

## 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

**Anonymous.** A Romanist View of the Fish-Eating Leprosy Theory. *Archives A Century Ago. Arch. Dermatol.* **140** (2004) 789.

Even in an age of masterful clinicians, Sir Jonathan Hutchinson was exceptional. In addition to his clinical work, Sir Jonathan was a prolific writer serving as editor for a time of the *British Medical Journal* . . . and, most incredibly, from 1889–1900 single-handedly writing every article in his 1-man journal, the *Archives of Clinical Surgery*. Sir Jonathan was a medical polymath, an expert in ophthalmology, neurology, pathology, surgery, and dermatology. It is in syphilology where his eponymous fame is most enduring . . . having seen over 1 million cases (1) of syphilis. . .

Unfortunately appended to his illustrious career will always be the enduring embarrassment of his *idée fixe*—that leprosy is caused by eating rotten fish. Notwithstanding the questionable geography or the less-than-obvious logical dietary leap, for the rest of his life Hutchinson espoused his doctrine despite universal rejection by contem-

porary leprologists. Even the discovery of the leprosy bacillus by his good friend Gerhard Hansen in 1874 couldn't dissuade Hutchinson. Hutchinson was mildly nonplused when no one could discover [the bacillus] in any fish. . . Hutchinson confessed that one reason he never sought to experimentally test his theory was "the assured conviction that the general facts were overwhelmingly conclusive and that they needed only to be clearly set forth." Indeed the only reason Hutchinson's theory gained any circulation at all, if never actual acceptance, was because of his otherwise impeccable reputation.—[Condensed from the essay . . . DMS] *Archives of Dermatology*

**Al Samie, A. R., and Al Qubati, Y.** Leprosy control in the Republic of Yemen: cooperation between government and non-government or ganizations, 1989–2003. *Lepr. Rev.* **75(2)** (2004) 164–170.

Although the prevalence rate of leprosy in the Republic of Yemen has dropped below the WHO elimination level of less than one

case per 10,000 of the population, it is still regarded as a serious public health problem calling for continued vigilance, notably in the detection and treatment of hidden and undiagnosed cases. In the past, religious misinterpretation has generated adverse behaviour patterns towards people affected by leprosy, characterized by aggression, negligence and isolation. Until about 1982, following a visit of a leprologist (Dr . S. K. Noordeen) from the World Health Organization, there was no leprosy control program and attempts to establish one remained ineffective until in 1989, when an agreement was signed between the Ministry of Public Health and Population and the German Leprosy Relief Association. This led to the development of a leprosy control program in four governorates, later extended to the rest of the country . This paper describes the progress made in the control of leprosy in the Yemen, 1989–2003, by the Ministry of Health and Population and the GLRA, in association with two local societies.—Authors' Abstract

**Donoghue, H. D., Spigelman, M., Greenblatt, C. L., Lev-Maor, G., Bar-Gal, G. K., Matheson, C., Vernon, K., Nerlich, A. G., and Zink, A. R.** Tuberculosis: from prehistory to Robert Koch, as revealed by ancient DNA. *Lancet Infect. Dis.* **4(9)** (2004) 584–592.

During the past 10 years palaeomicrobiology, a new scientific discipline, has developed. The study of ancient pathogens by direct detection of their DNA has answered several historical questions and shown changes to pathogens over time. However, ancient DNA (aDNA) continues to be controversial and great care is needed to provide valid data. Here we review the most successful application of the technology, which is the study of tuberculosis. This has provided direct support for the current theory of *Mycobacterium tuberculosis* evolution, and suggests areas of investigation for the interaction of *M. tuberculosis* with its host.—Authors' Abstract

**Hughes, M. S., James, G., Taylor, M. J., McCarroll, J., Neill, S. D., Chen, S. C.,**

**Mitchell, D. H., Love, D. N., and Malik, R.** PCR studies of feline leprosy cases. *J. Feline Med. Surg.* **6(4)** (2004) 235–243.

16S rRNA gene sequence analysis provided evidence for two different mycobacterial species, *Mycobacterium lepraemurium* and a potentially novel species, as causative agents of “feline leprosy.” Comparison of 16S rRNA gene sequence data obtained for *M. lepraemurium* and the potentially novel species indicated 12 nucleotide differences over a 446 bp region encompassing the V2 and V3 hypervariable regions. From available 16S rRNA gene sequence data, *M. lepraemurium* shared greatest nucleotide identity with *M. avium* subsp. *paratuberculosis* and *M. avium*. The novel species had a long helix 18 in the V3 region and shared greatest nucleotide identity with *M. leprae*, *M. haemophilum* and *M. malmoeense*. The novel species had an additional ‘A’ nucleotide at position 105 of the aligned 16S rRNA gene sequence, the only other mycobacterial database sequence having this same extra nucleotide being *M. leprae*. This nucleotide variation was exploited to develop specific PCR assays for the two species. These were found to be effective and specific when tested against a panel of mycobacteria including species found in feline leprosy lesions and closely related mycobacteria and also when applied directly to formalin-fixed, paraffin-embedded tissues from feline leprosy cases.—Authors' Abstract

**Jianping, S., Wenzhong, L., Meiwen, Y., Jun, Y., Longchao, Z., Rongmao, W., Lufang, H., Hongjiang, M., Fuchang, Y., Xinguo, H., and Liangde, P.** Analysis on the detection of new leprosy cases before, during and after the year of leprosy elimination campaigns. *Lepr. Rev.* **75(2)** (2004) 157–163.

To analyze the impact on of case finding of leprosy elimination campaigns (LECs), data on newly detected leprosy cases in a leprosy endemic area were collected before, during and after the year of LEC. The number of new leprosy cases detected during the year of LEC was significantly higher than previously. The number of newly detected cases after the year of LEC was similar to

that of detected before the year of LEC in counties with persisting case finding activities. However, the number of newly detected cases after the year of LEC significantly decreased in counties without active case finding activities. The average distance from the homes of leprosy cases detected during LEC to the leprosy control unit at the count town was 62.8 km, which is farther than that of other leprosy cases detected before and after the year of LEC. The average time from disease onset to diagnosis of leprosy cases detected after the year of LEC shortened. The results also showed that carrying out LECs is unlikely to have a significant impact on the trend of case finding within a short time in local areas, but it may improve some indicators of leprosy patients and so promote leprosy control in local areas.—Authors' Abstract

**Karthikeyan, K., and Thappa D. M.** Modular teaching program on leprosy. *Indian J. Lepr.* **75(4)** (2003) 317–325.

Though repeated attention has been drawn to a lack of proper teaching-learning modules in leprosy endemic countries, no satisfactory module exists. Keeping in view this fact, we attempted to draft a suitable module on leprosy that could be used to teach leprosy to undergraduate medical students in a simple and comprehensive manner. We used two different modules, Module A and Module B, to teach two different batches of students of the pre-final year (VI and VII semesters) of the MBBS course. Both these modules were conducted by the Department of Dermatology and STD, with participation by the Departments of Microbiology, Pathology and Preventive and Social Medicine. The drafts of the modules were discussed before hand in the Department, keeping in mind the number of days allotted to us. Both the modules were different in certain aspects, but the basic concept was the same. Because Module A had more time, certain practical aspects were also discussed. It was interesting to note that the percentage of increase in the post-test score was 17 for Module A and 15 for Module B, thus proving that both the modules were effective in conveying the core message about leprosy.—Authors' Abstract

**Meima, A., Smith, W. C., van Oortmarssen, G. J., Richardus, J. H., and Habbema, J. D.** The future incidence of leprosy: a scenario analysis. *Bull. World Health Organ.* **82(5)** (2004) 373–380.

**OBJECTIVE:** To investigate the impact of the current strategy for the elimination of leprosy on its incidence and to assess the consequences of failure to sustain this strategy. **METHODS:** Scenarios for assessing the impact of the elimination strategy were implemented in a computer simulation program. The scenarios reflected the assumptions made regarding contagiousness, transmission and bacille Calmette-Guerin (BCG) vaccination. The trend in case detection rate for the main countries in which leprosy was endemic during 1985–1998 was fitted, and incidence up to 2020 was projected. **FINDINGS:** Owing to the gradual shortening of delays in detection up to 1998, and because of the low relapse rate that occurs with multidrug treatment MDT, incidence is predicted to decrease beyond 2000 in all scenarios. The annual decline was a few per cent higher when favourable assumptions were made about protection and coverage of BCG vaccination. Overall, the predicted annual decline in incidences ranged from 2% to 12%. **CONCLUSION:** The elimination strategy reduces transmission, but the decline may be slow. Relaxation of control after 2005 is unjustified given the uncertainty about the rate of decline and the adverse effects of longer delays in detection. A long-term strategy for leprosy control should be adopted.—Authors' Abstract

**Rao, T. P., Krishnamurthy, P., Vijayakumar, P., Mishra, R. K., and Samy M. S.** "Instant" new leprosy case-detection: an experience in Bihar State in India. *Indian J. Lepr.* **75(1)** (2003) 9–15.

The National Leprosy Eradication Programme (NLEP) is based on survey, education and treatment, including coverage of all the registered cases with multi-drug therapy (MDT). The Government of India introduced MDT in all leprosy endemic districts through a vertical set-up, and through mobile leprosy treatment units in low endemic

districts. Anti-leprosy work has not been uniform in all the states and needed push-start in some, such as Bihar. There have been spurts of leprosy elimination activities and the entire populations of the regions have not been covered because of various administrative reasons and logistic problems. In Singhbhum district of Bihar, a successful attempt was made to cover the maximum population by campaign approach. The strategy was to involve all the field workers of the leprosy program in the district, supported by a small group of experienced personnel. The campaign, lasting for 39 working days, resulted in detecting leprosy cases equivalent to 64% of cases detected during the previous one full year. The entire operation helped the local staff to gain experience that would be useful for the future of the NLEP, and also provide an insight into working practices. Similar campaign approach can be used in situations where case-detection activities are feeble and the implementation of MDT is slow. If such campaigns are repeated at appropriate intervals, it will be a great support to achieving the goal of leprosy elimination.—Authors' Abstract

**Subramanian, M., Showkath Ali, M. K., Thorat, D. M., Muthukumar, M., Sathiskumar, E., Ramadoss, C., and Ali Khan, M.** Leprosy situation in endemic states of India and prospects of elimination of the disease. *Indian J. Lepr* **75(4)** (2003) 335–345.

In India there is a dramatic fall in the prevalence rate (PR) of leprosy, but the new case-detection rate (NCDR) has not been reduced concomitantly. It is the operational efficiency of the National Leprosy Eradication Programme (NLEP) that has led to a significant reduction in the NCDR in Andhra Pradesh and Tamil Nadu. The ratio of PR to NCDR has been declining in these two states and it reveals that elimination could be reached even with the high NCDR level of 3 to 4 per 10,000 population, particularly if single skin lesion (SSL) cases are discharged through single dose treatment of rifampicin, ofloxacin and minocycline (ROM). On the other hand, the significant number of cases detected in Bihar and Orissa during

modified leprosy elimination campaigns (MLECs) reveals that there are lacunae in operational activities in new case-detection resulting in a large number of undetected cases in the community. Only one-third of the cases are reporting voluntarily. Awareness of leprosy is not adequate to motivate the patients to report voluntarily and complete their treatment, thus underscoring the need for relying on active case-detection so that transmission can be broken and elimination of leprosy achieved. In addition, the influence of socio-economic factors on continued occurrence of leprosy cannot be ruled out. The establishment of a sentinel surveillance system along with a computerized simplified information system to gain in-depth knowledge on the functioning of the NLEP will ensure operational efficiency. In view of this situation, the NLEP should adopt a more realistic approach towards reaching the elimination goal.—Authors' Abstract

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

**Abstract.** 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.—*Cadernos de Saúde Pública*

## Chemotherapy

**Bermudez, L. E., Kolonoski, P., Seitz, L. E., Petrofsky, M., Reynolds, R., Wu, M., and Young, L. S.** SRI-286, a thiosemicarbazole, in combination with mefloquine and moxifloxacin for treatment of murine *Mycobacterium avium* complex disease. *Antimicrob. Agents Chemother.* **48(9)** (2004) 3556–3558.

Treatment of *Mycobacterium avium* disease remains challenging when macrolide resistance develops. We infected C57 beige mice and treated them with mefloquine, SRI-286, and moxifloxacin. SRI-286 (80 mg/kg) was bactericidal in the liver. Mefloquine plus moxifloxacin or mefloquine plus SRI-286 were better than mefloquine alone.—Authors' Abstract

**de Savary, N., Lee, R., and Vaidya, B.** Severe hypothyroidism after thalidomide treatment. *J. Roy. Soc. Med.* **97** (2004) 443–445.

An important but little recognized side-effect of thalidomide is hypothyroidism.

Three cases of thalidomide-induced hypothyroidism were reported during the early 1960s. In 2002, Badros and colleagues reported a patient who developed severe hypothyroidism about three months after starting thalidomide for multiple myeloma. Furthermore, they found that 14% of patients on thalidomide treatment were subclinically hypothyroid (TSH >10 µ/L) at three months, and suggested that thyroid dysfunction might contribute to some of the known side-effects of the drug, such as fatigue, constipation and bradycardia.—[Abstracted from text] *Journal of the Royal Society of Medicine*

**Douglas, J. T., Cellona, R. V., Fajardo, T. T. Jr., Abalos, R. M., Balagon, M. V., and Klatser, P. R.** Prospective study of serological conversion as a risk factor for development of leprosy among household contacts. *Clin. Diagn. Lab. Immunol.* **11(5)** (2004) 897–900.

See *Current Literature, Epidemiology and Prevention*, p. 556.

**Fernandez-Martinez, E., Morales-Rios, M. S., Perez-Alvarez, V., and Muriel, P.** Immunomodulatory effects of thalidomide analogs on LPS-induced plasma and hepatic cytokines in the rat. *Biochem. Pharmacol.* **68(7)** (2004) 1321–1329.

Thalidomide has shown to inhibit, selectively and mainly the cytokine tumor necrosis factor-alpha (TNF-alpha), thus, thalidomide has inhibitory consequences on other cytokines; this is ascribed as an immunomodulatory effect. Novel thalidomide analogs are reported with immunomodulatory activity. The aim of this work was to synthesize some of these analogs and to assess them as immunomodulatory agents in an acute model of LPS-induced septic challenge in rat. Animal groups received orally twice a day vehicle carboxymethylcellulose (0.9%), or thalidomide in suspension (100 mg/kg), or analogs in an equimolar dose. Two hours after last dose, rats were injected with saline (NaCl, 0.9%, i.p.) or LPS (5 mg/kg, i.p.). Groups were sacrificed 2 hr after injection and samples of blood and liver were obtained. TNF-alpha, interleukin-6, -1beta, and -10 (IL-6, IL-1beta, IL-10) were quantified by enzyme linked immunosorbent assay (ELISA) and studied in plasma and liver. After 2 hr of LPS-induction, different patterns of measured cytokines were observed with thalidomide analogs administration evidencing their immunomodulatory effects. Interestingly, some analogs decreased significantly plasma and hepatic levels of LPS-induced proinflammatory TNF-alpha and others increased plasma concentration of anti-inflammatory IL-10. Thalidomide analogs also showed slight effects on the remaining proinflammatory cytokines. Differences among immunomodulatory effects of analogs can be related to potency, mechanism of action, and half lives. Thalidomide analogs could be used as a pharmacological tool and in therapeutics in the future.—Authors' Abstract

**Makino, K., Nakajima, T., Shikamura, M., Ito, F., Ando, S., Kochi, C., Ina-**

**gawa, H., Soma, G., and Terada, H.** Efficient intracellular delivery of rifampicin to alveolar macrophages using rifampicin-loaded PLGA microspheres: effects of molecular weight and composition of PLGA on release of rifampicin. *Colloids Surf B Biointerfaces*. **36(1)** (2004) 35–42.

Monodispersed PLGA microspheres containing rifampicin (RFP) have been prepared by solvent evaporation method using a Shirasu porous glass (SPG) membrane. The microspheres were spherical and their average diameter was about 2 microm. The loading efficiency of rifampicin was dependent on the molecular weight of PLGA. The higher loading efficiency was obtained by the usage of PLGA with the lower molecular weight, which may be caused by the interaction of the amino groups of rifampicin with the terminal carboxyl groups of PLGA. PLGA with the monomer compositions of 50/50 and 75/25, of lactic acid/glycolic acid, were used in this study. From rifampicin-loaded PLGA microspheres formulated using PLGA with the molecular weight of 20,000, rifampicin was released with almost constant rate for 20 days after the lag phase was observed for the initial 7 days at pH 7.4. On the other hand, from rifampicin-loaded PLGA microspheres formulated using PLGA with the molecular weight of 5000 or 10,000, almost 90% of rifampicin-loaded in the microspheres was released in the initial 10 days. Highly effective delivery of rifampicin to alveolar macrophages was observed by the usage of rifampicin-loaded PLGA microspheres. Almost 19 times higher concentration of rifampicin was found to be incorporated in alveolar macrophages when rifampicin-loaded PLGA microspheres were added to the cell culture medium than when rifampicin solution was added.—Authors' Abstract

**Maurer, T., Poncelet, A., and Berger, T.**

Thalidomide treatment for prurigo nodularis in human immunodeficiency virus-infected subjects: efficacy and risk of neuropathy. *Arch. Dermatol.* **140(7)** (2004) 845–849.

OBJECTIVE: To evaluate safety and efficacy of thalidomide in the treatment of

prurigo nodularis in a group of human immunodeficiency virus (HIV)-infected patients whose condition was recalcitrant to standard treatment. DESIGN: Prospective study. SETTING: Outpatient dermatology and neurology clinic, both referral settings. PATIENTS: Eight HIV-infected patients with refractory prurigo nodularis; a total of 10 met inclusion criteria, but 2 could not be followed up. INTERVENTIONS: Treatment with thalidomide, 100 mg/d. Subjects were randomized after 1 month to receive 100 or 200 mg/d. If side effects were noted, the drug was reduced to a tolerable dose or discontinued. Subjects were monitored at baseline and monthly for degree of pruritus and total area of body involvement of prurigo nodularis. Sequential neurologic assessments were performed. MAIN OUTCOME MEASURES: Efficacy and toxic effects. RESULTS: The dosage of thalidomide ranged from 33 to 200 mg/d. Eight subjects had a greater than 50% response in reduction of itch over 3.4 months (average). Seven subjects had a greater than 50% reduction of skin involvement over 5 months (average). Three subjects developed thalidomide peripheral neuropathy (TPN). There was no correlation between duration of treatment, daily or cumulative dose, and TPN. A change in the Neuropathy Impairment Score of 10 points was a good marker of TPN, as was a greater than 50% decrease in the sural sensory nerve action potential amplitude. CONCLUSIONS: Thalidomide reduced the signs and symptoms of prurigo nodularis in HIV-infected subjects. One third of subjects developed TPN, underscoring the importance of careful neurologic assessment.—Authors' Abstract

**Noguchi, T., Sano, H., Shimazawa, R., Tanatani, A., Miyachi, H., and Hashimoto, Y.** Phenylhomophthalimide-type NOS inhibitors derived from thalidomide. *Bioorg. Med. Chem. Lett.* **14(16)** (2004) 4141–4145.

Thalidomide shows moderate inhibitory activity toward neuronal nitric oxide synthase (nNOS) and inducible NOS (iNOS), but not toward endothelial NOS (eNOS). Structural development studies of thalidomide yielded novel phenylhomophthalimide-type NOS in-

hibitors with enhanced activity and different subtype selectivity.—Authors' Abstract

**Seidel, V., and Taylor, P. W.** *In vitro* activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. *Int. J. Antimicrob. Agents.* **23(6)** (2004) 613–619.

Extracts of the roots of plants of the Geraniaceae family have been used for many years in South Africa as native herbal remedies and there is circumstantial evidence for efficacy in the treatment of pulmonary tuberculosis. We have examined dried roots of *Pelargonium reniforme* and *P. sidoides* for antibacterial activity against rapidly growing mycobacteria. Fractions with activity against *Mycobacterium aurum* and *M. smegmatis* were obtained from both plant species by bioassay-guided fractionation of n-hexane extracts and were found to contain mixtures of straight-chain fatty acids. Analysis by gas chromatography-mass spectrometry (GC-MS) of the corresponding fatty acid methyl esters revealed structures with chain lengths ranging from C12 to C26. Unsaturated compounds were analyzed as the corresponding dimethyl disulfide adducts to determine double-bond positions. Active mixtures differed in the relative abundance of their components, but all contained 16:0 (palmitic), Delta9-18:1 (oleic) and Delta9,12-18:2 (linoleic acid) as the major components. When tested against *M. aurum*, *M. smegmatis* and other rapidly growing mycobacteria (*M. fortuitum*, *M. abscessus* and *M. phlei*), all saturated compounds except 12:0 were devoid of antimycobacterial activity, whereas unsaturated compounds showed antimycobacterial activity related to their degree of unsaturation, their chain length and the bacterial species tested. The most potent compound was linoleic acid, with MIC of 2 mg/l against *M. aurum*.—Authors' Abstract

**Shimizu, S., Suzuki, M., Tomoda, A., Arai, S., Taguchi, H., Hanawa, T., and Kamiya, S.** Phenoxazine compounds produced by the reactions with bovine hemoglobin show antimicrobial activity against non-tuberculosis mycobacteria. *Tohoku J. Exp. Med.* **203(1)** (2004) 47–52.

We studied the anti-microbial effects of phenoxazines produced by the reaction of o-aminophenol or its derivatives with bovine hemoglobin, on seven species of mycobacteria such as *Mycobacterium tuberculosis*, *Mycobacterium marinum*, *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum*, *Mycobacterium fortuitum*, *Mycobacterium kansasii* and *Mycobacterium smegmatis* and some bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar Typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*. These phenoxazines, including 2-amino-4, 4alpha-dihydro-4alpha, 7-dimethyl-3H-phenoxazine-3-one (Phx-1), 3-amino-1, 4alpha-dihydro-4alpha, 8-dimethyl-2H-phenoxazine-2-one (Phx-2), and 2-aminophenoxazine-3-one (Phx-3), prevented the proliferation of four non-tuberculosis mycobacteria including *M. scrofulaceum*, *M. kansasii*, *M. marinum*, and *M. intracellulare* dose-dependently, though the inhibitory effects of these phenoxazines differed according to the species of mycobacteria. However these phenoxazines failed to prevent the proliferation of *M. tuberculosis*, *M. fortuitum*, and *M. smegmatis*, and the concerned bacteria other than mycobacteria. The present results may contribute to development of novel antibiotics against non-tuberculosis mycobacteria.—Authors' Abstract

**Ziglam, H. M., Daniels, I., and Finch, R. G.** Immunomodulating activity of rifampicin. *J. Chemother.* **16(4)** (2004) 357–361.

It has been shown that some antibiotics can modify cytokine production. We have examined the effect of rifampicin on secretion of interleukin-1beta (IL-1beta), IL-6, IL-10, and tumor necrosis factor alpha (TNF-alpha) by lipopolysaccharide (LPS)-stimulated or heat killed staphylococci (Pansorbin) stimulated monocytes. Secretion of IL-1beta and TNF-a were significantly inhibited ( $p < 0.002$ ) whereas secretion of IL-6 and IL-10 were significantly increased ( $p < 0.003$ ) by rifampicin treated mononuclear cells. Rifampicin had immunomodulatory effects through its capacity to alter the secretion of tested cytokines by human monocytes.—Authors' Abstract

## Clinical Sciences

**Arruda, A. P., Marques, W. Jr., Foss, N. T., Garbino, J. A., Virmond, M., and Barreira, A. A.** Near nerve potential of sural nerve in leprosy. *Arq. Neuropsiquiatr.* **62(3A)** (2004) 571–574. Epub 2004 Aug. 24.

Leprosy neuropathy is characterized by initial involvement of the small nerve fibers, later followed by involvement of the large fibers, when routine nerve conduction studies become abnormal. To increase the diagnostic yield and precocity of these studies, we applied the near nerve technique to the sural nerve of 8 leprosy patients. Contrary to our expectations, the main component of the sural nerve sensory action potential was abnormal in all patients, but the minimum conduction velocity originating from small 3–6 mm fibers was normal or only mildly involved in three patients. Also, although Schwann cells are the first to be involved in leprosy, the results are suggestive of axonal degeneration instead of demyelination. To better understand the neurophysiology and physiology of leprosy and to increase the accuracy and precocity of the diagnosis, it will be necessary to investigate patients in the very early stages of the disease and to correlate these findings with the corresponding nerve pathology.—Authors' Abstract

**Bhatia, A., Nanda, S., Gupta, U., Gupta, S., and Reddy, B.** Topical phenytoin suspension and normal saline in the treatment of leprosy trophic ulcers: arandomized, double-blind, comparative study. *J. Dermatolog. Treat.* **15(5)** (2004) 321–327.

**OBJECTIVE:** To evaluate and compare two strengths of topical phenytoin sodium suspension (2% and 4%) with normal saline in the healing of acute trophic ulcers in leprosy patients. **METHODS:** A prospective, parallel, double-blind, randomized study was conducted in 45 leprosy inpatients with acute trophic ulcers. Patients were randomized to receive 2%, 4% or normal saline dressing on their ulcers once daily for 4 weeks. Efficacy parameters such as a reduc-

tion in the surface area of the ulcer, bacterial culture of the ulcer swab, appearance of healthy granulation tissue, cessation of ulcer discharge and overall gradation of clinical healing and safety were assessed at weekly intervals. **RESULTS:** The ulcer area reduction was greater in the 2% and 4% phenytoin groups compared with the normal saline group ( $p < 0.001$ ). Appearance of healthy granulation tissue and cessation of ulcer discharge was also observed earlier in the two phenytoin groups. At the end of 4 weeks, 11 ulcers each had healed completely in both the 2% and 4% phenytoin groups compared with none in the control group. There were no statistical differences between the 2% and 4% phenytoin groups. No side effects were reported by any patient. **CONCLUSION:** Topical phenytoin appears to be an effective, safe and cheap therapeutic option for the healing of trophic ulcers in leprosy patients.—Authors' Abstract

**Boggild, A. K., Correia, J. D., Keystone, J. S., and Kain, K. C.** Leprosy in Toronto: an analysis of 184 imported cases. *Canadian Med. Assn. J.* **170(1)** (2004) 55–59.

**Background:** Leprosy is a rare but serious mycobacterial infection. Immigration from areas where the disease is endemic has resulted in the importation of leprosy into countries where it is not endemic and where physicians and health care workers have little or no experience in diagnosis and therapy. In this study we characterized leprosy patients seen in a tropical disease unit that manages most of the reported leprosy cases in Canada. **Methods:** We reviewed the clinical records of all 184 leprosy patients who were referred to the tropical Disease Unit at Toronto General Hospital, Toronto, Ontario, Canada, between 1979 and 2002 and abstracted demographic and clinical information. **Results:** Patients were more likely to be male (122 or 66.3%) and of Indian (44 or 23.9%), Filipino (49 or 22.6%) or Vietnamese (37 or 20.1%) origin. Patients experienced symptoms for a mean of 4.8 yrs before referral to the Tropical Disease Unit.



Most had no family history of leprosy (152/172 or 84%). Most patients presented either with borderline tuberculoid (80 or 43.5%) or borderline lepromatous (37 or 20.1%) disease. On average, patients presented with 5.8 skin lesions. Upper - and lower-extremity nerve dysfunction was common at presentation, with up to one-third of patients demonstrating either sense or motor loss. A significantly greater lag time to presentation was observed in patients who emigrated from low-prevalence regions ( $p < 0.001$ ). Interpretation: Leprosy is a chronic infectious disease that is associated with serious morbidity if left untreated. Leprosy is uncommon in developed countries, but it is important for physicians to have a high index of suspicion when a foreign-born patient presents with chronic dermatitis and peripheral nerve involvement.—Tropical Disease Bulletin

**Burman, K. D., Rijall, A., Agrawal, S., Agarwalla, A., and Verma, K. K.** Childhood leprosy in eastern Nepal: a hospital-based study. *Indian J. Lepr.* **75(1)** (2003) 47–52.

A hospital-based retrospective study on childhood leprosy was carried out at B.R. Koirala Institute of Health Sciences, Dharan, covering the period April 1998–April 2002. 20 (4.45%) leprosy patients were detected in children aged 6–14 years. The male:female ratio was 4:1. History of contact was found in 10% of the patients. The commonest type of leprosy was borderline tuberculoid leprosy (550%), followed by borderline lepromatous leprosy (301%). Most of the patients had more than one lesion. Nerve involvement and grade 2 deformity were noted in 55% and 20% of the patients, respectively. Slit skin smear was positive in 30% of patients.—Authors' Abstract

**Cortés, S. L., and Rodríguez, G.** Leprosy in children: association between clinical and pathological aspects. *J. Trop. Pediatr.* **50(1)** (2004) 12–15.

Leprosy among children is a public health problem reflecting the disease's transmis-

sion in the community and the efficiency of control programs. To evaluate some clinical, epidemiological and histopathological criteria, as well as the level of agreement between clinical and histopathological diagnoses, 207 biopsies were studied from patients less than 15 years old who were clinically diagnosed with leprosy between March 1994 and September 2000. Leprosy was confirmed by histopathology in 119 cases (57.5 per cent). Forty-seven per cent of children were 10 years old or more; 28.5 per cent shared their dwellings with leprosy patients; 35 per cent had only one lesion, and 43 per cent were multibacillary cases. Agreement between clinical and histopathological classification was 36 per cent; hypochromic chronic eczema and post-inflammatory incontinence of melanin pigment were the clinical lesions most frequently mistaken with leprosy. Leprosy among children represents 7 per cent of new leprosy cases in Colombia and the high percentage of multibacillary cases suggests that diagnosis is being made late. The disease must be investigated in all children living with leprosy patients and skin biopsy is recommended to avoid false-positive diagnoses.—Tropical Disease Bulletin

**Couppié, P., Abel, S., Voinchet, H., Rousel, M., Hélénon, R., Huerrero, M., Sainte-Marie, D., and Cabié, A.** Immune Reconstitution Inflammatory Syndrome Associated with HIV and Leprosy. *Arch. Dermatol.* **140** (2004) 997–1000.

**Background:** Immune reconstitution inflammatory syndrome (IRIS) is an unusual inflammatory reaction to an opportunistic infection that occurs in human immunodeficiency virus (HIV)-positive patients with profound immunosuppression during the reconstitution of the immune system in the initial months of highly active antiretroviral treatment.

**Observations:** We describe 3 cases of leprosy occurring in patients treated with a combination of 3 antiretroviral drugs who fulfilled the criteria for IRIS. A reactional state occurred in all 3 cases. Two of the 3 patients presented an unusual ulcerous progression of the lesions not generally observed in cases of leprosy. The outcome was

favorable in all 3 cases. The frequency of IRIS associated with leprosy in French Guiana and Martinique is estimated at 3 cases per 1000 HIV-positive patients receiving highly active antiretroviral treatment.

**Conclusions:** Leprosy should be recognized as an IRIS-associated infection with possibility of atypical presentation.—Archives of Dermatology

**Dave, S., Nori, A. V., Thappa, D. M., and Siddaraju, N.** Leprous osteitis presenting as bone cyst and erosions. *Dermatol. Online J.* **10(1)** (2004) 17.

A 30-year-old man presented to the Hansen outpatient department with swelling and ulceration of toes for 2 months and swelling of the right fifth and fourth fingers and the left second finger for 1 month. In addition to skin lesions of lepromatous leprosy (subpolar type), there was nontender, non-fluctuant swelling of the right fifth and fourth fingers and left second finger. Skin over the right fifth finger showed sinus-like openings with associated purulent discharge. He also had swelling and ulceration of second left toe. Slit-skin smear (SSS) showed a bacterial index of 6+ from the ear lobes and cutaneous nodules, 4+ from the patch, and 3+ from normal skin. Modified Ziehl-Neelsen staining of the discharge extruding from the sinuses on the right fifth finger also showed abundant acid-fast bacilli. Radiography of the hands and feet showed lytic lesions in the distal epimetaphyseal region of proximal phalanx of the right fifth finger and left second finger and erosion of distal end of proximal phalanges of both second toes. Histopathological examination of biopsy specimen from the patch (back) showed features of lepromatous leprosy, and Fite-Faraco stain for tissue acid-fast bacteria (AFB) was strongly positive. Fine-needle-aspiration cytology (FNAC) from the lytic lesion in the bone also showed predominantly foamy macrophages with strongly positive staining for AFB with a few interspersed lymphocytes, epithelioid cells and Langhans giant cells. On the basis of these features, a clinical diagnosis of subpolar lepromatous leprosy with leprous osteitis was made. In today's clinical era of

improved case detection and prompt treatment with effective multidrug regimens, advanced bone changes are rarely encountered. We describe this case of lepromatous leprosy that developed cavitating lesions of the phalanges of the hand, seen on x-ray as well-defined bone cyst and erosions.—Authors' Abstract

**Dawe, S., Lockwood, D. N., and Creamer, D.** A case of post-partum borderline tuberculoid leprosy complicated by a median nerve abscess, peptic ulceration and rifampicin-induced haemolytic renal failure. *Lepr. Rev.* **75(2)** (2004) 181–187.

We report a case of borderline tuberculoid leprosy complicated by a median nerve abscess, acute renal failure secondary to rifampicin-induced haemolysis and duodenal ulceration secondary to steroid use. Rifampicin induced haemolysis is a rare and probably under-reported complication of leprosy multi-drug therapy. It should be considered when patients complain of flu-like symptoms after taking their monthly rifampicin.—Authors' Abstract

**de Freitas, M. R., Nascimento, O. J., de Freitas, M. R., and Hahn, M. D.** Isolated superficial peroneal nerve lesion in pure neural leprosy: case report. *Arq. Neuropsiquiatr.* **62(2B)** (2004) 535–539.

Patients with leprosy may have only nerve involvement without skin changes. These cases are known as pure neural leprosy and can be seen in 10% of leprosy patients. Most patients have mononeuritic or multiple mononeuritic neuropathy patterns. The isolated lesion of the superficial peroneal nerve is uncommonly seen. We report a patient with involvement of this nerve in which there was no thickening of superficial nerves. The performed nerve biopsy showed inflammatory infiltration, loss of fibers and presence of *Mycobacterium leprae*. We believe that in prevalent leprosy countries we should take in mind the possibility of isolated pure neural leprosy in some patients without skin lesion. In these cases the diagnosis of leprosy is impossible on clinical

grounds and nerve biopsy is mandatory.—  
Authors' Abstract

**Helmer, K. A., Fleischfresser, I., Kucharski-Esmanhoto, L. D., Neto, J. F., and Santamaria, J. R.** The Lucio's Phenomenon (necrotizing erythema) in pregnancy. *An. Bras. Dermatol.* **79(2)** (2004) 205–210. [Article in Spanish].

**Summary:** The Lucio's phenomenon, a type 2 reactional condition in leprosy probably mediated by immune complexes, is a severe necrotizing skin reaction that occurs mainly in patients with non-nodular lepromatous leprosy. This report presents a 27-year-old woman, in her 32nd week of pregnancy, with a one-week history of painful skin lesions in extremities, reddish-purple, sharply delineated, confluent, with bullae and occasional necrosis and ulceration. The patient also referred fever. Bacilloscopy showed acid-fast bacilli and globi, and the histopathologic findings of a skin biopsy were consistent with lepromatous leprosy and Lucio's phenomenon. Prednisone and multidrug therapy with rifampin, clofazimine and dapsone were given, with remission. Pregnancy has been associated with a high incidence of first diagnosis of leprosy or with an exacerbation of symptoms in patients with the established disease because hormonal alterations cause immunological imbalance, particularly between the last three months of pregnancy and the first three months of lactation, when immunosuppression is higher. Despite the recommendation not to take drugs during pregnancy, the multidrug therapy regimen must be used, since the benefits achieved with the treatment surpass the risks.—*Anais Brasileiros de Dermatologia*

**Jain, R., Dogra, S., Kaur, I., and Kumar, B.** Leprosy and herpes zoster; an association or dissociation? *Indian J Lepr* **75(3)** (2003) 263–264.

Nerve involvement is common to the pathogenesis of both leprosy and herpes zoster. We report two cases of borderline leprosy in which the skin lesions character-

istically spared the healed zoster scar. Possible mechanisms and relationship are discussed.—Authors' Abstract

**Karthikeyan, K., and Thappa, D. M.** Squamous cell carcinoma in plantar ulcers in leprosy: a study of 11 cases. *Indian J. Lepr.* **75(3)** (2003) 219–224.

The objectives of our study were to describe and analyze the malignancies that occurred in plantar ulcers of leprosy patients. The possible predisposing conditions, duration and extent of the spread of the tumour were also studied. All patients with trophic ulcer of the foot attending the urban leprosy clinic in our hospital from January 1998 to January 2003 were screened for change to malignancy. During the study period, 79 cases of plantar ulcers in leprosy were seen. The mean age of these cases was 39.9 years with male-to-female ratio of 4:1. Eleven cases with plantar ulcers and malignant change were diagnosed in our hospital during the study period. The male-to-female ratio was 4.5:1. The mean age of these patients was 60.6 years. Their age ranged from 46 to 75 years. Nine of the cases were treated cases of borderline tuberculoid leprosy, while two had treated lepromatous leprosy. In our study, two distinct morphological types of malignant changes were seen. Histopathologically, all cases, except one, were of well-differentiated squamous cell carcinoma variation; one case had verrucous carcinoma. Though trophic ulcers are common in leprosy cases, only long-standing and neglected ones undergo malignancy.—Authors' Abstract

**Katoch, K., Katoch, V. M., Natrajan, M., Sreevatsa, Gupta, U. D., Sharma, V. D., and Shivanavar, C. T.** 10–12 years follow-up of highly bacillated BL/LL leprosy patients on combined chemotherapy and immunotherapy. *Vaccine.* **22(27–28)** (2004) 3649–3657.

This study reports the follow-up results of 36 highly bacillated untreated BL/LL cases who were serially allocated to three treat-

ment groups. Group I patients received a modified WHO regimen (Rifampicin 600 mg once a month supervised, 50 mg of Clofazimine and 100 mg of Dapsone daily unsupervised) and BCG 0.1 mg per dose 6 monthly; group II patients received the same multi-drug treatment (MDT) and Mw ( $2 \times 10^8$ ) killed bacilli per dose) 6 monthly; group III patients received the same MDT with 0.1 ml of distilled water 6 monthly and acted as a control. Treatment was continued till smear negativity. All these three groups were comparable by their initial clinical score, bacteriological index (BI), viable bacilli as assessed by the mouse footpad (MFP), bacillary adenosine triphosphate (ATP) content and also histologically at the time of starting treatment. All these parameters were evaluated every 6 months. The vaccines were well tolerated. All the patients in group I became smear negative by 3.5 years, in group II in 3 years whereas those in group III took 5 years. The incidence of reactions was the same in all the groups during the first 2 years, however, patients of group III (MDT + placebo) continued to have reactions up to 3 years. No viable bacilli could be detected in the local and distal sites as estimated by MFP and bacillary ATP after 12 months in both the immunotherapy groups. These could be detected in patients on MDT alone up to 24 months of therapy. Histologically patients in both the immunotherapy groups (groups I and II) showed accelerated granuloma clearance, histological upgrading and non-specific healing without granuloma formation both at the local and distal sites and this was achieved much earlier compared to the MDT + placebo group. Thus, by the addition of immunotherapy the effective treatment period of achieving bacteriological negativity could be reduced by about 40%, time period of reactions reduced by 33% and there were no reactions and/or relapses in the 10–12 years post-treatment follow-up.—  
Authors' Abstract

**Lomonte, C., Chiarulli, G., Cazzato, F., Giammaria, B., Marchio, G., Losurdo, N., Antonelli, M., Casucci, F., and Basile, C.** End-stage renal disease in leprosy. *J. Nephrol.* **17**(2) (2004) 302–305.

**BACKGROUND:** Leprosy or Hansen's disease (HAD) undoubtedly remains an emergency in certain countries. It is an ancient deforming disease caused by *Mycobacterium leprae*. The countries with the highest endemic leprosy rate in 2000 were Brazil, India and Madagascar. In Italy, the old epidemic has been defeated and there are approximately 400 patients under constant monitoring with three to four new cases per year involving Italian residents. The kidney is one of the target organs during the splanchnic localization of leprosy. The histopathological renal lesion spectrum includes glomerulonephritis (GN), renal amyloidosis (RA) and interstitial nephritis (IN). Both proteinuria and chronic renal failure are the main clinical expressions of renal damage in leprosy. To the best of our knowledge, very little is reported concerning end-stage renal disease (ESRD) in leprosy patients both in the most important national and international renal registries and in the available literature. This study aimed to report the long-term experience of our department in this field. **METHODS:** To achieve this, we analyzed retrospectively the HAD Center (Gioia del Colle) database at our hospital. **RESULTS:** Eight leprosy patients were dialyzed from 1980 to June 2003 (six males and two females), with a mean age of  $61.0 \pm 8.9$  S.D. yrs (range: 51–76) and a mean HAD duration of  $36.1 \pm 5.1$  yrs. The first clinical nephropathy manifestations were non-nephrotic proteinuria associated with chronic renal failure in four patients, and nephrotic proteinuria in four patients. Kidney biopsies performed in three patients showed two had RA, and one had IN. Two patients were treated initially by peritoneal dialysis; they were then switched to hemodialysis (HD) after 3 and 10 months because of recurrent peritonitis. HD treatment lasted  $40.6 \pm 31.4$  months (range: 9–101). Six patients died, one due to hyperkalemia, one because of a technical dialysis accident, and the remainder due to causes unrelated to the dialysis treatment. Two patients are still alive, treated with HD for 17 and 44 months. **CONCLUSIONS:** Uremia represents a late complication of leprosy and has a multifactorial genesis, although RA is among the most frequent causes, conventional bicarbonate HD appears to offer good results in

the treatment of uremia in leprosy patients.—Authors' Abstract

**Malaviya, G. N.** Palmaris longus—a muscle with multiple uses in leprosy-affected hands. *Indian J. Lepr.* **75(4)** (2003) 327–334.

The present paper reviews the anatomy of palmaris longus muscle and also the situations where palmaris longus muscle has been used as an independent motor or as a donor of tendon graft material. Its relevance in leprosy-affected hands is also discussed because the muscle is usually spared in hand palsies consequent to leprotic neural damage. The advantages and disadvantages of its use in different operative procedures have been analyzed. The author's experience with this muscle in the correction of hand deformities in leprosy is described.—Author's Abstract

**Ooi, W. W., and Srinivasan, J.** Leprosy and the peripheral nervous system: basic and clinical aspects. *Muscle Nerve.* **30(4)** (2004) 393–409.

Leprosy is one of the most common causes of nontraumatic peripheral neuropathy in the developing world. The causative agent, *Mycobacterium leprae*, has a predilection for Schwann cells, where the organism multiplies unimpeded by organism-specific host immunity, resulting in destruction of myelin, secondary inflammatory changes, and destruction of the nerve architecture. The cardinal diagnostic features of leprosy are anesthetic skin lesions, neuropathy, and positive skin smears for the bacilli. However, patients may rarely present without skin lesions in pure neuritic leprosy. Electrodiagnostic findings early in the disease reveal demyelinating features, such as slowing of conduction velocity and prolongation of latencies, but as the disease progresses secondary axonal damage commonly ensues. Electrodiagnostic studies are also useful to monitor for toxicity secondary to therapy, particularly thalidomide-associated neuropathy. Nerve biopsy of a sensory cutaneous nerve is sometimes es-

sential to confirm a diagnosis of leprosy. Significant advances in understanding of the pathogenesis, mapping of the genome, and other advances in molecular biology may result in better preventive and therapeutic modalities, and the goal of eradicating leprosy as a global problem may yet be realized.—Authors' Abstract

**Pais, A. V., Pereira, S., Garg, I., Stephen, J., Antony, M., and Inchara, Y. K.** Intra-abdominal, crystal-storing histiocytosis due to clofazimine in a patient with lepromatous leprosy and concurrent carcinoma of the colon. *Lepr. Rev.* **75(2)** (2004) 171–176.

We report a case with abdominal complications of clofazimine treatment which included blackish discoloration of the lymph nodes, omentum and peritoneum. A 44-year-old female with lepromatous leprosy and a history of adverse reaction to clofazimine 2 years previously, presented with rectosigmoid junction adenocarcinoma. Laparotomy revealed an inoperable tumour with pigmentation of the bowel, serosa and peritoneum. A second operation had to be performed for transverse loop colostomy and a mesenteric lymph node biopsy sent for frozen section showed typical clofazimine crystals. Despite widespread use for many years in the treatment of leprosy, this drug is not known to be carcinogenic and this case provides no evidence for an association or link between its use and the patient's cancer. Apart from its use in leprosy, clofazimine may be used in the treatment of disseminated *Mycobacterium avium*-intracellulare infection, Buruli ulcer due to *M. ulcerans* and occasionally in other mycobacterial infections. An awareness of the rare side-effect described above may help in the clinical assessment and management of such cases, including the avoidance of unnecessary laparotomy.—Authors' Abstract

**Pimentel, M. I., Nery, J. A., Borges, E., Goncalves, R. R., and Sarno, E. N.** Impairments in multibacillary leprosy; a study from Brazil. *Lepr. Rev.* **75(2)** (2004) 143–152.

This is a retrospective cohort study of 103 multibacillary leprosy patients (18% BB, 48% BL and 34% LL) followed during and after treatment, in a tertiary referral centre with an outpatient clinic in an endemic area in Brazil, for an average period of 65 months since the start of multidrug therapy (24-dose MDT). The objective of the study was to identify the role of overt neuritis (presence of pain in a peripheral nerve trunk, with or without enlargement or neural function damage), in the development of impairments. They were evaluated using the World Health Organization disability grade before treatment, at the end of the treatment, and at the end of the follow-up period. Thirty-four percent of patients presented overt neuritis during MDT, and 45% had overt neuritis episodes during the follow-up period; the most commonly affected nerves were ulnar, fibular and posterior tibial nerves, and the neuritic episodes were carefully treated with steroid therapy and physiotherapy. Impairments were associated with: affected (painful and/or thick) nerves at diagnosis ( $p < 0.005$ ); delay in diagnosis ( $p = 0.010$ ); impairments already present at the start of treatment ( $p = 0.00041$  at the end of MDT, and  $p = 0.000013$  at the end of follow-up); occurrence of overt neuritis episodes during MDT ( $p = 0.0016$ ) or the whole follow-up ( $p = 0.015$ ). These data draw attention to the importance of early diagnosis and of good neurological examination throughout the follow-up, as well as suggest the importance of neuritis in the induction of impairments in multibacillary leprosy.—Authors' Abstract

**Rath, N., and Kar, H. K.** Leprosy in HIV infection: a study of three cases. *Indian J. Lepr.* **75(4)** (2003) 355–359.

The course of leprosy in patients with HIV infection has been a controversial issue for a long time. It is still a matter of debate whether the HIV status of an individual has any impact on the natural history of leprosy and response to anti-leprosy treatment. We report here three HIV-positive leprosy cases (two BT and one BB) along with their CD4 counts and HIV staging with anti-leprosy therapeutic response. Both BT cases responded well to conventional WHO MDT

(PB) for 6 months, whereas the BB case relapsed 3 months after completion of MDT (MB) for one year. However, he became inactive again following a further one-year course of MDT (MB).—Authors' Abstract

**Richardus, J. H., Withington, S. G., Anderson, A. M., Croft, R. P., Nicholls, P. G., van Brakel, W. H., and Smith, W. C. S.** Adverse events of standardized regimens of corticosteroids for prophylaxis and treatment of nerve function impairment in leprosy: results from the "TRIPOD" trials. *Lepr. Rev.* **74(4)** (2003) 319–327.

Reactions in leprosy causing nerve function impairment (NFI) are increasingly treated with standardized regimens of corticosteroids, often under field conditions. Safety concerns led to an assessment of adverse events of corticosteroids, based on data of three trials studying prevention of NFI (the TRIPOD study). A multicenter, randomized, double-blind placebo-controlled trial was conducted in leprosy control programs in Nepal and Bangladesh. Treatment was with prednisolone according to fixed schedules for 16 weeks, starting in one trial with 20 mg/day (prophylactic regimen: total dosage 1.96 g) and in the other two trials with 40 mg/day (therapeutic regimen: total dosage 2.52 g). Minor adverse events were defined as moon face, fungal infections, acne, and gastric pain requiring antacid. Major adverse events were defined as psychosis, peptic ulcer, glaucoma, cataract, diabetes and hypertension. Also, the occurrence of infected plantar, palmar, and corneal ulceration was monitored, together with occurrence of TB. Considering all three trials together, minor adverse events were observed in 130/815 patients (16%). Of these, 51/414 (12%) were in the placebo group and 79/401 (20%) in the prednisolone group. The relative risk for minor adverse events in the prednisolone group was 1.6 ( $p = 0.004$ ). Adverse events with a significantly increased were acne, fungal infections and gastric pain. Major adverse events were observed in 15/815 patients (2%); 7/414 (2%) in the placebo group and 8/401 (2%) in the prednisolone group. No major adverse events had a significantly increased risk in

the prednisolone arm of the trials. No cases of TB were observed in 300 patients who could be followed-up for 24 months. Standardized regimens of corticosteroids for both prophylaxis and treatment of reactions and NFI in leprosy under field conditions in developing countries are safe when a standard pre-treatment examination is performed, treatment for minor conditions can be carried out by field staff, referral for specialized medical care is possible, and sufficient follow-up is done during and after treatment.—Tropical Disease Bulletin

**Rijal, A., Agrawal, S., Agarwalla, A., and Lakhey, M.** Bullous erythema nodosum leprosum: a case report from Nepal. *Lepr Rev.* **75(2)** (2004) 177–180.

A patient with lepromatous leprosy, while on WHO multidrug therapy (MDT) for multibacillary disease, was diagnosed as having dapsone syndrome with recurrent episodes of bullous lesions on the lower extremities for 4–5 years. The lesions were associated with high-grade fever. Examination revealed multiple hypopigmented macules on the limbs. Multiple atrophic scars were also found on the buttocks and lower limbs. Bilateral ulnar, radial cutaneous and lateral popliteal nerves were thickened. On day 10 of WHO-MB-MDT he developed a flaccid bulla on the lower leg. Skin slit smear showed a bacterial index (BI) of 3+ and the histopathology was consistent with type II reaction. High dose corticosteroid therapy was started but he continued to have new lesions, and was therefore referred to a centre where thalidomide was available. Clinical response was good and he remained symptom-free after gradual reduction in dosage. ENL should be differentiated from bullous drug reactions, pemphigus vulgaris, bullous pemphigoid and other blistering diseases.—Authors' Abstract

**Senturk, V., and Sagduyu, A.** [Psychiatric disorders and disability among leprosy patients; a review.] *Turk. Psikiyatri. Der g.* **15(3)** (2004) 236–243. [Article in Turkish]

As a gross estimate, leprosy currently affects 1–16 million patients worldwide

There are currently 3600 registered patients in Turkey. The social stigma connected to leprosy makes this disease completely different from others. Even nowadays people affected by leprosy have to leave their village or are socially isolated. The physical deformity ratio is approximately 25% in other countries whereas it is more than half in Turkey. The prevalence of mental disorders among leprosy patients is higher than that among the general population. Depression is the most common psychiatric disorder among leprosy patients. Another important finding is that the long duration of the illness and physical handicaps raise the risk of psychiatric disorders. Nevertheless, the results of two studies conducted in Turkey on this subject contradict the results of international studies. Leprosy patients experience functional disabilities that limit their lives and ability to establish relationships with others both in social and occupational fields. The physical disability rate is high (75%) in Turkey. A review of the literature revealed several papers on the psychosocial aspects of illness but few references to the degree or pattern of psychiatric disorders among leprosy patients. The main purpose of this paper is to review psychiatric disorders and disabilities in leprosy patients and to obtain concrete results.—Authors' Abstract

**Soysal, A., Atay, T., Ozu, T., and Arpacı, B.** Electrophysiological evaluation of peripheral and autonomic involvement in leprosy. *Can. J. Neurol. Sci.* **31(3)** (2004) 357–362.

**OBJECTIVE:** Motor and sensory nerve conductions, F responses, sympathetic skin responses and R-R interval variations (RRIV) were studied to determine the type of peripheral neuropathy among patients with leprosy. **METHODS:** Twenty-nine consecutive patients with leprosy (25 male, 4 female) hospitalized in the "Istanbul Leprosy Hospital" between January–December, 1999 were included in this study. Ten patients had borderline lepromatous leprosy, and 19 had lepromatous leprosy. None of the patients studied had the tuberculoid form. The mean age was  $55 \pm 12$  years. The control group consisted of 30 (26 male, 4 fe-

male) healthy volunteers (mean age:  $58.1 \pm 7.8$  years). All subjects included in the study underwent neurological examination and electrophysiological evaluation. Standard procedures were performed for evaluating sensory and motor conduction studies.

Motor studies were carried out on both left and right median, ulnar, tibial and common peroneal nerves while median, ulnar, sural and superficial peroneal nerves were examined for sensory studies. Sympathetic skin response recordings on both hands and RRIV recordings on precordial region were done in order to evaluate the autonomic involvement. **RESULTS:** The lower extremity was found to be more severely affected than the upper, and sensory impairment predominated over motor. Of 58 upper limbs examined, no sympathetic skin responses was recorded in 46 (79.3%). Compared with the controls, the RRIVs of the leprosy patients were found to be reduced during both resting and deep forced hyperventilation. **CONCLUSION:** Our results indicate that leprosy causes a predominantly axonal polyneuropathy that is more severe in the lower extremities. Sensory nerve damage is accompanied by autonomic involvement.—Authors' Abstract

**Taylor, N. L., Raj, A. D., Dick, H. M., and Solomon, S.** The correction of ulnar claw fingers: a follow-up study comparing the extensor-to-flexor with the palmaris longus 4-tailed tendon transfer in patients with leprosy. *J. Hand Surg. [Am]*. **29(4)** (2004) 595–604.

**PURPOSE:** The extensor to flexor 4-tailed tendon transfer (EF4T) and the palmaris longus 4-tailed tendon transfer (PL4T) are 2 surgical procedures used to correct intrinsic paralysis of the hand in leprosy. The EF4T traditionally is the more common procedure and requires the transfer of a wrist extensor muscle. The PL4T requires the transfer of the palmaris longus and morbidity is expected to be lower. A follow-up study was performed to determine whether the clinical outcome of the PL4T is superior to the EF4T procedure in leprosy patients with ulnar claw fingers that are considered mobile before surgery. **METHODS:** Fifty-five patients presented 65 affected hands, of which 40

hands had the PL4T and 25 had the EF4T procedure. Each hand was assessed before surgery and at follow-up evaluation by pre-determined angle measurements, standardized photographs, mechanical function, and patient satisfaction. Each hand was given an overall technical grade according to previously published standards. **RESULTS:** After an average follow-up period of 33 months there was no statistically significant difference in the technical outcome or patient satisfaction between the 2 tendon transfer procedures. **CONCLUSIONS:** Whenever the palmaris longus is available it may be considered to be the motor tendon of choice to undertake a many-tailed procedure for claw finger reconstruction in mobile hands paralyzed by leprosy. The palmaris longus should be considered as a possible motor tendon when correcting intrinsic muscle paralysis of the hand.—Authors' Abstract

**van Brakel, W. H., Anderson, A. M., Withington, S. G., Croft, R. P., Nicholls, P. G., Richardus, J. H., and Smith W. C. S.** The prognostic importance of detecting mild sensory impairment in leprosy: a randomized controlled trial (TRIPOD 2). *Lepr. Rev.* **74(4)** (2003) 300–310.

This study was designed to investigate whether leprosy patients diagnosed with mild sensory impairment have a better prognosis when treated with steroids than similarly impaired patients treated with placebo. A multicenter, randomized, double-blind, placebo-controlled trial was conducted in Nepal and Bangladesh [date not given]. Patients were eligible if they had a confirmed leprosy diagnosis, were between 15 and 50 years old, had mild sensory impairment of the ulnar or posterior tibial nerve of less than 6 months duration and did not require steroids for other reasons. 'Mild impairment' was defined as 'impaired on the Semmes-Weinstein monofilament test, but testing normal on the ballpen sensory test.' Subjects were randomized to either prednisolone treatment starting at 40 mg per day, tapering over 4 months, or placebo. Nerve function was monitored monthly. Any patient who deteriorated was taken out of the trial and was put on full-dose steroid treatment. Outcome assessment was done at 4, 6,



9 and 12 months from the start of the treatment. Outcome measures were the proportion of patients needing full-dose prednisolone and the Semmes-Wenstein sum scores. Each patient contributed only one nerve to the analysis. Seventy-five patients had nerves eligible for analysis, of whom 41 (55%) and 34 (45%) were allocated to the prednisolone and placebo arms, respectively. At 4 months, three patients in the prednisolone arm (7%) and six in the placebo arm (18%) had an outcome event requiring full-dose steroids. At 12 months, these proportions had almost reversed, 11 (27%) and 6 (18%) in the treatment and placebo arms, respectively. In the latter group, 75% had recovered sponta-

neously after 12 months. Prednisolone treatment of sensory impairment of the ulnar and posterior tibial nerves detectable with the monofilament test, but not with the balloon test, did not improve the long-term outcome in terms of recovery of touch sensibility, nor did it reduce the risk of leprosy reactions or nerve function impairment beyond the initial 4-month treatment phase. Two unexpected main findings were the strong tendency of mild sensory impairment to recover spontaneously and the fact that patients with mild sensory impairment without any other signs or symptoms of reaction or nerve function impairment are relatively rare.—Tropical Disease Bulletin

## Immunopathology

**Antas, P. R., Sales, J. S., Pereira, K. C., Oliveira, E. B., Cunha, K. S., Sarno, E. N., and Sampaio, E. P.** Patterns of intracellular cytokines in CD4 and CD8<sup>+</sup> T cells from patients with mycobacterial infections. *Braz. J. Med. Biol. Res.* **37(8)** (2004) 1119–1129. Epub 2004 Jul 20.

Using a short-term bulk culture protocol designed for an intracellular-staining method based on a flow cytometry approach to the frequencies of cytokine-producing cells from tuberculosis and leprosy patients, we found distinct patterns of T cell subset expression. The method also reveals the profile of peak cytokine production and can provide simultaneous information about the phenotype of cytokine-producing cells, providing a reliable assay for monitoring the immunity of these patients. The immune response of *Mycobacterium leprae* and purified protein derivative (PPD) *in vitro* to a panel of mycobacteria-infected patients from an endemic area was assessed in primary mononuclear cell cultures. The kinetics and source of the cytokine pattern were measured at the single-cell level. IFN-gamma-, TNF-alpha-, IL-4- and IL-10-secreting T cells were intracytoplasmic evaluated in an attempt to identify *M. leprae*- and PPD-specific cells directly from the peripheral blood. The analysis by this approach indicated that TNF-alpha was the first (8 hr) to be produced, followed by IFN-

gamma (16 hr), IL-10 (20 hr) and IL-4 (24 hr), and double-staining experiments confirmed that CD4<sup>+</sup> were a greater source of TNF-alpha than of CD8<sup>+</sup> T cells ( $p < 0.05$ ). Both T cell subsets secreted similar amounts of IFN-gamma. We conclude that the protocol permits rapid evaluation of cytokine production by different T cell populations. The method can also be used to define immune status in non-infected and contact individuals.—Authors' Abstract

**Bermudez, L. E., Petrofsky, M., and Sangari, F.** Intracellular phenotype of *Mycobacterium avium* enters macrophages primarily by a macropinocytosis-like mechanism and survives in a compartment that differs from that with extracellular phenotype. *Cell. Biol. Int.* **28(5)** (2004) 411–419.

*Mycobacterium avium* uptake by human macrophages differs between the phenotypes of bacterium grown in laboratory media (extracellular growth, EG) and bacterium grown within macrophages (intracellular growth, IG). Studies *in vivo* have confirmed that, when spreading, pathogenic mycobacteria enter macrophages by a complement receptor 3-independent pathway, in contrast to mycobacteria uptake *in vitro*. *M. avium*, grown in macrophages (IG) for 3 or more days, invade fresh macrophages by a macropinocytosis-

like mechanism, in contrast to bacteria grown in media (EG), confirmed by the inhibitory effect of wortmannin, an inhibitor of phosphoinositide-3-kinase, on the uptake of IG, but not EG, by macrophages. The IG phenotype was seen in vacuoles with lower pH than those inhabited by the EG phenotype. Incubation of macrophages with bafilomycin A1, an inhibitor of vacuole acidification, decreased the viability of intracellular IG, but not EG, phenotype, suggesting the importance of an acidic environment for the regulation of IG genes. In addition, the percentage of vacuoles that incorporate and retain LAMP-1 is smaller with EG than with IG bacteria. The formation of *M. avium* macropinosomes was also shown to be independent of microtubules. These data suggest that uptake of extracellular fluid is part of *M. avium* IG phenotype uptake by macrophages, and that the IG phenotype inhabits a slightly different vacuole than that of EG.—Authors' Abstract

**Cosma, C. L., Humbert, O., and Ramakrishnan, L.** Superinfecting mycobacteria home to established tuberculous granulomas. *Nat. Immunol.* **5(8)** (2004) 828–835. Epub 2004 Jun 27. Comment in: *Nat. Immunol.* **5(8)** (2004) 778–779.

A central paradox of tuberculosis immunity is that reinfection and bacterial persistence occur despite vigorous host immune responses concentrated in granulomas, which are organized structures that form in response to infection. Prevailing models attribute reinfection and persistence to bacterial avoidance of host immunity via establishment of infection outside primary granulomas. Alternatively, persistence is attributed to a gradual bacterial adaptation to evolving host immune responses. We show here that superinfecting *Mycobacterium marinum* traffic rapidly into preexisting granulomas, including their caseous (necrotic) centers, through specific mycobacterium-directed and host cell-mediated processes, yet adapt quickly to persist long term therein. These findings demonstrate a failure of established granulomas, concentrated foci of activated macrophages and antigen-specific immune effector cells, to eradicate newly deposited

mycobacteria not previously exposed to host responses.—Authors' Abstract

**Durai, M., Kim, H. R., and Moudgil, K.**

**D.** The regulatory C-terminal determinants within mycobacterial heat shock protein 65 are cryptic and cross-reactive with the dominant self homologs: implications for the pathogenesis of autoimmune arthritis. *J. Immunol.* **173(1)** (2004) 181–188.

The 65-kDa mycobacterial heat shock protein (Bhsp65) has been invoked in the pathogenesis of both adjuvant arthritis (AA) in the Lewis rat (R T.1(1)) and human rheumatoid arthritis. Arthritic Lewis rats in the late phase of AA show diversification of the T cell response to Bhsp65 C-terminal determinants (BCTD), and pretreatment of naive Lewis rats with a mixture of peptides representing these neoepitopes affords protection against AA. However, the fine specificity and physiologic significance of the BCTD-directed T cell repertoire, and the role of homologous self (rat) hsp65 (Rhsp65), if any, in spreading of the T cell response to Bhsp65 have not yet been examined. We observed that T cells primed by peptides comprising BCTD can adoptively transfer protection against AA to the recipient Lewis rats. However, these T cells can be activated by preprocessed (peptide) form of BCTD, but not native Bhsp65, showing that BCTD are cryptic epitopes. The BCTD-reactive T cells can be activated by the naturally generated (dominant) C-terminal epitopes of both exogenous and endogenous Rhsp65 and vice versa. Furthermore, certain individual peptides constituting BCTD and their self homologs can also induce protection against AA. These results support a model for the diversification of T cell response to Bhsp65 during the course of AA involving up-regulation of the display of cryptic BCTD coupled with spontaneous induction of T cell response to the cross-reactive dominant C-terminal epitopes of Rhsp65. The identification of disease-regulating cryptic determinants in Ags implicated in arthritis provides a novel approach for immunotherapy of rheumatoid arthritis.—Authors' Abstract

**Fischer, K., Scotet, E., Niemeyer, M., Koebnick, H., Zerrahn, J., Maillet, S., Hurwitz, R., Kursar, M., Bonneville, M., Kaufmann, S. H., and Schaible, U. E.** Mycobacterial phosphatidylinositol mannoside is a natural antigen for CD1d-restricted T cells. *Proc. Natl. Acad. Sci. U.S.A.* **101(29)** (2004) 10685–10690. Epub 2004 Jul 08.

A group of T cells recognizes glycolipids presented by molecules of the CD1 family. The CD1d-restricted natural killer T cells (NKT cells) are primarily considered to be self-reactive. By employing CD1d-binding and T cell assays, the following structural parameters for presentation by CD1d were defined for a number of mycobacterial and mammalian lipids: two acyl chains facilitated binding, and a polar head group was essential for T cell recognition. Of the mycobacterial lipids tested, only a phosphatidylinositol mannoside (PIM) fulfilled the requirements for CD1d binding and NKT cell stimulation. This PIM activated human and murine NKT cells via CD1d, thereby triggering antigen-specific IFN- $\gamma$  production and cell-mediated cytotoxicity, and PIM-loaded CD1d tetramers identified a subpopulation of murine and human NKT cells. This phospholipid, therefore, represents a mycobacterial antigen recognized by T cells in the context of CD1d.—Authors' Abstract

**Kudo, K., Sano, H., Takahashi, H., Kuronuma, K., Yokota, S., Fujii, N., Shimada, K., Yano, I., Kumazawa, Y., Voelker, D. R., Abe, S., and Kuroki, Y.** Pulmonary collectins enhance phagocytosis of *Mycobacterium avium* through increased activity of mannose receptor J. *Immunol.* **172(12)** (2004) 7592–7602.

Collectins, including surfactant proteins A (SP-A) and D (SP-D) and mannose binding lectin (MBL), are the important constituents of the innate immune system. *Mycobacterium avium*, a facultative intracellular pathogen, has developed numerous mechanisms for entering mononuclear phagocytes. In this study, we investigated the interactions of collectins with *M. avium* and the effects of these

lectins on phagocytosis of *M. avium* by macrophages. SP-A, SP-D, and MBL exhibited a concentration-dependent binding to *M. avium*. The binding of SP-A to *M. avium* was Ca(2+)-dependent but that of SP-D and MBL was Ca(2+)-independent. SP-A and SP-D but not MBL enhanced the phagocytosis of FITC-labeled *M. avium* by rat alveolar macrophages and human monocyte-derived macrophages. Excess mannan, zymosan, and lipoarabinomannan derived from the *M. avium*-intracellular complex, significantly decreased the collectin-stimulated phagocytosis of *M. avium*. Enhanced phagocytosis was not affected by the presence of cycloheximide or chelation of Ca(2+). The mutated collectin, SP-A(E195Q, R197D) exhibited decreased binding to *M. avium* but stimulated phagocytosis to a level comparable to wild-type SP-A. Enhanced phagocytosis by cells persisted even after preincubation and removal of SP-A or SP-D. Rat alveolar macrophages that had been incubated with SP-A or SP-D also exhibited enhanced uptake of (125)I-mannosylated BSA. Analysis by confocal microscopy and flow cytometry revealed that the lung collectins up-regulated the cell surface expression of mannose receptor on monocyte-derived macrophages. These results provide compelling evidence that SP-A and SP-D enhance mannose receptor-mediated phagocytosis of *M. avium* by macrophages.—Authors' Abstract

**Luo, Y., Yamada, H., Chen, X., Ryan, A. A., Evanoff, D. P., Triccas, J. A., and O'Donnell, M. A.** Recombinant *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) expressing mouse IL-18 augments Th1 immunity and macrophage cytotoxicity. *Clin. Exp. Immunol.* **137(1)** (2004) 24–34.

Interleukin-18 (IL-18) has been demonstrated to synergize with BCG for induction of a T-helper-type 1 (Th1) immune response. Since successful treatment of superficial bladder cancer with BCG requires proper induction of Th1 immunity, we have developed a recombinant (r) BCG strain that functionally secretes mouse (m) IL-18. This rBCG-mIL-18 strain significantly increased

production of the major Th1 cytokine IFN-gamma in splenocyte cultures, at levels comparable to that elicited by control BCG plus exogenous rIL-18. IFN-gamma production by splenocytes was eliminated by addition of neutralizing anti-IL-18 antibody. Endogenous IL-12 played a favorable role whereas IL-10 played an adverse role in rBCG-mIL-18-induced IFN-gamma production. Enhanced host antimycobacterial immunity was observed in mice infected with rBCG-mIL-18 which showed less splenic enlargement and reduced bacterial load compared to control mice infected with BCG. Further, splenocytes from rBCG-mIL-18-infected mice, in response to BCG antigen, displayed increased production of IFN-gamma and GM-CSF, decreased production of IL-10, elevated cellular proliferation and higher differentiation of IFN-gamma-secreting cells. rBCG-mIL-18 also enhanced BCG-induced macrophage cytotoxicity against bladder cancer MBT-2 cells in a dose-dependent manner. Neutralizing all endogenous macrophage-derived cytokines tested (IL-12, IL-18 and TNF-alpha) as well as IFN-gamma severely diminished the rBCG-mIL-18-induced macrophage cytolytic activity, indicating a critical role for these cytokines in this process. Cytokine analysis for supernatants of macrophage-BCG mixture cultures manifested higher levels of IFN-gamma and TNF-alpha in rBCG-mIL-18 cultures than in control BCG cultures. Taken together, this rBCG-mIL-18 strain augments BCG's immunostimulatory property and may serve as a better agent for bladder cancer immunotherapy and antimycobacterial immunization.—Authors' Abstract

**Mendez-Samperio, P., Palma-Barrios, J., Vazquez-Hernandez, A., and Garcia-Martinez, E.** Secretion of interleukin-8 by human-derived cell lines infected with *Mycobacterium bovis*. *Mediators Inflamm.* **13(1)** (2004) 45–49.

**BACKGROUND:** The variable efficacy of bacillus Calmette-Guérin (*Mycobacterium bovis* BCG) in protecting humans against tuberculosis has prompted a search for the mechanisms through which BCG induces chemokines. In this study, our exper-

iments were designed to determine the role of the transcription factor nuclear factor-kappaB (NF-kappaB) and intracellular calcium in the production of interleukin (IL)-8, a main chemotactic factor, by human-derived monocytic cell line U937 and by a human epithelial HEp-2 cell line infected with *M. bovis* BCG. **METHODS:** The concentrations of IL-8 in culture supernatants of U937 cells or HEp-2 cells infected with *M. bovis* BCG were determined by enzyme-linked immunosorbent assay. We used sulfasalazine and curcumin, which are well-described inhibitors of NF-kappaB activity, and we used ethylenediamine tetraacetic acid to deplete extracellular Ca<sup>2+</sup> or used the cell-permeable agent 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetra (acetoxymethyl) ester to chelate releasable intracellular stores of Ca<sup>2+</sup> in order to investigate the mechanisms through which *M. bovis* BCG induces IL-8 secretion in our system. **RESULTS:** The enzyme-linked immunosorbent assay showed that IL-8 protein secretion was elevated in *M. bovis*-infected cell lines. This effect was statistically significant ( $p < 0.01$ ). When calcium influx was suppressed in *M. bovis*-infected cell lines, IL-8 secretion was inhibited. Notably, specific inhibitors of NF-kappaB (sulfasalazine and curcumin) inhibited *M. bovis*-induced IL-8 secretion from U937 cells or HEp-2 cells. **CONCLUSIONS:** Collectively, these results indicate that activation of NF-kappaB is an important signal transduction pathway in *M. bovis*-induced IL-8 secretion in monocytic or epithelial cells. Furthermore, the results showed that calcium influx had a direct effect on IL-8 secretion in U937 cells or HEp-2 cells infected with *M. bovis*.—Authors' Abstract

**Miyamoto, Y., Mukai, T., Takeshita, F., Nakata, N., Maeda, Y., Kai, M., and Makino, M.** Aggregation of mycobacteria caused by disruption of fibronectin-attachment protein-encoding gene. *FEMS Microbiol. Lett.* **236(2)** (2004) 227–234.

The fibronectin-attachment protein (FAP) is conserved among several species of mycobacteria. Although this protein is associated with attachment and internalization of bacteria to host cells via fibronectin, the

physiological role of the protein still remains unclear. To investigate this point, we generated FAP gene disruptant in *Mycobacterium smegmatis*. The gene disruption, verified by Southern blot and PCR analysis, induced changes on the bacteria, which are associated with strong aggregation and alteration of cell surface properties. Increased hydrophobicity and Congo red accumulation was observed in the FAP gene disruptant. In addition, the complementation experiment demonstrated that the corresponding gene restored wild type morphology in the disruptant. These results indicate that the FAP affects the cell surface properties, and its deletion lead to enhanced aggregation of the *M. smegmatis*.—Authors' Abstract

**Pietersen, R., Thilo, L., and de Chastellier, C.** *Mycobacterium tuberculosis* and *Mycobacterium avium* modify the composition of the phagosomal membrane in infected macrophages by selective depletion of cell surface-derived glycoconjugates. *Eur. J. Cell Biol.* **83(4)** (2004) 153–158.

The growth of pathogenic mycobacteria in phagosomes of the host cell correlates with their ability to prevent phagosome maturation. The underlying molecular mechanism remains elusive. In a previous study, we have shown that *Mycobacterium avium* depletes the phagosome membrane of cell surface-derived glycoconjugates (de Chastellier and Thilo, *Eur. J. Cell Biol.* **81**, 17–25, 2002). We now extended these quantitative observations to the major human pathogen, *Mycobacterium tuberculosis* (H37Rv). At increasing times after infection of mouse bone marrow-derived macrophages, cell-surface glycoconjugates were labelled enzymatically with [<sup>3</sup>H]galactose. Subsequent endocytic membrane traffic resulted in a redistribution of this label from the cell surface to endocytic membranes, including phagosomes. The steady-state distribution was measured by quantitative autoradiography at the electron microscope level. Relative to early endosomes, with which phagosomes continued to fuse and rapidly exchange membrane constituents, the phagosome membrane was depleted

about 3-fold, starting during infection and in the course of 9 days thereafter. These results were in quantitative agreement with our previous observations for *Mycobacterium avium*. For the latter case, we now showed by cell fractionation that the depletion was selective, mainly involving glycoproteins in the 110–210 kDa range. Together, these results indicated that pathogenic mycobacteria induced and maintained a bulk change in phagosome membrane composition that could be of special relevance for survival of pathogenic mycobacteria within phagosomes.—Authors' Abstract

**Pozos, T. C., and Ramakrishan, L.** New models for the study of Mycobacterium-host interactions. *Curr. Opin. Immunol.* **16(4)** (2004) 499–505.

The outcome of Mycobacterium infection is determined by a series of complex interactions between the bacteria and host immunity. Traditionally, mammalian models and cultured cells have been used to study these interactions. Recently, amoeba (*Dictyostelium*), fruit flies (*Drosophila*) and zebrafish, amenable to forward genetic screens, have been developed as models for mycobacterial pathogenesis. Infection of these hosts with mycobacteria has allowed the dissection of intracellular trafficking pathways (*Dictyostelium*) and the roles of phagocytic versus antimicrobial peptide responses (*Drosophila*). Real-time visualization of the optically transparent zebrafish embryo/larva has elucidated mechanisms by which Mycobacterium-infected leukocytes migrate and subsequently aggregate into granulomas, the hallmark pathological structures of tuberculosis.—Authors' Abstract

**Quesniaux, V., Fremont, C., Jacobs, M., Parida, S., Nicolle, D., Yermeev, V., Bihl, F., Erard, F., Botha, T., Drennan, M., Soler, M. N., Le Bert, M., Schnyder, B., and Ryffel, B.** Toll-like receptor pathways in the immune responses to mycobacteria. *Microbes Infect.* **6(10)** (2004) 946–959.

The control of *Mycobacterium tuberculosis* infection depends on recognition of the

pathogen and the activation of both the innate and adaptive immune responses. Toll-like receptors (TLR) were shown to play a critical role in the recognition of several pathogens. Mycobacterial antigens recognise distinct TLR resulting in rapid activation of cells of the innate immune system. Recent evidence from *in vitro* and *in vivo* investigations, summarized in this review demonstrates TLR-dependent activation of innate immune response, while the induction of adaptive immunity to mycobacteria may be TLR independent.—Authors' Abstract

**Rambukkana, A.** *Mycobacterium leprae*-induced demyelination: a model for early nerve degeneration. *Curr Opin Immunol.* **16(4)** (2004) 511–518.

The molecular events that occur at the early phase of many demyelinating neurodegenerative diseases are unknown. A recent demonstration of rapid demyelination and axonal injury induced by *Mycobacterium leprae* provides a model for elucidating the molecular events of early nerve degeneration which might be common to neurodegenerative diseases of both infectious origin and unknown etiology. The identification of the *M. leprae*-targeted Schwann cell receptor, dystroglycan, and its associated molecules in myelination, demyelination and axonal functions suggests a role for these molecules in early nerve degeneration.—Author's Abstract

**Reed, M. B., Domenech, P., Manca, C., Su, H., Barczak, A. K., Kreiswirth, B. N., Kaplan, G., and Barry, C. E. 3rd.** A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. *Nature.* **431(7004)** (2004) 84–87.

Fifty million new infections with *Mycobacterium tuberculosis* occur annually, claiming 2–3 million lives from tuberculosis worldwide. Despite the apparent lack of significant genetic heterogeneity between strains of *M. tuberculosis*, there is mounting evidence that considerable heterogeneity exists in molecules important in disease pathogenesis. These differences may manifest in

the ability of some isolates to modify the host cellular immune response, thereby contributing to the observed diversity of clinical outcomes. Here we describe the identification and functional relevance of a highly biologically active lipid species—a polyketide synthase-derived phenolic glycolipid (PGL) produced by a subset of *M. tuberculosis* isolates belonging to the W-Beijing family that show “hyperlethality” in murine disease models. Disruption of PGL synthesis results in loss of this hypervirulent phenotype without significantly affecting bacterial load during disease. Loss of PGL was found to correlate with an increase in the release of the pro-inflammatory cytokines tumour-necrosis factor-alpha and interleukins 6 and 12 *in vitro*. Furthermore, the overproduction of PGL by *M. tuberculosis* or the addition of purified PGL to monocyte-derived macrophages was found to inhibit the release of these pro-inflammatory mediators in a dose-dependent manner.—Authors' Abstract

**Rook, G. A., Hernandez-Pando, R., Dheda, K., and Teng Seah, G.** IL-4 in tuberculosis: implications for vaccine design. *Trends Immunol.* **25(9)** (2004) 483–488.

Current attempts to find a vaccine for tuberculosis (TB) are based on the assumption that it must drive a Th1 response. We review the evidence that progressive disease might not be due to absence of Th1, but rather to the subversive effect of an unusual Th2-like response, involving interleukin-4 (IL-4) and IL-4delta2. This Th2-like response can impair bactericidal function and lead to toxicity of tumour necrosis factor-alpha (TNF-alpha) and to pulmonary fibrosis. If this is important, effective vaccines will need to suppress pre-existing Th2-like activity. Such vaccines are feasible and are active therapeutically in mouse TB.—Authors' Abstract

**Sendide, K., Deghmane, A. E., Reyrat, J. M., Talal, A., and Hmama, Z.** *Mycobacterium bovis* BCG urease attenuates major histocompatibility complex class II trafficking to the macrophage cell surface. *Infect. Immun.* **72(7)** (2004) 4200–4209.

We have previously shown that *Mycobacterium tuberculosis* attenuates cell surface expression of major histocompatibility complex class II molecules in response to gamma interferon (IFN-gamma) by a mechanism dependent on intracellular sequestration of alpha,beta dimers. In this study we examined whether intracellular alkalinization due to mycobacterial urease could account for the defect in intracellular trafficking of class II molecules. Phagocytosis of wild-type *Mycobacterium bovis* BCG was associated with secretion of ammonia intracellularly, which increased substantially upon addition of exogenous urea to the culture medium. Increased intracellular ammonia, due to urea degradation by the bacterium, correlated with inhibition of class II surface expression. Conversely, no ammonia was detected in cells infected with a urease-negative mutant strain of *M. bovis* BCG, which also displayed a reduced effect on surface expression of class II molecules. A direct cause-effect relationship between urease and class II molecule trafficking was established with experiments where cells ingesting beads coated with purified urease showed an increased ammonia level and decreased surface expression of class II in response to IFN-gamma. In contrast to BCG, infection of macrophages with *Mycobacterium smegmatis*, which expresses relatively greater urease activity in cell-free culture, had a marginal effect on both the intracellular level of ammonia and class II expression. The limited effect of *M. smegmatis* was consistent with a failure to resist intracellular killing, suggesting that urease alone is not sufficient to resist macrophage microbicidal mechanisms and that this is required for a more distal effect on cell regulation. Our results demonstrate that alkalization of critical intracellular organelles by pathogenic mycobacteria expressing urease contributes significantly to the intracellular

retention of class II dimers.—Authors' Abstract

**Temmerman, S., Pethe, K., Parra, M., Alonso, S., Rouanet, C., Pickett, T., Drowart, A., Debrie, A. S., Delogu, G., Menozzi, F. D., Sergheraert, C., Brennan, M. J., Mascart, F., and Locht, C.** Methylation-dependent T cell immunity to *Mycobacterium tuberculosis* heparin-binding hemagglutinin. *Nat. Med.* **10(9)** (2004) 935–941. Epub 2004 Aug 08.

Although post-translational modifications of protein antigens may be important components of some B cell epitopes, the determinants of T cell immunity are generally nonmodified peptides. Here we show that methylation of the *Mycobacterium tuberculosis* heparin-binding hemagglutinin (HBHA) by the bacterium is essential for effective T cell immunity to this antigen in infected healthy humans and in mice. Methylated HBHA provides high levels of protection against *M. tuberculosis* challenge in mice, whereas nonmethylated HBHA does not. Protective immunity induced by methylated HBHA is comparable to that afforded by vaccination with bacille Calmette et Guérin, the only available anti-tuberculosis vaccine. Thus, post-translational modifications of proteins may be crucial for their ability to induce protective T cell-mediated immunity against infectious diseases such as tuberculosis.—Authors' Abstract

**Ziglam, H. M., Daniels, I., and Finch, R. G.** Immunomodulating activity of rifampicin. *J. Chemother.* **16(4)** (2004) 357–361.

See *Current Literature, Chemotherapy*, p. 515.

## Immunopathology (Leprosy)

**Alves, L., de Mendonca Lima, L., da Silva Maeda, E., Carvalho, L., Holy, J., Sarno, E. N., Pessolani, M. C., and Barker, L. P.** *Mycobacterium leprae* infection of human Schwann cells depends on selective host kinases and pathogen-

modulated endocytic pathways. *FEMS Microbiol. Lett.* **238(2)** (2004) 429–437.

*Mycobacterium leprae*, an obligate intracellular pathogen, shows a unique tropism for Schwann cells (SC). This leads to the pe-

ripheral neuropathy disorder observed in leprosy. In this study, we investigated signal transduction events and the intracellular fate of *M. leprae* during the interaction of the microorganism with SC. First, we demonstrated that the human schwannoma cell line ST88-14 readily phagocytized the bacteria as observed by time-lapse microscopy, actin staining and electron microscopy. The effect of specific kinase inhibitors on *M. leprae* internalization was then investigated showing that functional protein tyrosine kinase, calcium-dependent protein kinase and phosphatidylinositol 3-kinase, but not cAMP-dependent protein kinase are essential for phagocytosis of the bacteria. Similar results were obtained when irradiated and live bacteria were compared and when *M. leprae* was pre-coated with recombinant histone-like-protein/ laminin binding protein, a bacterial adhesin. In addition, experiments were performed to analyze the bacterial trafficking within the endosomal network by labeling the acidified intracellular compartments of *M. leprae*-infected SC with the Lyso-tracker acidotrophic probe. Acidification of vesicles containing live *M. leprae* was minimal in both RAW murine macrophages and SC, although phagosomes containing heat-killed bacteria seem to follow normal endocytic maturation. These data indicate that the invading bacteria interfere with normal endocytic pathway maturation of bacteria-containing phagosomes within SC.—Authors' Abstract

**Hagge, D. A., Ray, N. A., Krahenbuhl, J. L., and Adams, L. B.** An *in vitro* model for the lepromatous leprosy granuloma: fate of *Mycobacterium leprae* from target macrophages after interaction with normal and activated effector macrophages. *J. Immunol.* **172(12)** (2004) 7771–7779.

The lepromatous leprosy granuloma is a dynamic entity requiring a steady influx of macrophages (Mphi) for its maintenance. We have developed an *in vitro* model to study the fate of *Mycobacterium leprae* in a LL lesion, with and without immunotherapeutic intervention. Target cells, consisting of granuloma Mphi harvested from the footpads of *M. leprae*-infected athymic nu/nu mice, were cocultured with normal or IFN-

gamma-activated (ACT) effector Mphi. The bacilli were recovered and assessed for viability by radiorespirometry. *M. leprae* recovered from target Mphi possessed high metabolic activity, indicating a viable state in this uncultivable organism. *M. leprae* recovered from target Mphi incubated with normal effector Mphi exhibited significantly higher metabolism. In contrast, bacilli recovered from target Mphi cocultured with ACT effector Mphi displayed a markedly decreased metabolic activity. Inhibition by ACT Mphi required an E:T ratio of at least 5:1, a coculture incubation period of 3–5 days, and the production of reactive nitrogen intermediates, but not reactive oxygen intermediates. Neither IFN-gamma nor TNF-alpha were required during the cocultivation period. However, cell-to-cell contact between the target and effector Mphi was necessary for augmentation of *M. leprae* metabolism by normal effector Mphi as well as for inhibition of *M. leprae* by ACT effector Mphi. Conventional fluorescence microscopy and confocal fluorescence microscopy revealed that the bacilli from the target Mphi were acquired by the effector Mphi. Thus, the state of Mphi infiltrating the granuloma may markedly affect the viability of *M. leprae* residing in Mphi in the lepromatous lesion.—Authors' Abstract

**Kang, T. J., Yeum, C. E., Kim, B. C., You, E. Y., and Chae, G. T.** Differential production of interleukin-10 and interleukin-12 in mononuclear cells from leprosy patients with a Toll-like receptor 2 mutation. *Immunology* **112(4)** (2004) 674–680.

Toll-like receptor 2 (TLR2) is a key mediator of the immune response to mycobacterial infections, and mutations in TLR2 have been shown to confer susceptibility to infection with mycobacteria. This study investigated the profiles of cytokines, such as interferon (IFN)-gamma, interleukin (IL)-10, IL-12 and tumour necrosis factor (TNF)-alpha in response to *Mycobacterium leprae* in peripheral blood mononuclear cells (PBMC) with the TLR2 mutation Arg677Trp, a recently reported polymorphism that is associated with lepromatous leprosy. In leprosy patients with the TLR2 mutation, production of IL-2, IL-12, IFN-



gamma, and TNF-alpha by *M. leprae* -stimulated PBMC were significantly decreased compared with that in groups with wild-type TLR2. However, the cells from patients with the TLR2 mutation showed significantly increased production of IL-10. There was no significant difference in IL-4 production between the mutant and wild-type during stimulation. Thus, these results suggest that the TLR2 signal pathway plays a critical role in the alteration of cytokine profiles in PBMC from leprosy patients and the TLR2 mutation Arg677Trp provides a mechanism for the poor cellular immune response associated with lepromatous leprosy.—Authors' Abstract

**Kim, B. C., Kang, T. J., Jin, S. H., Kim, S. K., Lee, S. B., and Chae, G. T.** Tissue-specific down-regulation of RIPK 2 in *Mycobacterium leprae*-infected nu/nu mice. *Mediators Inflamm.* **13(1)** (2004) 51–52.

RIPK 2 is adapter molecule in the signal pathway involved in Toll-like receptors. However, there has been no reported association between receptor-interacting serine/threonine kinase 2 (RIPK 2) expression and the infectious diseases involving mycobacterial infection. This study found that its expression was down-regulated in the footpads and skin but was up-regulated in the liver of *Mycobacterium leprae*-infected nu/nu mice compared with those of the *M. leprae* non-infected nu/nu mice. It was observed that the interleukin-12p40 and interferon-gamma genes involved in the susceptibility of *M. leprae* were down-regulated in the skin but were up-regulated in the liver. Overall, this suggests that regulation of RIPK 2 expression is tissue-specifically associated with *M. leprae* infection.—Authors' Abstract

**Kimura, H., Maeda, Y., Takeshita, F., Takaoka, L. E., Matsuoka, M., and Makino, M.** Upregulation of T-cell-stimulating activity of mycobacteria-infected macrophages. *Scand. J. Immunol.* **60(3)** (2004) 278–286.

Macrophages are one of the most abundant host cells to come in contact with mycobacte-

ria. However, the infected macrophages less efficiently stimulate autologous T cells *in vitro*. We investigated the effect of the induction of phenotypic change of macrophages on the host cell activities by using *Mycobacterium leprae* as a pathogen. The treatment of macrophages with interferon-gamma (IFN-gamma), GM-CSF and interleukin-4 deprived macrophages of CD14 antigen expression but instead provided them with CD1a, CD83 and enhanced CD86 antigen expression. These phenotypic features resembled those of monocyte-derived dendritic cells (DC). These macrophage-derived DC-like cells (MACDC) stimulated autologous CD4+ and CD8+ T cells when infected with *M. leprae*. Further enhancement of the antigen-presenting function and CD1a expression of macrophages was observed when treated with IFN-gamma. The *M. leprae*-infected and -treated macrophages expressed bacterial cell membrane-derived antigens on the surface and were efficiently cytolysed by the cell membrane antigen-specific CD8+ cytotoxic T lymphocytes (CTL). These results suggest that the induction of phenotypic changes in macrophages can lead to the upregulation of host defence activity against *M. leprae*.—Authors' Abstract

**Moraes, M. O., Pacheco, A. G., Schonkeren, J. J., Vanderborght, P. R., Nery, J. A., Santos, A. R., Moraes, M. E., Moraes, J. R., Ottenhoff, T. H., Sampaio, E. P., Huizinga, T. W., and Sarno, E. N.** Interleukin-10 promoter single-nucleotide polymorphisms as markers for disease susceptibility and disease severity in leprosy. *Genes Immun.* Epub 2004 Aug 12.

We have determined IL-10 promoter genotypes of five single-nucleotide polymorphisms (SNPs): T-3575A, A-2849G, C-2763A, -A-1082G and C-819T. The haplotype frequencies were defined in healthy subjects compared to leprosy patients, and analyzed for their occurrence in multi- (MB) vs paucibacillary (PB) as severe and mild forms of leprosy, respectively. Haplotypes defined by three SNP positions (-3575, -2849 and -2763) captured significant differences between controls and patients (p =

0.04). The haplotype carrying -3575A, -2849G and -2763C was associated with resistance to leprosy and to the development of severe forms of the disease using either a binomial (controls vs. cases,  $p = 0.005$ , OR = 0.35, CI = 0.13-0.91) or ordinal (controls vs PB vs MB,  $p = 0.006$ , OR = 0.32, CI = 0.12-0.83) model. By contrast, the IL-10 haplotype -3575T/-2849A/-2763C was found to be associated with susceptibility to leprosy *per se* ( $p = 0.027$ , OR = 2.37, CI = 1.04-5.39), but not leprosy type. The data suggest that the IL-10 locus contributes to the outcome of leprosy.—Authors' Abstract

**Ohyama, H., Kato, N., Takeuchi, K., Soga, Y., Uemura, Y., Nishimura, F., and Matsushita, S.** Monocytes of distinct clinical types of leprosy are differentially activated by cross-linking class II HLA molecules to secrete IL-12. *APMIS* **112(4-5)** (2004) 271-274.

Leprosy is characterized by a wide spectrum of clinical features depending on the individual differences in Th1-type immunity. The objective of this study was to evaluate whether monocyte activation by stimulus via class II HLA molecules would be correlated with the differences in cellular immune responses among diverse clinical forms of leprosy. IL-1 $\beta$  and IL-12 productivity in monocyte preparations obtained from PBMCs was estimated in patients with lepromatous- and tuberculoid-type leprosy. We found that monocytes from lepromatous patients produced significantly higher (about 4-fold higher) amounts of IL-12 as compared to in patients with tuberculoid type of leprosy when class II HLA molecules were cross-linked with anti-HLA class II antibodies, whereas almost equal amounts of IL-1 $\beta$  were produced from each monocyte preparation by stimulus via class II HLA molecules regardless of the clinical form of leprosy. These results suggest that monocyte activation differs between lepromatous and tuberculoid patients in terms of IL-12 secretion, which might be related to individual differences in the cellular immune responses according to the clinical type of leprosy.—Authors' Abstract

**Reddy, Y. N., Murthy, S. V., Krishna, D. R., and Prabhakar, M. C.** Oxidative stress and anti-oxidant status in leprosy patients. *Indian J. Lepr.* **75(4)** (2003) 307-316.

Severe oxidative stress has been reported in leprosy patients because of malnutrition and poor immunity. The purpose of this study was to investigate the serum lipid peroxidation products, serum LDH and important free radical scavenging enzymes, i.e. superoxide dismutase (SOD), and catalase and anti-oxidant glutathione levels and total anti-oxidant status, in different types of leprosy patients. The subjects for this study were normal human volunteers (NHVs, N = 14), paucibacillary leprosy patients (PB, N = 18), untreated MB patients (MB1, N = 18), MB patients under treatment (MB2, N = 19), and MB patients released from treatment (RFT) (MB3, N = 28). The levels of lipid peroxidation product, malondialdehyde (MDA), and LDH increased significantly ( $p < 0.001$ ) in MB (MB1, MB2, MB3) patients, and both gradually decreased with clinical improvement following MDT. The levels of SOD, catalase and glutathione, and the total anti-oxidant status decreased significantly in MB (MB1, MB2, MB3) patients ( $p < 0.001$ ), in comparison with NHVs. They gradually increased with clinical improvement with MDT. There was no significant variation of these parameters in PB leprosy patients in comparison with healthy volunteers. High free radical activity and low anti-oxidant levels observed in MB (MB1, MB2, MB3) leprosy patients indicate that there is an oxidative stress in MB cases, irrespective of the treatment status and suggest a suitable anti-oxidant therapy to prevent possible tissue injury.—Authors' Abstract

**Walsh, D. S., Lane, J. E., Abalos, R. M., and Myint, K. S.** TUNEL and limited immunophenotypic analyses of apoptosis in paucibacillary and multibacillary leprosy lesions. *FEMS Immunol. Med. Microbiol.* **41(3)** (2004) 265-269.

Some mycobacterial infections, such as tuberculosis, are characterized by apoptosis of infected or by-stander mononuclear immune cells. For localized (paucibacillary),

PB) and disseminated (multibacillary, MB) leprosy, characterized by polarized Th1-like vs. Th2-like immune responses, respectively, little is known about lesional apoptosis. We analyzed sections of paraffin-embedded, untreated leprosy lesions from 21 patients by an indirect immunofluorescent terminal deoxynucleotide-transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) assay. Some TUNEL (+) PB sections were then reacted with phycoerythrin-conjugated (red) antibodies against T cells, monocytes, or antigen-presenting (Langerhans) cells. TUNEL (+) bodies were detected in 9 of 16 PB lesions (56%) and in 1 of 5 MB lesions (20%). Some TUNEL (+) bodies in PB disease were CD3+ (T cell), as well as CD4+ (T-helper) or CD8+ (T-cytotoxic). Apoptosis characterizes PB and MB leprosy lesions and may be more frequent in PB disease. In PB disease, some TUNEL (+) bodies may derive from T cells.—Authors' Abstract

**Yamashita, Y., Maeda, Y., Takeshita, F., Brennan, P. J., and Makino, M.** Role of the polypeptide region of a 33kDa mycobacterial lipoprotein for efficient IL-12

production. *Cell. Immunol.* **229(1)** (2004) 13–20.

*Mycobacterium leprae* lipoprotein, LpK, induced IL-12 production from human monocytes. To determine the components essential for cytokine production and the relative role of lipidation in the activation process, we produced lipidated and non-lipidated truncated forms of LpK. While 0.5 nM of lipidated LpK-a having N-terminal 60 amino acids of LpK produced more than 700 pg/ml IL-12 p40, the non-lipidated LpK-b having the same amino acids as that of LpK-a required more than 20nM of the protein to produce an equivalent dose of cytokine. Truncated protein having the C-terminal 192 amino acids of LpK did not induce any cytokine production. Fifty nanomolar of the synthetic lipopeptide of LpK produced only about 200pg/ml IL-12. Among the truncated LpK, only LpK-a and lipopeptide stimulated NF- $\kappa$ B-dependent reporter activity in TLR-2 transfectant. However, when monocytes were stimulated with lipopeptide in the presence of non-lipidated protein, they produced IL-12 synergistically. Therefore, both peptide regions of LpK and lipid residues are necessary for efficient IL-12 production.—Authors' Abstract

## Immunopathology (Tuberculosis)

**Banerjee, S., Nandyala, A., Podili, R., Katoch, V. M., Murthy, K. J., and Hasnain, S. E.** *Mycobacterium tuberculosis* (Mtb) isocitrate dehydrogenases show strong B cell response and distinguish vaccinated controls from TB patients. *Proc. Natl. Acad. Sci. U.S.A.* **101(34)** (2004) 12652–12657. Epub 2004 Aug 16.

Proteins released from *Mycobacterium tuberculosis* (Mtb) during late logarithmic growth phase are often considered candidate components of immunogenic or autolysis markers. One such protein is isocitrate dehydrogenase (ICD), a key regulatory enzyme in the citric acid cycle. We have evaluated the immunogenic properties of two isoforms of Mtb ICD and compared them

with the control antigens heat-shock protein 60 and purified protein derivative (PPD). PPD lacks the sensitivity to distinguish between bacillus Calmette-Guérin (BCG)-vaccinated and tuberculosis (TB)-infected populations, and, therefore, epidemiological relevance of PPD in BCG-vaccinated regions is debatable. We show that Mtb ICDs elicit a strong B cell response in TB-infected populations and can differentiate between healthy BCG-vaccinated populations and those with TB. The study population (N = 215) was categorized into different groups, namely, patients with fresh infection (N = 42), relapsed TB cases (N = 32), patients with extrapulmonary TB (N = 35), clinically healthy donors (N = 44), nontuberculous mycobacteria patients (N = 30), and non-TB

patients (culture negative for acid-fast bacteria but carrying other infections, N = 32). The Mtb ICDs showed statistically significant antigenic distinction between healthy BCG-vaccinated controls and TB patients ( $p < 0.0001$ ) and those with other infections. Although extrapulmonary infections could not be discriminated from healthy controls by heat-shock protein 60 ( $p = 0.2177$ ), interestingly, the Mtb ICDs could significantly ( $p < 0.0001$ ) do so. Our results highlight the immunodominant, immunosensitive, and immunospecific nature of Mtb ICDs and point to an unusual property of this tricarboxylic acid energy cycle enzyme.—Authors' Abstract

**Boshoff, H. I., Myers, T. G., Copp, B. R., McNeil, M. R., Wilson, M. A., Barry, C. E. 3rd.** The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *J. Biol. Chem.* **279(38)** (2004) 40174–40184. Epub 2004 Jul 09.

The differential transcriptional response of *Mycobacterium tuberculosis* to drugs and growth-inhibitory conditions was monitored to generate a data set of 430 microarray profiles. Unbiased grouping of these profiles independently clustered agents of known mechanism of action accurately and was successful at predicting the mechanism of action of several unknown agents. These predictions were validated biochemically for two agents of previously uncategorized mechanism, pyridoacridones and phenothiazines. Analysis of this data set further revealed 150 underlying clusters of coordinately regulated genes offering the first glimpse at the full metabolic potential of this organism. A signature subset of these gene clusters was sufficient to classify all known agents as to mechanism of action. Transcriptional profiling of both crude and purified natural products can provide critical information on both mechanism and detoxification prior to purification that can be used to guide the drug discovery process. Thus, the transcriptional profile generated by a crude marine natural product recapitulated the mechanistic prediction from the pure active component. The underlying gene

clusters further provide fundamental insights into the metabolic response of bacteria to drug-induced stress and provide a rational basis for the selection of critical metabolic targets for screening for new agents with improved activity against this important human pathogen.—Authors' Abstract

**Briken, V., Porcelli, S. A., Besra, G. S., and Kremer, L.** Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. *Mol. Microbiol.* **53(2)** (2004) 391–403.

The cell wall component lipoarabinomannan (ManLAM) from *Mycobacterium tuberculosis* is involved in the inhibition of phagosome maturation, apoptosis and interferon (IFN)-gamma signalling in macrophages and interleukin (IL)-12 cytokine secretion of dendritic cells (DC). All these processes are important for the host to mount an efficient immune response. Conversely, LAM isolated from non-pathogenic mycobacteria (PILAM) have the opposite effect, by inducing a potent proinflammatory response in macrophages and DCs. LAMs from diverse mycobacterial species differ in the modification of their terminal arabinose residues. The strong proinflammatory response induced by PILAM correlates with the presence of phospho-myoinositol on the terminal arabinose. Interestingly, recent work indicates that the biosynthetic precursor of LAM, lipomannan (LM), which is also present in the cell wall, displays strong proinflammatory effects, independently of which mycobacterial species it is isolated from. Results from in vitro assays and knock-out mice suggest that LM, like PILAM, mediates its biological activity via Toll-like receptor 2. We hypothesize that the LAM/LM ratio might be a crucial factor in determining the virulence of a mycobacterial species and the outcome of the infection. Recent progress in the identification of genes involved in the biosynthesis of LAM is discussed, in particular with respect to the fact that enzymes controlling the LAM/LM balance might represent targets for new anti-tubercular drugs. In addition, inactivation of these genes may lead to attenuated strains of

*M. tuberculosis* for the development of new vaccine candidates.—Authors' Abstract

**Chua, J., and Deretic, V.** *Mycobacterium tuberculosis* reprograms waves of phosphatidylinositol 3-phosphate on phagosomal organelles. *J. Biol. Chem.* **275(35)** (2004) 36982–36992. Epub 2004 Jun 21.

The potent human pathogen *Mycobacterium tuberculosis* persists in macrophages within a specialized, immature phagosome by interfering with the pathway of phagolysosome biogenesis. The molecular mechanisms underlying this process remain to be fully elucidated. Here, using four-dimensional microscopy, we detected on model phagosomes, which normally mature into phagolysosomes, the existence of cyclical waves of phosphatidylinositol 3-phosphate (PI3P), a membrane trafficking regulatory lipid essential for phagosomal acquisition of lysosomal characteristics. We show that mycobacteria interfere with the dynamics of PI3P on phagosomal organelles by altering the timing and characteristics of the PI3P waves on phagosomes. The default program of cyclical PI3P waves on model phagosomes is composed of an initial stage (phase I), represented by a strong PI3P burst occurring only upon the completion of phagosome formation, and a subsequent stage (phase II) of recurring PI3P waves on maturing phagosomes with the average periodicity of 20 min. Mycobacteria alter this program in two ways: (i) by inducing, in a cholesterol-dependent fashion, a neophase I\* of premature PI3P production, coinciding with the process of mycobacterial entry into the macrophage, and (ii) by inhibiting the calmodulin-dependent phase II responsible for the acquisition of lysosomal characteristics. We conclude that the default pathway of phagosomal maturation into the phagolysosome includes temporally organized cyclical waves of PI3P on phagosomal membranes and that this process is targeted for reprogramming by mycobacteria as they prevent phagolysosome formation.—Authors' Abstract

**Ciaramella, A., Cavone, A., Santucci, M. B., Garg, S. K., Sanarico, N., Bocchino,**

**M., Galati, D., Martino, A., Auricchio, G., D'Orazio, M., Stewart, G. R., Neyrolles, O., Young, D. B., Colizzi, V., and Fraziano, M.** Induction of apoptosis and release of interleukin-1 beta by cell wall-associated 19-kDa lipoprotein during the course of mycobacterial infection. *J. Infect. Dis.* **190(6)** (2004) 1167–1176. Epub 2004 Aug. 11.

*Mycobacterium tuberculosis* induces apoptosis in human monocyte-derived macrophages (MDMs) during the early stages of infection. We investigated the proapoptotic role of cell wall-associated mycobacterial 19-kDa lipoprotein and the possible association between 19-kDa lipoprotein signaling and production of proinflammatory cytokines. Purified mycobacterial 19-kDa lipoprotein, 19-kDa lipoprotein-expressing *M. smegmatis* (*M. smegmatis* 19+), 19-kDa lipoprotein knockout (KO) *M. tuberculosis*, and 19-kDa lipoprotein KO *M. bovis* bacille Calmette-Guerin (BCG) strains were analyzed for their ability to induce apoptosis in MDMs. The 19-kDa lipoprotein and infection with *M. smegmatis* 19+ induced apoptosis in MDMs. *M. tuberculosis* and BCG KO strains had significantly decreased abilities to induce apoptosis. The 19-kDa lipoprotein proapoptotic signal was mediated by Toll-like receptor 2 but not by tumor necrosis factor-alpha. Only the release of interleukin (IL)-1 beta was decreased after infection with 19-kDa lipoprotein KO strains. These findings indicate that the 19-kDa lipoprotein is the main signal required to trigger both apoptosis and the release of IL-1 beta during the early stages of mycobacterial infection.—Authors' Abstract

**Cliff, J. M., Andrade, I. N., Mistry, R., Clayton, C. L., Lennon, M. G., Lewis, A. P., Duncan, K., Lukey, P. T., and Dockrell, H. M.** Differential gene expression identifies novel markers of CD4+ and CD8+ T cell activation following stimulation by *Mycobacterium tuberculosis*. *J. Immunol.* **173(1)** (2004) 485–493.

T cell activation in response to antigenic stimulation is a complex process, involving changes in the expression level of a large number of genes. We have used cDNA array

technology to characterize the differences in gene expression between human CD4+ and CD8+ T cells. PBMC from six healthy donors were stimulated with live *Mycobacterium tuberculosis*, and the gene expression profiles of each donor's CD4+ and CD8+ T cells were analyzed separately. ANOVA revealed 518 genes that were consistently differentially expressed between CD4+ and CD8+ T cells. These differentially expressed genes include a combination of well-known, previously characterized genes with a range of biological functions and unknown in silico predicted hypothetical genes. Where possible, the novel genes have been characterized using bioinformatics, and putative transcription factors, signaling molecules, transmembrane, and secreted factors have been identified. A subset of these differentially expressed genes could be exploited as markers of CD4+ and CD8+ T cell activation for use in vaccine trials. These observed differences in the gene expression profile of CD4+ and CD8+ T cells following activation by a human pathogen contribute to an increased understanding of T cell activation and differentiation and the roles these T cell subsets may play in immunity to infection.—Authors' Abstract

**Gehring, A. J., Dobos, K. M., Belisle, J.T., Harding, C. V., and Boom, W. H.** *Mycobacterium tuberculosis* LprG (Rv1411c): a novel TLR-2 ligand that inhibits human macrophage class II MHC antigen processing. *J. Immunol.* 173(4) (2004) 2660–2668.

MHC class II (MHC-II)-restricted CD4(+) T cells are essential for control of *Mycobacterium tuberculosis* infection. This report describes the identification and purification of LprG (Rv1411c) as an inhibitor of primary human macrophage MHC-II Ag processing. LprG is a 24-kDa lipoprotein found in the *M. tuberculosis* cell wall. Prolonged exposure (>16 hr) of human macrophages to LprG resulted in marked inhibition of MHC-II Ag processing. Inhibition of MHC-II Ag processing was dependent on TLR-2. Short-term exposure (<6 hr) to LprG stimulated TLR-2-dependent TNF- $\alpha$  production. Thus, LprG can exploit TLR-2 signaling to inhibit MHC-II Ag

processing in human macrophages. Inhibition of MHC-II Ag processing by mycobacterial lipoproteins may allow *M. tuberculosis*, within infected macrophages, to avoid recognition by CD4(+) T cells.—Authors' Abstract

**Glatman-Freedman, A., Casadevall, A., Dai, Z., Jacobs, W. R. Jr., Li, A., Morris, S. L., Navoa, J.A., Piperdi, S., Robbins, J. B., Schneerson, R., Schwebach, J. R., and Shapir o, M.** Antigenic evidence of prevalence and diversity of *Mycobacterium tuberculosis* arabinomannan. *J. Clin. Microbiol.* 42(7) (2004) 3225–3231.

Arabinomannan (AM) is a polysaccharide of the mycobacterial capsule. The capsular polysaccharides of various microorganisms are diverse, and this diversity is important for classification of organisms into serotypes and vaccine development. In the present study we examined the prevalence and diversity of AM among *Mycobacterium tuberculosis* strains using four AM-binding monoclonal antibodies (MAbs). One of these MAbs, MAb 9d8, is known to bind to AM specifically. By whole-cell enzyme-linked immunosorbent assay (ELISA), the AM recognized by MAb 9d8 was detected on the surfaces of 9 of 11 strains, while 2 strains showed no reactivity with MAb 9d8. However, the AM recognized by MAb 9d8 was found in the culture supernatants of all 11 *M. tuberculosis* strains tested, as demonstrated by capture ELISA. Other AM-binding MAbs reacted both with the surfaces and with the culture supernatants of all 11 strains. Mice immunized with an experimental AM-recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) conjugate vaccine had an increased antibody response to AM and a moderate reduction in the numbers of CFU in their organs 7 days after challenge. Our results indicate that AM was detected in all *M. tuberculosis* strains tested, with differences in epitope distributions of certain strains. In addition, our results suggest that an experimental AM-rEPA vaccine has a moderate effect on the numbers of CFU in organs early after infection.—Authors' Abstract

**Guidry, T. V., Olsen, M., Kil, K. S., Hunter, R. L. Jr., Geng, Y. J., and Actor, J. K.** Failure of CD1D<sup>-/-</sup> mice to elicit hypersensitive granulomas to mycobacterial cord factor trehalose 6,6'-dimycolate. *J. Interferon Cytokine Res.* **24(6)** (2004) 362–371.

The present study defines pathologic differences in acute and hypersensitive responses to *Mycobacterium tuberculosis* glycolipid trehalose-6,6'-dimycolate (TDM, cord factor) in normal BALB/c mice and those deficient in group II CD1 molecule CD1d1. Mice immunized against TDM demonstrate hypersensitive responses, yet the mechanisms for TDM presentation remain elusive. Mice lacking CD1d (CD1D<sup>-/-</sup>) demonstrate dysregulated granulomatous response to TDM, compared with CD1D<sup>+/-</sup> heterozygous controls. Because CD1d-restricted T cells can regulate macrophage immune functions at mucosal surfaces, we hypothesized that CD1D<sup>-/-</sup> mice would show deficient TDM-induced hypersensitive pulmonary granulomatous response in which T cells play a central role. Control CD1D<sup>+/+</sup> mice sensitized and subsequently challenged with TDM demonstrated aggressive inflammation defined by monocytic lesions contained by CD3(+) lymphocytic cuffing. CD1D<sup>-/-</sup> mice demonstrated distinctly different pathologies, with edema present concurrent with extended, nonfocal mononuclear cell-based granulomatous reactions. Furthermore, CD1D<sup>-/-</sup> mice did not demonstrate destructive lesions, and CD3(+) lymphocytes were only loosely organized in proximity to reactive pathology. The CD1d-deficient mice demonstrated rapid increases in proinflammatory mRNAs, with significant differences in interferon-gamma (IFN-gamma) compared to the wild-type group. IFN-gamma, interleukin-6 (IL-6), and IL-12 proteins were significantly elevated in the CD1D<sup>-/-</sup> group compared with wild-type mice ( $p < 0.05$ ) 2 days after TDM challenge. However, by 7 days postadministration, similar production for all cytokines and proinflammatory molecules examined was present in both groups of mice. These experiments provide evidence for a role for CD1d in mediation of pathology during hypersensitive responses to the mycobacterial glycolipid TDM.—Authors' Abstract

**Haydel, S. E., and Clark-Curtiss, J. E.** Global expression analysis of two-component system regulator genes during *Mycobacterium tuberculosis* growth in human macrophages. *FEMS Microbiol. Lett.* **236(2)** (2004) 341–347.

In the *Mycobacterium tuberculosis* H37Rv genome, there are 11 paired two-component regulatory system genes, two orphan histidine kinase genes, and six orphan response regulator genes. Expression of the 17 response regulator genes and the two orphan histidine kinase genes during growth of *M. tuberculosis* in human peripheral blood monocyte-derived macrophages has been analyzed by using cDNA mixtures prepared by the selective capture of transcribed sequences (SCOTS) technique. SCOTS probes were prepared from cDNA obtained from *M. tuberculosis* grown for 18, 48, and 110 hr in human macrophages. Based on expression profiles, the regulatory genes were assigned to three categories: (i) constitutively expressed during growth in macrophages (three genes); (ii) differentially expressed during growth in macrophages (nine genes) and (iii) no detectable expression during growth in macrophages (seven genes).—Authors' Abstract

**Islam, N., Kanost, A. R., Teixeira, L., Johnson, J., Hejal, R., Aung, H., Wilkinson, R. J., Hirsch, C. S., and Toossi, Z.** Role of cellular activation and tumor necrosis factor-alpha in the early expression of *Mycobacterium tuberculosis* 85B mRNA in human alveolar macrophages. *J. Infect. Dis.* **190(2)** (2004) 341–351. Epub 2004 Jun 18.

**BACKGROUND:** Infection of alveolar macrophages (AMs), which constitute the first line of defense against *Mycobacterium tuberculosis*, initiates an intense interaction between the host's innate immune response and mycobacteria that may assist in the successful intracellular parasitism of *M. tuberculosis*. **METHODS:** Expression of tumor necrosis factor (TNF)-alpha and *M. tuberculosis* 85B mRNA was studied in *M. tuberculosis*-infected AMs, to better delineate the role of macrophages in the early events in initiation of infection. **RESULTS:**

Both TNF- $\alpha$  mRNA and *M. tuberculosis* 85B were induced in AMs; at 24 hr, the time point of maximum TNF- $\alpha$  induction, the mRNA levels for TNF- $\alpha$  and *M. tuberculosis* 85B correlated with one another, and induction of either gene correlated strongly with their protein levels. Inhibition of endogenous TNF- $\alpha$  by soluble (s) TNF receptor (R) I and sTNFR II reduced expression of both TNF- $\alpha$  and *M. tuberculosis* 85B. The activation of nuclear factor- $\kappa$ B was found to underlie expression of both TNF- $\alpha$  and *M. tuberculosis* 85B. Exogenous TNF- $\alpha$  was slightly more potent than interleukin (IL)-6 and granulocyte-macrophage colony-stimulating factor and was significantly stronger than IL-1 in inducing expression of *M. tuberculosis* 85B. Interestingly, inhibition of bactericidal mediators, reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates (RNIs), reduced expression of TNF- $\alpha$  and *M. tuberculosis* 85B genes in *M. tuberculosis*-infected AMs. CONCLUSION: Activation of AMs by *M. tuberculosis* initiates a cascade of events whereby TNF- $\alpha$ , ROI, and RNI enhance the expression of the *M. tuberculosis* 85B gene.—Authors' Abstract

**Jang, S., Uematsu, S., Akira, S., and Salgame, P.** IL-6 and IL-10 induction from dendritic cells in response to *Mycobacterium tuberculosis* is predominantly dependent on TLR2-mediated recognition. *J. Immunol.* 173(5) (2004) 3392–3397.

The initial TLR-mediated interaction between *Mycobacterium tuberculosis* and dendritic cells is critical, since the cytokine production that ensues can greatly influence the class of adaptive immunity that is generated to the pathogen. In this study, we therefore determined the dependency on TLR2 and TLR4 for *M. tuberculosis*-induced cytokine production by murine dendritic cells. A key new finding of this study is that production of IL-6 and IL-10 from dendritic cells in response to *M. tuberculosis* is principally dependent on TLR2. The study also indicates that *M. tuberculosis* can induce IL-12 production in the absence of either TLR2 or TLR4, suggesting redundancy or possibly involvement of other receptors in IL-12 pro-

duction. In addition, the data also reveal that lack of TLR2 or TLR4 does not impact on dendritic cell maturation or on their ability to influence the polarity of differentiating naive T cells. Collectively, data presented here provide a mechanistic insight for the contribution of TLR2 and TLR4 to tuberculosis disease progression and offer strategies for regulating IL-6 and IL-10 production in dendritic cell-based vaccine strategies.—Authors' Abstract

**Keller, C., Lauber, J., Blumenthal, A., Buer, J., and Ehlers, S.** Resistance and susceptibility to tuberculosis analysed at the transcriptome level: lessons from mouse macrophages. *Tuberculosis* (Edinb). 84(3–4) (2004) 144–158.

Gene expression patterns associated with resistance and susceptibility to tuberculosis (TB) were investigated at the macrophage level in the well-defined mouse model of infection. Oligonucleotide microarrays were used to analyze the regulation of gene expression in murine bone marrow-derived macrophages infected with *Mycobacterium tuberculosis*. Four mouse strains, known to differ in terms of growth permissiveness for *M. tuberculosis* in infected tissues, in the development of pulmonary pathology, and in the rate of premature death due to tuberculosis, were compared: C57BL/6 and BALB/c representing resistant, DBA/2 and CBA/J representing susceptible mouse strains. Genes (55) were regulated more than two-fold in macrophages of all strains investigated following *M. tuberculosis* infection. Importantly, 18 genes were commonly regulated only in macrophages of the two resistant strains upon infection, and 102 genes were commonly regulated exclusively in macrophages of the two susceptible strains. Using this approach, we have therefore identified more than 100 genes potentially associated with resistance and susceptibility, respectively, to TB at the macrophage level. A tentative interpretation of our microarray data suggests that macrophages from susceptible mice predominantly stimulate the recruitment of cells that contribute disproportionately to tissue damage rather than to microbial elimination. In conclusion, microarray gene chips are use-



ful tools for generating new hypotheses about resistance and susceptibility to TB, and the mouse model can now be used to subject candidate genes identified by this approach to further functional analyses.—Authors' Abstract

**Manca, C., Reed, M. B., Freeman, S., Mathema, B., Kreiswirth, B., Barry, C. E. 3rd, and Kaplan, G.** Differential monocyte activation underlies strain-specific *Mycobacterium tuberculosis* pathogenesis. *Infect. Immun.* **72(9)** (2004) 5511–5514.

*In vitro* infection of monocytes with *Mycobacterium tuberculosis* HN878 and related W/Beijing isolates preferentially induced interleukin-4 (IL-4) and IL-13, which characterize Th2 polarized immunity. In contrast, CDC1551 induced more IL-12 and other molecules associated with phagocyte activation and Th1 protective immunity. The differential cytokine-chemokine response was mediated by extracted lipids, suggesting that these molecules regulate host responses to infection.—Authors' Abstract

**Marino, S., Pawar, S., Fuller, C. L., Reinhart, T. A., Flynn, J. L., and Kirschner, D. E.** Dendritic cell trafficking and antigen presentation in the human immune response to *Mycobacterium tuberculosis*. *J. Immunol.* **173(1)** (2004) 494–506.

*Mycobacterium tuberculosis* (Mtb) is an extraordinarily successful human pathogen, one of the major causes of death by infectious disease worldwide. A key issue for the study of tuberculosis is to understand why individuals infected with Mtb experience different clinical outcomes. To better understand the dynamics of Mtb infection and immunity, we coupled nonhuman primate experiments with a mathematical model we previously developed that qualitatively and quantitatively captures important processes of cellular priming and activation. These processes occur between the lung and the nearest draining lymph node where the key cells mediating this process are the dendritic cells (DC). The nonhuman primate experiments consist of bacteria and cell numbers

from tissues of 17 adult cynomolgus macaques (*Macaca fascicularis*) that were infected with Mtb strain Erdman (approximately 25 CFU/animal via bronchoscope). The main result of this work is that delays in either DC migration to the draining lymph node or T cell trafficking to the site of infection can alter the outcome of Mtb infection, defining progression to primary disease or latent infection and reactivated tuberculosis. Our results also support the idea that the development of a new generation of treatment against Mtb should optimally elicit a fast DC turnover at the site of infection, as well as strong activation of DCs for maximal Ag presentation and production of key cytokines. This will induce the most protective T cell response.—Authors' Abstract

**Mollenkopf, H. J., Kursar, M., and Kaufmann, S. H.** Immune response to post-primary tuberculosis in mice: *Mycobacterium tuberculosis* and *Mycobacterium bovis* bacille Calmette-Guérin induce equal protection. *J. Infect. Dis.* **190(3)** (2004) 588–597. Epub 2004 Jul 06.

We addressed the question of whether protective immunity induced by natural infection with *Mycobacterium tuberculosis* and that induced by vaccination with *Mycobacterium bovis* bacille Calmette-Guérin (BCG) differ in the murine model. We infected mice with *M. tuberculosis* Erdman, cured them by chemotherapy, and subsequently reinfected them with a low dose of *M. tuberculosis* H37Rv. The course of tuberculosis was compared with that in mice previously vaccinated with BCG Danish 1331. Protection against postprimary *M. tuberculosis* infection did not differ significantly between the 2 groups. After challenge infection, numbers of interferon- $\gamma$ -positive splenocytes did not differ between mice with primary infection and vaccinated mice. Splenocytes from primary *M. tuberculosis*-infected mice conferred marginally higher protection than did those from BCG-vaccinated mice. Serum transfer did not protect against reinfection in either group. Our data emphasize that natural infection with *M. tuberculosis* and vaccination with BCG do not differ in their capacity to induce pro-

tective immunity against tuberculosis and support the notions that reinfection contributes to the development of active disease and that any novel vaccine against tuberculosis has to perform better than both vaccination with BCG and immunity evoked by natural infection.—Authors' Abstract

**Peters, W., Cyster, J. G., Mack, M., Schlondorff, D., Wolf, A. J., Ernst, J. D., and Charo, I. F.** CCR2-dependent trafficking of F4/80dim macrophages and CD11c dim/intermediate dendritic cells is crucial for T cell recruitment to lungs infected with *Mycobacterium tuberculosis*. *J. Immunol.* **172(12)** (2004) 7647–7653.

We previously reported that CCR2(−/−) mice are susceptible to *Mycobacterium tuberculosis* infection. Susceptibility was associated with an early and sustained macrophage trafficking defect, followed by delayed recruitment of dendritic cells (DCs) and T cells to the lungs. However, the relative importance of the lack of CCR2 expression by macrophages and DCs vs T cells in susceptibility to infection was unclear. In this study, we used mixed bone marrow transplantation to create mice in which the genotype of the T cells was either CCR2(+ / +) or CCR2(− / −) while maintaining the genotype of the myeloid cells as CCR2(+ / +). After infection with *M. tuberculosis*, we found that the genotype of the macrophages and/or DCs, but not that of the T cells, was critical for both T cell and myeloid cell migration to the lungs. Further investigation revealed a critical role for CCR2 in the recruitment of F4/80(dim) macrophages and CD11c (dim/intermediate) DCs to the infected lung.—Authors' Abstract

**Sander, P., Rezwan, M., Walker, B., Rampini, S. K., Kroppenstedt, R. M., Ehlers, S., Keller, C., Keeble, J. R., Hagemeyer, M., Colston, M. J., Springer, B., and Bottger, E. C.** Lipoprotein processing is required for virulence of *Mycobacterium tuberculosis*. *Mol. Microbiol.* **52(6)** (2004) 1543–1552.

Lipoproteins are a subgroup of secreted bacterial proteins characterized by a lipi-

dated N-terminus, processing of which is mediated by the consecutive activity of lipoprotein diacylglycerol transferase (Lgt) and lipoprotein signal peptidase (LspA). The study of LspA function has been limited mainly to non-pathogenic microorganisms. To study a potential role for LspA in the pathogenesis of bacterial infections, we have disrupted *lspA* by allelic replacement in *Mycobacterium tuberculosis*, one of the world's most devastating pathogens. Despite the presence of an impermeable lipid outer layer, it was found that LspA was dispensable for growth under *in vitro* culture conditions. In contrast, the mutant was markedly attenuated in virulence models of tuberculosis. Our findings establish lipoprotein metabolism as a major virulence determinant of tuberculosis and define a role for lipoprotein processing in bacterial pathogenesis. In addition, these results hint at a promising new target for therapeutic intervention, as a highly specific inhibitor of bacterial lipoprotein signal peptidases is available.—Authors' Abstract

**Saunders, B. M., Briscoe, H., and Britton, W. J.** T cell-derived tumour necrosis factor is essential, but not sufficient, for protection against *Mycobacterium tuberculosis* infection. *Clin. Exp. Immunol.* **137(2)** (2004) 279–287.

Tumour necrosis factor (TNF) is critical for sustained protective immunity against *Mycobacterium tuberculosis* infection. To investigate the relative contributions of macrophage- and T cell-derived TNF towards this immunity T cells from wild-type (WT) or TNF−/− mice were transferred into RAG−/− or TNF−/− mice which were then infected with *M. tuberculosis*. Infected RAG−/− mice and RAG−/− recipients of TNF deficient T cells developed overwhelming infection, with extensive pulmonary and hepatic necrosis and succumbed with a median of only 16 days infection. By contrast, RAG−/− recipients of WT T cells showed a significant increase in survival with a median of 32 days. Although initial bacterial growth was similar in all groups of RAG−/− mice, the transfer of WT, but not TNF−/−, T cells led to the formation of discrete foci of leucocytes and macrophages

and delayed the development of necrotizing pathology. To determine requirements for macrophage-derived TNF, WT or TNF<sup>-/-</sup> T cells were transferred into TNF<sup>-/-</sup> mice at the time of *M. tuberculosis* infection. Transfer of WT T cells significantly prolonged survival and reduced the early tissue necrosis evident in the TNF<sup>-/-</sup> mice, however, these mice eventually succumbed indicating that T cell-derived TNF alone is insufficient to control the infection. Therefore, both T cell- and macrophage-derived TNF play distinct roles in orchestrating the protective inflammatory response and enhancing survival during *M. tuberculosis* infection.—Authors' Abstract

**Toossi, Z., Mayanja-Kizza, H., Kanost, A., Edmonds, K., McHugh, M., and Hirsch, C.** Protective responses in tuberculosis: induction of genes for interferon-gamma and cytotoxicity by *Mycobacterium tuberculosis* and during human tuberculosis. *Scand. J. Immunol.* **60(3)** (2004) 299–306.

The host effector mechanisms against *Mycobacterium tuberculosis* infection are not well understood, and this remains a problem in the development of new vaccines and immunotherapies in tuberculosis (TB). Here, we studied the expression of genes for interferon gamma (IFN-gamma) and molecules involved in lymphocyte-mediated cytotoxicity [granzyme B (grzB), perforin, granulysin and Fas ligand (FasL)] against *M. tuberculosis*-infected macrophages. The kinetics of expression of these molecules were first established in peripheral blood mononuclear cells (PBMC) of healthy donors, and then investigated in TB patients with and without HIV-1 coinfection and appropriate control groups. We found that only IFN-gamma and grzB were induced by *M. tuberculosis* in PBMC from healthy purified protein derivative skin test reactive subjects. However, expression of neither gene nor IFN-gamma protein correlated with intracellular *M. tuberculosis* growth containment by macrophages. *Mycobacterium tuberculosis* induction of IFN-gamma, but not grzB, mRNA expression was significantly lower ( $p < 0.03$ ) in TB patients as compared with healthy subjects.—Authors' Abstract

**Vergne, I., Chua, J., Singh, S. B., and Deretic, V.** Cell Biology of *Mycobacterium tuberculosis* Phagosome. *Annu. Rev. Cell. Dev. Biol.* Epub. 2004 Jun. 15 [ahead of print].

Phagocytosis and phagolysosome biogenesis represent fundamental biological processes essential for proper tissue homeostasis, development, elimination of invading microorganisms, and antigen processing and presentation. Phagosome formation triggers a pre-programmed pathway of maturation into the phagolysosome, a process controlled by Ca<sup>2+</sup> and the regulators of organellar trafficking centered around the small GTP-binding proteins Rabs and their downstream effectors, including lipid kinases, organellar tethering molecules, and membrane fusion apparatus. *Mycobacterium tuberculosis* is a potent human pathogen parasitizing macrophages. It interferes with the Rab-controlled membrane trafficking and arrests the maturing phagosome at a stage where no harm can be done to the pathogen while the delivery of nutrients and membrane to the vacuole continues harboring the microorganism. This process, referred to as the *M. tuberculosis* phagosome maturation arrest or inhibition of phagosome-lysosome fusion, is critical for *M. tuberculosis* persistence in human populations. It also provides a general model system for dissecting the phagolysosome biogenesis pathways. Here we review the fundamental trafficking processes targeted by *M. tuberculosis* and the mycobacterial products that interfere with phagosomal maturation. Expected online publication date for the Annual Review of Cell and Developmental Biology Volume 20 is October 6, 2004.—Authors' Abstract

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

We have developed a colorimetric microtiter plate hybridization assay in order to simplify detection of *Mycobacterium leprae* in clinical specimens. This system detects the products amplified by a sensitive R T-

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 seen at the ALERT Hospital in Addis Ababa, Ethiopia [date not given]. 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.—Tropical Disease Bulletin

## Microbiology

**Flint, J. L., Kowalski, J. C., Karnati, P. K., and Derbyshire, K. M.** The RD1 virulence locus of *Mycobacterium tuberculosis* regulates DNA transfer in *Mycobacterium smegmatis*. Proc. Natl. Acad. Sci. U.S.A. **101(34)** (2004) 12598–12603. Epub 2004 Aug. 16.

Conjugal DNA transfer occurs by an atypical mechanism in *Mycobacterium smegmatis*. The transfer system is chromosomally encoded and requires recipient recombination functions for both chromosome and plasmid transfer. Cis-acting sequences have been identified that confer mobility on non-transferable plasmids, but these are larger and have different properties to canonical oriT sites found in bacterial plasmids. To identify trans-acting factors required for mediating DNA transfer, a library of transposon insertion mutants was generated in the donor strain, and individual mutants were screened for their effect on transfer. From this screen, a collection of insertion mutants was isolated that increased conjugation frequencies relative to wild type. Remarkably, the mutations map to a 25-kb region of the *M. smegmatis* chromosome that is syntenous with the RD1 region of *Mycobacterium tuberculosis*, which is considered to be the primary attenuating deletion in the related vaccine strain *Mycobacterium bovis* bacillus Calmette-Guérin. The genes of the RD1 region encode a secretory apparatus responsible for exporting Cfp10- and Esat-6, both potent antigens and virulence factors. In

crosses using two *M. smegmatis* donors, we show that wild-type cells can suppress the elevated transfer phenotype of mutant donors, which is consistent with the secretion of a factor that suppresses conjugation. Most importantly, the RD1 region of *M. tuberculosis* complements the conjugation phenotype of the RD1 mutants in *M. smegmatis*. Our results indicate that the *M. tuberculosis* and *M. smegmatis* RD1 regions are functionally equivalent and provide a unique perspective on the role of this critical secretion apparatus.—Authors' Abstract

**Lee, R. E., Li, W., Chatterjee, D., and Lee, R. E.** Rapid structural characterization of the arabinogalactan and lipoarabinomannan in live mycobacterial cells using 2D and 3D HR-MAS NMR. Glycobiology. Epub 2004 Sep. 15 [ahead of print]

Mycobacteria possess a unique, highly evolved, carbohydrate and lipid-rich cell wall that is believed to be important for their survival in hostile environments. Until now our understanding of mycobacterial cell wall structure has been based upon destructive isolation and fragmentation of individual cell wall components. This study describes the observation of the major cell wall structures in live, intact mycobacteria using two-dimensional (2D) and three-dimensional (3D) High-Resolution Magic-Angle Spinning (HR-MAS) NMR. As little as 20 mg (wet weight) of [(13)C] enriched

cells were required to produce a whole-cell spectra in which discrete cross-peaks corresponding to specific cell wall components could be identified. The most abundant signals of the arabinogalactan (AG) and lipoarabinomannan (LAM) were assigned in the HR-MAS NMR spectra by comparing the 2D and 3D NMR whole-cell spectra with the spectra of purified cellular components. This study confirmed that the structures of the AG and LAM moieties in the cell wall of live mycobacteria are consistent with structural reports in the literature, which were obtained via degradative analysis. Most importantly, using intact cells, it was possible to directly demonstrate the effects of Ethambutol on the mycobacterial cell wall polysaccharides, characterize the effects of embB gene knockout in the *M. smegmatis* DeltaembB mutant, and observe differences in the cell wall structures of two mycobacterial species (*M. bovis* BCG and *M. smegmatis*). Herein, we show that HR-MAS NMR is a powerful, rapid, non-destructive technique to monitor changes in the complex, carbohydrate rich cell wall of live mycobacterial cells.—Authors' Abstract

**Mostowy, S., Onipede, A., Gagneux, S., Niemann, S., Kremer, K., Desmond, E. P., Kato-Maeda, M., and Behr, M.** Genomic analysis distinguishes *Mycobacterium africanum*. *Clin. Microbiol.* **42(8)** (2004) 3594–3599.

See *Current Literature, Other Mycobacterial Diseases*, p. 562.

**Nigou, J., Gilleron, M., Brando, T., and Puzo, G.** Structural analysis of mycobacterial lipoglycans. *Appl. Biochem. Biotechnol.* **118(1–3)** (2004) 253–267.

*Mycobacterium tuberculosis*, the causative agent of tuberculosis, is one of the most effective human pathogens. The mycobacterial cell envelope contains lipoglycans, and of particular interest is lipoarabinomannan (LAM), one of the most potent mycobacterial immunomodulatory molecules. The importance of lipoarabinomannan (LAM) in the immunopathogenesis of tuberculosis has incited structural studies on this molecule to

(1) establish a precise structural model of the molecule and (2) decipher the structure/function relationships. In recent years, we have focused on the two domains essential for LAM biologic activities: the mannosylphosphatidyl-myo-inositol anchor and the caps. We review here the recent procedures developed for the structural analysis of these domains.—Authors' Abstract

**Pethe, K., Swenson, D. L., Alonso, S., Anderson, J., Wang, C., and Russell, D. G.** Isolation of *Mycobacterium tuberculosis* mutants defective in the arrest of phagosome maturation. *Proc. Natl. Acad. Sci. U.S.A.* **101(37)** (2004) 13642–13647. Epub 2004 Aug. 31.

*Mycobacterium tuberculosis* resides within the phagocytes of its host. It ensures its continued survival through arresting the normal maturation of its phagosome, which is retained within the early endosomal system of the macrophage. Although individual bacterial components have been shown to modulate phagosome biogenesis, the mechanism(s) active in live, intact bacteria remain elusive. We have developed a genetic screen that facilitates the isolation of mutants defective in arresting the maturation of their phagosomes. Macrophages were incubated with iron-dextran that was chased into lysosomes. The cells were subsequently infected with *M. tuberculosis* from a library of transposon-mutagenized bacteria. After four rounds of enrichment, the majority of mutants isolated were unable to prevent acidification of their phagosomes and were attenuated for intracellular survival. The genes affected range in function from those with no known homologues to putative transporters and lipid synthesis enzymes. Further characterization of these bacteria is needed. In addition to clarifying the processes active in modulation of phagosome biogenesis by *M. tuberculosis*, this screen may be applicable to other pathogens that restrict the maturation of their phagosome.—Authors' Abstract

**Sarkola, A., Makinen, J., Marjamaki, M., Marttila, H. J., Viljanen, M. K., and Soini, H.** Prospective evaluation of the GenoType assay for routine identification

of mycobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **23(8)** (2004) 642–645. Epub 2004 Jul. 28.

In order to evaluate the proficiency of the GenoType Mycobacteria strip hybridization assay (Hain Lifescience, Nehren, Germany) for the routine identification of mycobacteria, the assay was used to identify 178 clinical isolates during a 6-month prospective study. The GenoType results were compared to the identification results obtained with AccuProbe (GenProbe, San Diego, CA, USA) or 16S rDNA sequencing, and an overall agreement of 89.3% between GenoType and the two reference methods was reached. The GenoType assay is, thus, a rapid and reliable method for the identification of clinically important mycobacteria, and it is well suited for use in a routine laboratory.—Authors' Abstract

**Semret, M., Zhai, G., Mostowy, S., Cleto, C., Alexander, D., Cangelosi, G., Cousins, D., Collins, D. M., van Soolingen, D., and Behr, M. A.** Extensive genomic polymorphism within *Mycobacterium avium*. J. Bacteriol. **186(18)** (2004) 6332–6334.

We have initiated comparative genomic analysis of *Mycobacterium avium* subspecies by DNA microarray, uncovering 14 large sequence polymorphisms (LSPs) comprising over 700 kb that distinguish *M. avium* subsp. *avium* from *M. avium* subsp. *paratuberculosis*. Genes predicted to encode metabolic pathways were overrepresented in the LSPs, and analysis revealed a polymorphism within the mycobactin biosynthesis operon that potentially explains the *in vitro* mycobactin dependence of *M. avium* subsp. *paratuberculosis*.—Authors' Abstract

**Torrelles, J. B., Khoo, K. H., Sieling, P. A., Modlin, R. L., Zhang, N., Marques, A. M., Treumann, A., Rithner, C. D., Brennan, P. J., and Chatterjee, D.** Truncated structural variants of lipoarabinomannan in *Mycobacterium leprae* and an ethambutol-resistant strain of *Mycobacterium tuberculosis*. J. Biol. Chem.

**279(39)** (2004) 41227–41239. Epub 2004 Jul. 19.

Current knowledge on the structure of lipoarabinomannan (LAM) has resulted primarily from detailed studies on a few selected laboratory strains of *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG, and *Mycobacterium smegmatis*. Our previous work was the first to report on the salient structural features of *M. tuberculosis* clinical isolates and demonstrated significant structural variations. A prime effort is to correlate a particular structural characteristic with observed differences in eliciting an immunobiological response, especially in the context of CD1-restricted presentation of LAM to T cells. T cell clones derived from the cutaneous lesions of leprosy patients have been shown to recognize specifically LAM from *Mycobacterium leprae* and not from *M. tuberculosis* Erdman or H37Rv. Herein we provide further fine structural data on LAM from *M. leprae* (LepLAM) and a tuberculosis clinical isolate, CSU20 (CSU20LAM), which was unexpectedly recognized by the supposedly LepLAM-specific CD1-restricted T cell clones. In comparison with the de facto laboratory LAM standard from *M. tuberculosis* H37Rv (RvLAM), LepLAM derived from *in vivo* grown *M. leprae* is apparently simpler in its arabinan architecture with a high degree of exposed, non-mannose-capped termini. On the other hand, CSU20, an ethambutol-resistant clinical isolate, makes a vastly heterogeneous population of LAM ranging from rather small and non-mannose-capped to full-length and fully capped variants. LepLAM and CSU20LAM contain a higher level of succinylation than RvLAM, which, in the context of truncated or less elaborated arabinan, may contribute to selective recognition by T cells. LAM from all species could be resolved into discrete forms by isoelectric focusing based apparently on their arabinan heterogeneity. In the light of our current and more recent findings, we reason that all immunobiological data should be cautiously interpreted and that the actual LAM variants that may be present *in vivo* during infection and pathogenesis need to be taken into consideration.—Authors' Abstract

## Microbiology (Leprosy)

**Marques, M. A., Espinosa, B. J., Xavier da Silveira, E. K., Pessolani, M. C., Chapeaurouge, A., Perales, J., Dobos, K. M., Belisle, J. T., Spencer, J. S., and Brennan, P. J.** Continued proteomic analysis of *Mycobacterium leprae* subcellular fractions. *Proteomics*. **4(10)** (2004) 2942–2953

Recently the sequence of the *Mycobacterium leprae* chromosome, the only known obligate intracellular mycobacterium, was completed. It has a dramatic reduction in functional genes, with a coding capacity of only 49.5%, the lowest one so far observed among bacterial genomes. The leprosy bacillus seems to preserve a minimal set of genes that allows its survival in the host. The identification of genes that are actually expressed by the bacterium is of high signifi-

cance in the context of mycobacterial pathogenesis. In this current study, a proteomic approach was undertaken to identify the proteins present in the soluble/cytosol and membrane subcellular fractions obtained from armadillo derived *M. leprae*. Proteins from each fraction were separated by two-dimensional gel electrophoresis (2-DE) and identified by mass spectrometry. A total of 147 protein spots were identified from 2-DE patterns and shown to comprise products of 44 different genes, twenty eight of them corresponding to new proteins. Additionally, two highly basic proteins (with p I >10.0) were isolated by heparin affinity chromatography and identified by N-terminal sequencing. This study constitutes the first application of proteomics to a host-derived Mycobacterium.—Authors' Abstract

## Microbiology (Tuberculosis)

**Ahmed, N., Alam, M., Rao, K. R., Kauser, F., Kumar, N. A., Qazi, N. N., Sangal, V., Sharma, V. D., Das, R., Katoch, V. M., Murthy, K. J., Suneetha, S., Sharma, S. K., Sechi, L. A., Gilman, R. H., and Hasnain, S. E.** Molecular genotyping of a large, multicentric collection of tubercle bacilli indicates geographical partitioning of strain variation and has implications for global epidemiology of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **42(7)** (2004) 3240–3247.

See *Current Literature, Molecular and Genetic Studies*, p. 565.

**Bacon, J., James, B. W., Wernisch, L., Williams, A., Morley, K. A., Hatch, G. J., Mangan, J. A., Hinds, J., Stoker, N. G., Butcher, P. D., and Marsh, P. D.** The influence of reduced oxygen availability on pathogenicity and gene expression in *Mycobacterium tuberculosis*. *Tuberculosis (Edinb.)* **84(3–4)** (2004) 205–217.

We investigated how *Mycobacterium tuberculosis* responded to a reduced oxygen tension in terms of its pathogenicity and gene expression by growing cells under either aerobic or low-oxygen conditions in chemostat culture. The chemostat enabled us to control and vary the oxygen tension independently of other environmental parameters, so that true cause-and-effect relationships of reduced oxygen availability could be established. Cells grown under low oxygen were more pathogenic for guinea pigs than those grown aerobically. The effect of reduced oxygen on global gene expression was determined using DNA microarray. Spearman rank correlation confirmed that microarray expression profiles were highly reproducible between repeat cultures. Using microarray analysis we have identified genes that respond to a low-oxygen environment without the influence of other parameters such as nutrient depletion. Some of these genes appear to be involved in the biosynthesis of cell wall precursors and their induction may have contributed to increased

infectivity in the guinea pig. This study has shown that a combination of chemostat culture and microarray presents a biologically robust and statistically reliable experimental approach for studying the effect of relevant and specific environmental stimuli on mycobacterial virulence and gene expression.—Authors' Abstract

**Bahk, Y. Y., Kim, S. A., Kim, J. S., Euh, H. J., Bai, G. H., Cho, S. N., and Kim, Y. S.** Antigens secreted from *Mycobacterium tuberculosis*: Identification by proteomics approach and test for diagnostic marker. *Proteomics*. Epub 2004 Sep. 20 [ahead of print]

Tuberculosis caused by mycobacteria, mainly *Mycobacterium tuberculosis*, is a major infectious disease of the respiratory system. An early diagnosis followed by chemotherapy is the major control strategy. In an effort to identify the antigens suitable for immunodiagnosis and vaccines, the proteins secreted in a culture medium from the *M. tuberculosis* K-strain, which is the most prevalent among the clinical isolates in Korea and belongs to the Beijing family, were analyzed by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and compared with those from the *M. tuberculosis* H37Rv and CDC1551 strains. Eight proteins, Rv0652, Rv1636, Rv2818c, Rv3369, Rv3865, Rv0566c, MT3304, and Rv3160, were identified by matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) or liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) and found to be relatively abundant in the culture medium from the *M. tuberculosis* K-strain but less so from the CDC1551 or H37Rv strains. In addition, Rv3874 (CFP-10), Rv-0560c and Rv3648c, which were expressed increasingly in the K and CDC1551 strains, were also identified using the same proteomics technology. All proteins were prepared by molecular cloning, expression in *Escherichia coli* followed by affinity purification. Among them, three proteins, rRv3369, rRv0566c, and rRv3874, were selected by prescreening and examined for their potential as serodiagnostic antigens using an enzyme-linked immunosorbent

assay. When 100 sera from tuberculosis patients and 100 sera from the healthy controls were analyzed, rR V3369, rRv3874, and rRv0566c showed a sensitivity of 60%, 74%, and 43%, and a specificity of 96%, 97%, and 84%, respectively. These results suggest that the rRv3369 and rRv3874 proteins, which were expressed more abundantly in the more recently obtained clinical isolates of *M. tuberculosis* than in the laboratory-adapted H37Rv strain, are promising for use in the serodiagnosis of tuberculosis.—Authors' Abstract

**Choudhary, R. K., Pullakhandam, R., Ehtesham, N. Z., and Hasnain, S. E.** Expression and characterization of Rv2430c, a novel immunodominant antigen of *Mycobacterium tuberculosis*. *Protein Expr. Purif.* **36(2)** (2004) 249–253.

About 10% of the coding sequence of *Mycobacterium tuberculosis* corresponds to hitherto unknown members of the PE and PPE protein families which display significant sequence and length variation at their C-terminal region. It has been suggested that this could possibly represent a rich source of antigenic variation within the pathogen. We describe the purification and biophysical characterization of the recombinant PPE protein coded by hypothetical ORF Rv2430c, a member of the PPE gene family that was earlier shown to induce a strong B cell response. Expression of the recombinant PPE protein in *Escherichia coli* led to its localization in inclusion bodies and subsequent refolding using dialysis after its extraction from the same resulted in extensive precipitation. Therefore, an on-column refolding strategy was used, after which the protein was found to be in the soluble form. CD spectrum of the recombinant protein displayed predominantly alpha helical content (81%) which matched significantly with in silico and web-based secondary structure predictions. Furthermore, fluorescence emission spectra revealed that aromatic amino acids are buried inside the protein, which are exposed to aqueous environment under 8M urea. These results, for the first time, provide evidence on the structural features of PPE family protein which, viewed with its reported immunodominant charac-



teristics, have implications for other proteins of the PE/PPE family.—Authors' Abstract

**Hampshire, T., Soneji, S., Bacon, J., James, B. W., Hinds, J., Laing, K., Stabler, R. A., Marsh, P. D., and Butcher, P. D.** Stationary phase gene expression of *Mycobacterium tuberculosis* following a progressive nutrient depletion: a model for persistent organisms? Tuberculosis (Edinb.) **84(3–4)** (2004) 228–238.

The majority of individuals infected with TB develop a latent infection, in which organisms survive within the body while evading the host immune system. Such persistent bacilli are capable of surviving several months of combinatorial antibiotic treatment. Evidence suggests that stationary phase bacteria adapt to increase their tolerance to environmental stresses. We have developed a unique *in vitro* model of dormancy based on the characterization of a single, large volume fermenter culture of *M. tuberculosis*, as it adapts to stationary phase. Cells are maintained in controlled and defined aerobic conditions (50% dissolved oxygen tension), using probes that measure dissolved oxygen tension, temperature, and pH. Microarray analysis has been used in conjunction with viability and nutrient depletion assays to dissect differential gene expression. Following exponential phase growth the gradual depletion of glucose/glycerol resulted in a small population of survivors that were characterized for periods in excess of 100 days. Bacilli adapting to nutrient depletion displayed characteristics associated with persistence *in vivo*, including entry into a non-replicative state and the up-regulation of genes involved in beta-oxidation of fatty acids and virulence. A reduced population of non-replicating bacilli went on to adapt sufficiently to re-initiate cellular division.—Authors' Abstract

**Hisert, K. B., Kirksey, M. A., Gomez, J. E., Sousa, A. O., Cox, J. S., Jacobs, W. R. Jr., Nathan, C. E., and McKinney, J. D.** Identification of *Mycobacterium tuberculosis* counterimmune (cim) mutants in immunodeficient mice by differential screening. Infect. Immun. **72(9)** (2004) 5315–5321.

Tuberculosis (TB) is characterized by lifetime persistence of *Mycobacterium tuberculosis*. Despite the induction of a vigorous host immune response that curtails disease progression in the majority of cases, the organism is not eliminated. Subsequent immunosuppression can lead to reactivation after a prolonged period of clinical latency. Thus, while it is clear that protective immune mechanisms are engaged during *M. tuberculosis* infection, it also appears that the pathogen has evolved effective countermechanisms. Genetic studies with animal infection models and with patients have revealed a key role for the cytokine gamma interferon (IFN-gamma) in resistance to TB. IFN-gamma activates a large number of antimicrobial pathways. Three of these IFN-gamma-dependent mechanisms have been implicated in defense against *M. tuberculosis*: inducible nitric oxide synthase (iNOS), phagosome oxidase (phox), and the phagosome-associated GTPase LRG-47. In order to identify bacterial genes that provide protection against specific host immune pathways, we have developed the strategy of differential signature-tagged transposon mutagenesis. Using this approach we have identified three *M. tuberculosis* genes that are essential for progressive *M. tuberculosis* growth and rapid lethality in iNOS-deficient mice but not in IFN-gamma-deficient mice. We propose that these genes are involved in pathways that allow *M. tuberculosis* to counter IFN-gamma-dependent immune mechanisms other than iNOS.—Authors' Abstract

**Karakousis, P. C., Yoshimatsu, T., Lamichhane, G., Woolwine, S. C., Nuermberger, E. L., Grosset, J., and Bishai, W. R.** Dormancy phenotype displayed by extracellular *Mycobacterium tuberculosis* within artificial granulomas in mice. J. Exp. Med. **200(5)** (2004) 647–657.

*Mycobacterium tuberculosis* residing within pulmonary granulomas and cavities represents an important reservoir of persistent organisms during human latent tuberculosis infection. We present a novel *in vivo* model of tuberculosis involving the encapsulation of bacilli in semidiffusible hollow

fibers that are implanted subcutaneously into mice. Granulomatous lesions develop around these hollow fibers, and in this microenvironment, the organisms demonstrate an altered physiologic state characterized by stationary-state colony-forming unit counts and decreased metabolic activity. Moreover, these organisms show an antimicrobial susceptibility pattern similar to persistent bacilli in current models of tuberculosis chemotherapy in that they are more susceptible to the sterilizing drug, rifampin, than to the bactericidal drug isoniazid. We used this model of extracellular persistence within host granulomas to study both gene expression patterns and mutant survival patterns. Our results demonstrate induction of dosR (Rv3133c) and 20 other members of the DosR regulon believed to mediate the transition into dormancy, and that rel(Mtb) is required for *Mycobacterium tuberculosis* survival during extracellular persistence within host granulomas. Interestingly, the dormancy phenotype of extracellular *M. tuberculosis* within host granulomas appears to be immune mediated and interferon-gamma dependent.—Authors' Abstract

**Lightbody, K. L., Renshaw, P. S., Collins, M. L., Wright, R. L., Hunt, D. M., Gordon, S. V., Hewinson, R. G., Buxton, R. S., Williamson, R. A., and Carr, M. D.** Characterisation of complex formation between members of the *Mycobacterium tuberculosis* complex CFP-10/ESAT-6 protein family: towards an understanding of the rules governing complex formation and thereby functional flexibility. *FEMS Microbiol. Lett.* **238(1)** (2004) 255–262.

We have previously shown that the secreted *M. tuberculosis* complex proteins CFP-10 and ESAT-6 form a tight, 1:1 complex, which may represent their functional form. In the work reported here a combination of yeast two-hybrid and biochemical analysis has been used to characterise complex formation between two other pairs of CFP-10/ESAT-6 family proteins (Rv0287/Rv0288 and Rv3019c/Rv3020c) and to determine whether complexes can be formed between non-genome paired members of the family. The results clearly demonstrate that Rv0287/Rv0288 and Rv3019c/3020c form

tight complexes, as initially observed for CFP-10/ESAT-6. The closely related Rv0287/Rv0288 and Rv3019c/Rv3020c proteins are also able to form non-genome paired complexes (Rv0287/Rv3019c and Rv0288/Rv3020c), but are not capable of binding to the more distantly related CFP-10/ESAT-6 proteins.—Authors' Abstract

**Liu, X., Tiwari, R. K., Geliebter, J., Wu, J. M., and Godfrey, H. P.** Interaction of a *Mycobacterium tuberculosis* repetitive DNA sequence with eukaryotic proteins. *Biochem. Biophys. Res. Commun.* **320(3)** (2004) 966–972.

*Mycobacterium tuberculosis* infects one-third of the world's population and causes two million deaths annually. Its intracellular residence raises the possibility that bacterial nucleic acids might interact with key host proteins and contribute to the pathophysiology of infection. To test this hypothesis, we searched for motifs closely resembling eukaryotic transcription factor binding sites in the *M. tuberculosis* H37Rv genome and found activator protein-2 and zinc finger protein-5 binding motifs in a 36-nucleotide repetitive mycobacterial DNA sequence (RPT1). RPT1 bound specifically to nuclear extract proteins from U937, A549, and HeLa cells in electrophoretic mobility shift assays but not to activator protein-2. Several nuclear and cytosolic proteins showing at least partial binding specificity for RPT1 were isolated from U937 and A549 cells by pull-down assays, including Ku70 (DNA-dependent protein kinase subunit) and poly(ADP-ribose) polymerase-1. RPT1 also specifically activated DNA-dependent protein phosphorylation. These results suggest that mycobacterial nucleic acid fragments may interact specifically with eukaryotic regulatory proteins which might contribute to bacterial life-cycle maintenance.—Authors' Abstract

**Mostowy, S., Cleto, C., Sherman, D. R., and Behr, M. A.** The *Mycobacterium tuberculosis* complex transcriptome of attenuation. *Tuberculosis (Edinb.)* **84(3–4)** (2004) 197–204.

Although the deletion of RD1 is likely correlated to attenuation from virulence for members of the *Mycobacterium tuberculosis* (MTB) complex, the reasons for this phenotype remain to be fully explained. As genomic variation is responsible for at least a component of variability in gene expression, we looked to the *in vitro* global expression profile of the RD1 artificial knockout from *M. tuberculosis* H37Rv (H37Rv:deltaRD1) for clues to elucidate its phenotypic shift towards attenuation. By comparing the transcriptome of H37Rv:deltaRD1 to that of virulent H37Rv, 15 regulated genes located in nine different regions outside of RD1 have been identified, capturing an effect of RD1's deletion on the rest of the genome. To assess whether these regulations are characteristic of attenuated MTB in general, expression profiles of natural RD1 mutants (BCG Russia, BCG Pasteur, and *M. microti*) as well as the "avirulent" *M. tuberculosis* H37Ra, whose RD1 region is genomically intact, were obtained. Results indicate that attenuated strains lack the expression of RD1 genes including *cfp10* and *esat6*, whether through deletion or reduced expression. Furthermore, comparative transcriptomics reveals the concurrent down-regulation of several gene neighborhoods beyond RD1. The potential relevance of these other expression changes towards MTB virulence is discussed.—Authors' Abstract

**Waddell, S. J., Stabler, R. A., Laing, K., Kremer, L., Reynolds, R. C., and Besra, G. S.** The use of microarray analysis to determine the gene expression profiles of *Mycobacterium tuberculosis* in response to anti-bacterial compounds. *Tuberculosis* (Edinb.) **84**(3–4) (2004) 263–274.

The response of *Mycobacterium tuberculosis* to six anti-microbial agents was determined by microarray analysis in an attempt to define mechanisms of innate resistance in *M. tuberculosis*. The gene expression profiles of *M. tuberculosis* after treatment at the minimal inhibitory concentration (MIC) for 4 hr with isoniazid, isoxyl, tetrahydrolipstatin, SRI#221, SR1#967 and SR1#9190 were compared to untreated *M. tuberculosis*. A common response to drug exposure was defined, and this expression profile overlapped with a number of other mycobacterial stress responses recently identified by microarray analysis. Compound-specific responses were also distinguished including a number of putative transcriptional regulators and translocation-related genes. These genes may contribute to the intrinsic resistance of *M. tuberculosis* to anti-microbial compounds. Further investigation into these mechanisms may elucidate novel pathways contributing to mycobacterial drug resistance and influence anti-mycobacterial drug development strategies.—Authors' Abstract

## Experimental Infections

**Algood, H. M., and Flynn, J. L.** CCR5-deficient mice control *Mycobacterium tuberculosis* infection despite increased pulmonary lymphocytic infiltration. *J Immunol.* **173**(5) (2004) 3287–3296.

The control of *Mycobacterium tuberculosis* infection is dependent on the development of an adaptive immune response, which is mediated by granulomas. The granuloma is a dynamic structure that forms in the lung and consists primarily of macrophages and lymphocytes. For this structure to be effective in containment of the bacillus, it must develop in an organized and timely manner. The formation of the granu-

loma is dependent on recruitment of activated cells through adhesion molecules and chemokines. *M. tuberculosis* infection causes an increase in the expression of beta-chemokines CCL3, CCL4, and CCL5, and their receptor CCR5, in the lungs. In this study, we demonstrate that CCR5-transgenic knockout mice were capable of recruiting immune cells to the lung to form granulomas. CCR5(–/–) mice successfully induced a Th1 response and controlled infection. Unexpectedly, *M. tuberculosis* infection in these mice resulted in greater numbers of lymphocytes migrating to the lung and higher levels of many inflammatory cytokines, compared with wild-type mice,

without apparent long-term detrimental effects. In the absence of CCR5, there were more dendritic cells in the lung-draining lymph nodes and more primed T lymphocytes in these mice. Bacterial numbers in the lymph nodes were also higher in CCR5(−/−) mice. Therefore, CCR5 may play a role in the migration of dendritic cells to and from the lymph nodes during *M. tuberculosis* infection.—Authors' Abstract

**Brennan, M. J., Morris, S. L., and Sizemore, C. F.** Tuberculosis vaccine development: research, regulatory and clinical strategies. *Expert. Opin. Biol. Ther.* **4(9)** (2004) 1493–1504.

In the past decade, while the global tuberculosis (TB) epidemic has continued to devastate mankind, considerable progress has nevertheless been made in the development of new and improved vaccines for this ancient disease. Recombinant bacillus Calmette-Guérin strains, DNA-based vaccines, live attenuated *Mycobacterium tuberculosis* vaccines and subunit vaccines formulated with novel adjuvants have shown promise in preclinical animal challenge models. Three of these vaccines are being evaluated at present in human clinical studies, and several other vaccine preparations are being targeted for clinical trials in the near future. Although the preclinical characterisation and testing of new TB vaccines has clearly led to exciting new findings, complex regulatory and clinical trial design issues remain as a challenge to TB vaccine development. This report reviews some of the exciting advances in TB research that have led to the development of new TB vaccines, and addresses the unique regulatory and clinical issues associated with the testing of novel anti-TB preparations in human populations.—Authors' Abstract

**Brodin, P., Majlessi, L., Brosch, R., Smith, D., Bancroft, G., Clark, S., Williams, A., Leclerc, C., and Cole, S. T. J.** Enhanced protection against tuberculosis by vaccination with recombinant *Mycobacterium microti* vaccine that induces T cell immunity against region of

difference 1 antigens. *Infect. Dis.* **190(1)** (2004) 115–122. Epub 2004 Jun. 04.

*Mycobacterium microti*, the vole bacillus, which was used as a live vaccine against tuberculosis until the 1970s, confers the same protection in humans as does *Mycobacterium bovis* bacille Calmette-Guérin (BCG). However, because the efficacy of the BCG vaccine varies considerably, we have tried to develop a better vaccine by reintroducing into *M. microti* the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, *M. microti* OV254::RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8(+) T lymphocytes that had strong expression of the CD44(hi) activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with *M. microti* alone or with BCG. The *M. microti* OV254::RD1-2F9 vaccine was less virulent and persistent in mice and than was BCG::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.—Authors' Abstract

**Gagliardi, M. C., Teloni, R., Mariotti, S., Iona, E., Pardini, M., Fattorini, L., Orefici, G., and Nisini, R.** Bacillus Calmette-Guérin shares with virulent *Mycobacterium tuberculosis* the capacity to subvert monocyte differentiation into dendritic cell: implication for its efficacy as a vaccine preventing tuberculosis. *Vaccine.* **22(29–30)** (2004) 3848–3857.

The only available vaccine against tuberculosis (TB) is Bacillus Calmette-Guérin (BCG) whose efficacy in preventing pulmonary tuberculosis is however controversial. Here, we show that BCG infection of monocytes causes their differentiation into mature dendritic cells (DCs) lacking CD1 molecules expression, coupled with suboptimal up-regulation of HLA class II, CD80 and CD40 molecules and a marked unresponsiveness to lipopolysaccharide stimula-

tion. In addition, alloreactive naïve T lymphocytes primed by these subverted DCs did not undergo defined functional polarization, as witnessed by their inability to produce IFN-gamma. Since efficient antigen presentation and IFN-gamma production by mycobacterial-specific T lymphocytes are required for protection against *Mycobacterium tuberculosis*, our data might provide additional explanation for the low efficacy of BCG vaccination.—Authors' Abstract

**Harth, G., Maslesa-Galic, S., and Horwitz, M. A.** A two-plasmid system for stable, selective-pressure-independent expression of multiple extracellular proteins in mycobacteria. *Microbiology* **150**(Pt. 7) (2004) 2143–2151.

Recombinant mycobacteria expressing *Mycobacterium tuberculosis* extracellular proteins are leading candidates for new vaccines against tuberculosis and other mycobacterial diseases, and important tools both in antimycobacterial drug development and basic research in mycobacterial pathogenesis. Recombinant mycobacteria that stably overexpress and secrete major extracellular proteins of *M. tuberculosis* in native form on plasmids pSMT3 and pNBV1 were previously constructed by the authors. To enhance the versatility of this plasmid-based approach for mycobacterial protein expression, the *Escherichia coli* /mycobacteria shuttle plasmid pGB9 was modified to accommodate mycobacterial genes expressed from their endogenous promoters. Previous studies showed that the modified plasmid, designated pGB9.2, derived from the cryptic *Mycobacterium fortuitum* plasmid pMF1, was present at a low copy number in both *E. coli* and mycobacteria, and expression of recombinant *M. tuberculosis* proteins was found to be at levels paralleling its copy number, that is, approximating their endogenous levels. Plasmid pGB9.2 was compatible with the shuttle vectors pSMT3 and pNBV1 and in combination with them it simultaneously expressed the *M. tuberculosis* 30 kDa extracellular protein FbpB. Plasmid pGB9.2 was stably maintained in the absence of selective pressure in three mycobacterial species: *Mycobacterium bovis*

BCG, *M. tuberculosis* and *M. smegmatis*. Plasmid pGB9.2 was found to be self-transmissible between both fast- and slow-growing mycobacteria, but not from mycobacteria to *E. coli* or between *E. coli* strains. The combination of two compatible plasmids in one BCG strain allows expression of recombinant mycobacterial proteins at different levels, a potentially important factor in optimizing vaccine potency.—Authors' Abstract

**Mark Doherty, T.** New vaccines against tuberculosis. *Trop. Med. Int. Health.* **9**(7) (2004) 818–826

In September 2000, recognizing the effect of communicable diseases as obstacles to development in poorer countries, the European Commission assembled a special round table on “accelerated action targeted at major communicable diseases within the context of poverty reduction.” The three major communicable diseases discussed were tuberculosis (TB), malaria and HIV. One outcome of this discussion was a workshop examining issues related to the fight against TB in Africa, which took place in Gorée, Sénégal, in May 2001. The timing was propitious, as new vaccines for TB (recombinant MVA and BCG, and adjuvanted recombinant fusion proteins or peptide constructs), are just beginning to enter human clinical trials. All but the last of these have shown promise in animal models, up to and including non-human primates, and all are strongly immunogenic and apparently safe. Humans trials for safety and efficacy are thus the logical next step. This review summarizes recent advances in tuberculosis vaccine development, with a special emphasis on issues raised at the Gorée meeting about testing and deploying new generation vaccines in TB-endemic areas such as Africa.—Authors' Abstract

**Mollenkopf, H. J., Dietrich, G., Fensterle, J., Grode, L., Diehl, K. D., Knapp, B., Singh, M., O'Hagan, D. T., Ulmer, J. B., and Kaufmann, S. H.** Enhanced protective efficacy of a tuberculosis DNA vaccine by adsorption onto cationic PLG

microparticles. *Vaccine* **22(21–22)** (2004) 2690–2695.

Immunization with plasmid DNA vectors represents a promising new approach to vaccination. It has been shown to elicit humoral and cellular immunity and protection in various infection models. Here, we assessed the immunogenicity and protective efficacy of a DNA vaccine vector encoding the antigen 85A (Ag85A) of *Mycobacterium tuberculosis*. Since intramuscular (i.m.) immunization with naked DNA requires considerable amounts of DNA in order to be effective, we evaluated a strategy to reduce the amount of DNA needed. To this end, we used Ag85A DNA adsorbed onto cationic poly(DL-lactide-co-glycolide) (PLG) microparticles and observed similar levels of protection against aerosol challenge in mice using doses of PLG-DNA two orders of magnitude lower than with naked DNA itself.—Authors' Abstract

**Sharma, N., and Agrewala, J. N.** Potent role of vaccines prepared from macrophages infected with live bacteria in protection against *Mycobacterium tuberculosis* and *Salmonella typhimurium* infections. *J. Infect Dis.* **190(1)** (2004) 107–114. Epub 2004 Jun. 08.

The present study describes a novel and simple vaccination strategy that involves the culturing of live *Mycobacterium tuberculosis* and *Salmonella typhimurium* in syngeneic, allogeneic, and xenogeneic macrophages, followed by drug treatment and gamma irradiation, to kill the bacteria. Notable observations were that the lymphocytes obtained from the vaccinated mice proliferated and secreted mainly interferon-gamma and IgG2a, but not interleukin-4 and IgG1. The enumeration of viability of *M. tuberculosis* indicated a significant level of protection in the vaccinated mice after challenge with live *M. tuberculosis*. This vaccination strategy worked successfully for tuberculosis but also showed a significant decrease in mortality of mice challenged with live *S. typhimurium*.—Authors' Abstract

**Skeiky, Y. A., Alderson, M. R., Owendale, P. J., Guderian, J. A., Brandt, L., Dillon, D. C., Campos-Neto, A., Lobet, Y.,**

**Dalemans, W., Orme, I. M., and Reed, S. G.** Differential immune responses and protective efficacy induced by components of a tuberculosis polyprotein vaccine, Mtb72F, delivered as naked DNA or recombinant protein. *J. Immunol.* **172(12)** (2004) 7618–7628.

Key Ags of *Mycobacterium tuberculosis* initially identified in the context of host responses in healthy purified protein derivative-positive donors and infected C57BL/6 mice were prioritized for the development of a subunit vaccine against tuberculosis. Our lead construct, Mtb72F, codes for a 72-kDa polyprotein genetically linked in tandem in the linear order Mtb32(C)-Mtb39-Mtb32(N). Immunization of C57BL/6 mice with Mtb72F DNA resulted in the generation of IFN-gamma responses directed against the first two components of the polyprotein and a strong CD8(+) T cell response directed exclusively against Mtb32(C). In contrast, immunization of mice with Mtb72F protein formulated in the adjuvant AS02A resulted in the elicitation of a moderate IFN-gamma response and a weak CD8(+) T cell response to Mtb32c. However, immunization with a formulation of Mtb72F protein in AS01B adjuvant generated a comprehensive and robust immune response, resulting in the elicitation of strong IFN-gamma and Ab responses encompassing all three components of the polyprotein vaccine and a strong CD8(+) response directed against the same Mtb32(C) epitope identified by DNA immunization. All three forms of Mtb72F immunization resulted in the protection of C57BL/6 mice against aerosol challenge with a virulent strain of *M. tuberculosis*. Most importantly, immunization of guinea pigs with Mtb72F, delivered either as DNA or as a rAg-based vaccine, resulted in prolonged survival (>1 year) after aerosol challenge with virulent *M. tuberculosis* comparable to bacillus Calmette-Guérin immunization. Mtb72F in AS02A formulation is currently in phase I clinical trial, making it the first recombinant tuberculosis vaccine to be tested in humans.—Authors' Abstract

**Wang, Q. M., Sun, S. H., Hu, Z. L., Zhou, F. J., Yin, M., Xiao, C. J., and Zhang, J.**

C. Epitope DNA vaccines against tuberculosis: spacers and ubiquitin modulates cellular immune responses elicited by epitope DNA vaccine. *Scand. J. Immunol.* **60(3)** (2004) 219–225.

Cell-mediated immune responses are crucial in the protection against tuberculosis. In this study, we constructed epitope DNA vaccines (p3-M-38) encoding cytotoxic T lymphocyte (CTL) epitopes of MPT64 and 38 kDa proteins of *Mycobacterium tuberculosis*. In order to observe the influence of spacer sequence (Ala-Ala-Tyr) or ubiquitin (UbGR) on the efficacy of the two CTL epitopes, we also constructed DNA vaccines, p3-M-S(spacer)-38, p3-Ub (UbGR)-M-S-38 and p3-Ub-M-38. The immune responses elicited by the four DNA vaccines were

tested in C57BL/6 (H-2b) mice. The cytotoxicity of T cells was detected by LDH-release method and by enzyme-linked immunospot assay for epitope-specific cells secreting interferon-gamma. The results showed that DNA immunization with p3-M-38 vaccine could induce epitope-specific CD8+ CTL response and that the spacer sequence (AA Y) only enhanced M epitope presentation. The protein-targeting sequence (UbGR) enhanced the immunogenicity of the two epitopes. The finding that defined spacer sequences at C-terminus and protein-targeting degradation modulated the immune response of epitope string DNA vaccines will be of importance for the further development of multi-epitope DNA vaccines against tuberculosis.—Authors' Abstract

## Epidemiology and Prevention

**Bakker, M. I., Hatta, M., Kwenang, A., Faber, W. R., Van Beers, S. M., Klatser, P. R., and Oskam, L.** Population survey to determine risk factors for *Mycobacterium leprae* transmission and infection. *Int. J. Epidemiol.* Epub 2004 Jul. 15.

**BACKGROUND:** Not every leprosy patient is equally effective in transmitting *Mycobacterium leprae*. We studied the spatial distribution of infection (using seropositivity as a marker) in the population to identify which disease characteristics of leprosy patients are important in transmission. **METHODS:** Clinical data and blood samples for anti-*M. leprae* ELISA were collected during a cross-sectional survey on five Indonesian islands highly endemic for leprosy. A geographic information system (GIS) was used to define contacts of patients. We investigated spatial clustering of patients and seropositive people and used logistic regression to determine risk factors for seropositivity. **RESULTS:** Of the 3986 people examined for leprosy, 3271 gave blood. Seroprevalence varied between islands (1.7–8.7%) and correlated significantly with leprosy prevalence. Five clusters of patients and two clusters of seropositives were detected. In multivariate analysis, seropositivity significantly differed by leprosy status,

age, sex, and island. Serological status of patients appeared to be the best discriminator of contact groups with higher seroprevalence: contacts of seropositive patients had an adjusted odds ratio (aOR) of 1.75 (95% CI 0.92–3.31). This increased seroprevalence was strongest for contact groups living  $\leq 75$  m of two seropositive patients (aOR = 3.07; 95% CI 1.74–5.42). **CONCLUSIONS:** In this highly endemic area for leprosy, not only household contacts of seropositive patients, but also people living in the vicinity of a seropositive patient were more likely to harbour antibodies against *M. leprae*. Through measuring the serological status of patients and using a broader definition of contacts, higher risk groups can be more specifically identified.—Authors' Abstract

**Deps, P. D., Faria, L. V., Gonçalves, V. C., Silva, D. A., Ventura, C. G., and Zandonade, E.** Epidemiological features of the leprosy transmission in relation to armadillo exposure. *Hansen. Int.* **28(2)** (2003) 138–144. [Article in Portuguese].

**Introduction and purpose:** Some authors demonstrated the possibility of the armadillos, *Dasypus novemcinctus*, being an envi-

ronmental source of *Mycobacterium leprae*. An epidemiologic survey was done to check the correlation between the human contact with armadillos and the incidence of leprosy. It discusses some features that could be involved in the dynamic process of the leprosy development. The objective of this research is to check the frequency of cases of leprosy contacts with armadillos and also the interhuman contact before their diagnosis to establish the possibility of the *M. leprae* transmission to the human being through the contact with armadillos. Methods: One hundred and seven leprosy patients were surveyed (leprosy patients that had finished the MTD treatment) who lived in the Pedro Fontes Colony-Hospital, in Cariacica, Espirito Santo State, Brazil, 29 leprosy patients and 173 non leprosy patients from a dermatology service of the city of Vitória, Brazil. The survey included data about the armadillo meat consumption before leprosy diagnosis, the existence of known cases and/or familial leprosy cases. The data were analyzed by Qui-square test, correlation and Exact Fischer Test. Results: 90.4% of the leprosy patients or cured leprosy patients had once eaten armadillo meat before their leprosy diagnosis, while 15% of the non leprosy patients had already eaten armadillo meat. In a group without leprosy contact before the diagnosis, 96,1% ate armadillo meat, and 3,9% didn't eat. This study supposes a possible source of the *M. leprae* by the armadillo meat consumption, mainly, in a leprosy patients without previous leprosy contact.—Hansenologia Internationalis

**Douglas, J. T., Cellona, R. V., Fajardo, T. T. Jr., Abalos, R. M., Balagon, M. V., and Klatser, P. R.** Prospective study of serological conversion as a risk factor for development of leprosy among household contacts. Clin. Diagn. Lab. Immunol. **11(5)** (2004) 897–900.

Although the prevalence of leprosy has declined over the years, there is no evidence that incidence rates are falling. A method of early detection of those people prone to develop the most infectious form of leprosy would contribute to breaking the chain of transmission. Prophylactic treatment of serologically identified high-risk contacts of inci-

dent patients should be an operationally feasible approach for routine control programs. In addition, classification of high-risk household contacts will allow control program resources to be more focused. In this prospective study, we examined the ability of serology used for the detection of antibodies to phenolic glycolipid I of *Mycobacterium leprae* to identify those household contacts of multibacillary leprosy patients who had the highest risk of developing leprosy. After the start of multidrug therapy for the index case, a new case of leprosy developed in one in seven of the 178 households studied. In households where new cases appeared, the seropositivity rates were significantly higher ( $p < 0.001$ ) than those in households without new cases. Seropositive household contacts had a significantly higher risk of developing leprosy (relative hazard adjusted for age and sex [aRH], 7.2), notably multibacillary leprosy (aRH = 24), than seronegative contacts.—Authors' Abstract

**Mak, D. B., Platt, E. M., and Heath, C. H.** Leprosy transmission in the Kimberley, Western Australia. Med. J. Austral. **179(8)** (2003) 452.

Between 1986 and 2002, a total of 28 new leprosy cases were notified to the Kimberly Public Health Unit in Western Australia, Australia. At diagnosis, the patients were aged 8–63 years. In several recent cases, diagnosis was delayed despite multiple presentations to primary health care staff and medical specialists. Eleven patients (39%) had multibacillary disease, and can transmit the disease. The need for the proper management of leprosy patients and to control the increasing population movement in and out of leprosy-endemic areas to prevent leprosy transmission to other parts of Australia is discussed.—Tropical Diseases Bulletin

**Meima, A., Smith, W. C., van Oortmarsen, G. J., Richardus, J. H., and Habbema, J. D.** The future incidence of leprosy: a scenario analysis. Bull. World Health Organ. **82(5)** (2004) 373–380.

See *Current Literature, General and Historical*, p. 511.



## Rehabilitation and Social Concerns

**Deepak, S.** Answering the rehabilitation needs of leprosy-affected persons in integrated setting through primary health care services and community-based rehabilitation. *Indian J. Lepr.* **75(2)** (2003) 127–142.

This article aims to discuss the strategies for answering the rehabilitation needs of persons with leprosy-related disabilities in integrated settings through primary health care (PHC) services and community-based rehabilitation (CBR). While the provision of rehabilitation services through the PHC system remains problematic in most developing countries, the article concludes that CBR programs have the potential for rehabilitation of leprosy-affected persons in integrated settings. However, the limited coverage of CBR programs may pose an obstacle to such an approach. The author suggests the use of existing specific rehabilitation infrastructures meant only for leprosy-affected persons for initiating, sustaining and extending the CBR coverage to the surrounding communities. At the same time, the author asks for support and strengthening of organizations of leprosy-affected persons, promoting their active involvement in all rehabilitation processes.—Authors' Abstract

**Kaur, H., and Gandhi, A.** People's perception of leprosy—a study in Delhi. *Indian J. Lepr.* **75(1)** (2003) 37–46.

Leprosy is considered to cause more social than medical problems. The present study focussed on this aspect in order to investigate the level of awareness among people—about their attitude towards the disease and the afflicted. The results are based on interviews with 104 persons in Delhi. The sample data revealed that the level of knowledge of leprosy was inadequate. The cause of the disease was known to 44.2% of those interviewed, while 31.7% were com-

pletely ignorant; 6.7% believed it to be the consequence of an individual's past misdeeds, and 1.9% believed it to have been caused by divine curse. 63.1% were aware that the disease is curable. 73.1% of the persons interviewed sympathised with leprosy-afflicted beggars. 61.5% favored leprosy patients to stay with their families and within their communities. 67.3% felt that the cured could marry, while 25% felt that the leprosy-afflicted should stay in leprosy colonies away from the society. 54.8% were reluctant to employ the leprosy-afflicted as domestic help, and 31.7% were reluctant to establish matrimonial relationship with a family having a leprosy-afflicted person. The data call for intensification of public awareness regarding the aetiology of leprosy. Positive and scientific information should be disseminated to minimize the social prejudices associated with the disease.—Authors' Abstract

**Qian, J. G., Yan, L. B., Li, W. Z., and Zhang, G. C.** Posterior tibialis transfer for foot-drop due to leprosy: a case with 40 years follow-up. *Br. J. Plast. Sur. g.* **57(5)** (2004) 450–452.

A 56-year-old male was transferred to our centre because of a relapse of leprosy neuritis in the hands. We found that the patient had received a posterior tibialis tendon transfer for correction of his left dropped foot 40 years previously. On examination active dorsiflexion of the left ankle joint was close to 0 degrees with grade 4 power of dorsiflexion, and the plantar flexion was about 35 degrees. Walking gait was almost normal. There were some scars on the plantar surface of the left metatarsal area; but with the continuous use of a soft dressing pad under the middle part of the sole, plantar ulceration has been avoided for many years even with active daily activities of the patient. The patient is very satisfied with the operative results.—Authors' Abstract

## Other Mycobacterial Diseases

**Arend, S. M., Cerda de Palou, E., de Haas, P., Janssen, R., Hoeve, M. A., Verhard, E. M., Ottenhoff, T. H., van Soolingen, D., and van Dissel, J. T.** Pneumonia caused by *Mycobacterium kansasii* in a series of patients without recognised immune defect. *Clin. Microbiol. Infect.* **10(8)** (2004) 738–748.

The clinical and epidemiological characteristics of 17 patients diagnosed with *Mycobacterium kansasii* pneumonia within a limited geographical region over a period of 10 years are described. An in-depth evaluation of the innate and adaptive immune systems was performed for five available patients. A comparison was made of the genetic fingerprint patterns of the isolates obtained by restriction fragment length polymorphism (RFLP) analysis, with the major polymorphic tandem repeat (MPTR) as a probe. Predisposing factors consisted of smoking, airway abnormalities, substance abuse, diabetes or poor general condition, but in two patients no risk factor was identified. In the five patients tested, no abnormalities or deficiencies were detected in the innate or adaptive type-1 immunity. All *M. kansasii* isolates had identical MPTR RFLP patterns, although no epidemiological connection could be established, and these were identical to those of clinical isolates from Australian patients. These data do not support the theory that defects in the innate or adaptive type-1 immunity have a role in the pathogenesis of invasive *M. kansasii* infections. The identical fingerprint patterns of the isolates suggested the existence of a virulent strain of *M. kansasii*.—Authors' Abstract

**Debacker, M., Aguiar, J., Steunou, S. Z., Meyers, W. M., Guédénon, Scott, J. T., Dramaix, M., and Portaels, F.** *Mycobacterium ulcerans* disease (Buruli Ulcer) in rural hospital, Southern Benin, 1997–2001. *Emerg. Infect. Dis.* **10(8)** (2004) 1391–1398.

Data from 1700 patients living in southern Benin were collected at the Centre San-

itaire et Nutritionnel Gbemoten, Zagnanado, Benin, from 1997 through 2001. In the Zou region in 1999, Buruli ulcer (BU) had a higher detection rate (2.15/100,000) than leprosy (13.4/100,000) and tuberculosis (20.0/100,000). More than 13% of the patients had osteomyelitis. Delay in seeking treatment declined from 4 months in 1989 to 1 month in 2001, and median hospitalization time decreased from 9 months in 1989 to 1 month in 2001. This reduction is attributed, in part, to implementing an international co-operation program, creating a national BU program, and making advances in patient care.—Emerging Infectious Diseases

**Desmond, E., Ahmed, A. T., Probert, W. S., Ely, J., Jang, Y., Sanders, C. A., Lin, S. Y., and Flood, J.** *Mycobacterium africanum* cases, California. *Emerg. Infect. Dis.* **10(5)** (2004) 921–923.

Five *Mycobacterium tuberculosis* complex isolates in California were identified as *M. africanum* by spoligotyping, single nucleotide polymorphisms, a deletion mutation, and phenotypic traits, confirming it as a cause of tuberculosis in the United States. Three of the five patients from whom *M. africanum* was isolated had lived in Africa.—Authors' Abstract

**Drummond, C., and Butler, J. R.** *Mycobacterium ulcerans* treatment costs, Australia. *Emerg. Infect. Dis.* **10(6)** (2004) 1038–1043.

*Mycobacterium ulcerans* gives rise to severe skin ulceration that can be associated with considerable illness. The cost of diagnosis, treatment, and lost income has never been assessed in Australia. A survey of 26 confirmed cases of the disease in Victoria was undertaken. Data were collected on demographic details, diagnostic tests, treatment, time of work, and travel to obtain treatment. All costs are reported in Australian dollars in 1997–1998 prices. The cost varies considerably with disease severity. For mild cases, the average direct cost is

6803 Australian dollars, and for severe cases 27,681 Australian dollars. Hospitalization accounts for 61% to 90% of costs, and indirect costs amount to 24% of the total per case. *M. ulcerans* can be an expensive disease to diagnose and treat. Costs can be reduced by early diagnosis and definitive treatment. Research is needed to find cost-effective therapies for this disease.—Authors' Abstract

**Eddyani, M., Ofori-Adjei, D., Teugels, G., De Weirtdt, D., Boakye, D., Meyers, W. M., and Portaels, F.** Potential role for fish in transmission of *Mycobacterium ulcerans* disease (Buruli ulcer): an environmental study. *Appl. Environ. Microbiol.* **70(9)** (2004) 5679–5681.

This study reports a potential role that fish may play in the transmission of *Mycobacterium ulcerans* disease (Buruli ulcer). Fish found positive for *M. ulcerans* DNA all appear to feed on insects or plankton and are believed to concentrate *M. ulcerans* from this usual food source. These observations provide additional data supporting our previous hypothesis on sources of *M. ulcerans* and modes of transmission.—Authors' Abstract

**Field, S. K., Fisher, D., and Cowie, R. L.** *Mycobacterium avium* complex pulmonary disease in patients without HIV infection. *Chest* **126(2)** (2004) 566–581.

*Mycobacterium avium* complex (MAC) is ubiquitous. It is found in various freshwater and saltwater sources around the world, including hot water pipes. Although the organism was identified in the 1890s, its potential to cause human disease was only recognized 50 years later. Only a minority of people exposed to the organism will acquire MAC lung disease, usually those with underlying lung disease or immunosuppression. MAC may, however, cause progressive parenchymal lung disease and bronchiectasis in patients without underlying lung disease, particularly in middle-aged and elderly women. Preliminary data suggest that the interferon-gamma pathways may be deficient in elderly women with MAC lung dis-

ease. Other groups of patients who are more likely to harbor MAC in their lungs include patients with a cystic fibrosis or an abnormal alpha(1)-antiproteinase gene and patients with certain chest wall abnormalities. Treatment results continue to be disappointing, and the mortality of patients with MAC lung disease remains high. A PubMed search identified 38 reports of the treatment of MAC lung disease. Apart from the British Thoracic Society study, the only published controlled investigation, the studies published since 1994 have included a macrolide, either clarithromycin or azithromycin, usually in combination with ethambutol and a rifamycin. If success is defined as eradication of the organism without relapse over a period of several years after treatment has been discontinued, the reported treatment success rate with the macrolide containing regimens is approximately 55%. The prolonged treatment period, side effects, and possibly reinfection rather than relapse are responsible for the high failure rate.—Authors' Abstract

**Fujita, J., Kishimoto, T., Ohtsuki, Y., Shigeto, E., Ohnishi, T., Shiode, M., Yamaji, Y., Suemitsu, I., Yamadori, I., Bando, S., Nishimura, K., Matsushima, T., and Ishida, T.** Clinical features of eleven cases of *Mycobacterium avium*-intracellulare complex pulmonary disease associated with pneumoconiosis. *Respir. Med.* **98(8)** (2004) 721–725.

The relationship between silicosis and tuberculosis is well known. Also other mycobacteria such as *Mycobacterium kansasii* often occur in association with pneumoconiosis. However, there are few reports describing an association of *M. avium* -intracellulare complex (MAC) lung disease and pneumoconiosis. The purpose of the present study is to describe clinical features of MAC respiratory infection associated with pneumoconiosis. Eleven patients with MAC respiratory infection associated with pneumoconiosis (all men, 6 with silicosis and 5 with welders' pneumoconiosis) were collected. A determination of whether or not MAC caused pulmonary disease was made using the 1997 criteria required by the American Thoracic Society. Radiologically,

cavity formation as well as upper lung field predominance of MAC disease were observed in 8 of 11 cases (72.7%). Two of 11 patients died of respiratory failure. Our present study clearly demonstrates that clinical features of MAC respiratory infection associated with pneumoconiosis were different from MAC without underlying diseases.—Authors' Abstract

**Garcia-Pelayo, M. C., Caimi, K. C., Inwald, J. K., Hinds, J., Bigi, E., Romano, M. I., van Soolingen, D., Hewinson, R. G., Cataldi, A., and Gordon, S. V.** Microarray analysis of *Mycobacterium microti* reveals deletion of genes encoding PE-PPE proteins and ESAT-6 family antigens. *Tuberculosis (Edinb.)* **84(3-4)** (2004) 159–166.

*Mycobacterium microti* is the agent of tuberculosis in wild voles and has been used as a live vaccine against tuberculosis in man and cattle. To explore the *M. microti* genome in greater detail, we used a *M. tuberculosis* H37Rv genomic DNA microarray to detect gene deletions among *M. microti* isolates. A number of deletions were identified that correlated with those described previously (*Infect. Immun.* **70** (2002) 5568) but a novel *M. microti* deletion was also found (MiD4) which removes 5 genes that code for ESAT-6 family antigens and PE-PPE proteins. Southern blot experiments showed that this region was also deleted from *M. pinnipedii*, a mycobacterium isolated from seals that is closely related to *M. microti*. Genes encoding ESAT-6 antigens and PE-PPE proteins appear to be frequently deleted from *M. microti*, and the implications of this are discussed.—Authors' Abstract

**Haverkamp, M. H., Arend, S. M., Lindeboom, J. A., Hartwig, N. G., and van Dissel, J. T.** Nontuberculous mycobacterial infection in children: a 2-year prospective surveillance study in the Netherlands. *Clin. Infect. Dis.* **39(4)** (2004) 450–456. Epub 2004 Jul. 23.

We performed a prospective, 2-year nationwide study to assess incidence and disease characteristics of suspected infections

with nontuberculous mycobacteria (NTM) in children, via the Netherlands Pediatric Surveillance Unit. Data for 61 children were reported (median age, 31 months; interquartile range, 22–50 months; female sex, 37 subjects); 2 subjects had an underlying disease. Most children (53 [87%] of 61) had cervical lymph node enlargement, with abscess in 25 (47%) and fistula in 11 (21%). The estimated annual incidence of NTM infection was 77 cases per 100,000 children. In 16 children, the diagnosis was based solely on the results of skin tests with mycobacterial antigens. Cultures were performed in 36 cases and yielded mycobacteria in 27 (75%); *Mycobacterium avium* was isolated from 18 cultures. Children with a culture positive for mycobacteria did not differ in presentation, complications, or treatment from those whose cultures showed no growth. Thirty children underwent surgery, and chemotherapy was the single treatment in 24 (39%) of the cases. The treatment of localized NTM infection in immunocompetent children by antimycobacterial drugs should be evaluated further.—Authors' Abstract

**Heckman, G. A., Hawkins, C., Morris, A., Burrows, L. L., and Bergeron, C.** Rapidly progressive dementia due to *Mycobacterium neoaurum* meningoencephalitis. *Emerg. Infect. Dis.* **10(5)** (2004) 924–927.

Dementia developed in a patient with widespread neurologic manifestations; she died within 5 months. Pathologic findings showed granulomatous inflammation with caseation necrosis, foreign body-type giant cells, and proliferative endarteritis with vascular occlusions. Broad-range polymerase chain reaction identified *Mycobacterium neoaurum* as the possible pathogen. Central nervous system infection by *M. neoaurum* may result in rapidly progressive dementia.—Authors' Abstract

**Hyon, J. Y., Joo, M. J., Hose, S., Sinha, D., Dick, J. D., and O'Brien, T. P.** Comparative efficacy of topical gatifloxacin with ciprofloxacin, amikacin, and clarithromycin in the treatment of experi-

mental *Mycobacterium chelonae* keratitis. Arch. Ophthalmol. **122(8)** (2004) 1166–1169.

**OBJECTIVE:** To determine the comparative efficacy of topical gatifloxacin with ciprofloxacin, fortified amikacin, and clarithromycin against *Mycobacterium chelonae* keratitis in an animal model. **METHODS:** Experimental *M. chelonae* keratitis was induced via intrastromal inoculation in a rabbit model. Thirty-five rabbits were randomly divided into 5 groups and each group was treated hourly for 12 hours with topical 0.9% balanced salt solution, 0.3% gatifloxacin, 0.3% ciprofloxacin hydrochloride, a combination of topical fortified amikacin sulfate (50 mg/mL) and clarithromycin (10 mg/mL), or a triple combination of topical 0.3% gatifloxacin, fortified amikacin sulfate (50 mg/mL), and clarithromycin (10 mg/mL). Antibacterial efficacy of each regimen was determined by quantitative bacteriologic analysis. **RESULTS:** Treatment with 0.3% gatifloxacin or the triple combination of 0.3% gatifloxacin, topical fortified amikacin sulfate (50 mg/mL), and clarithromycin (10 mg/mL) reduced the number of mycobacterial or ganisms more significantly than the controls that were treated with a topical balanced salt solution (both  $p < .001$ ). Therapy with 0.3% gatifloxacin was more effective than 0.3% ciprofloxacin alone ( $p < .001$ ) and demonstrated synergy by enhancing the efficacy of the combination of fortified amikacin (50 mg/mL) and clarithromycin (10 mg/mL) ( $p < .001$ ). Neither 0.3% ciprofloxacin nor the combination of fortified amikacin (50 mg/mL) and clarithromycin (10 mg/mL) demonstrated a significant difference in activity against mycobacteria compared with the topical balanced salt solution. **CONCLUSION:** These results suggest that topical 0.3% gatifloxacin ophthalmic solution can be a new initial treatment agent against *M. chelonae* keratitis. **Clinical Relevance** Topical gatifloxacin 0.3% may provide an initial alternative in therapy of *M. chelonae* keratitis.—Authors' Abstract

**Kapur, N., Schuster, H., Parker, N., Kelsey, M. C., and Goldsmith, P. C.** Severe sporotrichoid infection with *Myco-*

*bacterium szulgai*. Clin. Exp. Dermatol. **29(4)** (2004) 377–379.

*Mycobacterium szulgai* is a nontuberculous, acid-fast bacillus or atypical mycobacteria, which prior to 1972 was not thought of as a pathogen. Since then most cases reported in the literature have been of pulmonary disease with only a few case reports of cutaneous disease. Our patient, who had an underlying, uncategorized, immunosuppressive condition, presented with multiple severe ulcers spreading proximally up the arms in a sporotrichoid pattern with more scattered lesions on his legs. He made a full recovery with appropriate antimicrobial treatment.—Authors' Abstract

**Lewis, C. G., Wells, M. K., and Jennings, W. C.** *Mycobacterium fortuitum* breast infection following nipple-piercing, mimicking carcinoma. Breast J. **10(4)** (2004) 363–365.

We reviewed a rare breast infection occurring 4 months after nipple piercing. Clinical examination suggested carcinoma. *Mycobacterium fortuitum* was eventually isolated after surgical biopsy and debridement. Antibiotic therapy was initiated intravenously using two drugs and oral therapy was continued for 6 months. A contralateral mycobacterial lesion emerged and was excised along with a residual fibrotic nodule at the original biopsy site. When adequate sampling of a complex and suspicious breast mass is benign and initial bacterial cultures are sterile, mycobacterial infection should be considered, particularly when there is a history of previous nipple piercing procedures.—Authors' Abstract

**Lopez-Calleja, A. I., Lezcano, M. A., Samper, S., de Juan, F., and Revillo, M. J.** *Mycobacterium malmoense* lymphadenitis in Spain: first two cases in immunocompetent patients. Eur. J. Clin. Microbiol. Infect. Dis. **23(7)** (2004) 567–569. Epub 2004 Jun. 26.

Reported here are two cases of *Mycobacterium malmoense* lymphadenitis that occurred in two immunocompetent children in

Spain. To the best of our knowledge, these are the first documented cases of extrapulmonary infection by *M. malmoense* in Spain. This report serves to draw attention to this emerging nontuberculous mycobacterium that is gaining increasing recognition as a pulmonary and extrapulmonary pathogen in different countries.—Authors' Abstract

**Majoor, C. J., Schreurs, A. J., and Weers-Pothoff, G.** *Mycobacterium xenopi* infection in an immunosuppressed patient with Crohn's disease. *Thorax*. **59(7)** (2004) 631–632.

A 48 year old patient with active Crohn's disease presented with bilateral nodules over his lungs resembling malignant metastasis. Bronchoscopic and pathological examination of the airways and sputum did not show any malignancy. After 6 weeks *Mycobacterium xenopi* was cultured from his bronchial washings while all other cultures remained negative. Treatment was started with rifampicin, ethambutol, and clarithromycin and, after 9 months of treatment, there was an almost complete resolution of his chest radiograph.—Authors' Abstract

**Marras, T. K., and Daley, C. L.** A Systematic Review of the Clinical Significance of Pulmonary *Mycobacterium kansasii* Isolates in HIV Infection. *J. Acquir. Immune Defic. Syndr*. **36(4)** (2004) 883–889.

**SUMMARY:** Guidelines recommend treating HIV-infected patients with pulmonary *Mycobacterium kansasii* infection only in the presence of multiple positive cultures and clinical and radiographic abnormalities. Some authors suggest a single positive culture warrants treatment. A systematic literature review was done to determine whether HIV-infected patients who had *M. kansasii* isolated from respiratory specimens may have an indolent infection (often referred to as colonization) not requiring treatment and to determine the utility of diagnostic criteria in distinguishing disease from indolent infection. Sixteen studies were included, with at least 573 patients: mean age 44 years; 91% male; 64%

men who had sex with men; 35% injection drug users; and median CD4 lymphocyte count of 2–381 cells/microL. The median rate of indolent infection was 8%. Of the few patients who did not satisfy diagnostic criteria and were left untreated, outcomes were generally favorable. Overall, survival was longer in treated patients (mean 12 vs. 4 months). Indolent pulmonary infection with *M. kansasii* may exist in the setting of HIV, but published data do not provide adequate information to identify such patients. It is unclear whether unfulfilled diagnostic criteria necessarily imply the absence of disease in this context.—Authors' Abstract

**Mostowy, S., Onipede, A., Gagneux, S., Niemann, S., Kremer, K., Desmond, E. P., Kato-Maeda, M., and Behr, M.** Genomic analysis distinguishes *Mycobacterium africanum*. *Clin. Microbiol.* **42(8)** (2004) 3594–3599.

*Mycobacterium africanum* is thought to comprise a unique species within the *Mycobacterium tuberculosis* complex. *M. africanum* has traditionally been identified by phenotypic criteria, occupying an intermediate position between *M. tuberculosis* and *M. bovis* according to biochemical characteristics. Although *M. africanum* isolates present near-identical sequence homology to other species of the *M. tuberculosis* complex, several studies have uncovered large genomic regions variably deleted from certain *M. africanum* isolates. To further investigate the genomic characteristics of organisms characterized as *M. africanum*, the DNA content of 12 isolates was interrogated by using Affymetrix GeneChip. Analysis revealed genomic regions of *M. tuberculosis* deleted from all isolates of putative diagnostic and biological consequence. The distribution of deleted sequences suggests that *M. africanum* subtype II isolates are situated among strains of "modern" *M. tuberculosis*. In contrast, other *M. africanum* isolates (subtype I) constitute two distinct evolutionary branches within the *M. tuberculosis* complex. To test for an association between deleted sequences and biochemical attributes used for speciation, a phenotypically diverse panel of "*M. africanum*-like" isolates from Guinea-Bissau was tested for these

deletions. These isolates clustered together within one of the *M. africanum* subtype I branches, irrespective of phenotype. These results indicate that convergent biochemical profiles can be independently obtained for *M. tuberculosis* complex members, challenging the traditional approach to *M. tuberculosis* complex speciation. Furthermore, the genomic results suggest a rational framework for defining *M. africanum* and provide tools to accurately assess its prevalence in clinical specimens.—Authors' Abstract

**Phillips, R., Adjei, O., Lucas, S., Benjamin, N., and Wansbrough-Jones, M.** Pilot randomized double-blind trial of treatment of *Mycobacterium ulcerans* disease (Buruli ulcer) with topical nitrogen oxides. *Antimicrob. Agents Chemother.* **48(8)** (2004) 2866–2870.

*Mycobacterium ulcerans* disease (Buruli ulcer) is a serious ulcerating skin disease which is common in many tropical countries. Standard treatment, by extensive excision and skin grafting, is not available in rural communities where the disease is common. We evaluated the efficacy and safety of treatment with topical nitrogen oxides. Thirty-seven patients with a clinical diagnosis of Buruli ulcer caused by *M. ulcerans* disease were randomly assigned to one of two groups. In one group, two creams containing sodium nitrite (6%, wt/wt) or citric acid monohydrate (9%, wt/wt) were applied daily for 6 weeks, while the other group received a placebo. In the second 6 weeks, both groups received the nitrogen oxide-generating combination of creams. Treatment was continued for another 4 weeks for patients whose ulcers were not healed after 12 weeks. The ulcer surface area was monitored by weekly tracings made by assessors blinded to the treatment. In the first 6 weeks, patients on sodium nitrite and citric acid monohydrate (group I, active treatment) showed a rapid decrease in ulcer size from  $28.6 \pm 5.6 \text{ cm}^2$  (mean  $\pm$  standard error) to  $12.6 \pm 3.2 \text{ cm}^2$ , a decrease significantly greater than that in group II (from  $15.3 \pm 3.1$  to  $11.7 \pm 3.7 \text{ cm}^2$ ;  $p = 0.03$ ). Five ulcers in the placebo group enlarged during this period, compared with one in the active group. In the second 6 weeks (both groups on ac-

tive treatment), the rates of healing were similar for the two groups and there was a significant reduction in ulcer size in group II (previously on placebo) compared to the first 6 weeks. Yellow pigmentation of the skin, which disappeared 3 days after treatment was stopped, was the only side effect to date. We conclude that creams releasing nitrogen oxides increase the healing rate of ulcers caused by *M. ulcerans* infection with minimal adverse events. This is the first controlled trial of any form of therapy which demonstrates efficacy in treating this disease.—Authors' Abstract

**Phillips, R., Kuijper, S., Benjamin, N., Wansbrough-Jones, M., Wilks, M., and Kolk, A. H.** *In vitro* killing of *Mycobacterium ulcerans* by acidified nitrite. *Antimicrob. Agents Chemother.* **48(8)** (2004) 3130–3132.

*Mycobacterium ulcerans*, which causes Buruli ulcer, was exposed to acidified nitrite or to acid alone for 10 or 20 min. Killing was rapid, and viable counts were reduced below detectable limits within 10 min of exposure to 40 mM acidified nitrite. *M. ulcerans* is highly susceptible to acidified nitrite *in vitro*.—Authors' Abstract

**Piersimoni, C., Goteri, G., Nista, D., Martiotti, A., Mazzarelli, G., and Bornigia, S.** *Mycobacterium lentiflavum* as an emerging causative agent of cervical lymphadenitis. *J. Clin. Microbiol.* **42(8)** (2004) 3894–3897.

A lymph node excision was performed on a 45-year-old woman with left cervical swelling. The disorder which developed after the patient had undergone oral surgery for a severe periodontal disease failed to respond to antimicrobial chemotherapy. A mycobacterial strain subsequently identified by high-performance liquid chromatography analysis of cell wall mycolic acids as *Mycobacterium lentiflavum* grew from the excised specimen. This case and previously published reports highlight the relevance of *M. lentiflavum* as an emerging causative agent of mycobacterial cervical lymphadenitis.—Authors' Abstract

**Reddy, V. M., Suleman, F. G., and Hayworth, D. A.** *Mycobacterium avium* binds to mouse intestinal mucus aldolase. *Tuberculosis (Edinb.)* **84(5)** (2004) 303–310.

SETTING: *Mycobacterium avium* complex (MAC) is known to colonize the gastrointestinal tract of human immunodeficiency virus (HIV) infected patients before causing bacteremia and disseminated disease. However, the mechanism involved in the gastrointestinal colonization is not known. OBJECTIVE: To identify putative intestinal mucus receptors which serve as anchor for MAC colonization. DESIGN: C57BL/6 mouse intestinal mucus was subjected to single and two-dimensional electrophoresis and blotted on nitrocellulose membranes. MAC specific mucus proteins were identified by probing the mucus western blots with biotinylated proteins derived from *M. avium* strain 101 (MAC101). RESULTS: Biotinylated MAC 101 proteins recognized a 39 kDa intestinal mucus glycoprotein. The protein displaying an isoelectric point (pI) of 9.0, was found to be periodate sensitive but resistant to sialidase, heparinase I and chondroitinase ABC. The internal amino acid sequence of the 39 kDa protein displayed homology with fructose-1-6-bisphosphate aldolase B (aldolase). The proclivity between MAC adhesins and aldolase was confirmed by probing rabbit muscle aldolase with MAC proteins. Furthermore, both 25 and 31 kDa MAC adhesins, superoxide dismutase and heparin binding protein, respectively, were found to bind to aldolase. CONCLUSIONS: MAC binds to intestinal mucus aldolase, conceivably facilitating intestinal colonization of the organism.—Authors' Abstract

**Schulzke, S., Adler, H., Bar, G., Heining, U., and Hammer, J.** *Mycobacterium bohemicum*—a cause of paediatric cervical lymphadenitis. *Swiss. Med. Wkly.* **134(15–16)** (2004) 221–222.

We report on two toddlers suffering from *Mycobacterium bohemicum* lymphadenitis. Acid-fast bacilli were cultured from submandibular lymph nodes and identified by molecular methods as *Mycobacterium bo-*

*hemicum*. Surgical treatment was successful and complemented by oral treatment with clarithromycin and rifampicin.—Authors' Abstract

**Sedlacek, L., Rifai, M., Feldmann, K., and Bange, F. C.** LightCycler-based differentiation of *Mycobacterium abscessus* and *Mycobacterium chelonae*. *J. Clin. Microbiol.* **42(7)** (2004) 3284–3287.

In this study we introduce a rapid procedure to identify *Mycobacterium abscessus* (types I and II) and *M. chelonae* using LightCycler-based analysis of the hsp65 gene. Results from 36 clinical strains were compared with hsp65 gene restriction analysis and biochemical profiles of bacilli. As all three methods yielded identical results for each isolate, this procedure offers an excellent alternative to previously established nucleic acid amplification-based techniques for the diagnosis of mycobacterial diseases.—Authors' Abstract

**Tan, H. H., Tan, A., Theng, C., and Ng, S. K.** Cutaneous *Mycobacterium haemophilum* infections in immunocompromised patients in a dermatology clinic in Singapore. *Ann. Acad. Med. Singapore.* **33(4)** (2004) 532–536.

INTRODUCTION: *Mycobacterium haemophilum*, a nontuberculous mycobacterium (NTM) that was first described in 1978, is a pathogen that can cause an array of symptoms in immunocompromised patients, predominantly cutaneous. CLINICAL PICTURE: We report our hospital's experience with the first 3 patients diagnosed with this infection from 1994 to 2002. All were women; one had systemic lupus erythematosus (SLE), one had mycosis fungoides and the last had Sjogren's syndrome with recurrent bacterial infections, although the specific nature of her immunocompromised state has not been defined. All were HIV negative. All 3 women presented with cutaneous lesions—the first with recurrent erythematous plaques on the limbs and back, the second with tender nodules and abscesses on the knees, and the third with papular eruptions on the cheek. TREAT-



MENT/OUTCOME: All responded to a combination of antibiotics and are presently still under going treatment and follow-up.

CONCLUSION: Infections caused by *M. haemophilum* occur mainly in immunocompromised patients. They can present with a variety of cutaneous manifestations, which require a high index of suspicion and coordination between the treating physician and the laboratory for diagnosis. Combination antibiotic treatment is recommended, and patients should be followed up after treatment to survey for possible relapse.—Authors' Abstract

**Tortoli, E., Rindi, L., Garcia, M. J., Chiaradonna, P., Dei, R., Garzelli, C., Kroppenstedt, R. M., Lari, N., Mattei, R., Mariottini, A., Mazzarelli, G., Murcia, M. I., Nanetti, A., Piccoli, P., and Scarparo, C.** Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. *Int. J. Syst. Evol. Microbiol.* **54(Pt. 4)** (2004) 1277–1285.

The possibility that the strains included within the *Mycobacterium avium* complex (MAC), but not belonging either to *M. avium* or to *Mycobacterium intracellulare*, may be members of undescribed taxa, has already been questioned by several taxonomists. A very homogeneous cluster of 12 strains characterized by identical nucleotide sequences both in the 16S rDNA and in the 16S–23S internal transcribed spacer was investigated. Similar strains, previously reported in the literature, had been assigned either to the species *M. intracellulare* on the basis of the 16S rDNA similarity or to the group of MAC intermediates. However, several phenotypical and epidemiological characteristics seem to distinguish these strains

from all other MAC organisms. The unique mycolic acid pattern obtained by HPLC is striking as it is characterized by two clusters of peaks, instead of the three presented by all other MAC organisms. All of the strains have been isolated from humans and all but one came from the respiratory tract of elderly people. The clinical significance of these strains, ascertained for seven patients, seems to suggest an unusually high virulence. The characteristics of all the strains reported in the literature, genotypically identical to the ones described here, seem to confirm our data, without reports of isolations from animals or the environment or, among humans, from AIDS patients. Therefore, an elevation of the MAC variant was proposed and characterized here, with the name *Mycobacterium chimaera* sp. nov.; this increases the number of species included in the *M. avium* complex. The type strain is FI-01069T (=CIP 107892T=DSM 44623T).—Authors' Abstract

**van Hest, R., van der Zanden, A., Boeree, M., Kremer, K., Dessens, M., Westendorp, P., Maraha, B., van Uffelen, R., Schutte, R., and de Lange, W.** *Mycobacterium heckeshornense* infection in an immunocompetent patient and identification by 16S rRNA sequence analysis of culture material and a histopathology tissue specimen. *J. Clin. Microbiol.* **42(9)** (2004) 4386–4389.

*Mycobacterium heckeshornense* is a rare isolate in clinical specimens. We performed simultaneous 16S rRNA sequence analysis of a mycobacterium culture and a histopathology specimen to determine the relevance of *M. heckeshornense* infection in an immunocompetent patient initially presenting with pneumothorax.—Authors' Abstract

## Molecular and Genetic Studies

**Ahmed, N., Alam, M., Rao, K. R., Kauser, F., Kumar, N. A., Qazi, N. N., Sangal, V., Sharma, V. D., Das, R., Katoch, V. M., Murthy, K. J., Suneetha, S.,**

**Sharma, S. K., Sechi, L.A., Gilman, R. H., and Hasnain, S. E.** Molecular genotyping of a large, multicentric collection of tubercle bacilli indicates geographical

partitioning of strain variation and has implications for global epidemiology of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **42(7)** (2004) 3240–3247.

Tuberculosis continues to be a major killer disease, despite an all-out effort launched against it in the postgenomic era. We describe here the population structure of *Mycobacterium tuberculosis* strains, as revealed by a chromosome-wide scan of fluorescent amplified fragment length polymorphisms (FAFLPs), for more than 1 100 independent isolates from 11 different countries. The bacterial strains were genotyped based on a total of  $136 \pm 1$  different FAFLP markers at the genome sequence interface, with details on IS6110 profiles, drug resistance status, clinicopathological observations, and host status integrated into the analysis process. The strains were found to cluster with possible geographic affinities, including the parameters of host species type, IS6110 profile, and drug susceptibility status. Of the five most commonly amplified fragment sets (or amplicons), type A predominated in strains of mixed origin, deposited in The Netherlands; type B was exclusively observed for Indian isolates; type C was found mainly in strains from Peru and Australia; and types D and E predominated in European strains from France and Italy. The amplicons were independent of certain large sequence polymorphisms representing two important deletions, TbD1 and Rd9. It appears that *M. tuberculosis* has a high genomic diversity with a possible geographic evolution. This may have occurred due to specific genomic deletions and synonymous substitutions selected rigorously against host defenses and environmental stresses on an evolutionary timescale. The genotypic data reported here are additionally significant for genotype-phenotype correlations and for determining whether pathogen diversity is a reflection of the host population diversity.—Authors' Abstract

**Butcher, P. D.** Microarrays for *Mycobacterium tuberculosis*. *Tuberculosis (Edinb.)* **84(3–4)** (2004) 131–137.

This special microarray issue of *Tuberculosis* recognises the important contributions

of *M. tuberculosis* whole genome DNA microarrays to tuberculosis research by bringing together a range of papers that address *M. tuberculosis* physiology, host-pathogen interactions, mechanisms of drug action, *in vitro* and *in vivo* gene expression, host responses, comparative genomics and functional analysis of particular genes. A number of complete datasets of *M. tuberculosis* mRNA expression levels are provided to facilitate multiple cross-condition comparison. Microarrays represent one of the new functional genomics technologies exploiting genome sequence information that will bring us closer to realising the scientific and moral imperatives of better vaccines, diagnostics and new drugs for the control of tuberculosis throughout the world.—Author's Abstract

**Fabre, M., Koeck, J. L., Le Fleche, P., Simon, F., Herve, V., Vergnaud, G., and Pourcel, C.** High genetic diversity revealed by variable-number tandem repeat genotyping and analysis of hsp65 gene polymorphism in a large collection of "*Mycobacterium canettii*" strains indicates that the *M. tuberculosis* complex is a recently emerged clone of "*M. canettii*." *J. Clin. Microbiol.* **42(7)** (2004) 3248–3255.

We have analyzed, using complementary molecular methods, the diversity of 43 strains of "*Mycobacterium canettii*" originating from the Republic of Djibouti, on the Horn of Africa, from 1998 to 2003. Genotyping by multiple-locus variable-number tandem repeat analysis shows that all the strains belong to a single but very distant group when compared to strains of the *Mycobacterium tuberculosis* complex (MTBC). Thirty-one strains cluster into one large group with little variability and five strains form another group, whereas the other seven are more diverged. In total, 14 genotypes are observed. The DR locus analysis reveals additional variability, some strains being devoid of a direct repeat locus and others having unique spacers. The hsp65 gene polymorphism was investigated by restriction enzyme analysis and sequencing of PCR amplicons. Four new single nucleotide polymorphisms were discovered. One strain was characterized by three nucleotide changes in

441 bp, creating new restriction enzyme polymorphisms. As no sequence variability was found for hsp65 in the whole MTBC, and as a single point mutation separates *M. tuberculosis* from the closest "*M. canettii*"

strains, this diversity within "*M. canettii*" subspecies strongly suggests that it is the most probable source species of the MTBC rather than just another branch of the MTBC.—Authors' Abstract