altogether eliminated. To test the viability of potentially infectious mycobacteria in formalin-fixed tissue, tissue specimens from autopsy lungs fixed in formalin were cultured for mycobacteria. Of 138 cases with histologic evidence of acid-fast bacilli, 12 grew mycobacteria, including 3 *Mycobacterium tuberculosis* isolates, suggesting that there is a risk of contracting tuberculosis from tissue that has been fixed in formalin, if aerosolization or accidental inoculation should occur.—Authors' Abstract

**Hafner, B., Haag, H., Geiss, H. K., and Nolte, O.** Different molecular methods for the identification of rarely isolated non-tuberculous mycobacteria and description of new hsp65 restriction fragment length polymorphism patterns. *Mol. Cell. Probes.* **18**(1) (2004) 59–65.

*See Current Literature, Molecular and Genetic Studies, p. 417.*

**Haile, Y., and Ryon, J. J.** Colorimetric microtitre plate hybridization assay for the detection of *Mycobacterium leprae* 16S rRNA in clinical specimens. *Lep. Rev.* **75**(1) (2004) 40–49.

We have developed a colorimetric microtitre plate hybridization assay in order to simplify detection of *Mycobacterium leprae* in clinical specimens. This system detects the products amplified by a sensitive RT-PCR assay targeting a species-specific sequence of the bacterial 16S rRNA. The assay detected as few as 10 bacilli isolated from infected nude mouse lymph nodes or human skin biopsies. Sensitivity for diagnosis of clinical specimens was assessed for 58 tissue biopsies from untreated leprosy patients. The assay detected *M. leprae* RT-PCR products in 100% of biopsies from patients with multibacillary disease and 80% of biopsies from patients with paucibacillary disease, for an overall sensitivity of 91.3%. The test was highly specific as no RT-PCR products were amplified from skin biopsies of normal individuals or patients with skin diseases other than leprosy. The colorimetric assay is faster, more sensitive, and simplifies detection of RT-PCR products compared to Southern blot analysis. It may be useful for diagnosis of difficult cases of leprosy, and, since RNA is rapidly degraded after cell death, it may be appropriate for assessing response to therapy and for distinguishing relapse from reaction.—Authors' Abstract

**Hazbon, M. H., and Alland, D.** Hairpin primers for simplified single-nucleotide polymorphism analysis of *Mycobacterium tuberculosis* and other organisms. *J. Clin. Microbiol.* **42**(3) (2004) 1236–1242.

*See Current Literature, Molecular and Genetic Studies, p. 418.*

**Krzywinska, E., Krzywinski, J., and Schorey, J. S.** Naturally occurring horizontal gene transfer and homologous recombination in *Mycobacterium*. *Microbiology* **150**(Pt 6) (2004) 1707–1712.

Acquisition of genetic information through horizontal gene transfer (HGT) is an important evolutionary process by which microorganisms gain novel phenotypic characteristics. In pathogenic bacteria, for example, it facilitates maintenance and enhancement of virulence and spread of drug resistance. In



the genus *Mycobacterium*, to which several primary human pathogens belong, HGT has not been clearly demonstrated. The few existing reports suggesting this process are based on circumstantial evidence of similarity of sequences found in distantly related species. Here, direct evidence of HGT between strains of *Mycobacterium avium* representing two different serotypes is presented. Conflicting evolutionary histories of genes encoding elements of the glycopeptidolipid (GPL) biosynthesis pathway led to an analysis of the GPL cluster genomic sequences from four *Mycobacterium avium* strains. The sequence of *M. avium* strain 2151 appeared to be a mosaic consisting of three regions having alternating identities to either *M. avium* strains 724 or 104. Maximum-likelihood estimation of two breakpoints allowed a approximately 4100 bp region horizontally transferred into the strain 2151 genome to be pinpointed with confidence. The maintenance of sequence continuity at both breakpoints and the lack of insertional elements at these sites strongly suggest that the integration of foreign DNA occurred by homologous recombination. To our knowledge, this is the first report to demonstrate naturally occurring homologous recombination in *Mycobacterium*. This previously undiscovered mechanism of genetic exchange may have major implications for the understanding of *Mycobacterium pathogenesis*.—Authors' Abstract

**Lanigan, M. D., Vaughan, J. A., Shiell, B. J., Beddome, G. J., and Michalski, W. P.** *Mycobacterial proteome extraction: comparison of disruption methods. Proteomics* **4**(4) (2004) 1094–1100.

*Mycobacterium avium* subsp. paratuberculosis has long been recognized as the causative agent of Johne's disease, a chronic inflammatory intestinal disease of sheep, cattle and other ruminants. *Mycobacterial* cells are extremely hardy, and proteomic analyses require the use of harsh conditions to effect their disruption. We compared the effectiveness of bead beating and sonication as cell lysis methods for the extraction of the proteomes of *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *paratuberculosis*. Broad and narrow range

two-dimensional gel electrophoresis was used to compare the numbers of silver stained protein spots that were observed in mycobacterial lysates. Despite differences in the yield of total protein from either species, and at different ages, the two methods appeared to give similar representations of the mycobacterial proteomes analyzed. Bead beating therefore represents a rapid and effective method of extracting the proteomes of mycobacterial species without the risks associated with an open tube sonication procedure.—Authors' Abstract

**Li, M. S., Waddell, S. J., Monahan, I. M., Mangan, J. A., Martin, S. L., Everett, M. J., and Butcher, P. D.** Increased transcription of a potential sigma factor regulatory gene Rv1364c in *Mycobacterium bovis* BCG while residing in macrophages indicates use of alternative promoters. *FEMS Microbiol. Lett.* **233**(2) (2004) 333–339.

Alternative sigma factors are key global regulators that coordinate bacterial responses to environmental changes necessary for adaptation and survival. In turn these sigma factors are controlled by regulators such as anti-sigma and anti-anti-sigma factors. In this report, using a cDNA-total RNA subtractive hybridization strategy that we have developed previously, we identified increased transcription of a potential sigma factor regulatory gene, Rv1364c, in *Mycobacterium bovis* BCG upon phagocytosis by macrophages and this was confirmed by Northern blot analysis. Primer extension analysis revealed the use of alternative promoters, P1 and P2, and that the increased expression inside macrophages coincided with promoter switching from P2 to P1. Rv1364c (653 amino acids), originally annotated as RsbU, contains structural domains homologous to the PAS redox sensor, the protein phosphatases anti-anti-sigma factor RsbU/SpoIIE, the protein kinase anti-sigma factor RsbW/SpoIIAB and the anti-anti-sigma factor RsbV/SpoIIAA found in other bacteria. These findings have important implications for understanding coordination of the expression of sigma factors under intra-macrophage conditions. Other potentially differentially expressed genes,

including genes for fatty acid metabolism, membrane transporters, heat shock proteins, potential sigma factors and energy metabolic pathways are also listed and their biological significance discussed.—Authors' Abstract

**Liu, C., Flamoe, E., Chen, H. J., Carter, D., Reed, S. G., and Campos-Neto, A.** Expression and purification of immunologically reactive DPPD, a recombinant *Mycobacterium tuberculosis* skin test antigen, using *Mycobacterium smegmatis* and *Escherichia coli* host cells. *Can. J. Microbiol.* **50(2)** (2004) 97–105.

DPPD is a *Mycobacterium tuberculosis* recombinant antigen that elicits specific delayed type hypersensitivity reactions similar in size and morphological aspects to that elicited by purified protein derivative, in both guinea pigs and humans infected with *M. tuberculosis*. In addition, earlier clinical studies with DPPD suggested that this molecule could improve the specificity of the tuberculin skin test, which is used as an important aid for the diagnosis of tuberculosis. However, these studies could only be performed with DPPD engineered as a fusion molecule with another *Mycobacterium* spp. protein because no expression of DPPD could be achieved as a single molecule or as a conventional fusion protein in any commercial system. Although recombinant fusion proteins are in general suitable for several biological studies, they are by definition not ideal for studies involving highly purified and defined polypeptide sequences. Here, we report two alternative approaches for the expression of immunologically reactive recombinant genuine DPPD. The first approach used the rapidly growing, non-pathogenic *Mycobacterium smegmatis* as host cells transformed with the pSMT3 plasmid vector containing the full-length DPPD gene. The second approach used *Escherichia coli* transformed with the pET-17b plasmid vector containing the DPPD gene engineered in a three-copy fusion manner in tandem with itself. Though at low levels, expression and purification of immunologically reactive DPPD in *M. smegmatis* could be achieved. More abundant expression and purification of DPPD as a homo-trimer mol-

ecule was achieved in *E. coli* ( $\geq 2$  mg/L of bacterial broth cultures). Interestingly, expression could only be achieved in host cells transformed with the DPPD gene containing its leader peptide. However, the expressed proteins lacked the leader sequence, which indicates that processing of the *M. tuberculosis* DPPD gene was accurately achieved and necessary in both *M. smegmatis* and *E. coli*. More importantly, the delayed type hypersensitivity reactions elicited by purified molecules in guinea pigs infected with *M. tuberculosis* were indistinguishable from that elicited by purified protein derivative. Because the DPPD gene is present only in the tuberculosis-complex organisms of the *Mycobacterium* genus, these highly purified molecules should be helpful in identifying individuals sensitized with tubercle bacilli.—Authors' Abstract

**Mailaender, C., Reiling, N., Engelhardt, H., Bossmann, S., Ehlers, S., and Niederweis, M.** The MspA porin promotes growth and increases antibiotic susceptibility of both *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis*. *Microbiology* **150(Pt 4)** (2004) 853–864.

Porins mediate the diffusion of hydrophilic solutes across the outer membrane of mycobacteria, but the efficiency of this pathway is very low compared to Gram-negative bacteria. To examine the importance of porins in slow-growing mycobacteria, the major porin MspA of *Mycobacterium smegmatis* was expressed in *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Approximately 20 and 35 MspA molecules per microm<sup>2</sup> cell wall were observed in *M. tuberculosis* and *M. bovis* BCG, respectively, by electron microscopy and quantitative immunoblot experiments. Surface accessibility of MspA in *M. tuberculosis* was demonstrated by flow cytometry. Glucose uptake was twofold faster, indicating that the outer membrane permeability of *M. bovis* BCG to small and hydrophilic solutes was increased by MspA. This significantly accelerated the growth of *M. bovis* BCG, identifying very slow nutrient uptake as one of the determinants of slow growth in mycobacteria. The suscep-

tibility of both *M. bovis* BCG and *M. tuberculosis* to zwitterionic beta-lactam antibiotics was substantially enhanced by MspA, decreasing the minimal inhibitory concentration up to 16-fold. Furthermore, *M. tuberculosis* became significantly more susceptible to isoniazid, ethambutol and streptomycin. Fluorescence with the nucleic acid binding dye SYTO 9 was 10-fold increased upon expression of mspA. These results indicated that MspA not only enhanced the efficiency of the porin pathway, but also that of pathways mediating access to large and/or hydrophobic agents. This study provides the first experimental evidence that porins are important for drug susceptibility of *M. tuberculosis*.—Authors' Abstract

**Muttucumaru, D. G., and Parish, T.** The molecular biology of recombination in mycobacteria: what do we know and how can we use it? *Curr. Issues Mol. Biol.* **6(2)** (2004) 145–157.

Recombination is a ubiquitous genetic process which results in the exchange of DNA between two substrates. Homologous recombination occurs between DNA species with identical sequence whereas illegitimate recombination can occur between DNA with very little or no homology. Site-specific recombination is often used by temperate phages to stably integrate into bacterial chromosomes. Characterisation of the mechanisms of recombination in mycobacteria has mainly focussed on RecA-dependent homologous recombination and phage-directed site-specific recombination. In contrast the high frequency of illegitimate recombination in slow-growing mycobacteria has not been explained. The role of DNA repair in dormancy and infection have not yet been fully established, but early work suggests that RecA-mediated pathways are not required for virulence. All three recombination mechanisms have been utilized in developing genetic techniques for the analysis of the biology and pathogenesis of mycobacteria. A recently developed method for studying essential genes will generate further insights into the biology of these important organisms.—Authors' Abstract

**Onwueme, K. C., Ferreras, J. A., Buglino, J., Lima, C. D., and Quadri, L. E.** Mycobacterial polyketide-associated proteins are acyltransferases: proof of principle with *Mycobacterium tuberculosis* PapA5. *Proc. Natl. Acad. Sci. USA* **101(13)** (2004) 4608–4613.

*Mycobacterium tuberculosis* (Mt) produces complex virulence-enhancing lipids with scaffolds consisting of phthiocerol and phthiodiolone dimycocerosate esters (PDIMs). Sequence analysis suggested that PapA5, a so-called polyketide-associated protein (Pap) encoded in the PDIM synthesis gene cluster, as well as PapA5 homologs found in Mt and other species, are a subfamily of acyltransferases. Studies with recombinant protein confirmed that PapA5 is an acetyltransferase. Deletion analysis in Mt demonstrated that papA5 is required for PDIM synthesis. We propose that PapA5 catalyzes diesterification of phthiocerol and phthiodiolone with mycocerosate. These studies present the functional characterization of a Pap and permit inferences regarding roles of other Paps in the synthesis of complex lipids, including the antibiotic rifamycin.—Authors' Abstract

**Pathak, A. K., Pathak, V., Riordan, J. M., Gurcha, S. S., Besra, G. S., and Reynolds, R. C.** Synthesis of manno-pyranose disaccharides as photoaffinity probes for mannosyltransferases in *Mycobacterium tuberculosis*. *Carbohydr. Res.* **339(3)** (2004) 683–691.

Mannosyltransferases play a crucial role in mycobacterial cell-wall biosynthesis and are potential new drug targets for the treatment of tuberculosis. Herein, we describe the synthesis of alpha-(1→2)- and alpha-(1→6)-linked mannopyranosyl disaccharides possessing a 5-azidonaphthlene-1-sulfonamidoethyl group as photoaffinity probes for active-site labeling studies of mannosyltransferases in *Mycobacterium tuberculosis*.—Authors' Abstract

**Sethi, S., Sharma, M., Sengupta, C., Mohandas, K., and Sharma, S. K.** Enhanced detection of *Mycobacteria* stained

with rhodamine auramine at 37 degrees C. Indian J. Pathol. Microbiol. **46(3)** (2003) 521–523.

Sputum smear microscopy is the most efficient and rapid technique for detection of acid-fast bacilli (AFB). Fluorochrome method of staining is preferred for Mycobacteria in the overburdened laboratories as the fluorescing bacilli are more readily detected than the fuchsin stained bacilli in shorter period of time. A total of 300 sputum samples obtained from suspected cases of Tuberculosis were collected and were subjected to staining by rhodamine auramine at 37 degrees C and also at room temperature (conventional method). The smears were then blindly evaluated. Fifty-eight samples were positive by both methods and 5 were positive at 37 degrees C only. Staining at 37 degrees C increased the smear positivity by 8.6% over conventional staining at room temperature. No smears were positive only with staining at room temperature alone. Out of 58 smears positive by both methods, 25 had equal number of AFB in both smears, 22 had more AFB in smear stained at 37 degrees C and 11 had greater number of AFB in smears stained at room temperature. Our study, therefore, indicates that rhodamine auramine staining at 37 degrees C is superior to conventional auramine method at room temperature for detecting AFB in sputum smears.—Authors' Abstract

**Sharma, K., Chandra, H., Gupta, P. K., Pathak, M., Narayan, A., Meena, L. S., D'Souza, R. C., Chopra, P., Ramachandran, S., and Singh, Y.** PknH, a transmembrane Hank's type serine/threonine kinase from *Mycobacterium tuberculosis* is differentially expressed under stress conditions. FEMS Microbiol. Lett. **233(1)** (2004) 107–113.

Serine/threonine protein kinases (STPKs) represent a burgeoning concept in prokaryotic signaling and have been implicated in a range of control mechanisms. This paper describes the enzymatic and molecular characterization of PknH, a mycobacterial STPK. After cloning and expression as a Glutathione-S-transferase fusion protein in *E. coli*, PknH was found to phosphorylate it-

self and exogenous substrates like myelin basic protein and histone. The kinase activity of PknH was inhibited by the kinase inhibitors staurosporine and H-7. The results confirmed that PknH is a transmembrane protein and is restricted to members of the *Mycobacterium tuberculosis* complex. In addition, transcriptional analysis of *pknH* in *M. tuberculosis* under various stress conditions revealed that exposure to low pH and heat shock decreased the level of *pknH* transcription significantly. This is the first report describing differential expression of a mycobacterial kinase in response to stress conditions which can indicate its ability to regulate cellular events promoting bacterial adaptation to environmental change.—Authors' Abstract

**Stermann, M., Sedlacek, L., Maass, S., and Bange, F. C.** A promoter mutation causes differential nitrate reductase activity of *Mycobacterium tuberculosis* and *Mycobacterium bovis*. J. Bacteriol. **186(9)** (2004) 2856–2861.

The recent publication of the genome sequence of *Mycobacterium bovis* showed >99.95% identity to *M. tuberculosis*. No genes unique to *M. bovis* were found. Instead numerous single-nucleotide polymorphisms (SNPs) were identified. This has led to the hypothesis that differential gene expression due to SNPs might explain the differences between the human and bovine tubercle bacilli. One phenotypic distinction between *M. tuberculosis* and *M. bovis* is nitrate reduction, which not only is an essential diagnostic tool but also contributes to mycobacterial pathogenesis. We previously showed that *narGHJI* encodes a nitrate reductase in both *M. tuberculosis* and *M. bovis* and that *NarGHJI*-mediated nitrate reductase activity was substantially higher in the human tubercle bacillus. In the present study we used a genetic approach to demonstrate that an SNP within the promoter of the nitrate reductase gene cluster *narGHJI* is responsible for the different nitrate reductase activity of *M. tuberculosis* and *M. bovis*. This is the first example of an SNP that leads to differential gene expression between the human and bovine tubercle bacilli.—Authors' Abstract



**Tansuphasiri, U., Boonrat, P., and Rienthong, S.** Direct identification of *Mycobacterium tuberculosis* from sputum on Ziehl-Neelsen acid fast stained slides by use of silica-based filter combined with polymerase chain reaction assay. *J. Med. Assoc. Thai.* **87**(2) (2004) 180–189.

This paper describes a method for isolation of deoxyribonucleic acid (DNA) from Ziehl-Neelsen stained sputum smears on glass slides; and isolated DNA was used for the IS6110 polymerase chain reaction (PCR)-based identification of *M. tuberculosis*. A total of 221 samples from newly diagnosed suspected tuberculosis cases were first examined by microscopic examination. For DNA extraction by silica-based filter, a home-made modified spin column gave the efficacy as did the nucleospin tissue reagent kit and therefore was selected for PCR template preparation. The extracted DNA was amplified by the IS6110 PCR using a primer pair that amplifies a 377-bp target, and the product was analyzed by agarose gel elec-

trophoresis with confirmation by Southern blot hybridization. In comparison with culture, PCR with template prepared by the silica based filter showed overall sensitivity and specificity of 91.7 and 100 per cent, respectively. This study used the over one year and less than one year slides samples to study the effect of storage time. In the more than one year storage group, PCR assay gave a sensitivity and specificity of 83.3 and 100 per cent, respectively. In conclusion, the applicability of the PCR directly to DNA extracted from Ziehl-Neelsen stained smears could become a valuable alternative approach for rapid identification of *M. tuberculosis*, and could be used to evaluate quality of the control of local laboratories in tuberculosis (TB) screening and solve the problem of specimen transportation. In addition, the method could be used in retrospective studies involving a wide range of PCR-based analyses, such as detection of rifampicin resistant gene in multidrug-resistant tuberculosis (MDR-TB) study.—Authors' Abstract

## Microbiology, Leprosy

**Groathouse, N. A., Rivoire, B., Kim, H., Lee, H., Cho, S. N., Brennan, P. J., and Vissa, V. D.** Multiple polymorphic loci for molecular typing of strains of *Mycobacterium leprae*. *J. Clin. Microbiol.* **42**(4) (2004) 1666–1672.

The need for molecular tools for the differentiation of isolates of *Mycobacterium leprae*, the organism that causes leprosy, is urgent in view of the continuing high levels of new case detection, despite years of aggressive chemotherapy and the consequent reduction in the prevalence of leprosy. The slow onset of leprosy and the reliance on physical examination for detection of disease have restricted the epidemiological tracking necessary to understand and control transmission. Two genetic loci in several isolates of *M. leprae* have previously been demonstrated to contain variable-number tandem repeats (VNTRs). On the basis of these reports and the availability of the full genome sequence, multiple-locus VNTR analysis for strain typing has been under-

taken. A panel of 11 short tandem repeat (STR) loci with repeat units of 1, 2, 3, 6, 12, 18, 21, and 27 bp from four clinical isolates of *M. leprae* propagated in armadillo hosts were screened by PCR. Fragment length polymorphisms were detected at 9 of the 11 loci by agarose gel electrophoresis. Sequencing of representative DNA products confirmed the presence of VNTRs between isolates. The application of nine new polymorphic STRs in conjunction with automated methods for electrophoresis and size determination allows greater discrimination between isolates of *M. leprae* and enhances the potential of this technique to track the transmission of leprosy.—Authors' Abstract

**Maeda, Y., Brennan, P. J., and Makino, M.** Studies of lipoproteins of *Mycobacterium leprae*. *Nihon Hansenbyo Gakkai Zasshi.* **73**(1) (2004) 15–21.

The deciphering of the genomic sequence of *Mycobacterium leprae* has made possible

to predict the possible lipoproteins. The consensus sequence at the N-terminal region of the protein, including the cysteine residue to which the lipid moiety gets attached, provides a clue to the search. As such, more than 20 putative lipoproteins have been identified from *Mycobacterium leprae* genomic sequence. Lipoprotein LpK (Accession no. ML0603) which encodes for 371 amino acid precursor protein, was identified. Expression of the protein, in *Escherichia coli* revealed a 33 kD protein, and metabolic labeling experiments proved that the protein was lipidated. The purified lipoprotein was found to induce production of IL-12 in human peripheral blood monocytes which may imply that *M. leprae* LpK is involved in protective immunity against leprosy. Pursuit of such lipoproteins may reveal insights into the pathogenesis of the disease.—Authors' Abstract

**Sampson, S. L., Dascher, C. C., Sambandamurthy, V. K., Russell, R. G., Jacobs, W. R., Jr., Bloom, B. R., and Hondalus, M. K.** Protection elicited by a double leucine and pantothenate auxotroph of *Mycobacterium tuberculosis* in guinea pigs. *Infect. Immun.* **72**(5) (2004) 3031–3037.

We developed a live, fully attenuated *Mycobacterium tuberculosis* vaccine candidate strain with two independent attenuating auxotrophic mutations in leucine and pantothenate biosynthesis. The deltaleuD deltapanCD double auxotroph is fully attenuated in the SCID mouse model and highly immunogenic and protective in the extremely sensitive guinea pig tuberculosis model, reducing both bacterial burden and disease pathology.—Authors' Abstract

**Truman, R., Fontes, A. B., De Miranda, A. B., Suffys, P., and Gillis, T.** Genotypic variation and stability of four variable-number tandem repeats and their suitability for discriminating strains of *Mycobacterium leprae*. *J. Clin. Microbiol.* **42**(6) (2004) 2558–2565.

It has not been possible to distinguish different strains of *Mycobacterium leprae* according to their genetic sequence. However,

the genome contains several variable-number tandem repeats (VNTR), which have been used effectively in strain typing of other bacteria. To determine their suitability for differentiating *M. leprae*, we developed PCR systems to amplify 5 different VNTR loci and examined a battery of 12 *M. leprae* strains derived from patients in different regions of the United States, Brazil, Mexico, and the Philippines, as well as from wild armadillos and a sooty mangabey monkey. We found diversity at four VNTR (D = 0.74), but one system (C(16)G(8)) failed to yield reproducible results. Alleles for the GAA VNTR varied in length from 10 to 16 copies, those for AT(17) varied in length from 10 to 15 copies, those for GTA varied in length from 9 to 12 copies, and those for TA(18) varied in length from 13 to 20 copies. Relatively little variation was seen with interspecies transfer of bacilli or during short-term passage of strains in nude mice or armadillos. The TA(18) locus was more polymorphic than other VNTR, and genotypic variation was more common after long-term expansion in armadillos. Most strain genotypes remained fairly stable in passage, but strain Thai-53 showed remarkable variability. Statistical cluster analysis segregated strains and passage samples appropriately but did not reveal any particular genotype associable with different regions or hosts of origin. VNTR polymorphisms can be used effectively to discriminate *M. leprae* strains. Inclusion of additional loci and other elements will likely lead to a robust typing system that can be used in community-based epidemiological studies and select clinical applications.—Authors' Abstract

**Youn, J. H., Myung, H. J., Liav, A., Chatterjee, D., Brennan, P. J., Choi, I. H., Cho, S. N., and Shin, J. S.** Production and characterization of peptide mimotopes of phenolic glycolipid-I of *Mycobacterium leprae*. *FEMS Immunol. Med. Microbiol.* **41**(1) (2004) 51–57.

Phenolic glycolipid-I (PGL-I), a *Mycobacterium leprae*-specific antigen, has been widely used for the serodiagnosis of leprosy and has been implicated in the pathogenesis of leprosy. In an effort to produce an al-

ternate antigen of PGL-I, the mimotope peptides of PGL-I, W(T/R)LGPY(V/M), were obtained using a monoclonal antibody, III603.8, specific to PGL-I by a phage library. The biotin-labeled predominant mimotope peptide of PGLP1, WTLGPYV, bound to III603.8 in a dose-dependent manner in an immunoassay. However, PGLP1 did not bind to anti-PGL-I antibodies in the

serum samples from leprosy patients that were reactive to PGL-I. Although the mimotope peptide of WTLGPYV was not effective as an alternate antigen of PGL-I for the serodiagnosis of leprosy, but it would be of interest to know how the mimotope peptides mimic the role of PGL-I antigen in the pathogenesis of *M. leprae* infection.—Authors' Abstract

## Microbiology, Tuberculosis

**Bigi, F., Gioffre, A., Klepp, L., de la Paz Santangelo, M., Alito, A., Caimi, K., Meikle, V., Zumarraga, M., Taboga, O., Romano, M. I., and Cataldi, A.** The knockout of the *lprG*-Rv1410 operon produces strong attenuation of *Mycobacterium tuberculosis*. *Microbes Infect.* **6**(2) (2004) 182–187.

P27 lipoprotein was previously described as an antigen in the *Mycobacterium tuberculosis* complex, encoded by the *lprG* gene, also named Rv1411 in the TubercuList (<http://genolist.pasteur.fr/TubercuList>) gene bank. It forms an operon with Rv1410 that encodes for an efflux pump, P55. A mutant of the H37Rv strain of *M. tuberculosis* not producing P27 (strain DeltaP27) was obtained by two-step mutagenesis using the counterselectable marker *sacB* and a thermosensitive origin of replication in the shuttle plasmid pPR27. By RT-PCR, we observed no *lprG* or Rv1410 mRNA in the DeltaP27 mutant strain compared with the wild type and complemented strains. Western blot experiments using anti-P27 polyclonal sera showed that the P27 protein was present both in the parental and in a complemented strain, in which the entire *lprG*-Rv1410 operon was reintroduced, but absent in the mutant strain. The three strains showed similar growth kinetics and characteristics in culture broth. To study the effect of the *lprG* mutation on *M. tuberculosis* virulence, BALB/c mice were inoculated to determine bacterial loads in spleens. At days 15 and 35 after infection, decreases of 1.5 and 2.5 logs in the bacterial load were found, respectively, in animals inoculated with the DeltaP27 mutant strain or with the wild type. This attenuation was reverted in the comple-

mented strain. These results demonstrated that *lprG* gene is required for growth of *M. tuberculosis* in immunocompetent mice. The reversion of attenuation in the complemented strain indicates that the attenuated phenotype resulted from disruption of the *lprG*-Rv1410 operon.—Authors' Abstract

**Dhiman, R. K., Schulbach, M. C., Mahapatra, S., Baulard, A. R., Vissa, V., Brennan, P. J., and Crick, D. C.** Identification of a novel class of omega,E,E-farnesyl diphosphate synthase from *Mycobacterium tuberculosis*. *J. Lipid. Res.* **45**(6) (2004) 1140–1147.

We have identified an omega,E,E-farnesyl diphosphate (omega,E,E-FPP) synthase, encoded by the open reading frame Rv3398c, from *Mycobacterium tuberculosis* that is unique among reported FPP synthases in that it does not contain the type I (eukaryotic) or the type II (eubacterial) omega,E,E-FPP synthase signature motif. Instead, it has a structural motif similar to that of the type I geranylgeranyl diphosphate synthase found in Archaea. Thus, the enzyme represents a novel class of omega,E,E-FPP synthase. Rv3398c was cloned from the *M. tuberculosis* H37Rv genome and expressed in *Mycobacterium smegmatis* using a new mycobacterial expression vector (pVV2) that encodes an in-frame N-terminal affinity tag fusion with the protein of interest. The fusion protein was well expressed and could be purified to near homogeneity, allowing facile kinetic analysis of recombinant Rv3398c. Of the potential allylic substrates tested, including dimethylallyl diphosphate, only geranyl diphosphate served as an acceptor for isopentenyl diphosphate.

The enzyme has an absolute requirement for divalent cation and has a  $K(m)$  of 43  $\mu M$  for isopentenyl diphosphate and 9.8  $\mu M$  for geranyl diphosphate and is reported to be essential for the viability of *M. tuberculosis*.—Authors' Abstract

**Hirsh, A. E., Tsolaki, A. G., DeRiemer, K., Feldman, M. W., and Small, P. M.** Stable association between strains of *Mycobacterium tuberculosis* and their human host populations. *Proc. Natl. Acad. Sci. U. S. A.* **101(14)** (2004) 4871–4876.

*Mycobacterium tuberculosis* is an important human pathogen in virtually every part of the world. Here we investigate whether distinct strains of *M. tuberculosis* infect different human populations and whether associations between host and pathogen populations are stable despite global traffic and the convergence of diverse strains of the pathogen in cosmopolitan urban centers. The recent global movement and transmission history of 100 *M. tuberculosis* isolates was inferred from a molecular epidemiologic study of tuberculosis that spans 12 years. Genetic relationships among these isolates were deduced from the distribution of large genomic deletions, which were identified by DNA microarray and confirmed by PCR and sequence analysis. Phylogenetic analysis of these deletions indicates that they are unique event polymorphisms and that horizontal gene transfer is extremely rare in *M. tuberculosis*. In conjunction with the epidemiological data, phylogenies reveal three large phylogeographic regions. A host's region of origin is predictive of the strain of tuberculosis he or she carries, and this association remains strong even when transmission takes place in a cosmopolitan urban center outside of the region of origin. Approximate dating of the time since divergence of East Asian and Philippine clades of *M. tuberculosis* suggests that these lineages diverged centuries ago. Thus, associations between host and pathogen populations appear to be highly stable.—Authors' Abstract

**Kocincova, D., Sonden, B., Bordat, Y., Pivert, E., de Mendonca-Lima, L., Gicquel, B., and Reyat, J. M.** The hy-

drophobic domain of the Mycobacterial Erp protein is not essential for the virulence of *Mycobacterium tuberculosis*. *Infect. Immun.* **72(4)** (2004) 2379–2382.

Erp (exported repetitive protein) is a member of a mycobacterium-specific family of extracellular proteins. A hydrophobic region that is localized at the C-terminal domain and that represents a quarter of the protein is highly conserved across species. Here we show that this hydrophobic region is not essential for restoring the virulence and tissue damage of an *erp::aph* mutant strain of *M. tuberculosis* as assessed by bacterial counts and lung histology analysis in a mouse model of tuberculosis.—Authors' Abstract

**Okkels, L. M., and Andersen, P.** Protein-protein interactions of proteins from the ESAT-6 family of *Mycobacterium tuberculosis*. *J. Bacteriol.* **186(8)** (2004) 2487–2491.

In the present study, we demonstrate that, in analogy with the genes encoding ESAT-6 and CFP-10, the genes *rv0287* and *rv0288* from the ESAT-6 gene family are cotranscribed. Using Western-Western blotting and protein-print overlay methodologies, we demonstrate that ESAT-6 and CFP-10, as well as the protein pair *Rv0288/Rv0287*, interact pairwise in a highly specific way. Most notably, the ESAT-6 proteins interact directly with *Rv3873*, a possible cell envelope component of the ESAT-6 secretion pathway.—Authors' Abstract

**Olakanmi, O., Schlesinger, L. S., Ahmed, A., and Britigan, B. E.** The nature of extracellular iron influences iron acquisition by *Mycobacterium tuberculosis* residing within human macrophages. *Infect. Immun.* **72(4)** (2004) 2022–2028.

We have reported that *Mycobacterium tuberculosis* residing within the phagosomes of human monocyte-derived macrophages (MDM) can acquire Fe from extracellular transferrin (TF) and sources within the MDM. In the lung, Fe is also bound to lactoferrin (LF) and low-molecular-weight



chelates. We therefore investigated the ability of intraphagosomal *M. tuberculosis* to acquire Fe from these sources. *M. tuberculosis* acquired 30-fold and 3-fold more Fe from LF and citrate, respectively, compared to TF, in spite of similar MDM-associated Fe. *M. tuberculosis* infection decreased MDM-associated Fe relative to uninfected MDM as follows: TF (38.7%), citrate (21.1%), and LF (15.3%). *M. tuberculosis* Fe acquisition from extracellular chelates (exogenous source) and from endogenous MDM Fe initially acquired from the three chelates (endogenous source) was compared. *M. tuberculosis* Fe acquisition was similar from exogenous and endogenous sources supplied as Fe-TF. In contrast, there was much greater intracellular *M. tuberculosis* Fe uptake from LF and citrate from the exogenous than endogenous source. Gamma interferon (IFN-gamma) reduced MDM Fe uptake from each chelate by approximately 50% and augmented the *M. tuberculosis*-induced decrease in MDM Fe uptake from exogenous TF, but not from LF or citrate. IFN-gamma minimally decreased intracellular *M. tuberculosis* Fe acquisition from exogenous Fe-TF but significantly increased Fe uptake from LF and citrate. Intraphagosomal *M. tuberculosis* Fe acquisition from both exogenous and endogenous MDM sources, and the effect of IFN-gamma on this process, is influenced by the nature of the extracellular Fe chelate. *M. tuberculosis* has developed efficient mechanisms of acquiring Fe from a variety of Fe chelates that it likely encounters within the human lung.—Authors' Abstract

**Pinto, R., Tang, Q. X., Britton, W. J., Leyh, T. S., and Triccas, J. A.** The *Mycobacterium tuberculosis* *cysD* and *cysNC* genes form a stress-induced operon that encodes a tri-functional sulfate-activating complex. *Microbiology* **150**(Pt 6) (2004) 1681–1686.

Sulfur metabolism has been implicated in the virulence, antibiotic resistance and antioxidant defence of *Mycobacterium tuberculosis*. Despite its human disease relevance, sulfur metabolism in mycobacteria has not yet been fully characterized. ATP sulfurylase catalyses the synthesis of activated sulfate

(adenosine 5'-phosphosulfate, APS), the first step in the reductive assimilation of sulfate. Expression of the *M. tuberculosis* *cysD* gene, predicted to encode the adenyllyl-transferase subunit of ATP sulfurylase, is up-regulated by the bacilli inside its preferred host, the macrophage. This study demonstrates that *cysD* and *cysNC* orthologues exist in *M. tuberculosis* and constitute an operon whose expression is induced by sulfur limitation and repressed by the presence of cysteine, a major end-product of sulfur assimilation. The *cysDNC* genes are also induced upon exposure to oxidative stress, suggesting regulation of sulfur assimilation by *M. tuberculosis* in response to toxic oxidants. To ensure that the *cysDNC* operon encoded the activities predicted by its primary sequence, and to begin to characterize the products of the operon, they were expressed in *Escherichia coli*, purified to homogeneity, and tested for their catalytic activities. The CysD and CysNC proteins were shown to form a multifunctional enzyme complex that exhibits the three linked catalytic activities that constitute the sulfate activation pathway.—Authors' Abstract

**Prabhakar, S., Mishra, A., Singhal, A., Katoch, V. M., Thakral, S. S., Tyagi, J. S., and Prasad, H. K.** Use of the *hupB* gene encoding a histone-like protein of *Mycobacterium tuberculosis* as a target for detection and differentiation of *M. tuberculosis* and *M. bovis*. *J. Clin. Microbiol.* **42**(6) (2004) 2724–2732.

The gene for histone-like protein (*hupB* [Rv2986c]) of *Mycobacterium tuberculosis* has been identified as a singular target which allows differentiation of two closely related mycobacterial species, namely, *M. tuberculosis* and *M. bovis* of the MTB complex, by a PCR assay. The N and S primer-generated PCR amplicons differed in *M. tuberculosis* and *M. bovis*; these amplicons were determined to be 645 and 618 bp, respectively. This difference was localized to the C-terminal part of the gene by using primers M and S. The C-terminal PCR amplicons of *M. tuberculosis* and *M. bovis* were determined to be 318 and 291 bp, respectively. The differences in the C-terminal portion of the gene were confirmed by re-

striction fragment length polymorphism analysis and sequencing. Sequence analysis indicated that in *M. bovis* there was a deletion of 27 bp (9 amino acids) in frame after codon 128 in the C-terminal part of the hupB gene. In the present study 104 mycobacterial strains and 11 nonmycobacterial species were analyzed for hupB gene sequences. Of the 104 mycobacterial strains included, 62 belonged to the MTB complex and 42 were non-MTB complex strains and species. Neither the hupB gene-specific primers (N and S) nor the C-terminal primers (M and S) amplify DNA from any other mycobacteria, making the assay suitable for distinguishing members of the MTB complex from other mycobacterial species, as well as for differentiating between members of the MTB complex, namely, *M. tuberculosis* and *M. bovis*.—Authors' Abstract

MTB patient isolate (ICC154) which possesses a unique genotypic signature. MATERIALS AND METHODS: The isolate ICC154 was tested for drug sensitivity. Over-expression of Rv1258c as a function of drug pressure was analyzed by RT-PCR and the strain was typed using fluorescent amplified fragment length polymorphism (FAFLP). RESULT: In the presence of rifampicin and ofloxacin, this isolate shows increased transcription of the gene Rv1258c. Genotypic fingerprinting revealed the presence of unique FAFLP markers. CONCLUSION: A clear association between drug resistance and overexpression of an efflux protein is evident from our studies. The presence of specific markers has implications in rapid identification of MDR clinical isolates and consequent disease management.—Authors' Abstract

**Shi, L., North, R., and Gennaro, M. L.** Effect of growth state on transcription levels of genes encoding major secreted antigens of *Mycobacterium tuberculosis* in the mouse lung. *Infect. Immun.* **72**(4) (2004) 2420–2424.

Arrest of the multiplication of *Mycobacterium tuberculosis* caused by expression of adaptive immunity in mouse lung was accompanied by a 10- to 20-fold decrease in levels of mRNAs encoding the secreted Ag85 complex and 38-kDa lipoprotein. *esat-6* mRNA levels were high throughout infection. The data imply that multiplying and nonreplicating tubercle bacilli have different antigen compositions.—Authors' Abstract

**Siddiqi, N., Das, R., Pathak, N., Banerjee, S., Ahmed, N., Katoch, V. M., and Hasnain, S. E.** *Mycobacterium tuberculosis* isolate with a distinct genomic identity overexpresses a tap-like efflux pump. *Infection.* **32**(2) (2004) 109–111.

BACKGROUND: One mechanism proposed for drug resistance in *Mycobacterium tuberculosis* (MTB) is by efflux of the drugs by membrane located pumps. We report a novel and definite association between drug resistance and transcription levels of a tap-like pump (Rv1258c) in a multi-drug resistant

**Wu, S., Howard, S. T., Lakey, D. L., Kipnis, A., Samten, B., Safi, H., Gruppo, V., Wizel, B., Shams, H., Basaraba, R. J., Orme, I. M., and Barnes, P. F.** The principal sigma factor sigA mediates enhanced growth of *Mycobacterium tuberculosis* in vivo. *Mol. Microbiol.* **51**(6) (2004) 1551–1562.

The ability of *Mycobacterium tuberculosis* to grow in macrophages is central to its pathogenicity. We found previously that the widespread 210 strain of *M. tuberculosis* grew more rapidly than other strains in human macrophages. Because principal sigma factors influence virulence in some bacteria, we analysed mRNA expression of the principal sigma factor, sigA, in *M. tuberculosis* isolates during growth in human macrophages. Isolates of the 210 strain had higher sigA mRNA levels and higher intracellular growth rates, compared with other clinical strains and the laboratory strain H37Rv. SigA was also upregulated in the 210 isolate TB294 during growth in macrophages, compared with growth in broth. In contrast, H37Rv sigA mRNA levels did not change under these conditions. Overexpression of sigA enhanced growth of recombinant *M. tuberculosis* in macrophages and in lungs of mice after aerosol infection, whereas recombinant strains expressing antisense transcripts to sigA showed decreased

growth in both models. In the presence of superoxide, sense sigA transformants showed greater resistance than vector controls, and the antisense sigA transformant did not grow. We conclude that *M. tuberculosis* sigA modulates the expression of genes

that contribute to virulence, enhancing growth in human macrophages and during the early phases of pulmonary infection *in vivo*. This effect may be mediated in part by increased resistance to reactive oxygen intermediates.—Authors' Abstract

## Experimental Infections

**Adams, V. C., Hunt, J. R., Martinelli, R., Palmer, R., Rook, G. A., and Brunet, L. R.** *Mycobacterium vaccae* induces a population of pulmonary CD11c+ cells with regulatory potential in allergic mice. *Eur. J. Immunol.* **34**(3) (2004) 631-638.

The hygiene hypothesis proposes that common, harmless microorganisms, present throughout our evolutionary history, have helped to develop immunoregulatory mechanisms that prevent inappropriate immune responses by the host. Using a mouse model of allergic pulmonary inflammation, we report that treatment with an ubiquitous saprophytic mycobacterium, *Mycobacterium vaccae*, significantly reduces allergic inflammation by decreasing type 2 responses such as eosinophilia and IL-4 expression. Rather than observing an increase in type-1 cytokine expression, we found elevated production of IL-10 in the lungs suggesting a role for regulatory T cells. Since induction of these cells may be dependent on APC, we investigated the effects of *M. vaccae* treatment on pulmonary CD11c+ cells. Increased levels of IL-10, TGF-beta and IFN-alpha mRNA were detected in CD11c+ cells from *M. vaccae*-treated allergic mice. We propose that *M. vaccae*-induced CD11c+ cells have a potential regulatory role at the site of inflammation through their secretion of immunomodulatory cytokines.—Authors' Abstract

**Arruda, M. S., Richini, V. B., Oliveira, S. M., and Vilani-Moreno, F. R.** Experimental murine mycobacteriosis: evaluation of the functional activity of alveolar macrophages in thalidomide-treated mice. *Braz. J. Med. Biol. Res.* **37**(4) (2004) 485-492.

Thalidomide is a selective inhibitor of tumor necrosis factor-alpha (TNF-alpha), a cytokine involved in mycobacterial death mechanisms. We investigated the role of this drug in the functional activity of alveolar macrophages in the presence of infection induced by intranasal inoculation of *Mycobacterium avium* in thalidomide-treated and untreated adult Swiss mice. Sixty animals were inoculated with  $5 \times 10^6$  *M. avium* by the respiratory route. Thirty animals received daily thalidomide (30 mg/kg mouse) and 30 received water by gavage up to sacrifice. Ten non-inoculated mice were used as a control group. Lots of animals from each group were evaluated until 6 weeks after inoculation. Infection resulted in an increased total number of inflammatory cells as well as increased activity of pulmonary macrophages. Histologically, intranasal inoculation of bacilli resulted in small mononuclear infiltrates located at the periphery of the organ. Culture of lung fragments revealed the presence of bacilli only at the beginning and at the end of the experimental period. Thalidomide administration did not affect the microbiological or histological features of the infection. Thalidomide-treated and untreated animals showed the same amount of *M. avium* colonies 3 weeks after infection. Although it did not affect bacillary clearance, thalidomide administration resulted in a decreased percent of spread cells and release of hydrogen peroxide, suggesting that factors other than TNF-alpha play a role in the killing of mycobacteria by alveolar macrophages. Thalidomide administration also reduced the number of spread cells among resident macrophages, suggesting a direct effect of the drug on this phenomenon.—Authors' Abstract

**Belley, A., Alexander, D., Di Pietrantonio, T., Girard, M., Jones, J., Schurr, E., Liu, J., Sherman, DR, Behr MA.** Impact of methoxymycolic acid production by *Mycobacterium bovis* BCG vaccines. *Infect. Immun.* **72(5)** (2004) 2803–2809.

BCG vaccines are a family of closely related daughter strains of an attenuated isolate of *Mycobacterium bovis* derived by *in vitro* passage from 1908 to 1921. During subsequent laboratory propagation of the vaccine strain until its lyophilization in 1961, BCG Pasteur underwent at least seven further genomic mutations. The impact of these mutations on the properties of the vaccine is currently unknown. One mutation, a glycine-to-aspartic acid substitution in the *mmaA3* gene, occurred between 1927 and 1931 and impairs methoxymycolic acid synthesis in BCG strains obtained from the Pasteur Institute after this period. Mycolic acids of the cell wall are classified into three functional groups (alpha-, methoxy-, and keto-mycolic acids), and together these lipids form a highly specialized permeability barrier around the bacterium. To explore the impact of methoxymycolic acid production by BCG strains, we complemented the functional gene of *mmaA3* into BCG Denmark and tested a number of *in vitro* and *in vivo* phenotypes. Surprisingly, restoration of methoxymycolic acids alone had no effect on cell wall permeability, resistance to antibiotics, or growth in cultured macrophages and C57BL/6 mice. Our results demonstrate that the loss of methoxymycolic acid production did not apparently affect the virulence of BCG strains.—Authors' Abstract

**Bivas-Benita, M., van Meijgaarden, K. E., Franken, K. L., Junginger, H. E., Borchard, G., Ottenhoff, T. H., and Geluk, A.** Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A\*0201-restricted T-cell epitopes of *Mycobacterium tuberculosis*. *Vaccine* **22(13–14)** (2004) 1609–1615.

In this study, we used an HLA-A2 transgenic mouse model to investigate the effects of pulmonary delivery of a new DNA plasmid encoding eight HLA-A\*0201-restricted

T-cell epitopes from *Mycobacterium tuberculosis* formulated in chitosan nanoparticles. It was shown that the chitosan-DNA formulation was able to induce the maturation of dendritic cells (DCs) while chitosan solution alone could not, indicating the DNA was released from the particles and able to stimulate DCs. Pulmonary administration of the DNA plasmid incorporated in chitosan nanoparticles was shown to induce increased levels of IFN-gamma secretion compared to pulmonary delivery of plasmid in solution or the more frequently used intramuscular immunization route. These results indicate that pulmonary delivery of DNA vaccines against tuberculosis may provide an advantageous delivery route compared to intramuscular immunization, and that increased immunogenicity can be achieved by delivery of this DNA encapsulated in chitosan nanoparticles.—Authors' Abstract

**Chambers, M. A., Wright, D. C., Brisker, J., Williams, A., Hatch, G., Gavier-Widen, D., Hall, G., Marsh, P. D., and Glyn Hewinson, R.** A single dose of killed *Mycobacterium bovis* BCG in a novel class of adjuvant (Novasome) protects guinea pigs from lethal tuberculosis. *Vaccine* **22(8)** (2004) 1063–1071.

The only vaccine currently available for the prevention of tuberculosis in man is a live attenuated vaccine, bacille Calmette-Guerin (BCG), derived from *Mycobacterium bovis*. Concerns over the lack of the universal efficacy and safety of BCG have resulted in efforts to develop a new generation of TB vaccines. Historically, killed whole-cell preparations of mycobacteria have been ineffective vaccines. We revisited the potential of killed whole-cell vaccines by comparing their efficacy with live BCG Pasteur in a guinea pig challenge model. BCG Pasteur was inactivated with a low concentration of formalin and showed to be non-viable in culture or severe combined immunodeficient mice. Formalin-inactivated BCG was mixed with non-phospholipid liposome adjuvants (Novasomes) and administered to guinea pigs as a single subcutaneous inoculation. All formulations were well tolerated and one conferred a significant survival advan-



tage against lethal aerogenic challenge with *M. bovis*.—Authors' Abstract

**Chambers, M. A., Gavier-Widen, D., and Glyn Hewinson, R.** Antibody bound to the surface antigen MPB83 of *Mycobacterium bovis* enhances survival against high dose and low dose challenge. FEMS Immunol. Med. Microbiol. **41**(2) (2004) 93–100.

Tuberculosis caused by infection with *Mycobacterium tuberculosis* or *Mycobacterium bovis* is a significant disease of man and animals. Whilst cellular immunity is the major immunological component required for protection against these organisms, recent reports have suggested that monoclonal antibodies can modify infection with *M. tuberculosis*. To test whether the same was true for *M. bovis* infection, we determined the effect of preincubation of *M. bovis* with a monoclonal antibody on subsequent intravenous infection of mice. Antibodies bound to the surface of *M. bovis* increased the survival time of mice infected with *M. bovis* and changed the morphology of granulomas and the distribution of acid-fast bacilli in the lung. These studies suggest that antibodies directed to the surface of virulent mycobacteria can modulate their virulence *in vivo*.—Authors' Abstract

**Evans, J. T., Ward, J. R., Kern, J., Johnson, M. E.** A single vaccination with protein-microspheres elicits a strong CD8 T-cell-mediated immune response against *Mycobacterium tuberculosis* antigen Mtb8.4. Vaccine **22**(15-16) (2004) 1964–1972.

Efficient protein-based vaccine delivery systems are needed to achieve a persistent memory immune response capable of detecting and eliminating intracellular pathogens such as *Mycobacterium tuberculosis* (TB). We have developed a novel protein-microsphere formulation using the recently discovered TB antigen Mtb8.4. Immunization of mice with a single dose of this Mtb8.4-microsphere formulation resulted in both humoral and cellular responses against Mtb8.4. The Mtb8.4-specific CD8 T-cell

responses following a single administration of Mtb8.4-microspheres exceeded that elicited by protein plus adjuvant following multiple immunizations. These results demonstrate the efficacy of a single dose protein-microsphere vaccine for the induction of strong cell-mediated and humoral immune responses against *M. tuberculosis* antigens.—Authors' Abstract

**Falkinham, J. O., III, Gross, W. B., and Pierson, F. W.** Effect of different cell fractions of *Mycobacterium avium* and vaccination regimens on *Mycobacterium avium* infection. Scand. J. Immunol. **59**(5) (2004) 478–484.

Because of the availability of uniform genetic stocks and the ability to modulate stress levels, chickens were investigated as a host for the development of an antimycobacterial vaccine. The imposition and the timing of stress significantly influenced the outcome of *Mycobacterium avium* infection in chickens. Simple, whole cell or lysate vaccines and combinations of vaccine preparations were identified that led to high levels of protection. In addition, short-term stress at the time of vaccination significantly increased the protective efficacy of *M. avium* vaccine preparations. Post-infection vaccination of *M. avium*-infected chickens was also shown to significantly reduce the number of lesions and colony counts.—Authors' Abstract

**Fan, X. L., Wang, L. M., Wang, F. X., Shi, C. H., Li, Y., Xue, Y., Bai, Y. L., and Xu, Z. K.** [Effects of Th1 cytokine gene on anti-CFP10 antibody production in BALB/c mice induced by *Mycobacterium tuberculosis* DNA vaccine] Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. **19**(3) (2003) 260–262. [Article in Chinese]

AIM: To investigate the effects of plasmid containing mouse IL-12 and human IL-18 genes on the humoral immune response of mice immunized by CFP10 gene of *Mycobacterium tuberculosis* (MTB) H(37)R(v) strain. METHODS: Human IL-18 cDNA was amplified from RNA of PBMCs by RT-PCR and cloned into the pGEM-Teasy vector.

After sequencing it was subcloned into the the sites of BamH I and EcoR I digestion of pcDNA3.1. BALB/c mice were injected intramuscularly by eukaryotic expression plasmid pcIL12 and pcIL18, together with MTB CFP10 DNA vaccine, respectively. The same immunization repeated three times at intervals of two weeks. Mouse sera were collected at two weeks after the each injection. The titer of anti-CFP10 antibody was detected by ELISA. RESULTS: IL-18 cDNA was amplified successfully from RNA of human PBMCs by RT-PCR and the result of sequencing was correct. The IL-18 gene was correctly inserted into the vector pcDNA3.1 by being confirmed with BamH I and EcoR I digestion analysis, positive plasmid was called pcIL18. After being immunized with pcCFP10 three times, the end point titer of anti-CFP10 was 1:4 000, while the titer obtained by being immunized with pcIL18 + pcCFP10 was 1:8 000, but yet, after being immunized with pcIL12+pcCFP10, the end point titer of anti-CFP10 antibody was only 1:200. CONCLUSION: Combination of IL-18 gene with MTB CFP10 DNA vaccine can enhance the humoral immune responses to pcCFP10, whereas the immunization with IL-12 gene plus pcCFP10 made humoral immune response markedly descent. As for whether IL-18 gene plus MTB CFP10 DNA vaccine can induce markedly the cellular mediated immune response to CFP10 or not remains to be further investigated.—Authors' Abstract

mice (C57BL/6 and BALB/c) challenged with virulent *Mycobacterium tuberculosis* (strain Harlingen). The H-kBCG vaccine alone, in accordance with earlier studies, did not give any or only gave slight protection compared to sham-vaccinated controls. However, the same vaccine given with Eurocine L3 adjuvant, either formulated as a suspension or as an emulsion, afforded significant levels of protection. This protection was at least as good as that of the control live attenuated BCG vaccine. The Eurocine L3 adjuvant is approved for human use as a nasal vaccine adjuvant and a successful phase I trial with nasal immunization with diphtheria vaccine has recently been performed in Sweden. Here we show that, in mice, intranasal priming with H-kBCG in Eurocine L3 adjuvant followed by intranasal booster resulted in the same level of protection as subcutaneous priming followed by intranasal booster. All H-kBCG formulations in the Eurocine L3 adjuvant elicited mycobacterial antigen-specific serum IgG and IFN gamma responses. In general, among the different vaccine formulation(s) in the Eurocine L3 adjuvant those that produced a relatively high Th2 response, as measured by IgG1/IgG2a ratio and IFN gamma production *in vitro*, were the most protective. In conclusion, H-kBCG in Eurocine L3 adjuvant could represent a safe and a more stable alternative to the conventional live BCG vaccine.—Authors' Abstract

**Haile, M., Schroder, U., Hamasur, B., Pawlowski, A., Jaxmar, T., Kallenius, G., and Svenson, S. B.** Immunization with heat-killed *Mycobacterium bovis* bacille Calmette-Guerin (BCG) in Eurocine L3 adjuvant protects against tuberculosis. *Vaccine* **22(11-12)** (2004) 1498-1508.

The current live attenuated vaccine against tuberculosis, BCG, poses a risk of disseminated infections in immunocompromised subjects. Therefore, in this study we compared the protective effect of a heat-killed bacille Calmette-Guerin (H-kBCG) vaccine given in a new adjuvant (Eurocine L3) with the protection provided by the conventional live attenuated BCG vaccine in

**Hendricks, E. E., Lin, K. C., Boisvert, K., Pauley, D., and Mansfield, K. G.** Alterations in expression of monocyte chemotactic protein-1 in the simian immunodeficiency virus model of disseminated *Mycobacterium avium* complex. *J. Infect. Dis.* **189(9)** (2004) 1714-1720.

*Mycobacterium avium* complex (MAC) infection is the most common disseminated bacterial infection in untreated patients with acquired immunodeficiency syndrome (AIDS). We investigated the potential role of monocyte chemotactic protein-1 (MCP-1) in the pathogenesis of disseminated MAC, using the simian immunodeficiency virus (SIV)/macaque model of AIDS. Macaques were inoculated with SIV, followed by challenge

with a pathogenic AIDS isolate of *M. avium* 14 days later. After challenge with *M. avium*, marked increases in serum MCP-1 levels were detected in SIV-infected macaques, a finding that was duplicated in coinoculated bronchoalveolar macrophages. MCP-1 levels were significantly higher in SIV-infected macaques than in non-SIV-infected controls (327.1 vs. 151.5 pg/mL, respectively;  $p = 0.04$ ), suggesting that up-regulation of MCP-1 contributes to the development of progressive mycobacterial disease. Similarly, morphometric analysis revealed increased expression of MCP-1 in hepatic microgranulomas from SIV-infected macaques. We conclude that the pronounced increases in MCP-1 levels demonstrated in tissue and serum samples after *M. avium* inoculation may play a role in the development of disseminated mycobacterial disease.—Authors' Abstract

**Hovav, A. H., Davidovitch, L., Nussbaum, G., Mullerad, J., Fishman, Y., and Bercovier, H.** Mitogenicity of the recombinant mycobacterial 27-kilodalton lipoprotein is not connected to its antiprotective effect. *Infect. Immun.* **72**(6) (2004) 3383–3390.

We reported previously that even though immunization with the recombinant mycobacterial 27-kDa lipoprotein (r27) induced a Th1-type response in mice, the vaccinated mice became more susceptible to challenge with *Mycobacterium tuberculosis*. In this study we show that r27 stimulates naive splenocytes to proliferate. Acylation of r27 was crucial for this effect, since a nonacylated mutant of r27, termed r27DeltaSP, failed to stimulate splenocytes either *in vitro* or *in vivo*. Depletion experiments indicated that only B cells were proliferating in a T-cell-independent manner. We also found that r27 is recognized by TLR2, which is involved in mitogenic stimulation. Interestingly, r27 but not r27DeltaSP induced high gamma interferon levels in splenocyte supernatants, whereas no significant interleukin-2 levels were detected. Since B-cell polyclonal activation might aggravate pathogen infection, we asked whether the antiprotective effect of the r27 lipoprotein is associated with its mitogenicity. We showed that, as in the case

of r27, immunization of mice with the non-mitogenic r27DeltaSP lipoprotein resulted in increased *M. tuberculosis* multiplication. We conclude that the antiprotective effect of the r27 lipoprotein must be linked to properties of the polypeptide portion of the lipoprotein rather than to its lipid moiety and its mitogenicity.—Authors' Abstract

**Hussain, R., Shahid, F., Zafar, S., Dojki, M., and Dockrell, H. M.** Immune profiling of leprosy and tuberculosis patients to 15-mer peptides of *Mycobacterium leprae* and *M. tuberculosis* GroES in a BCG vaccinated area: implications for development of vaccine and diagnostic reagents. *Immunology* **111**(4) (2004) 462–471.

*Mycobacterium leprae* (ML) GroES has been shown to induce strong T cell responses in tuberculoid as well as in exposed healthy contacts of leprosy patients, and therefore this antigen has been the focus of study as a potential vaccine candidate. Paradoxically, we have shown that ML GroES also induces extremely high titres of IgG1 antibody in leprosy patients across the disease spectrum, a response associated with disease progression. IgG1 antibodies in leprosy also show a negative association with interferon-gamma, a critical T cell cytokine responsible for macrophage activation and intracellular killing of mycobacteria. We therefore queried if antibody and T cell responses were being evoked by different epitopes in ML GroES proteins. To address the issue of epitope recognition in mycobacterial diseases, we have analysed 16 peptides (15-mer peptides) spanning the entire ML and *M. tuberculosis* GroES protein in leprosy (N = 19) and tuberculosis (N = 9) patients and healthy endemic controls (N = 8). Our analysis demonstrates clearly that the dominant peptides evoking T cell and IgG subclass antibodies were different. The target of both T and B cell responses were cross-reactive epitopes in all groups. Differences in disease and healthy states related to the strength (mean intensity) of the T cell and antibody response. IgG1 and IgG3 antibodies were associated with disseminated disease and IgG 2 and IgG4 with disease limitation. Such comprehensive immune

profiling of antigen-specific responses is critical to understanding the disease pathogenesis and also if these reagents are to be exploited for either diagnostic or vaccine purposes.—Authors' Abstract

**Lim, J. H., Kim, H. J., Lee, K. S., Jo, E. K., Song, C. H., Jung, S. B., Kim, S. Y., Lee, J. S., Paik, T. H., and Park, J. K.** Identification of the new T-cell-stimulating antigens from *Mycobacterium tuberculosis* culture filtrate. *FEMS Microbiol. Lett.* **232(1)** (2004) 51–59.

The proteins secreted by *Mycobacterium tuberculosis* are an important target for vaccine development. To identify the antigens from *M. tuberculosis* culture filtrate (CF) that strongly stimulate T-cells, the CF was fractionated by ion-exchange chromatography and then non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis with mini-whole gel elution. Each fraction was screened for its ability to induce interferon-gamma (IFN-gamma) production in peripheral blood mononuclear cells isolated from healthy tuberculin reactors. The protein bands that strongly induced IFN-gamma production were subjected to N-terminal sequencing. Two new proteins, a 17-kDa protein (Rv0164, MTSP17) and an 11-kDa (Rv3204, MTSP11) protein, were identified. The recombinant MTSP17 (rMTSP17) and rMTSP11 induced significant production of IFN-gamma and interleukin (IL)-12p40 in peripheral blood mononuclear cells from healthy tuberculin reactors. Interestingly, IL-12p40 production in response to rMTSP11 was significantly higher than that in response to rMTSP17 or the three components of the antigen 85 complex. These results suggest that MTSP11 antigen should be further evaluated as a component of a subunit vaccine.—Authors' Abstract

**Luo, X. D., Zhu, D. Y., Jiang, S., Chen, Q., and Jiang, Y.** [The estimation of protective efficacy of the fusion gene vaccine encoding tubercle antigen 85B and MPT64 in mice challenged with *Mycobacterium tuberculosis*]. *Zhonghua Yi Xue Za Zhi* **84(8)** (2004) 687–691. [Article in Chinese]

**OBJECTIVE:** To evaluate the protective efficacy of the fusion DNA vaccine (AM) encoding tubercle Ag85B and MPT64 in mice infected with *Mycobacterium tuberculosis*. **METHODS:** C57BL/6 mice were intramuscularly immunized with the DNA vaccines. The mice were challenged with 10(6) CFU H37Rv via lateral tail vein 35 days later after the third immunization for DNA vaccine groups and 100 days later for BCG vaccinated group. The mice in vaccinated groups and control groups were sacrificed 42 days later following challenge. The lungs and spleens were removed respectively, and the number of CFU in organs and histopathologic changes was determined. The antibody level, IFN-gamma, IL-4 and the survival time in all of the mice were evaluated. **RESULTS:** Antibody titer of pcDNA/Ag85B + pcDNA/MPT64 group and pcDNA/AM group was higher than that of other groups ( $p < 0.05$ ). The level of IFN-gamma produced by spleen lymphocytes and spleen lymphocyte proliferation from BCG group, pcDNA/Ag85B, pcDNA/Ag85B + pcDNA/MPT64 group and pcDNA/AM group was higher than that of other groups ( $p < 0.05$ ). No IL-4 was found in all groups. The number of bacterial colonies in the lungs and spleens was significantly decreased at 6th week postchallenge in all the vaccinated groups ( $p < 0.05$ ), especially in BCG group ( $p < 0.01$ ). The pulmonary histopathological changes were observed 6 weeks later following challenge with *M. tuberculosis* H37Rv. In PBS and pcDNA3.1 groups, the lesion was characterized by seroplastic inflammatory infiltration and lung tissue necrosis, in BCG group by granulomas and numerous macrophages, lymphocytes and a few epithelioid cells. The lesion in pcDNA/Ag85B groups was characterized by seroplastic inflammatory infiltration and a few macrophages, in pcDNA/Ag85B + pcDNA/MPT64 group and pcDNA/AM group, by granulomas, numerous macrophages and lymphocytes. The lesion in spleen was different from the lung and characterized by proliferative lymphocytes and inflammatory infiltration. The results in spleen were similar to those in lung. The survival time of BCG vaccinated mice after challenge with *M. tuberculosis* H37Rv was longer than that of other groups. The survival time of AM group was longer than that of other DNA vaccine



groups. **CONCLUSION:** The pcDNA/AM can improve the protective efficacy in immunized mice against *M. tuberculosis*.—Authors' Abstract

**McShane, H.** Developing an improved vaccine against tuberculosis. *Expert. Rev. Vaccines*. **3(3)** (2004) 299–306.

Despite the availability of a vaccine for over 80 years, the tuberculosis epidemic continues to be a major cause of mortality and morbidity throughout the world. The factors contributing to the resurgence of tuberculosis and the possible explanations for the failure of the current vaccine, bacille Calmette-Guerin, are discussed. The nature of protective immunity to *Mycobacterium tuberculosis* and how this relates to the development of new candidate vaccines is then considered. The issues surrounding the progression of the most promising candidates into Phase I clinical trials are also discussed.—Authors' Abstract

**Miki, K., Nagata, T., Tanaka, T., Kim, Y. H., Uchijima, M., Ohara, N., Nakamura, S., Okada, M., and Koide, Y.** Induction of protective cellular immunity against *Mycobacterium tuberculosis* by recombinant attenuated self-destructing *Listeria monocytogenes* strains harboring eukaryotic expression plasmids for antigen 85 complex and MPB/MPT51. *Infect. Immun.* **72(4)** (2004) 2014–2021.

We report here the induction of specific protective cellular immunity against *Mycobacterium tuberculosis* by the employment of vaccination with recombinant attenuated *Listeria monocytogenes* strains. We constructed self-destructing attenuated *L. monocytogenes* Delta 2 strains carrying eukaryotic expression plasmids for the antigen 85 complex (Ag85A and Ag85B) and for MPB/MPT51 (mycobacterial protein secreted by *M. bovis* BCG/mycobacterial protein secreted by *M. tuberculosis*) molecules. Infection of these recombinant bacteria allowed expression of the genes in the J774A.1 murine macrophage cell line. Intraperitoneal vaccination of C57BL/6 mice with these recombinant bacteria was capa-

ble of inducing purified protein derivative-specific cellular immune responses, such as foot pad reactions, proliferative responses of splenocytes, and gamma interferon production from splenocytes, suggesting the efficacy of vaccination against mycobacterial infection by use of these recombinant *L. monocytogenes* strains. Furthermore, intravenous vaccination with recombinant bacteria carrying expression plasmids for Ag85A, Ag85B, or MPB/MPT51 in BALB/c mice elicited significant protective responses, comparable to those evoked by a live *Mycobacterium bovis* BCG vaccine. Notably, this is the first report to show that MPB/MPT51 is a major protective antigen in addition to Ag85A and Ag85B, which have been reported to be major mycobacterial protective antigens.—Authors' Abstract

**Nolt, D., and Flynn, J. L.** Interleukin-12 therapy reduces the number of immune cells and pathology in lungs of mice infected with *Mycobacterium tuberculosis*. *Infect. Immun.* **72(5)** (2004) 2976–2988.

Alternate modalities for the treatment of *Mycobacterium tuberculosis* are needed due to the rise in numbers of immunosuppressed individuals at risk for serious disease and the increasing prevalence of multidrug-resistant isolates. Interleukin-12 (IL-12) has been shown to improve immune responses against *M. tuberculosis* infection in both humans and mice. Previous studies using high-dose IL-12 in various disease models reported a paradoxical immunosuppression. We demonstrate here that exogenous administration of IL-12 for 8 weeks after an aerosolized low dose of *M. tuberculosis* results in increased survival and decreased pulmonary bacterial loads for CD4-T-cell-deficient mice, most likely due to an early increase in gamma interferon. IL-12 treatment did not impair or enhance the ability of the wild-type mice to control infection, as measured by bacterial numbers. Two novel findings are reported here regarding exogenous IL-12 therapy for *M. tuberculosis* infections: (i). IL-12 treatment resulted in decreased numbers of immune cells and reduced frequencies of lymphocytes (CD8(+), CD4(+), and NK cells) in the lungs of infected mice and (ii). IL-12 therapy reduced the pathology of *M.*

tuberculosis-infected lungs, as granulomas were smaller and less numerous. These studies support an immunoregulatory role for IL-12 in tuberculosis.—Authors' Abstract

**Romano, M., Denis, O., D'Souza, S., Wang, X. M., Ottenhoff, T. H., Brulet, J. M., and Huygen, K.** Induction of *in vivo* functional Db-restricted cytolytic T cell activity against a putative phosphate transport receptor of *Mycobacterium tuberculosis*. *J. Immunol.* **172**(11) (2004) 6913–6921.

Using plasmid vaccination with DNA encoding the putative phosphate transport receptor PstS-3 from *Mycobacterium tuberculosis* and 36 overlapping 20-mer peptides spanning the entire PstS-3 sequence, we determined the immunodominant Th1-type CD4(+) T cell epitopes in C57BL/10 mice, as measured by spleen cell IL-2 and IFN- $\gamma$  production. Furthermore, a potent IFN- $\gamma$ -inducing, D(b)-restricted CD8(+) epitope was identified using MHC class I mutant B6.C-H-2(bm13) mice and intracellular IFN- $\gamma$  and whole blood CD8(+) T cell tetramer staining. Using adoptive transfer of CFSE-labeled, peptide-pulsed syngeneic spleen cells from naive animals into DNA vaccinated or *M. tuberculosis*-infected recipients, we demonstrated a functional *in vivo* CTL activity against this D(b)-restricted PstS-3 epitope. IFN- $\gamma$  ELISPOT responses to this epitope were also detected in tuberculosis-infected mice. The CD4(+) and CD8(+) T cell epitopes defined for PstS-3 were completely specific and not recognized in mice vaccinated with either PstS-1 or PstS-2 DNA. The H-2 haplotype exerted a strong influence on immune reactivity to the PstS-3 Ag, and mice of the H-2(b, p, and f) haplotype produced significant Ab and Th1-type cytokine levels, whereas mice of H-2(d, k, r, s, and q) haplotype were completely unreactive. Low responsiveness against PstS-3 in MHC class II mutant B6.C-H-2(bm12) mice could be overcome by DNA vaccination. IFN- $\gamma$ -producing CD8(+) T cells could also be detected against the D(b)-restricted epitope in H-2(p) haplotype mice. These results highlight the potential of DNA vaccination for the induction and characterization

of CD4(+) and particularly CD8(+) T cell responses against mycobacterial Ags. Authors' Abstract

**Sampson, S. L., Dascher, C. C., Sambandamurthy, V. K., Russell, R. G., Jacobs, W. R., Jr., Bloom, B. R., and Hondalus, M. K.** Protection elicited by a double leucine and pantothenate auxotroph of *Mycobacterium tuberculosis* in guinea pigs. *Infect. Immun.* **72**(5) (2004) 3031–3037.

We developed a live, fully attenuated *Mycobacterium tuberculosis* vaccine candidate strain with two independent attenuating auxotrophic mutations in leucine and pantothenate biosynthesis. The  $\Delta$ leuD  $\Delta$ panCD double auxotroph is fully attenuated in the SCID mouse model and highly immunogenic and protective in the extremely sensitive guinea pig tuberculosis model, reducing both bacterial burden and disease pathology.—Authors' Abstract

**Shi, C. H., Fan, X. L., Xu, Z. K., Li, Y., Bai, Y. L., and Xue Y.** [*Mycobacterium tuberculosis* secreting protein Ag85B-ESAT6 fused expression and purification]. *Zhonghua Jie He He Hu Xi Za Zhi.* **27**(2) (2004) 89–92. [Article in Chinese]

**OBJECTIVE:** To investigate the fused expression of secreted protein Ag85B-ESAT6 of *Mycobacterium tuberculosis*, and to provide a promising preventive subunit vaccine against tuberculosis. **METHODS:** The gene encoding Ag85B and ESAT6 protein was amplified by PCR from genome of *Mycobacterium tuberculosis* H(37)Rv strain, and inserted into cloning vector P(GEM)-T-easy. After sequence analysis, and digestion by restriction endonuclease, Ag85B-ESAT6 was cloned into corresponding sites of the expression vector P(PRO) EXHT, and the recombinant plasmid was transformed into expressive strain *E. coli* DH5  $\alpha$ , induced with IPTG and fusion protein was purified by Ni-NTA purification system. The specific antibody titer in the sera of BALB/c mouse immunized with two fusion protein was detected by ELISA. **RESULTS:** The sequences of Ag85B and ESAT6 by PCR amplification

were identical to those reported by Gen-Bank. The recombinant plasmid fused expression protein of Ag85B-ESAT6 with relative molecular mass (Mr) of 37,000, which was confirmed by Western-blot analysis with specific monoclonal antibody against  $6 \times \text{HismAb}$ . The fused expression protein was insoluble. It could be purified by Ni-NTA purification system. The specific antibody titer in the sera of BALB/c mouse immunized with fusion Ag85B-ESAT6 was 1:1000 and that of mouse immunized with fusion protein ESAT6-Ag85B was 1:5000. CONCLUSIONS: Secreted protein Ag85B-ESAT6 of *Mycobacterium tuberculosis* was successfully fused expressed in E. Coli DH5 alpha. It may become a new type of vaccine against tuberculosis.—Authors' Abstract

**Tian, X., Cai, H., and Zhu, Y. X.** Protection of Mice with a Divalent Tuberculosis DNA Vaccine Encoding Antigens Ag85B and MPT64. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai). **36(4)** (2004) 269–276.

DNA vaccine may be a promising tool for controlling tuberculosis development. However, vaccines encoding single anti-

gens of mycobacterium did not produce protective effect as BCG did. In the present study, we evaluated the immunogenicity and protective efficacy of a divalent DNA vaccine encoding two immunodominant antigens Ag85B and MPT64 of *Mycobacterium tuberculosis*. We found that both humoral and Th1-type (high IFN-kappa, low IL-4) cellular responses obtained from the divalent DNA vaccine group were significantly higher than that conferred by BCG. RT-PCR results showed that antigens were expressed differentially in various organs in divalent DNA vaccine group. The survival rate for mice treated with the divalent DNA vaccine after challenging with high doses of virulent *M. tuberculosis* H37Rv was significantly higher than that of the BCG group or any of the single DNA vaccine group. Significant differences were also found between the single and divalent DNA vaccinated mice in terms of body, spleen and lung weight. Bacterial loading decreased about 2000-fold in lungs and about 100-fold in spleens of divalent DNA vaccinated mice when compared with that of the control group. We conclude that our divalent DNA vaccine may be a better choice for controlling tuberculosis disease in animals.—Authors' Abstract

## Epidemiology

**Chisi, J. E., Nkhoma, A., Zverev, Y., Misiri, H., and Komolafe, O. O.** Leprosy in Nkhotakota District Hospital. East Afr. Med. J. **80(12)** (2003) 635–639

OBJECTIVE: To study the profile of leprosy cases at Nkhotakota District Hospital in Central Region of Malawi. DESIGN: Retrospective cross-sectional study of all registered cases of leprosy from records over a nine year period (January 1992 to April 2001) SETTING: Nkhotakota District Hospital-Central Region of Malawi. RESULTS: In total 526 cases of leprosy were identified from the records. The prevalence rates gradually increased from 0.998 per 10,000 cases in 1992 to 3.39 cases per 10,000 in 1995. There was however a gradual decline of prevalence rates from

1997/1998 that had 3.17 cases per 10,000 to 1.3 cases per 10,000 in 2001. 1996 registered 2.34 cases per 10,000. Fifty seven cases (10.8%) were found with children of the age of 14 or below and 469 (89.2%) cases were of adults. Paucibacillary leprosy presented with more cases than multibacillary leprosy ( $p < 0.0000001$ ). There were 80 (15.2%) cases of multibacillary leprosy compared to 446 (84.8%) cases of paucibacillary leprosy. In addition more males were affected by multibacillary leprosy than females ( $p < 0.0001$ ) and females were more affected by paucibacillary leprosy ( $p < 0.01$ ) than males. CONCLUSION: The results show that paucibacillary leprosy though minor in Malawi can become endemic as paucibacillary leprosy is a reflection of leprosy contacts in the population. We therefore

recommend continued epidemiological surveys of leprosy. Training in leprosy detection should be encouraged so that this disease can be totally eradicated in Malawi.—Authors' Abstract

**Dissanayake, S.** Relative lack of clinical disease among household contacts of tuberculosis patients compared to leprosy households. *Trans. R. Soc. Trop. Med. Hyg.* **98**(3) (2004) 156–164.

The incidence of clinical tuberculosis and clinical leprosy among household members of tuberculosis and leprosy patients in Sri Lanka was studied. The study period was approximately 20 years (January 1981 to December 2001) and the total number of patients and contacts were 325 and 968 for tuberculosis and 726 and 3066 for leprosy, respectively. While none of the tuberculosis patient households had more than 1 patient nor any contacts who developed clinical disease during the observation period, 20% (148/726) of the leprosy patients had more than 1 patient in the family and 0.9% (13/1403) of their contacts who were followed-up developed clinical leprosy during the observation period. Although the tuberculosis patient household contacts did not develop clinical disease, in 79% (88/112) of contacts who were tested by Western blot analysis, there was serologic evidence of *Mycobacterium tuberculosis* infection. These data show that in populations of comparable socio-economic, environmental and geographic locations, tuberculosis and leprosy show very different transmission patterns. In general, in tuberculosis household contacts, in spite of exposure, infection did not proceed to clinical disease. In contrast, a significant number of leprosy household contacts developed clinical leprosy. These findings have implications in the design and implementation of control programs for these two diseases.—Authors' Abstract

**Grover, C., Kubba, S., Nanda, S., Kumar, V., and Reddy, B. S.** Leprosy with Guillain Barre Syndrome: a new neurologic manifestation? *J. Dermatol.* **31**(2) (2004) 119–123.

A 19-year-old female patient of lepromatous leprosy with Type II reaction, on multidrug therapy and prednisolone, presented with acute onset flaccid quadriplegia. The cerebrospinal fluid examination revealed albumino-cytologic dissociation. Nerve biopsy showed infiltration with lepra bacilli, features of vasculitis, and demyelination. There were no other identifiable precipitating factors for Guillain Barre Syndrome in this patient. Her condition improved without any steroid therapy. This case emphasizes the hypothesis that cell injury caused by Type II reaction can expose neural antigens and incite an autoimmune reaction in the form of Guillain Barre Syndrome.—Authors' Abstract

**Gupte, M. D., Murthy, B. N., Mahmood, K., Meeralakshmi, S., Nagaraju, B., and Prabhakaran, R.** Application of lot quality assurance sampling for leprosy elimination monitoring—examination of some critical factors. *Int. J. Epidemiol.* **33**(2) (2004) 344–348.

**BACKGROUND:** The concept of elimination of an infectious disease is different from eradication and in a way from control as well. In disease elimination programs the desired reduced level of prevalence is set up as the target to be achieved in a practical time frame. Elimination can be considered in the context of national or regional levels. Prevalence levels depend on occurrence of new cases and thus could remain fluctuating. There are no ready pragmatic methods to monitor the progress of leprosy elimination programs. We therefore tried to explore newer methods to answer these demands. With the lowering of prevalence of leprosy to the desired level of 1 case per 10,000 population at the global level, the program administrators' concern will be shifted to smaller areas e.g., national and sub-national levels. For monitoring this situation, we earlier observed that lot quality assurance sampling (LQAS), a quality control tool in industry was useful in the initially high endemic areas. However, critical factors such as geographical distribution of cases and adoption of cluster sampling design instead of simple random sampling design deserve attention before LQAS could



generally be recommended. The present exercise was aimed at validating applicability of LQAS, and adopting these modifications for monitoring leprosy elimination in Tamil Nadu state, which was highly endemic for leprosy. **METHODS:** A representative sample of 64,000 people drawn from eight districts of Tamil Nadu state, India, with maximum allowable number of 25 cases was considered, using LQAS methodology to test whether leprosy prevalence was at or below 7 per 10,000 population. Expected number of cases for each district was obtained assuming Poisson distribution. Goodness of fit for the observed and expected cases (closeness of the expected number of cases to those observed) was tested through chi(2). Enhancing factor (design effect) for sample size was obtained by computing the intraclass correlation. **RESULTS:** The survey actually covered a population of 62,157 individuals, of whom 56,469 (90.8%) were examined. Ninety-six cases were detected and this number far exceeded the critical value of 25. The number of cases for each district and the number of cases in the entire surveyed area both followed Poisson distribution. The intraclass correlation coefficients were close to zero and the design effect was observed to be close to one. **CONCLUSIONS:** Based on the LQAS exercises leprosy prevalence in the state of Tamil Nadu in India was above 7 per 10,000. LQAS method using clusters was validated for monitoring leprosy elimination in high endemic areas. Use of cluster sampling makes this method further useful as a rapid assessment procedure. This method needs to be tested for its applicability in moderate and low endemic areas, where the sample size may need increasing. It is further possible to consider LQAS as a monitoring tool for elimination programs with respect to other disease conditions.—Authors' Abstract

**Izumi, S., and Agusni, I.** [Global situation of leprosy and recent progress in molecular epidemiology of the disease]. *Nihon Hansenbyo Gakkai Zasshi* **73**(1) (2004) 37–46. [Article in Japanese]

Recent discovery of genetic diversity of *Mycobacterium leprae* such as variable

number of tandem repeats opened a new era in molecular epidemiology of leprosy infection. It was revealed that the leprosy bacillus in residential environment of endemic villages is an important source of infection. The global elimination strategy will be revised taking new molecular epidemiological knowledge into account. Responsibility of leprosy specialist is to propose feasible control program to local administration based on the epidemiological analysis on transmission of the disease.—Authors' Abstract

**Kerr-Pontes, L. R., Montenegro, A. C., Barreto, M. L., Werneck, G. L., and Feldmeier, H.** Inequality and leprosy in Northeast Brazil: an ecological study. *Int. J. Epidemiol.* **33**(2) (2004) 262–269.

**BACKGROUND:** Leprosy is an important public health problem in many developing countries and many features of its determinants are still obscure. **METHODS:** To investigate whether the incidence of leprosy is related to certain environmental and socioeconomic determinants, an ecological study was undertaken in 165 municipalities of the state of Ceara, Brazil. Social, economic, education, sanitation, demography, meteorology, and health data were collected. The dependent variable was the average incidence rate of leprosy from 1991 to 1999. Simple and multiple linear regressions were performed to assess the relationship between the dependent and the independent variables. **RESULTS:** The average incidence rate for all the municipalities for the 1991–1999 period, varied from 0.06 to 14.68 per 10,000 persons per year. The level of inequality ( $\beta = 1.67$ ,  $p = 0.011$ ), the mean years of study among the population  $\geq 25$  years old ( $\beta = 1.35$ ,  $p < 0.001$ ), the population growth from 1991 to 1996 ( $\beta = 0.02$ ,  $p = 0.007$ ), the percentage of children 7–14 years old that did not go to the school ( $\beta = 0.02$ ,  $p = 0.028$ ), and the presence of a railroad in the municipality ( $\beta = 0.45$ ,  $p = 0.038$ ) were found to be predictors of the incidence rate of leprosy in Ceara. **CONCLUSION:** Our findings fit the assumption that, in Ceara, leprosy is associated with a high level of poverty and uncontrolled urbanization. We put forward the hypothesis that urbanization increases not

only social inequality eventually leading to strong polarization, but also excludes people from social and material opportunities. Apparently, such deprivations render them susceptible for leprosy.—Authors' Abstract

**Matsuoka, M., and Zhang, L.** [Molecular epidemiology of the leprosy]. *Nihon Hansenbyo Gakkai Zasshi* **73**(1) (2004) 7–14. [Article in Japanese]

Application of molecular biological techniques to the epidemiological study of leprosy is described. Studies of detecting *Mycobacterium leprae* DNA in samples of the nasal mucus are discussed in terms of the epidemiology and the significance of high prevalence. Epidemiological studies on the transmission of leprosy and correlation between geographic distribution of different *M. leprae* rpoT genotypes and prehistoric spread of the leprosy by genotyping based on the genomic polymorphism are introduced.—Authors' Abstract

**Meima, A., Richardus, J. H., and Habbema, J. D.** Trends in leprosy case detection worldwide since 1985. *Lepr Rev.* **75**(1) (2004) 19–33.

Trends in case detection and case detection rate (CDR) since 1985 are described at regional and national levels. Annual case detection by WHO Region was available for 1994–2000. Using different sources, complete time series for case detection were constructed for 1985–1998 for a group of 33 endemic countries cumulatively (top 33), and for 14 individual countries (top 14). Population statistics were used to derive CDRs. India contributed 79% to global case detection in 1998. Africa, the Americas and South-East Asia each contributed about 30% when India is excluded. During 1994–2000, case detection did not decrease in these three WHO Regions. The 33 countries contributed 99% and 98% to global case detection in 1994 and 1998, respectively. Cumulative case detection for the top 33 minus India gradually increased, overall almost doubling. The contribution of the top 14 to case detection of the top 33 hardly changed over time, equalling 96% in 1998 (81%

when India is excluded). In terms of annual case detection, Brazil was always ranked second after India; it accounted for 27% of 1998 case detection in the top 33 except India. In 1998, seven of the top 14 countries—including India and Brazil—had CDRs above 2 per 10,000. The CDR did not exceed 1 per 10,000 for the other half. Decreasing tendencies in CDR, either for the whole period or in the 1990s, are observed for four of the top 14 countries (Guinea and three Western Pacific countries: China, Vietnam and the Philippines). In conclusion, there is no general decline in case detection to date, and several important countries still have high CDRs. Prevalence is an irrelevant indicator for monitoring epidemiological changes in leprosy. Trends in the transmission and incidence of leprosy are still completely unclear, necessitating further research. The target to eliminate leprosy as a public health problem, defined as a prevalence of less than 1 per 10,000, is therefore also an inadequate yardstick for decision making on leprosy control.—Authors' Abstract

**Norman, G., Joseph, G. A., Udayasuriyan, P., Samuel, P., and Venugopal, M.** Leprosy case detection using school-children. *Lepr. Rev.* **75**(1) (2004) 34–39.

An innovative method that combined awareness creation with screening of high school students by their peers was undertaken in 26 randomly selected schools in the project area of the Schieffelin Leprosy Research and Training Center, Karigiri, Vellore, India. This method entailed educating teachers and student leaders in grades 8–12 about leprosy and how to suspect leprosy among their peers. The student leaders in turn conducted a similar awareness programme for their peers and encouraged them to report if they suffered from any skin problem or skin lesion. Based on the reporting by their peers, the class leaders prepared a 'suspect list'. Within a fortnight of the awareness program, a trained leprosy worker visited the school and examined all the students on the 'suspect list'. Those diagnosed to have leprosy were referred to a medical officer, who then confirmed the diagnosis and initiated treatment. Among the 23,125 students enrolled in the

26 randomly selected schools, 234 student leaders were educated about leprosy and trained to detect suspect lesions among their peers. A total of 2200 (9.5%) children reported with skin lesions to their leaders and after screening by a leprosy supervisor and confirmation by a medical officer, 14 new cases (NCDR 6.05/10,000) were detected. This rate was found to be comparable with

case detection rates of annual school surveys done during the National Leprosy Eradication Programme (NLEP), when all school-children were examined. The paper suggests that schoolchildren can be used effectively in leprosy case detection and this method has the additional advantage of creating awareness among them, their teachers and communities.—Authors' Abstract

## Rehabilitation

**Tsutsumi, A., Izutsu, T., Islam, M. D., Amed, J. U., Nakahara, S., Takagi, F., and Wakai, S.** Depressive status of leprosy patients in Bangladesh: association with self-perception of stigma. *Lepr. Rev.* **75**(1) (2004) 57–66.

Stigmatization by the general population and their negative attitudes towards leprosy negatively impacts on patients' mental health, and so too does patients' perception of that stigma. The objective of this present study is to assess the depressive status of leprosy patients, the patient perception of that stigma, and its association with their depressive status in Dhaka, Bangladesh. Subjects were 140 patients, and a selected comparison group of 135 local people without any chronic diseases. To evaluate depressive status, the Center for Epidemiologic Studies Depression scale (CES-D) Bengali version was applied. The patient group's depressive

status was significantly more severe than that of the comparison group. Depressive status of those who answered affirmatively was significantly more severe than that of those who answered negatively for three responses to questions: 1) 'I have been physically attacked by people', 2) 'I feel people regard me as strange' and 3) 'I have been refused the purchase of something by a shopkeeper'. The results showed that the depressive status in leprosy patients was greater than that of the general public. Further, actual experiences of discrimination based on stigma associated with the depressive status of leprosy patients. Mental health care for patients, regulation of discriminatory action and education that would decrease social stigma among the general population, especially people who might often have contact with patients, seem necessary to improve the mental health of Bangladeshi leprosy patients.—Authors' Abstract

## Other Mycobacterial Diseases

**Abshire, R., Cockrum, P., Crider, J., and Schlech, B.** Topical antibacterial therapy for mycobacterial keratitis: potential for surgical prophylaxis and treatment. *Clin. Ther.* **26**(2) (2004) 191–196.

**BACKGROUND:** *Mycobacterium chelonae* and *Mycobacterium fortuitum* are the 2 most commonly implicated species of nontuberculous mycobacteria in cases of bacterial keratitis. **OBJECTIVES:** This article summarizes available data on the *in vitro* antibacterial activity against *M. chelonae* or *M. fortuitum* of 2 agents—amikacin and clarithromycin—that have been used in the treatment of bacterial keratitis. In addition,

the article reviews the *in vitro* activity of 5 commercially available topical ocular fluoro-quinolones (in order of availability, ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin) that may have potential in the surgical prophylaxis and treatment of keratitis caused by *M. chelonae* or *M. fortuitum*. **METHODS:** A search of the English-language literature indexed on the MEDLINE, Life Sciences, EMBASE, BIOSIS, and Pharmaprojects databases from 1966 to October 7, 2003, was conducted using the terms *Mycobacterium chelonae*, *Mycobacterium fortuitum*, bacterial keratitis, topical antibiotic therapy, ocular infection-mycobacteria, and LASIK infec-

tions. Data on the minimum concentrations at which 90% of isolates were inhibited (MIC(90)s) were reviewed and compared. RESULTS: In the literature reviewed, the MIC(90) against *M. fortuitum* was from 1 to 16 microg/mL for amikacin, from  $\leq 2$  to  $\geq 8$  microg/mL for clarithromycin, from 0.1 to 1 microg/mL for ciprofloxacin, from 0.5 to 3.13 microg/mL for ofloxacin, and  $\leq 2$  microg/mL for levofloxacin. The results were similar against *M. chelonae*. The fourth-generation fluoroquinolones—gatifloxacin and moxifloxacin—had similar MIC(90)s against *M. fortuitum* (both, 0.2 to 1 microg/mL); however, moxifloxacin had greater activity than gatifloxacin against *M. chelonae* (minimum inhibitory concentration range: moxifloxacin,  $\leq 1$  to 1.6 microg/mL; gatifloxacin, 3.2 to 6.25 microg/mL). CONCLUSIONS: Topical fluoroquinolones may be beneficial for ocular surgical prophylaxis and for the treatment of keratitis caused by *M. chelonae* or *M. fortuitum*. Based on their reported MIC(90)s, none of the antibacterials reviewed had greater *in vitro* activity than moxifloxacin. In addition, moxifloxacin had greater *in vitro* activity than gatifloxacin against *M. chelonae*, one of the predominant nontuberculous mycobacterial species involved in bacterial keratitis. Pending the conduct of controlled clinical studies, these findings suggest that moxifloxacin may have utility in the prevention and treatment of atypical mycobacterial keratitis.—Authors' Abstract

Angeli, K., Lacour, J. P., Mantoux, F., Roujeau, J. C., Andre, P., Truffot-Perrot, C., and Ortonne, J. P. [*Mycobacterium fortuitum* skin infection occurring after a facelift]. Ann. Dermatol. Venerol. 131(2) (2004) 198–200. [Article in French]

INTRODUCTION: *Mycobacterium fortuitum* skin infections are rare and usually iatrogenic. We report a case with cervical involvement following a facelift. OBSERVATION: A 65 year-old woman, without past history, underwent bilateral surgical facelift, complicated by cutaneous necrosis and treated with directed healing at home. Six weeks later, an abscessed nodule appeared under the left maxillary and was drained sur-

gically. Then other pre-auricular and left cervical inflammatory nodules appeared without adenopathy or fever. *M. fortuitum* was isolated in bacteriological samples. The initially probabilistic antibiotherapy with clarithromycin, subsequently adapted with amikacine and ciprofloxacin and then imipenem for a total duration of 3 months, led to the clinical cure. DISCUSSION: *Mycobacterium fortuitum* is a rapidly growing, ubiquitous, mycobacteria responsible for nosocomial infections in immunocompetent patients, notably following plastic surgery. Contamination occurs where there has been a rupture in the skin barrier through contact with a vector (water, surgical material, antiseptic.). Treatment, which is not codified, consists in the association of surgery and antibiotics for several months.—Authors' Abstract

Belzunegui, J., Santisteban, M., Gorordo, M., Barastay, E., Rodriguez-Escalera, C., Lopez-Dominguez, L., Gonzalez, C., and Figueroa, M. Osteoarticular mycobacterial infections in patients with the human immunodeficiency virus. Clin. Exp. Rheumatol. 22(3) (2004) 343–345.

OBJECTIVE: Data about the characteristics of patients with the human immunodeficiency virus (HIV) and concomitant mycobacterial skeletal infection are scarce. Thus, our aim was to describe this condition in a cohort of 11 patients. METHODS: A review of the records of 11 HIV-positive individuals with microbiological confirmation of mycobacterial osteoarticular infection was conducted. The studied data included: age, sex, risk factor for the HIV days between the onset of symptoms and diagnosis, evidence of previous tuberculosis, location of the infection, isolated organism, diagnostic method, laboratory data (erythrocyte sedimentation rate, haemoglobin, leukocyte count), number of CD4+ lymphocytes, antiretroviral therapy, treatment and outcome. RESULTS: Eight patients were men and 3 were women. The median age was 34.2 years (range 20 to 46 years). Previous tuberculosis was present in 5 cases. Mean days between the onset of symptoms and diagnosis was 124 (range 20 to 365 days). Infections involved the knee (4 cases), spine



(3 cases), hip (2 cases), elbow (1 case) and tibia (1 case). ESR was frequently elevated. The CD4 count ranged from 0.03 to  $0.779 \times 10(9)/l$  (mean  $0.245 \times 10(9)/l$ ). *M. tuberculosis* was the responsible organism in 9 cases, *Mycobacterium tuberculosis* plus *Staphylococcus aureus* in one case and *M. Kansasii* in one case. Patients received specific treatments with good results. Surgery was necessary in 4 cases. No deaths occurred. Four patients were anti-retroviral naive at the moment the diagnosis was made. The remainder 8 were on zidovudine therapy. **CONCLUSION:** The immunologic status of patients with HIV and concomitant mycobacterial skeletal infections is quite variable. The outcome of this condition seems to be good.—Authors' Abstract

**Bormann, G., and Marsch, W. C.** [Sporotrichoid atypical mycobacterial infections. Antibiotic monotherapy] *Hautarzt*. **55(3)** (2004) 292–295. [Article in German]

Sporotrichoid forms of atypical mycobacterial infections usually do not show a tendency to spontaneous healing. The therapy of choice in such cases is systemic antibiotics. We report three cases of sporotrichoid atypical mycobacterial infections of the skin which healed completely under long-term monotherapy with modern antibiotics (levofloxacin, clarithromycin, minocycline). We recommend confirming the diagnosis by means of culture, followed by monotherapy with low side-effect antibiotics, based on sensitivity studies. If pathogen proof is obtained by PCR, antibiotic therapy should be based on the known sensitivity of the pathogen in question.—Authors' Abstract

**Bruijnesteijn Van Coppenraet, E. S., Lindeboom, J. A., Prins, J. M., Peeters, M. F., Claas, E. C., and Kuijper, E. J.** Real-time PCR assay using fine-needle aspirates and tissue biopsy specimens for rapid diagnosis of mycobacterial lymphadenitis in children. *J. Clin. Microbiol.* **42(6)** (2004) 2644–2650.

A real-time PCR assay was developed to diagnose and identify the causative agents

of suspected mycobacterial lymphadenitis. Primers and probes for the real-time PCR were designed on the basis of the internal transcribed spacer sequence, enabling the recognition of the genus *Mycobacterium* and the species *Mycobacterium avium* and *M. tuberculosis*. The detection limit for the assay was established at 1100 CFU/ml of pus, and the specificity tests showed no false-positive reaction with other mycobacterial species and other pathogens causing lymphadenitis. From 67 children with suspected mycobacterial lymphadenitis based on a positive mycobacterial skin test, 102 samples (58 fine-needle aspirates [FNA] and 44 tissue specimens) were obtained. The real-time PCR assay detected a mycobacterial infection in 48 patients (71.6%), whereas auramine staining and culturing were positive for 31 (46.3%) and 28 (41.8%) of the patients. The addition of the real-time PCR assay to conventional diagnostic tests resulted in the recognition of 13 more patients with mycobacterial disease. These results indicate that the real-time PCR is more sensitive than conventional staining and culturing techniques ( $p = 0.006$ ). The *M. avium*-specific real-time PCR was positive for 38 patients, and the *M. tuberculosis*-specific real-time PCR was positive for 1 patient. Analysis of 27 patients from whom FNA and tissue biopsy specimens were collected revealed significantly more positive real-time PCR results for FNA than for tissue biopsy specimens ( $p = 0.003$ ). Samples from an age-matched control group of 50 patients with PCR-proven cat scratch disease were all found to be negative by the real-time PCR. We conclude that this real-time PCR assay with a sensitivity of 72% for patients with lymphadenitis and a specificity of 100% for the detection of atypical mycobacteria can provide excellent support for clinical decision making in children with lymphadenitis.—Authors' Abstract

**Buckingham, S. J., Haddow, L. J., Shaw, P. J., and Miller, R. F.** Immune reconstitution inflammatory syndrome in HIV-infected patients with mycobacterial infections starting highly active anti-retroviral therapy. *Clin. Radiol.* **59(6)** (2004) 505–513.

**AIM:** To describe the radiological appearances of immune reconstitution inflammatory syndrome (IRIS) in human immunodeficiency virus (HIV)-infected patients with mycobacterial infections starting highly active anti-retroviral therapy (HAART). **MATERIALS AND METHODS:** Five consecutive HIV infected patients with IRIS due to mycobacterial infection were studied. Inter-current infection and poor drug compliance were excluded as causes of presentation. The chest radiological appearances at the time of starting HAART and at the time of diagnosis of IRIS were compared. **RESULTS:** In these five patients there was clinical and radiological deterioration, occurring between 10 days and 7 months after starting HAART, leading to unmasking of previously undiagnosed mycobacterial infection or to worsening of mycobacterial disease. All five patients had HAART-induced increases in CD4+ T lymphocyte counts and reductions in peripheral blood HIV "viral load." Chest radiographic abnormalities due to IRIS included marked mediastinal lymphadenopathy in three patients—severe enough to produce tracheal compression in two patients (one of whom had stridor)—and was associated with new pulmonary infiltrates in two patients. The other two patients had new infiltrates, which in one patient was associated with a pleural effusion. **CONCLUSION:** These cases illustrate the diverse chest radiographic appearances of IRIS occurring after HAART in patients with mycobacterial and HIV co-infection. Marked mediastinal lymphadenopathy occurred in three of these five patients (with associated tracheal narrowing in two patients); four patients developed pulmonary infiltrates and one had an effusion. The cases further highlight that the onset of IRIS may be delayed for several months after HAART is started.—Authors' Abstract

**Christiansen, D. C., Roberts, G. D., and Patel, R.** *Mycobacterium celatum*, an emerging pathogen and cause of false positive amplified *Mycobacterium tuberculosis* direct test. *Diagn. Microbiol. Infect. Dis.* **49**(1) (2004) 19–24.

*Mycobacterium celatum* is a recently described organism. Herein we describe a case

of *M. celatum* lymphadenitis in an immunocompetent child and the first reported case of a *M. celatum* infection (lung abscess) in a transplant recipient. A literature review identified 19 other cases of *M. celatum* infection. Fifteen occurred in patients with the acquired immunodeficiency syndrome. Of these, nine were disseminated and six were localized (primarily to the lungs). The remaining 4 patients were immunocompetent and had localized infection (pneumonitis or lymphadenitis). Diagnosis of *M. celatum* infection can be challenging as *M. celatum* can cause false-positive results with the current version of the Amplified *Mycobacterium tuberculosis* Direct Test (Gen-Probe, San Diego, CA.). Definitive identification is available by DNA sequencing or high-performance liquid chromatography. *M. celatum* can cause infection in immunocompromised and immunocompetent hosts.—Authors' Abstract

**Cordonnier, C., Martino, R., Trabasso, P., Held, T. K., Akan, H., Ward, M. S., Fabian, K., Ullmann, A. J., Wulffraat, N., Ljungman, P., Alessandrino, E. P., Pretnar, J., Gmur, J., Varela, R., Vitek, A., Sica, S., Rovira, M.; and the European Blood and Marrow Transplant Group Infectious Diseases Working Party.** Mycobacterial infection: a difficult and late diagnosis in stem cell transplant recipients. *Clin. Infect. Dis.* **38**(9) (2004) 1229–1236.

The Infectious Diseases Working Party of the European Blood and Marrow Transplant Group conducted a survey to obtain information about the frequency, presentation, and treatment of mycobacterial infection (MBI) in stem cell transplant (SCT) recipients. Among 29 centers, MBI was diagnosed in 0.79% of 1513 allogeneic and 0.23% of 3012 autologous SCT recipients during 1994–1998 a median of 160 days after transplantation. The mean interval between first symptoms and diagnosis was 29 days and was still longer for patients with atypical MBI or recipients of corticosteroid therapy. The prevalence of MBI was highest among those who received matched unrelated or mismatched STCs from related donors. Of 31 patients, 20 had tuberculosis, 8 had atyp-

ical MBI, and 3 had diagnoses based on histological findings only. Five patients (16%) died, all of whom had received an allogeneic SCT. Because of the increased numbers of unmatched donors and transplantation programs in countries with a high prevalence of tuberculosis, constant vigilance is required to early detect MBI in SCT recipients.—Authors' Abstract

**Darje, H.** [*Mycobacterium ulcerans* infection: epidemiological, clinical and therapeutical aspects]. Bull. Soc. Pathol. Exot. **96(5)** (2003) 368–371. [Article in French]

*Mycobacterium ulcerans* causing Buruli ulcer is an environmental mycobacteria responsible for an infectious necrotizing panniculitis. The epidemiology of this disabling disease is strongly linked to the aquatic ecosystem. Occurring mainly in children, it is an emergent public health threat in many humid rural tropical areas. Human contamination probably follows a direct percutaneous route from humid environment, but some insects may play a role in transmission. The clinical features develop in three phases: pre-ulcer, ulcer with unstick margins, healing leading to functional sequelae. Treatment relies on antibiotics in order to sterilize the infectious focus, together with the surgical repair of lost skin and joint deformities, as well as early physiotherapy. Despite uncertainties of *in vivo* efficacy of antibiotics, it seems logical to administer chemotherapy with both Rifampicin and Aminoglycosid or Fluroquinolon and Aminoglycosid. Surgical treatment depends on the size of the ulcer, as well as available techniques and skills on the field. Wide excision and graft are often recommended, however limited excision followed by small islet grafts may be successful.—Author's Abstract

**Davis, J. P., Prinsley, P. R., and Robinson, P. J.** Cervical lymphadenopathy due to mycobacterial infection: a diagnostic protocol. J. Laryngol. Otol. **107(7)** (1993) 614–617.

Mycobacterial cervical lymphadenopathy is relatively uncommon in the United King-

dom; when cases do occur opportunities for early diagnosis and treatment may be missed. We have reviewed twenty-three cases of mycobacterial cervical lymphadenopathy presenting to an urban general hospital over a four-year period. We discuss the techniques available to aid a diagnosis of mycobacterial disease and suggest a protocol to allow efficient use of these techniques.—Authors' Abstract

**Doucette, K., and Fishman, J. A.** Nontuberculous mycobacterial infection in hematopoietic stem cell and solid organ transplant recipients. Clin. Infect. Dis. **38(10)** (2004) 1428–1439.

Nontuberculous mycobacteria (NTM) are ubiquitous environmental organisms. In immunocompetent hosts, they are a rare cause of disease. In immunocompromised hosts, disease due to NTM is well documented. Reports of NTM disease have increased in hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients. This increase may reflect increased numbers of transplants, intensification of immune suppressive regimens, prolonged survival of transplant recipients, and/or improved diagnostic techniques. The difficulty of diagnosis and the impact associated with infections due to NTM in HSCT and SOT recipients necessitates that, to ensure prompt diagnosis and early initiation of therapy, a high level of suspicion for NTM disease be maintained. The most common manifestations of NTM infection in SOT recipients include cutaneous and pleuropulmonary disease, and, in HSCT recipients, catheter-related infection. Skin and pulmonary lesions should be biopsied for histologic examination, special staining, and microbiologic cultures, including cultures for bacteria, *Nocardia* species, fungi, and mycobacteria. Mycobacterial infections associated with catheters may be documented by tunnel or blood (isolator) cultures. Susceptibility testing of mycobacterial isolates is an essential component of optimal care. The frequent isolation of NTM other than *Mycobacterium avium* complex (MAC) from transplant recipients limits the extrapolation of therapeutic data from human immunodeficiency virus-infected individuals to the population of

transplant recipients. Issues involved in the management of NTM disease in transplant recipients are characterized by a case of disseminated infection due to *Mycobacterium avium* complex in a lung transplant recipient, with a review of the relevant literature.—Authors' Abstract

**Duttaroy, B., Agrawal, C., and Pendse, A.** Spinal tuberculosis due to dissemination of atypical mycobacteria. *Indian J. Med. Sci.* **58(5)** (2004) 203–205.

There has been an increase in disease caused by Non Tuberculous Mycobacteria (NTM) since the early 1980s. Though ubiquitous in environment, they may act as clinically important pathogens in various conditions. More importantly they are resistant to the conventional anti-tuberculous therapy (ATT) and respond to antibiotics such as quinolones and aminoglycosides and need an aggressive surgical intervention. Missing these atypical mycobacteria may lead to unnecessary administration of ATT and hence delay in proper management of the case. We report a case of spinal tuberculosis due to a Non Tuberculous Mycobacteria, *M. fortuitum* (Rapid grower). Relevant literature is also reviewed.—Authors' Abstract

**Edmonson, B. C., Morris, W. R., and Osborn, F. D.** Bacillary angiomatosis with cytomegaloviral and mycobacterial infections of the palpebral conjunctiva in a patient with AIDS. *Ophthalm. Plast. Reconstr. Surg.* **20(2)** (2004) 168–170.

We report the clinical and histopathologic findings of bacillary angiomatosis involving the palpebral conjunctiva with concomitant infection by cytomegalovirus and *Mycobacterium* species in a patient with acquired immune deficiency syndrome. After debulking, the conjunctival tissue was studied with the use of light and electron microscopy; stains for bacteria, acid-fast bacilli, and *Bartonella* species; and immunohistochemical studies for cytomegalovirus and herpes simplex virus. We observed the typical histopathologic findings of bacillary angiomatosis, the presence of bacilli stained by the Steiner and Steiner method,

and the electron microscopic demonstration of bacilli consistent with *Bartonella* species. Immunohistochemistry confirmed infection with cytomegalovirus, which had been suggested by characteristic cytologic abnormalities. Acid-fast bacilli were also found in the excised tissue. Patients with bacillary angiomatosis of the conjunctiva may have infections with multiple additional microorganisms.—Authors' Abstract

**Ergan, B., Coplu, L., Alp, A., and Artvinli, M.** *Mycobacterium smegmatis* pneumonia. *Respirology* **9(2)** (2004) 283–285.

*Mycobacterium smegmatis* is a non-tuberculous mycobacterium that is usually associated with soft tissue or wound infections in humans. Pulmonary infections secondary to this pathogen are rarely seen and occur only in patients with an underlying condition, such as lipoid pneumonia. This report presents the first case of *M. smegmatis* pneumonia in an otherwise healthy individual who had no predisposing condition.—Authors' Abstract

**Gira, A. K., Reisenauer, A. H., Hammock, L., Nadiminti, U., Macy, J. T., Reeves, A., Burnett, C., Yakus, M. A., Toney, S., Jensen, B. J., Blumberg, H. M., Caughman, S. W., and Nolte, F. S.** Furunculosis due to *Mycobacterium mageritense* associated with footbaths at a nail salon. *J. Clin. Microbiol.* **42(4)** (2004) 1813–1817.

We report two cases of lower-extremity furunculosis caused by *Mycobacterium mageritense*. Both patients were patrons of the same nail salon, where they received footbaths prior to pedicures. *M. mageritense* bacteria isolated from two whirlpool footbaths were determined to be closely related to the patient isolates by pulsed-field gel electrophoresis.—Authors' Abstract

**Henry, M. T., Inamdar, L., O'Riordain, D., Schweiger, M., and Watson, J. P.** Nontuberculous mycobacteria in non-HIV patients: epidemiology, treatment and response. *Eur. Respir. J.* **23(5)** (2004) 741–746.



Recent international guidelines published in 1997 and 1999 have proposed diagnostic and treatment criteria for disease caused by nontuberculous mycobacteria (NTM). In this paper, the epidemiological data, diagnostic criteria, treatment regimens and outcomes from 117 HIV-negative patients who had a positive culture for NTM between 1995–1999 are reviewed. The authors wished to identify factors associated with improved outcome in these patients. A total of 71 patients were believed to have a clinical disease caused by NTM, as defined by international criteria. A total of 72% patients were found to have had pulmonary disease. There was a rise in infections between 1995–1999, with a peak in infections in 1997. The most striking rise was in *Mycobacterium avium* intracellular complex infections (1995: 33% infections; 1996: 36% infections; 1997: 41% infections; 1998: 61% infections; 1999: 57% infections). There was a link between deprivation and number of positive NTM isolates (34.4% isolates occurred in the areas of lowest Carstairs deprivation index versus 20.6% isolates from areas of least deprivation). There was a significant association between appropriate therapy, defined by American Thoracic Society and British Thoracic Society guidelines, and successful outcome (74%) in contrast to those who received inappropriate treatment prior to the publication of these guidelines. Nontuberculous mycobacteria infections remain a significant problem in non-HIV patients. Adherence to published guidelines may improve patient outcomes.—Authors' Abstract

**Hesseling, A. C., Schaaf, H. S., Victor, T., Beyers, N., Marais, B. J., Cotton, M. F., Wiid, I., Gie, R. P., van Helden, P., and Warren, R. M.** Resistant *Mycobacterium bovis* Bacillus Calmette-Guerin disease: implications for management of Bacillus Calmette-Guerin Disease in human immunodeficiency virus-infected children. *Pediatr. Infect. Dis. J.* **23**(5) (2004) 476–479.

Guidelines for the diagnosis and management of Bacillus Calmette-Guerin (BCG) disease in children are lacking, and there are

limited data on drug resistance of *Mycobacterium bovis* BCG. A 6-month-old HIV-infected infant presented with right axillary adenitis ipsilateral to the site of BCG immunization. *M. tuberculosis* complex was cultured from axillary lymph nodes and gastric aspirates, and *M. bovis* BCG was isolated. Susceptibility testing before initiation of therapy demonstrated inherent resistance to isoniazid. The organism acquired rifampin resistance during therapy. This was confirmed by the presence of a mutation in codon 531 (Ser531Tyr) of the *rpoB* gene. Treatment guidelines for BCG disease with consideration of inherent and possible acquired drug resistance should be established in settings with high rates of vertical HIV transmission and routine BCG vaccination.—Authors' Abstract

**Kiska, D. L., Turenne, C. Y., Dubansky, A. S., and Domachowske, J. B.** First case report of catheter-related bacteremia due to "*Mycobacterium lacticola*." *J. Clin. Microbiol.* **42**(6) (2004) 2855–2857.

This is the first report of infection caused by "*Mycobacterium lacticola*," a rapidly growing, scotochromogenic mycobacterium that was isolated from the blood of an immunosuppressed child. The organism was identified by sequence analysis of >1400 bp of the 16S rRNA gene. The clinical relevance of this isolate, coupled with its unique 16S rRNA gene sequence, should prompt further investigation to establish this organism as a valid mycobacterial species.—Authors' Abstract

**Lamb, S. R., Stables, G. I., and Merchant, W.** Disseminated cutaneous infection with *Mycobacterium chelonae* in a patient with steroid-dependent rheumatoid arthritis. *Clin. Exp. Dermatol.* **29**(3) (2004) 254–257.

*Mycobacterium chelonae* is a rapidly growing atypical mycobacterium that is a normal commensal of water and soil. We report a case of a 61-year-old man with seronegative rheumatoid arthritis and fibrosing alveolitis on long-term prednisolone

who presented with a number of tender, red, subcutaneous nodules on his upper arms and a pustule on his left cheek. Histopathologic examination revealed dense neutrophilic collections within the deep dermis and subcutaneous fat with abscess formation. Long filamentous organisms were seen within these collections and were subsequently identified by special stains and PCR as *Mycobacterium chelonae*. Treatment was not possible as the patient developed bacteria bronchopneumonia before identification of the organism and he subsequently died. Post-mortem revealed no extra-cutaneous evidence of mycobacterium infection.—Authors' Abstract

**Lange, C. G., Woolley, I. J., and Brodt, R.**

H. Disseminated *Mycobacterium avium*-intracellular complex (MAC) infection in the era of effective antiretroviral therapy: is prophylaxis still indicated? *Drugs* **64**(7) (2004) 679–692.

Before highly active antiretroviral therapies (HAART) were available for the treatment of persons with HIV infection, disseminated *Mycobacterium avium*-intracellular complex (MAC) infection was one of the most common opportunistic infections that affected people living with AIDS. Routine use of chemoprophylaxis with a macrolide has been advocated in guidelines for the treatment of HIV-infected individuals if they have a circulating CD4+ cell count of  $\leq 50$  cells/microL. In addition, lifelong prophylaxis for disease recurrence has been recommended for those with a history of disseminated MAC infection. The introduction of HAART has resulted in a remarkable decline in the incidence of opportunistic infections and death among persons living with AIDS. Considerable reconstitution of functional immune responses against opportunistic infections can be achieved with HAART. In the case of infection with MAC, there has been a substantial reduction in the incidence of disseminated infections in the HAART era, even in countries where the use of MAC prophylaxis was never widely accepted. Moreover, the clinical picture of MAC infections in patients treated with potent antiretroviral therapies has shifted from a disseminated

disease with bacteraemia to a localised infection, presenting most often with lymphadenopathy and osteomyelitis. Data from several recently conducted randomised, double-blind, placebo-controlled trials led to the current practice of discontinuing primary and secondary prophylaxis against disseminated MAC infections at stable CD4+ cell counts  $>100$  cells/microL. These recommendations are still conservative as primary or secondary disseminated MAC infections are only rarely seen in patients who respond to HAART, despite treatment initiation at very low CD4+ cell counts. Potential adverse effects of macrolide therapy and drug interactions with antiretrovirals also metabolised via the cytochrome P450 enzyme system must be critically weighed against the marginal benefit that MAC prophylaxis may provide in addition to treatment with HAART. These authors feel that, unless patients who initiate HAART at low CD4+ cell counts do not respond to HIV-treatment, routine MAC prophylaxis should not be recommended. Nevertheless, the patient population for whom MAC prophylaxis may still be indicated in the era of HAART needs to be identified in prospectively designed clinical trials.—Authors' Abstract

**Mastroianni, A.** *Mycobacterium flavescens* vertebral osteomyelitis in an immunocompetent host. *Infez. Med.* **11**(2) (2003) 97–101.

The aim of this paper is to describe a rare case of vertebral osteomyelitis caused by *Mycobacterium flavescens* in an immunocompetent patient. *Mycobacterium flavescens* is a rapidly growing, pigmented, non-tuberculous mycobacterium, usually described as non-pathogenic for humans but occasionally reported as the cause of serious infectious complications recognized in clinical practice. This study stressed the importance of recent reports that describe the occurrence of vertebral osteomyelitis due to non-tuberculous mycobacteria that have also been recognized with an increasing incidence among immunocompetent hosts. A brief review of the current literature on human disease caused by *Mycobacterium flavescens* is also reported.—Author's Abstract

**Menard, A., Couppie, P., Sainte-Marie, D., and Pradinaud, R.** [Diagnosis of *Mycobacterium ulcerans* infection by PCR: about three cases observed in French Guiana]. *Bull. Soc. Pathol. Exot.* **96**(5) (2003) 403–405. [Article in French]

*Mycobacterium ulcerans* infection is the third most important mycobacterial infection in the world. It has been described in many different countries including French Guiana. The diagnosis of *M. ulcerans* infection by culture is often difficult because culture is hard to perform in endemic areas and their sensitivity is not reliable. As a result the diagnosis of this infection is often delayed. However, molecular methods are now available to diagnose rapidly infections by *M. ulcerans* and distinguish it from other mycobacteria. We report three cases of skin infection due to *M. ulcerans* observed in French Guiana. Diagnosis was initially made by polymerase chain reaction and was confirmed later by culture (in two patients) and inoculation to mice (in one patient). A faster diagnosis of *M. ulcerans* infection should lead to a better prognosis of this infection.—Authors' Abstract

**Minami, S., Suzuki, K., Tsuyuguchi, K., and Sakatani, M.** [Four cases of *Mycobacterium xenopi* pulmonary disease]. *Kekkaku* **79**(4) (2004) 313–320. [Article in Japanese]

*Mycobacterium xenopi* is very rare pathogen in Japan. We reported herein four cases of *M. xenopi* pulmonary disease. These patients were all male and their ages ranged from 53 to 72. They all had past history of pulmonary tuberculosis, including two cases who had been also treated for *Mycobacterium kansasii* pulmonary disease later. The bacilli could be cultured in Mycobacteria Growth Indicator Tube (MGIT) system from 10 sputum samples, but they could not be cultured on Ogawa egg media except for two samples. All four cases fulfilled the criteria for the diagnosis of nontuberculous mycobacteria pulmonary disease proposed by the Japanese Society for Tuberculosis. Combination chemotherapy including isoniazid, rifampicin, and ethambu-

tol was started in all four cases when mycobacteria were detected under tentative diagnosis of the relapse of tuberculosis or *Mycobacterium kansasii* disease. Sputum converted to culture negative by the chemotherapy in two cases. In one case, the chemotherapeutic regimen was changed to rifampicin, ethambutol, and clarithromycin after the bacteriological identification of *M. xenopi*, and the new regimen was found to be effective. In the final case, both of the regimens were finally ineffective.—Authors' Abstract

*MMWR Morb. Mortal. Wkly. Rep.* **53**(9) (2004) 192–194. Erratum in: *MMWR Morb. Mortal. Wkly. Rep.* **53**(11) (2004) 246. *Mycobacterium chelonae* infections associated with face lifts—New Jersey, 2002–2003. Centers for Disease Control and Prevention (CDC).

In March 2003, the New Jersey Department of Health and Senior Services (NJDHSS) was notified about three patients who acquired surgical-site infections caused by *Mycobacterium chelonae* after having face lifts (i.e., rhytidectomies) performed at an outpatient surgical center. NJDHSS learned subsequently of another patient with *M. chelonae* infection who had a rhytidectomy performed at a second surgical center. The four patients received diagnoses of *M. chelonae* infection during March 2002–February 2003. NJDHSS conducted an epidemiologic, environmental, and microbiologic investigation. This report summarizes the results of that investigation, which identified contaminated methylene blue used as a tissue-marking agent as the source of infection. Surgeons should use only sterile, single-use, tissue-marking agents during procedures that require aseptic technique, and clinicians should consider *M. chelonae* when evaluating surgical-site infections.—Authors' Abstract

**Okenu, D. M., Ofielu, L. O., Easley, K. A., Guarner, J., Spotts Whitney, E. A., Raghunathan, P. L., Stienstra, Y., Asamo, K., van der Werf, T. S., van der Graaf, W. T., Tappero, J. W., Ashford,**

**D. A., and King, C. H.** Immunoglobulin M antibody responses to *Mycobacterium ulcerans* allow discrimination between cases of active Buruli ulcer disease and matched family controls in areas where the disease is endemic. Clin. Diagn. Lab. Immunol. **11**(2) (2004) 387–391.

Buruli ulcer disease (BUD) is an emerging disease caused by *Mycobacterium ulcerans*. In the present study we have characterized the serological reactivities of sera from volunteer case patients with laboratory-confirmed BUD and controls living in three different regions of Ghana where the disease is endemic to determine if serology may be useful for disease confirmation. Our results showed highly reactive immunoglobulin G (IgG) responses among patients with laboratory-confirmed disease, healthy control family members of the case patients, and sera from patients with tuberculosis from areas where BUD is not endemic. These responses were represented by reactivities to multiple protein bands found in the *M. ulcerans* culture filtrate (CF). In contrast, patient IgM antibody responses to the *M. ulcerans* CF (MUCF) proteins were more distinct than those of healthy family members living in the same village. A total of 84.8% (56 of 66) of the BUD patients exhibited strong IgM antibody responses against MUCF proteins (30, 43 and 70 to 80 kDa), whereas only 4.5% (3 of 66) of the family controls exhibited such responses. The sensitivity of the total IgM response for the patients was 84.8% (95% confidence interval [CI], 74.3 to 91.6%), and the specificity determined with sera from family controls was 95.5% (95% CI, 87.5 to 98.4%). These studies suggest that the IgM responses of patients with BUD will be helpful in the identification and production of the *M. ulcerans* recombinant antigens required for the development of a sensitive and specific serological assay for the confirmation of active BUD.—Authors' Abstract

**Redfern, D. J., Coleridge, S. D., and Bendall, S. P.** AIDS-related ankle arthropathy: *Mycobacterium avium*-intracellulare infection. J. Bone Joint Surg. Br. **86**(2) (2004) 279–281.

We present a case of *Mycobacterium avium*-intracellulare (MAI) infection of the ankle joint in a patient with HIV infection. The patient presented with a painful, destructive arthropathy of the ankle. Initial microbiological studies were negative but infection with MAI was later identified from biopsies taken during hindfoot fusion. Antibiotic triple therapy was given and the patient remains pain-free without evidence of active infection. To our knowledge, this is the first case of MAI infection of the ankle reported in the literature. A high index of suspicion of (atypical) Mycobacterial infection should be maintained in patients with HIV infection presenting with an indolent but destructive arthropathy of the ankle joint.—Authors' Abstract

**Rose, M., Kitz, R., Mischke, A., Enzensberger, R., Schneider, V., and Zielen, S.** Lymphadenitis cervicalis due to *Mycobacterium interjectum* in immunocompetent children. Acta. Paediatr. **93**(3) (2004) 424–426.

This report presents two cases of cervical lymphadenitis due to *Mycobacterium interjectum* in healthy young children, identified by sequencing of the 16S rRNA gene. Surgical resection combined with chemotherapy resulted in cure. CONCLUSION: The attention of clinicians needs to be drawn to an emerging mycobacterial pathogen which might be overlooked or misidentified in routine laboratory testing.—Authors' Abstract

**Smith, R. M., Drobniewski, F., Gibson, A., Montague, J. D., Logan, M. N., Hunt, D., Hewinson, G., Salmon, R. L., and O'Neill, B.** *Mycobacterium bovis* infection, United Kingdom. Emerg. Infect. Dis. **10**(3) (2004) 539–541.

We describe the first documented spillover of bovine tuberculosis from animals into the human population of the United Kingdom since the resurgence of the disease in cattle in the country. This finding suggests that there may be a small risk for transmission to humans, making continued vigilance particularly necessary.—Authors' Abstract



**Sohail, M. R., and Smilack, J. D.** Hernia repair mesh-associated *Mycobacterium goodii* infection. *J. Clin. Microbiol.* **42**(6) (2004) 2858–2860.

We report on a patient with an abdominal wall abscess that developed after an inguinal hernia repair that utilized synthetic mesh. *Mycobacterium goodii*, a recently recognized, rapidly growing mycobacterium related to *M. smegmatis*, was isolated both from the abdominal wall aspirate and from surgically drained material. Infection resolved following thorough debridement, mesh removal, and prolonged antimicrobial therapy. This case report extends our understanding of the spectrum of *M. goodii* infection.—Authors' Abstract

**Southern, P. M., Jr.** Tenosynovitis caused by *Mycobacterium kansasii* associated with a dog bite. *Am. J. Med. Sci.* **327**(5) (2004) 258–261.

A 68-year-old man with adult-onset diabetes mellitus suffered an accidental puncture wound to the palm of his hand while playing with his pet dog. He received cephalosporin prophylaxis for 1 week. No inflammation occurred. Six months later, a mass developed near his elbow. It was removed. Histopathology revealed granulomas containing acid-fast bacilli (AFB). No culture was done. Swelling and decreased motion of the wrist and fingers developed. Magnetic resonance imaging revealed inflammation of the flexor compartment of the hand, wrist, and forearm. Surgical incision and drainage yielded purulent material, granulomatous inflammation, with AFB. Cultures yielded *Mycobacterium kansasii*. Several surgical procedures were required; *M. kansasii* was recovered. He received isoniazid and rifampin for 1 year and prolonged rehabilitation. After 4 years, he was relatively asymptomatic, with good function of wrist and fingers. We believe this to be the first report of tenosynovitis caused by *M. kansasii* in association with a dog bite.—Authors' Abstract

**Takada, K., Suzuki, K., Kataharada, K., Okada, M., Nakashima, M., Nakanishi,**

**T., Ohsuzu, F., and Yoshiyama, T.** A patient with a *Mycobacterium avium* complex infection complicated by systemic lupus erythematosus. *J. Infect. Chemother.* **10**(1) (2004) 49–52.

A 41-year-old woman was admitted to our hospital because of fever and polyarthralgia. A diagnosis of systemic lupus erythematosus (SLE) was made based on the findings of polyarthritis, leukocytopenia, lymphocytopenia, proteinuria, and positive reactions for antinuclear antibody (ANA) and anti-double strand (ds)DNA antibody. She had also been suffering from a pulmonary *Mycobacterium avium* complex (MAC) infection with such symptoms as cough and sputum for the past 3 years. Antimicrobial drugs for MAC infection were administered first, and later she was given cyclophosphamide pulse therapy, consisting of methylprednisolone (8 mg/day) and mizoribine (100 mg/day). Owing to these therapeutic regimens, SLE was successfully treated without an exacerbation of the MAC infection. The risk factors for MAC infection and SLE are also discussed.—Authors' Abstract

**Turenne, C. Y., Thibert, L., Williams, K., Burdz, T. V., Cook, V. J., Wolfe, J. N., Cockcroft, D. W., and Kabani, A.** *Mycobacterium saskatchewanense* sp. nov., a novel slowly growing scotochromogenic species from human clinical isolates related to *Mycobacterium interjectum* and Accuprobe-positive for *Mycobacterium avium* complex. *Int. J. Syst. Evol. Microbiol.* **54**(Pt 3) (2004) 659–667.

A pigmented, slowly growing *Mycobacterium avium* complex AccuProbe-positive organism was isolated from the sputum and pleural fluid of a 72-year-old female with bronchiectasis. The unusual morphology of the organism prompted further identification by 16S rRNA gene sequencing, revealing a perfect identity with previously uncharacterized strain *Mycobacterium* sp. MCRO 8 (GenBank accession no. X93034), with the closest established species by 16S rDNA analysis being *Mycobacterium interjectum*. HPLC of the organism corresponded to previously obtained patterns identified as *M. interjectum*-like and, upon sequence evalua-

tion of a selection of strains with a similar profile, more were subsequently identified as MCRO 8. A total of 16 strains isolated from human respiratory samples were evaluated in the characterization of this novel species, for which the name *Mycobacterium saskatchewanense* sp. nov. is proposed. The type strain is strain 00-250(T) (=ATCC BAA-544(T)=DSM 44616(T)=CIP 108114(T)).—Authors' Abstract

**Yim, K., Nazeer, S. H., Kiska, D., Rose, F. B., Brown, D., and Cynamon, M. H.** Recurrent *Mycobacterium xenopi* infection in a patient with rheumatoid arthri-

tis receiving etanercept. *Scand. J. Infect. Dis.* **36(2)** (2004) 150–154.

A case of recurrent *Mycobacterium xenopi* infection presenting as Pott's disease in a patient receiving etanercept for severe rheumatoid arthritis is described. A 49-yr-old Caucasian male had received a total of 11 months of anti-mycobacterial therapy for hip infection acquired 15 months earlier; he presented with progressive back pain, which was diagnosed as Pott's disease. He had been treated with etanercept in addition to his prior immunosuppressive agents after the diagnosis of hip infection.—Authors' Abstract

## Molecular and Genetic Studies

**Arnvig, K. B., Pennell, S., Gopal, B., and Colston, M. J.** A high-affinity interaction between NusA and the *rrn* nut site in *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U. S. A.* **101(22)** (2004) 8325–8330.

The bacterial NusA protein enhances transcriptional pausing and termination and is known to play an essential role in antitermination. Antitermination is signaled by a nut-like cis-acting RNA sequence comprising boxB, boxA, and boxC. In the present study, we demonstrate a direct, specific high-affinity interaction between the *rrn* leader nut-like sites and the NusA proteins of *Mycobacterium tuberculosis* and *Escherichia coli*. This NusA-RNA interaction relies on the conserved region downstream of boxA, the boxC region, thus demonstrating a key function of this element. We have established an *in vivo* assay for antitermination in mycobacteria and use this to show that the *M. tuberculosis* *rrn* nut-like site enhances transcriptional read-through of untranslated RNA consistent with an antitermination signal within this site. Finally, we present evidence that this NusA-RNA interaction affects transcriptional events further downstream.—Authors' Abstract

**Barouni, A. S., Augusto, C. J., Lopes, M. T., Zanini, M. S., and Salas, C. E. A**

pncA polymorphism to differentiate between *Mycobacterium bovis* and *Mycobacterium tuberculosis*. *Mol. Cell. Probes.* **18(3)** (2004) 167–170.

The pyrazinamidase gene coding for the enzyme that activates the bactericidal drug pyrazinamide contains a polymorphic site that is preserved in *Mycobacterium bovis*. We synthesized two sets of primers, one encompassing a 180 bp fragment, and the second spanning a 726 bp fragment including the full pncA gene. Following PCR of *Mycobacterium tuberculosis* and *M. bovis* samples, it is possible to discriminate by this polymorphism between these species by digestion with Eco065 I. Digestion of the 180 bp fragment results in two fragments of 101 and 79 bp, specific for *M. tuberculosis*. Alternatively, digestion of the 726 bp fragment yields three fragments of 452, 165 and 109 bp for *M. tuberculosis*, but only two fragments of 561 and 165 bp for *M. bovis*.—Authors' Abstract

**Cangelosi, G. A., Freeman, R. J., Lewis, K. N., Livingston-Rosanoff, D., Shah, K. S., Milan, S. J., and Goldberg, S. V.** Evaluation of a high-throughput repetitive-sequence-based PCR system for DNA fingerprinting of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex strains. *J. Clin. Microbiol.* **42(6)** (2004) 2685–2693.

Repetitive-sequence-based PCR (rep-PCR) is useful for generating DNA fingerprints of diverse bacterial and fungal species. Rep-PCR amplicon fingerprints represent genomic segments lying between repetitive sequences. A commercial system that electrophoretically separates rep-PCR amplicons on microfluidic chips, and provides computer-generated readouts of results has been adapted for use with *Mycobacterium* species. The ability of this system to type *M. tuberculosis* and *M. avium* complex (MAC) isolates was evaluated. *M. tuberculosis* strains (N = 56) were typed by spoligotyping with rep-PCR as a high-resolution adjunct. Results were compared with those generated by a standard approach of spoligotyping with IS6110-targeted restriction fragment length polymorphism (IS6110-RFLP) as the high-resolution adjunct. The sample included 11 epidemiologically and genotypically linked outbreak isolates and a population-based sample of 45 isolates from recent immigrants to Seattle, Wash., from the African Horn countries of Somalia, Eritrea, and Ethiopia. Twenty isolates exhibited unique spoligotypes and were not analyzed further. Of the 36 outbreak and African Horn isolates with nonunique spoligotypes, 23 fell into four clusters identified by IS6110-RFLP and rep-PCR, with 97% concordance observed between the two methods. Both approaches revealed extensive strain heterogeneity within the African Horn sample, consistent with a predominant pattern of reactivation of latent infections in this immigrant population. Rep-PCR exhibited 89% concordance with IS1245-RFLP typing of 28 *M. avium* subspecies *avium* strains. For *M. tuberculosis* as well as *M. avium* subspecies *avium*, the discriminative power of rep-PCR equaled or exceeded that of RFLP. Rep-PCR also generated DNA fingerprints from *M. intracellulare* (N = 8) and MAC(x) (N = 2) strains. It shows promise as a fast, unified method for high-throughput genotypic fingerprinting of multiple *Mycobacterium* species.—Authors' Abstract

**Chen, Q., Zhu, D. Y., Luo, X. D., Jiang, Y., and Jiang, S.** [Preparation and application of recombinant *Mycobacterium tuberculosis* CFP10-ESAT-6 fusion pro-

tein]. *Zhonghua Jie He He Hu Xi Za Zhi*. **27(4)** (2004) 244–248. [Article in Chinese]

**OBJECTIVE:** To prepare the recombinant CFP10-ESAT-6 fusion protein, and to study its immunological characteristics, and its potential for serodiagnosis of tuberculosis. **METHODS:** The *hsp*-ESAT-6 fusion gene was amplified by Gene SOEing, and then cloned into pQE30 plasmid. The recombinant CFP10-ESAT-6 fusion protein was expressed and purified. Its antigenicity was confirmed by Western blot. Animal models infected with *M. tuberculosis* H(37)Rv strain and *M. bovis* BCG respectively were made to evaluate the potential value of the fusion protein in the serodiagnosis of tuberculosis. **RESULTS:** The sequence of recombinant plasmid pQE30-CFP10-ESAT-6 was identical to the predicted sequence. The recombinant protein (rCFP10-ESAT-6), about 26,000, existed in the cytoplasm of DH5alpha in soluble form and represented 40% of the total bacterial protein. The purity and concentration of the final product was 98% and 1.2 g/L, respectively. Western blot showed that the rCFP10-ESAT-6 had good immunoreactivity with sera from patients with active tuberculosis and rabbits immunized with CFP10 and ESAT-6 respectively. The positive cutoff value was A(490) plus 2 standard deviation from negative guinea pig sera detected by ELISA. Serological reactivity to rCFP10-ESAT-6 was observed in 11 of the serum samples from guinea pigs with tuberculosis and 1 of sera from guinea pigs infected with BCG, while the serological reactivity to PPD was observed in 11 of sera from guinea pigs with tuberculosis and in 11 of sera from guinea pigs infected with BCG. **CONCLUSIONS:** The rCFP10-ESAT-6 fusion protein was highly expressed in soluble form in *E. coli*. It had antigenicity of both CFP10 and ESAT-6, and could be used to differentiate infection with *M. tuberculosis* H(37)Rv strain from immunization with *M. bovis* BCG. The study provided experimental data for potential application of rCFP10-ESAT-6 in the diagnosis of tuberculosis.—Authors' Abstract

**Delogu, G., Pusceddu, C., Bua, A., Fadda, G., Brennan, M. J., and Zanetti, S.**

Rv1818c-encoded PE\_PGRS protein of *Mycobacterium tuberculosis* is surface exposed and influences bacterial cell structure. *Mol. Microbiol.* **52**(3) (2004) 725–733.

Identification of the novel PE multigene family was an unexpected finding of the genomic sequencing of *Mycobacterium tuberculosis*. Presently, the biological role of the PE and PE\_PGRS proteins encoded by this unique family of mycobacterial genes remains unknown. In this report, a representative PE\_PGRS gene (Rv1818c/PE\_PGRS33) was selected to investigate the role of these proteins. Cell fractionation studies and fluorescence analysis of recombinant strains of *Mycobacterium smegmatis* and *M. tuberculosis* expressing green fluorescent protein (GFP)-tagged proteins indicated that the Rv1818c gene product localized in the mycobacterial cell wall, mostly at the bacterial cell poles, where it is exposed to the extracellular milieu. Further analysis of this PE\_PGRS protein showed that the PE domain is necessary for subcellular localization. In addition, the PGRS domain, but not PE, affects bacterial shape and colony morphology when Rv1818c is overexpressed in *M. smegmatis* and *M. tuberculosis*. Taken together, the results indicate that PE\_PGRS and PE proteins can be associated with the mycobacterial cell wall and influence cellular structure as well as the formation of mycobacterial colonies. Regulated expression of PE genes could have implications for the survival and pathogenesis of mycobacteria within the human host and in other environmental niches.—Authors' Abstract

**Frota, C. C., Hunt, D. M., Buxton, R. S., Rickman, L., Hinds, J., Kremer, K., van Soolingen, D., and Colston, M. J.** Genome structure in the vole bacillus, *Mycobacterium microti*, a member of the *Mycobacterium tuberculosis* complex with a low virulence for humans. *Microbiology* **150**(Pt 5) (2004) 1519–1527.

*Mycobacterium microti*, a member of the *Mycobacterium tuberculosis* complex, is phylogenetically closely related to *M. tuberculosis*, differing in a few biochemical properties. However, these species have dif-

ferent levels of virulence in different hosts; most notably *M. microti* shows lower virulence for humans than *M. tuberculosis*. This report presents genomic comparisons using DNA microarray analysis for an extensive study of the diversity of *M. microti* strains. Compared to *M. tuberculosis* H37Rv, 13 deletions were identified in 12 strains of *M. microti*, including the regions RD1 to RD10, which are also missing in *Mycobacterium bovis* BCG. In addition, four new deleted regions, named MiD1, RD1beta, MiD2 and MiD3, were identified. DNA sequencing was used to define the extent of most of the deletions in one strain. Although RD1 of *M. bovis* BCG and *M. microti* is thought to be crucial for attenuation, in this study, three of the four *M. microti* strains that were isolated from immunocompetent patients had the RD1 deletion. In fact, only the RD3 deletion was present in all of the strains examined, although deletions RD7, RD8 and MiD1 were found in almost all the *M. microti* strains. These deletions might therefore have some relation to the different host range of *M. microti*. It was also noticeable that of the 12 strains studied, only three were identical; these strains were all isolated from immunocompetent humans, suggesting that they could have arisen from a single source. Thus, this study shows that it is difficult to ascribe virulence to any particular pattern of deletion in *M. microti*.—Authors' Abstract

**Hafner, B., Haag, H., Geiss, H. K., and Nolte, O.** Different molecular methods for the identification of rarely isolated non-tuberculous mycobacteria and description of new hsp65 restriction fragment length polymorphism patterns. *Mol. Cell. Probes* **18**(1) (2004) 59–65.

Analysis of heat shock protein 65 (hsp65) gene restriction fragment length polymorphism (RFLP) is done frequently to identify non-tuberculous mycobacteria (NTM) on a genetic basis. Here we report the results of analysing the hsp65 patterns of some rarely isolated NTM for which patterns have not been published before (*Mycobacterium bohemicum*, *Mycobacterium hassiacum*, *Mycobacterium heckeshornense*, *Mycobacterium monacense*, and *Mycobacterium triplex*). Furthermore new hsp65-variants



for *Mycobacterium interjectum* (type II), *Mycobacterium mucogenicum* (type V), *Mycobacterium gordonae* (type VIII) and *Mycobacterium paraffinicum* (perhaps synonymous to *Mycobacterium scrofulaceum*) are described. All species were characterised by hsp65-RFLP, sequencing a 441-bp fragment of the hsp65 gene and sequencing the hypervariable region of the 16S rDNA. Additional data for less frequently isolated mycobacteria are provided and the hitherto described data for the *Mycobacterium gordonae* complex are summarised. Although the hsp65-RFLP analysis turned out to be a useful method a number of restraints (lack of standardisation, slight variability in fragment length) limits its broader use. Reliable identification of NTM needs, however, more than one molecular method. Identification results obtained by applying different methods yielded conflicting results. Confusion may be caused by older data base entries which are not updated and not longer reflect the actual systematic and taxonomy of the genus *Mycobacterium*.—Authors' Abstract

**Hazbon, M. H., and Alland, D.** Hairpin primers for simplified single-nucleotide polymorphism analysis of *Mycobacterium tuberculosis* and other organisms. *J. Clin. Microbiol.* **42**(3) (2004) 1236–1242.

We describe a novel, simple, rapid, and highly sensitive method to detect single-nucleotide polymorphisms (SNPs) in *Mycobacterium tuberculosis* and other organisms. Amplification refractory mutation (ARMS) SNP assays were modified by converting the SNP-detecting linear primers in the ARMS assay to hairpin-shaped primers (HPs) through the addition of a 5' tail complementary to the 3' end of the linear primer. The improved ability of these primers to detect SNPs in *M. tuberculosis* was compared in a real-time PCR with SYBR-I green dye. Linear primers resulted in incorrect or indeterminate allele designation for 6 of the 13 SNP alleles tested in seven different SNP assays, while HPs determined the correct SNP in all cases. We compared the cycle threshold differences (DeltaC(t)) between the reactions containing primer-template matches and the reactions containing primer-template mismatches (where a larger DeltaC(t) indicates

a more robust assay). The use of HPs dramatically improved the mean DeltaC(t) values for the SNP assays (7.6 for linear primers and 11.2 for HPs). We designed 98 different HP assays for SNPs previously associated with resistance to the antibiotic isoniazid to test the large-scale utility of the HP approach. Assay design was successful in 72.4%, 83.7%, 88.8%, and 92.9% of the assays after one to four rounds of assay design, respectively. HP SNP assays are simple, sensitive, robust, and inexpensive. These advantages favor the application of this technique for SNP assays of *M. tuberculosis* and other organisms.—Authors' Abstract

**Helguera-Repetto, C., Cox, R. A., Munoz-Sanchez, J. L., and Gonzalez-Y-Merchand, J. A.** The pathogen *Mycobacterium marinum*, a faster growing close relative of *Mycobacterium tuberculosis*, has a single rRNA operon per genome. *FEMS Microbiol. Lett.* **235**(2) (2004) 281–288.

Although *Mycobacterium marinum* and *Mycobacterium tuberculosis* are very closely related they differ significantly in their growth rates. The Type strain of *M. marinum* and one clinical isolate were investigated and, like *M. tuberculosis*, were found to have a single rRNA (rrn) operon per genome located downstream from murA gene and controlled by two promoters. No sequence differences were found that account for the difference in the growth rates of the two species. We infer that *M. tuberculosis* has the capacity to synthesize rRNA much faster than it actually does; and propose that the high number of insertion sequences in this species attenuate growth rate to lower values.—Authors' Abstract

**Krzywinska, E., Krzywinski, J., and Schorey, J. S.** Phylogeny of *Mycobacterium avium* strains inferred from glycopeptidolipid biosynthesis pathway genes. *Microbiology* **150**(Pt 6) (2004) 1699–1706.

The *Mycobacterium avium* complex (MAC) encompasses two species, *M. avium*

and *Mycobacterium intracellulare*, which are opportunistic pathogens of humans and animals. The standard method of MAC strain differentiation is serotyping based on a variation in the antigenic glycopeptidolipid (GPL) composition. To elucidate the relationships among *M. avium* serotypes a phylogenetic analysis of 13 reference and clinical *M. avium* strains from 8 serotypes was performed using as markers two genomic regions (890 bp of the *gtfB* gene and 2150 bp spanning the *rtfA*-*mtfC* genes) which are associated with the strains' serological properties. Strains belonging to three other known *M. avium* serotypes were not included in the phylogeny inference due to apparent lack of the marker sequences in their genomes, as revealed by PCR and Southern blot analysis. These studies suggest that serotypes prevalent in AIDS patients have multiple origins. In trees inferred from both markers, serotype 1 strains, known to have the simplest and shortest GPLs among all other serotypes, were polyphyletic. Likewise, comparisons of the inferred phylogenies with the molecular typing results imply that the existing tools used in epidemiological studies may be poor estimators of *M. avium* strain relatedness. Additionally, trees inferred from each marker had significantly incongruent topologies due to a well supported alternative placement of strain 2151, suggesting a complex evolutionary history of this genomic region.—Authors' Abstract

**Kurabachew, M., Enger, O., Sandaa, R. A., Skuce, R., and Bjorvatn, B.** A multiplex polymerase chain reaction assay for genus-, group- and species-specific detection of mycobacteria. *Diagn. Microbiol. Infect. Dis.* **49(2)** (2004) 99–104.

We developed and evaluated a single-step, multiplex polymerase chain reaction (PCR) assay for distinguishing (1) between the *Mycobacterium tuberculosis* complex (MTBC) and mycobacteria other than tuberculosis (MOTT) and (2) between *M. tuberculosis* and *M. bovis* species. The assay targeted the 16S and the 23S rDNA to distinguish between MTBC and MOTT species, and the *oxyR* gene to distinguish between *M. tuberculosis* and *M. bovis*

strains. Clinical samples and reference strains (N = 156) comprised 93 strains of *M. tuberculosis*, 44 of *M. bovis*, 1 *M. africanum* strain, and 18 strains representing 9 different species of MOTTs. MOTTs generated only a single PCR product of about 2.5 kilobase; however, all of the MTBC strains produced a 118 base pair (bp) fragment and an additional 270 bp fragment was obtained for *M. tuberculosis* and *M. africanum* when the primer pair *oxyRTB*-2.1/*oxyRMT*-1 was used. When *oxyRTB*-2.1/*oxyRMB*-1 primers were used, the 270 bp fragment was obtained for only *M. bovis*. The assay needed as little as 1 pg of purified genomic DNA to make a positive identification.—Authors' Abstract

**McGarvey, J. A., Wagner, D., and Bermudez, L. E.** Differential gene expression in mononuclear phagocytes infected with pathogenic and non-pathogenic mycobacteria. *Clin. Exp. Immunol.* **36(3)** (2004) 490–500.

See Current Literature, Immunopathology, p. 360.

**Muttucumaru, D. G., and Parish, T.** The molecular biology of recombination in mycobacteria: what do we know and how can we use it? *Curr. Issues Mol. Biol.* **6(2)** (2004) 145–157.

See Current Literature, Microbiology, p. 384.

**Stermann, M., Sedlacek, L., Maass, S., and Bange, F. C.** A promoter mutation causes differential nitrate reductase activity of *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *J. Bacteriol.* **186(9)** (2004) 2856–2861.

See Current Literature, Microbiology, p. 385.

**Sun, R., Converse, P. J., Ko, C., Tyagi, S., Morrison, N. E., and Bishai, W. R.** *Mycobacterium tuberculosis* ECF sigma factor sigC is required for lethality in mice

and for the conditional expression of a defined gene set. *Mol. Microbiol.* **52**(1) (2004) 25–38.

Bacterial alternative RNA polymerase sigma factors are key global adaptive response regulators with a likely role in *Mycobacterium tuberculosis* pathogenesis. We constructed a mutant lacking the sigma factor gene, sigC, by allelic exchange, in the virulent CDC1551 strain of *M. tuberculosis* and compared the resulting mutant with the isogenic wild-type strain and complemented mutant strain. *In vitro*, compared to the wild-type and complemented strains, the mutant was found to have similar ability to survive in both murine bone marrow-derived macrophages and activated J774 macrophages. In time-to-death experiments in the mouse model, the DeltasigC mutant was significantly attenuated, causing no death in infected mice whereas the wild-type and complemented strains caused 100% mortality within 235 days after aerosol infection with a median time to death of 170 days. Mouse organ bacterial burdens indicated that the mutant proliferated and persisted at the same level as the wild-type and complemented strains in lung tissue and was able to persist in mice without causing death for >300 days. A complete genomic microarray study demonstrated that SigC modulates the expression of several key virulence-associated genes including hspX, senX3 and mtrA, encoding the alpha-crystallin homologue, a two-component sensor kinase and a two-component response regulator respectively. Altered expression of a subset of these genes was confirmed by quantitative RT-PCR analysis. Analysis of genes modulated by SigC also revealed a putative consensus DNA recognition sequence for SigC of SSSAAT-N(16-20)-CGTSSS (S = C or G). Promoter recognition for one of these genes was confirmed by *in vitro* transcription analysis after purification of recombinant SigC and reconstitution of an Esigma(C) RNA polymerase holoenzyme. These data indicate that the *M. tuberculosis* transcription factor SigC governs expression of an important *M. tuberculosis* regulon and is essential for lethality in mice, but is not required for bacterial survival in this species. These observations place the DeltasigC mutant in a class of *M. tuberculosis* mutants which persist in tissues

but are attenuated in their ability to elicit lethal immunopathology.—Authors' Abstract

**Talaat, A. M., Lyons, R., Howard, S. T., and Johnston, S. A.** The temporal expression profile of *Mycobacterium tuberculosis* infection in mice. *Proc. Natl. Acad. Sci. U. S. A.* **101**(13) (2004) 4602–4607.

Infection with *Mycobacterium tuberculosis* causes the illness tuberculosis with an annual mortality of approximately 2 million. Understanding the nature of the host-pathogen interactions at different stages of tuberculosis is central to new strategies for developing chemotherapies and vaccines. Toward this end, we adapted microarray technology to analyze the change in gene expression profiles of *M. tuberculosis* during infection in mice. This protocol provides the transcription profile of genes expressed during the course of early tuberculosis in immune-competent (BALB/c) and severe combined immune-deficient (SCID) hosts in comparison with growth in medium. The microarray analysis revealed clusters of genes that changed their transcription levels exclusively in the lungs of BALB/c, SCID mice, or medium over time. We identified a set of genes (N = 67) activated only in BALB/c and not in SCID mice at 21 days after infection, a key point in the progression of tuberculosis. A subset of the lung-activated genes was previously identified as induced during mycobacterial survival in a macrophage cell line. Another group of *in vivo*-expressed genes may also define a previously unreported genomic island. In addition, our analysis suggests the similarity between mycobacterial transcriptional machinery during growth in SCID and in broth, which questions the validity of using the SCID model for assessing mycobacterial virulence. The *in vivo* expression-profiling technology presented should be applicable to any microbial model of infection.—Authors' Abstract

**Tsolaki, A. G., Hirsh, A. E., DeRiemer, K., Enciso, J. A., Wong, M. Z., Hannan, M., Goguet de la Salmoniere, Y. O., Aman, K., Kato-Maeda, M., and Small, P. M.** Functional and evolutionary

genomics of *Mycobacterium tuberculosis*: insights from genomic deletions in 100 strains. *Proc. Natl. Acad. Sci. U. S. A.* **101**(14) (2004) 4721–4722; 4865–4870.

To better understand genome function and evolution in *Mycobacterium tuberculosis*, the genomes of 100 epidemiologically well characterized clinical isolates were interrogated by DNA microarrays and sequencing. We identified 68 different large-sequence polymorphisms (comprising 186,137 bp, or 4.2% of the genome) that are present in H37Rv, but absent from one or more clinical isolates. A total of 224 genes (5.5%), including genes in all major functional categories, were found to be partially or completely deleted. Deletions are not distributed randomly throughout the genome but instead tend to be aggregated. The distinct deletions in some aggregations appear in closely related isolates, suggesting a genomically disruptive process specific to an individual mycobacterial lineage. Other genomic aggregations include distinct deletions that appear in phylogenetically unrelated isolates, suggesting that a genomic region is vulnerable throughout the species. Although the deletions identified here are evidently inessential to the causation of disease (they are found in active clinical cases), their frequency spectrum suggests that most are weakly deleterious to the pathogen. For some deletions, short-term evolutionary pressure due to the host immune system or antibiotics may favor the elimination of genes, whereas longer-term physiological requirements maintain the genes in the population.—Authors' Abstract

**Wu, S., Howard, S. T., Lakey, D. L., Kipnis, A., Samten, B., Safi, H., Gruppo,**

**V., Wizel, B., Shams, H., Basaraba, R. J., Orme, I. M., and Barnes, P. F.** The principal sigma factor sigA mediates enhanced growth of *Mycobacterium tuberculosis* *in vivo*. *Mol. Microbiol.* **51**(6) (2004) 1551–1562.

The ability of *Mycobacterium tuberculosis* to grow in macrophages is central to its pathogenicity. We found previously that the widespread 210 strain of *M. tuberculosis* grew more rapidly than other strains in human macrophages. Because principal sigma factors influence virulence in some bacteria, we analysed mRNA expression of the principal sigma factor, sigA, in *M. tuberculosis* isolates during growth in human macrophages. Isolates of the 210 strain had higher sigA mRNA levels and higher intracellular growth rates, compared with other clinical strains and the laboratory strain H37Rv. SigA was also upregulated in the 210 isolate TB294 during growth in macrophages, compared with growth in broth. In contrast, H37Rv sigA mRNA levels did not change under these conditions. Overexpression of sigA enhanced growth of recombinant *M. tuberculosis* in macrophages and in lungs of mice after aerosol infection, whereas recombinant strains expressing antisense transcripts to sigA showed decreased growth in both models. In the presence of superoxide, sense sigA transformants showed greater resistance than vector controls, and the antisense sigA transformant did not grow. We conclude that *M. tuberculosis* sigA modulates the expression of genes that contribute to virulence, enhancing growth in human macrophages and during the early phases of pulmonary infection *in vivo*. This effect may be mediated in part by increased resistance to reactive oxygen intermediates.—Authors' Abstract