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CURRENT LITERATURE

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

General and Historical

Alves Moreira, T. [A panorama of Hansen's disease: present status and perspectives]. Hist. Cienc. Saude Manguinhos. **10(Suppl. 1)** (2003) 291–307.

The interviewee speaks about the endemic disease, which at present contaminates 4.4 out of ten thousand inhabitants in Brazil, the country with the second highest number of patients. When Tadiana speaks about the Brazilian participation in the program launched by the World Health Organization, she explains that WHO's objective is to extinguish Hansen's disease on the planet until 2005. However, she says that Brazilian main goal is to reduce the occurrence of the disease to less than one case per ten thousand people. In our country, the structure and the organization of this program, which comprehends the education and specialization of professionals in order to guarantee early diagnoses, as well as patients' follow-up during treatment, is developed by Sistema Unico de Saude (SUS) and has been implemented in all the states of the federation. The chemotherapy treatment lasts about a year, when taken seriously. In many cases, the disease comes back a while later. Tadiana comments on the differences of the disease according to the different regions of the country. It has been extinguished in the South,whereas the rates in the North and Central East regions almost reach endemic peaks. Working close to patients and ex-patients associations, first as a nurse and later in the implementation of policies for Hansen's disease issues, Tadiana Alves Moreira stresses the importance of early diagnoses, which avoid the physical damages and deformities that take place in the advanced stages of the disease, so reducing the stigma over the diseased.—Author's Abstract

Benchimol, J. L., Sa, M. R., Alves da Cruz, Mde. S., and Magalhaes de Andrade, M. Fight for survival: the life of a Hansen's disease sufferer through his correspondence with Adolpho Lutz. Hist Cienc Saude Manguinhos. **10(Suppl. 1)** (2003) 361–396. [Article in English, Spanish]

This project presents the complete set of letters between the family of a Hansen's disease (leprosy) sufferer in the state of Maranhao, in the Northeast of Brazil, and the doctor and bacteriologist Adolpho Lutz. For more than twenty years Fabricio Caldas de Oliveira and Numa Pires de Oliveira, father and son, exchanged a steady flow of letters with the scientist in pursuit of a cure for the

disease that had assailed Numa since childhood. The 24 letters compiled here paint a unique portrait of the medical and social drama confronted by this family, and the results of the use of chaulmoogra oil and other medications in their search for alternative treatments.—Authors' Abstract

Benchimol, J. L., and Romero Sa, M. Adolpho Lutz and controversies over the transmission of leprosy by mosquitoes. Hist. Cienc. Saude Manguinhos. **10(Suppl. 1)** (2003) 49–93.

During his years of study in Switzerland and Germany, Adolpho Lutz published his first articles on zoology, clinical practice, and therapeutics. In Limeira, Sao Paulo, he began studies on animal and human diseases caused by germs and parasites. In 1885–1886, Lutz traveled to Hamburg to study the morphology of germs related to skin diseases, in conjunction with Paul Gerson Unna, one of Germany's foremost dermatologists. He proposed the inclusion of Hansen's and Koch's bacilli in a new genus. In 1889, Unna nominated his student as physician-in-chief of the Leper Settlement on Molokai Island, Hawaii. From then on, Lutz sustained the theory that the disease was transmitted by mosquitos. He conducted research to prove this theory when he was head of the Instituto Bacteriologico de Sao Paulo (1893–1908) and, later, after he moved to the Instituto Oswaldo Cruz (1908–1940). Although this research was not successful, on commissions and at congresses in which he participated until his death in October 1940, he still held to his conviction that leprosy was transmitted by mosquitoes.—Authors' Abstract

Cueto, M., and de la Puente, J. C. Vida de leprosa: the testimony of a woman living with Hansen's disease in the Peruvian Amazon, 1947. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 337–360. [Article in English, Spanish]

This is the narrative of a patient made before, during and after being incarcerated in an agricultural colony in the Peruvian Amazon. In a vivid style, it narrates the decay of the body, the stigma and the compulsive segregation, as well as the hope for a better life. It is the perspective of a patient, something that is difficult to find when researching the history of health. The original publication was possible thanks to the German physician Maxime H. Kuczynski-Godard who was a German medical doctor that arrived in Peru in the mid 1930s and organized valuable activities in the Peruvian jungle as a part of an effort, which eventually failed, of the Peruvian State to colonize, or really to "civilize" the Amazon.—Authors' Abstract

Levison, J. H. Beyond quarantine: a history of leprosy in Puerto Rico, 1898–1930s. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 225–245.

From biblical times to the modern period, leprosy has been a disease associated with stigma. This mark of disgrace, physically present in the sufferers' sores and disfigured limbs, and embodied in the identity of a "leper," has cast leprosy into the shadows of society. This paper draws on primary sources, written in Spanish, to reconstruct the social history of leprosy in Puerto Rico when the United States annexed this island in 1898. The public health policies that developed over the period of 1898 to the 1930s were unique to Puerto Rico because of the interplay between political events, scientific developments and popular concerns. Puerto Rico was influenced by the United States' priorities for public health, and the leprosy control policies that developed were superimposed on vestiges of the colonial Spanish public health system. During the United States' initial occupation, extreme segregation sacrificed the individual rights and liberties of these patients for the benefit of society. The lives of these leprosy sufferers were irrevocably changed as a result.—Author's Abstract

Manton, J. Global and local contexts: the Northern Ogoja Leprosy Scheme, Nigeria, 1945–1960. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 209–23.

Deriving funding from missionary sources in Ireland, Britain and the USA, and from

international leprosy relief organizations such as the British Empire Leprosy Relief Association (BELRA) and drawing on developing capacities in international public health under the auspices of WHO and UNICEF through the 1950s, the Roman Catholic Mission Ogoja Leprosy Scheme applied international expertise at a local level with ever-increasing success and coverage. This paper supplements the presentation of a successful leprosy control program in missionary narratives with an appreciation of how international medical politics shaped the parameters of success and the development of therapeutic understanding in the late colonial period in Nigeria.—Author's Abstract

Monteiro, Y. N. Prophylaxis and exclusion: compulsory isolation of Hansen's disease patients in Sao Paulo. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 95–121.

This article aims to retrieve the history of Hansen's disease in Brazil, analyzing the medical thinking of the time and the shaping of health policies that permitted the implementation, in Sao Paulo, of a prophylactic policy of compulsory exclusion for all Hansen's disease patients. It also analyzes how the structuring and implementation of this policy led to a "Sao paulo model" that strongly influenced the rest of the country. It addresses the creation of the state's network of leper colonies, their characteristics and the emergence of a veritable "parallel state" that endured until 1967, with complete disregard of all the changes taking place in both national and international prophylactic policymaking.—Author's Abstract

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

There was a village which was called Yunosawa, lots of leprosy patients lived, existed from 1887 to 1941, Kusatu town, Gunnma Prefecture, Japan. It was the only place continued securing self-government to the last as area was free from the isolation policy of State in prewar days there. The aim of this study will make clear the dynamism of "The protection from the tension of the society of leprosy patient currently persecuted" to "The defense of the society from the leprosy patient who is a source of infection." In this study, explained the factor of confusion to a National Leprosarium Kuryu Rakusen-en during World War II and considered relation between patient movement and residents of Yunosawa village at the postwar period.—Authors' Abstract

Obregon, D. The anti-leprosy campaign in Colombia: the rhetoric of hygiene and science, 1920–1940. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 179–207.

Since the 1920's, the medical community realized that the strategy of leprosy control based on segregation and persecution of patients was inefficient and expensive. In the 1930's the new liberal government incorporated leprosy within the general sanitary institutions, by merging the Bureau of Lazarettos and the National Department of Hygiene. The disease-apart approach started to be replaced by a more general public health strategy, which involved controlling other illnesses. Prevention and research played a more influential role, and the new sanitary officials saw leprosy in the light of the economic rationality of expenditures, placing more emphasis on therapies and making them mandatory for all patients. Improvements in leprosy treatment became widely known and available. However, the image of leprosy as a special condition and the practice of segregation were deeply entrenched within the Colombian culture and institutions. The rhetoric changed, but to break with several decades of persecution was a difficult task.—Author's Abstract

Oliveira, M. L., Mendes, C. M., Tardin, R. T., Cunha, M. D., and Arruda, A. Social representation of Hansen's disease thirty years after the term "leprosy" was replaced in Brazil. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 41–48.

Based on the theories of social representation (SC) and Central Core (CC), a structural study was undertaken regarding the neologism hanseniase (Hansen's disease), the term adopted by Brazil's Ministry of Health in the 1970s. Carried out during 2001, this study interviewed eight hundred housewives residing in the Rio de Janeiro and Duque de Caxias municipalities. It found that Hansen's disease is part of a process of modernization of common thinking, anchored in the additional representation of leprosy. This finding is understandable from the perspective that the central structure of a social representation has a historical determination, so short- and middle-term changes are not to be expected. Furthermore, there has been no ongoing investment in social marketing to make the new terminology more widely known. The authors discuss the relation between social representation and the concept of the history of mentalities.—Authors' Abstract

Pandya, S. S. The first international leprosy conference, Berlin, 1897: the politics of segregation. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 161–177.

The present paper examines the first attempts to internationalize the problem of leprosy, a subject hitherto overlooked by historians of imperialism and disease. The last decade of the nineteenth century saw many in the "civilized countries" of the imperialist West gripped by a paranoia about an invasion of leprosy via germ-laden immigrants and returning expatriates who had acquired the infection in leprosy-endemic colonial possessions. Such alarmists clamoured for the adoption of vigorous leper segregation policies in such colonies. But the contagiousness of leprosy did not go unquestioned by other westerners. The convocation in Berlin of the first international meeting on leprosy revealed the interplay of differing and sometimes incompatible views about the containment of leprosy by segregation. The roles of officials from several countries, as well as the roles of five protagonists (Albert Ashmead, Jules Goldschmidt, Edvard Ehlers, Armauer Hansen, and Phineas Abraham) in the shaping of the Berlin Conference are here examined.—Author's Abstract

Porter, J. D., Ogden, J. A., Rao, P. V., Rao, V. P., Rajesh, D., Buskade, R. A., and Soutar, D. Introducing operations research into management and policy practices of a non-governmental organization (NGO): a partnership between an Indian leprosy NGO and an international academic institution. Health Policy Plan. **19(2)** (2004) 80–87.

This paper reports on a partnership between LEPRA, a non-governmental organization (NGO), and the London School of Hygiene and Tropical Medicine (LSHTM) to explore the feasibility and appropriateness of incorporating operations research into the management and decision-making of a leprosy NGO. A pilot study in Orissa was used to determine the advantages and disadvantages of introducing operations research to assist in decision-making and programme implementation within the organization. The results highlight the difficulty and complexity of the process, but point to several important themes: partnership, changing perspectives, use of time and priority-setting, identification of gaps in systems, and building institutional and personal capabilities. The results of the study provide support to encourage NGOs to become actively involved in research. Because of their work and service to local communities, NGOs have the opportunity to collect information about the perceptions, resources and constraints of individuals, families and the communities themselves in accessing appropriate care. Their proximity to communities gives them a feeling of responsibility for ensuring that this information is translated to the district, national and ultimately international level. This will help to ensure the creation of appropriate infectious disease control policies that support the needs of patients. "Outside" academic institutions can help NGOs to facilitate this up-stream flow of information from the local to the national and international level, to help to ensure that international disease control policies are appropriately serving local communities.—Authors' Abstract

Robertson, J. The papers of Stanley Browne: leprologist and medical missionary (1907–1986). Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 427–433.

This article elaborates a significant archival acquisition that supplement the collection documents related to the life and work of Stanley George Browne held at the Wellcome Library for the History and Understanding of Medicine in London, specifically his work in the Belgian Congo (from 1936 to 1959), at Uzuakoli in Nigeria (1959 to 1966), in London with the Leprosy Study Centre (1966–1980), and also in his international capacity as leprosy consultant. It also briefly refers to an endangered collection of documents, photographs, files and correspondence held in a small museum in Culion Sanatorium, The Philippines. This research is part of the International Leprosy Association Global Project on the History of Leprosy. Its results can be accessed at the site http://www.leprosyhistory.org.—Author's Abstract

Robertson, J. Leprosy and the elusive *M. leprae*: colonial and Imperial medical exchanges in the nineteenth century. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 13–40.

In the 1800's, humoral understandings of leprosy successively give way to disease models based on morbid anatomy, physiopathology, and bacteriology. Linkages between these disease models were reinforced by the ubiquitous seed/soil metaphor deployed both before and after the identification of *M. leprae*. While this metaphor provided a continuous link between medical descriptions, Henry Vandyke Carter's *On Leprosy* (1874) marks a convergence of different models of disease. Simultaneously, this metaphor can be traced in popular medical debates in the late nineteenth century, accompanying fears of a resurgence of leprosy in Europe. Later the mapping of the genome ushers in a new model of disease but, ironically, while leprosy research draws its logic from a view of the world in which a seed and soil metaphor expresses many different aspects of the activity of the disease, the bacillus itself continues to be unreceptive to cultivation.—Author's Abstract

Rosa Maciel, L., Oliveira, M. L., Gallo, M. E., and Damasco, M. S. Memories and history of Hansen's disease in Brazil told by witnesses (1960–2000). Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 308–335.

This report is a preliminary result of a survey on memories and history of Hansen's disease, or 'hanseniasis,' prepared by the Fundacao Oswaldo Cruz (Fiocruz) and the Universidade Federal do Rio de Janeiro (UFRJ) using statements from those who have been afflicted by the disease or those that have fought against it. It outlines the methodology used by the authors and gives a succinct history of Hansen's disease in Brazil, together wish information on the stage of the survey with extracts from our archives of statements. The founding and the role of Movement for the Reintegration of People Afflicted by Hansen's Disease (Morhan) are explained in the testimony of Thomas Frist, a social scientist who worked in Brazil in the 1970s and 1980s, when the country's old colonies were being restructured, and Cristiano Torres, a former patient who spent time in prevention centers and leproseries in Para state and who is now active in proposing new policy for the control of Hansen's disease.—Authors' Abstract

Santos, V. S. [Researching documents on the history of hansen's disease in Brazil]. Hist. Cienc. Saude Manguinhos. **10(Suppl 1)** (2003) 415–426. [Article in Spanish]

This article corresponds to part of the results of a research on leprosy-related sources developed in several institutions in the city of Rio de Janeiro. At Real Gabinete Portugues de Leitura, Arquivo Nacional and Biblioteca Nacional, banks, indexes, official documents and photos on the administration of leprosaria and articles on the treatment of the disease have been investigated. At Centro de Pesquisa e Documentacao de Historia Contemporanea do Brazil (CPDOC-FGV), several files have been researched, mainly those with information on the health policies of the first Vargas administration (1930–1945). This research is part of the International Leprosy Association Global Project on the History of Leprosy. Its results can be accessed at the site http://www.leprosyhistory.org—Author's Abstract

Smith, T. H. A monument to Lazarus: the leprosy hospital of Rio de Janeiro. Hist. Cienc. Saude Manguinhos **10(Suppl 1)** (2003) 143–160.

Soon after the Portuguese made landfall in 1500, Europeans and, later, African slaves introduced leprosy, and Saint Lazarus, the patron saint of its victims, into Brazil. Social and political pressure mounted by the middle of the eighteenth century in the city of Rio de Janeiro to remove those unfortunates from the city's streets even before the move of Brazil's capital in 1763. Frei Antoniom the bishop of Rio, founded the venerable hospital that year in the neighborhood of Sao Cristovao, He requested that the Irmandade do Santissimo Sacramento da Candelaria provide oversight and administration. The brotherhood continues to honor its covenant of 239 years ago. The history of this hospital provides insight into the complex relationships that existed between the citizenry and church and state. Rio's leprosy hospital, now the Hospital Frei Antonio, had an important role in the evolution of the health care professions, progress in medical science, and the genesis of the hygienic movement in Brazil. This study also contributes to the history of a disease that persists in 2002 Brazil as a public health issue.—Author's Abstract

Staples, J. Delineating disease: self-management of leprosy identities in South India. Med. Anthropol. **23(1)** (2004) 69–88.

The national and international agencies working to eliminate leprosy are also dominant in setting the boundaries of official discourse on the issue. Within these boundaries the disease is commonly represented as a medical problem with negative social consequences, and it is believed that both problem and consequences will be resolved if leprosy is eliminated and its victims treated and (if necessary) reintegrated within their social groups. For those affected by leprosy the issues are frequently different, elimination in some respects representing a problem as much as a solution. Against this background, which I describe with reference to a group of leprosy-affected people in South India and their position vis-à-vis leprosy organizations, I explore some of the contexts in which leprosy patients actively manage their own situations, often in defiance of prevailing development orthodoxies. I conclude that closer observation and analysis of the strategies patients use to manage their disease status have important policy implications.—Author's Abstract

Taylor, G. M., Stewart, G. R., Cooke, M., Chaplin, S., Ladva, S., Kirkup, J., Palmer, S., and Young, D. B. Koch's bacillus—a look at the first isolate *of Mycobacterium tuberculosis* from a modern perspective. Microbiology **149(Pt 11)** (2003) 3213–3220.

Using molecular methods the authors have studied mycobacterial DNA taken from a 19th century victim of tuberculosis. This was the case from which Robert Koch first isolated and cultured the organism responsible for tuberculosis. The mycobacteria were preserved within five glass culture tubes as abundant bacterial colonies on slopes of a gelatinous culture medium of unknown composition. Originally presented by Koch to surgical laryngologist Walter Jobson Horne in London in 1901, the relic has, since 1983, been in the care of the Royal College of Surgeons of England. Light and electron microscopy established the presence of acid-fast mycobacteria but showed that morphological preservation was generally poor. Eleven different genomic loci were successfully amplified by PCR. This series of experiments confirmed that the organisms were indeed *Mycobacterium tuberculosis* and further showed that the original strain was in evolutionary terms similar to 'modern' isolates, having undergone the TB D1 deletion. Attempts to determine the genotypic group of the isolate were only partially successful, due in part to the degraded nature of the DNA and possibly also to a truncation in the katG gene, which formed part of the classification scheme. Spoligotyping resulted in amplification of DR spacers consistent with *M. tuberculosis* but with discrepancies between independent extracts, stressing the limitations of this typing method when applied to poorly preserved material.—Authors' Abstract

Zhang, L. Leprosy in China. Nihon Hansenbyo Gakkai Zasshi. **72(3)** (2003) 209–215.

Leprosy has definitely been present in China for at least 2000 years. Through painstaking efforts over the past half century, China has put leprosy under control and reached the WHO's target at elimination of leprosy at the national and subnational level. But difficulties as well as problems like disabilities, discrimination, drug-resistance and dismissing of research still remain in the control of leprosy. Highly attention should be continuously paid on to attain the prevalence rate of less than 0.1/10,000 and the incidence rate below 0.5/100,000 in all counties (cities) throughout the country by the year 2010.—Author's Abstract

Chemotherapy

Ashitani, J., Yanagi, S., Arimura, Y., Sano, A., and Mukae, H. Acute respiratory distress syndrome induced by rifampicin with high levels of neutrophil and eosinophil products in bronchoalveolar lavage fluid. Respiration **70(5)** (2003) 541–543.

We reported a case with acute respiratory distress syndrome (ARDS) caused by rifampicin during therapy for pulmonary tuberculosis. A high level of eosinophil cationic protein in bronchoalveolar lavage fluid (BALF) was detected as well as interleukin-8 and neutrophil elastase. Based on these results together with the positive result of the drug lymphocyte-stimulating test, we concluded that rifampicin was the causative drug leading to ARDS. Corticosteroid therapy resulted in clinical improvement and resolution of the pulmonary infiltrates on the chest radiograph without the recurrence of pulmonary tuberculosis.—Authors' Abstract

Brown, C. W., Liu, S., Klucik, J., Berlin, K. D., Brennan, P. J., Kaur, D., and Benbrook, D. M. Novel heteroarotinoids as potential antagonists of *Mycobacterium bovis* BCG. J. Med. Chem. **47(4)** (2004) 1008–1017.

A series of 15 heteroarotinoids has been prepared and evaluated for activity against *Mycobacterium bovis* BCG with the thiourea-containing isoxyl (7) (0.5 microg/mL) as the standard. 2,2,4-Trimethyl-2H-chromen-7-yl 4-(methoxycarbonyl)benzoate (8) displayed the most significant activity (2.0–4.0 microg/mL) in terms of the lowest concentration (microg/mL) (MIC, minimum inhibitory concentration) required to produce a 99% reduction in the number of colonies on a plate as compared to that system free of the agent at the same dilution of the culture suspension. Ethyl 4-[[N-(2,2,4,4-tetramethylchroman-6-yl)thiocarbamoyl]amino] benzoate (9) and [[(1E,3Z,5E)-1-aza-4-methyl-6-(1,2,2,4-tetramethyl(1,2-dihydroquinolyl))hexa-1,3,5-trienyl]amino]aminomethane-1-thione (10) exhibited activity at 5.0–10.0 and 10.0–20.0 microg/mL, respectively, while the other examples had MIC values of 20 microg/mL or greater. The inhibitory ability of 8 may occur via the inhibition of mycolic acid synthesis in a like manner as found with 7, but this requires further study. The heteroarotinoids are the first examples to exhibit inhibitory ability against the growth of *Mycobacterium bovis* BCB.—Authors' Abstract

Bucaretchi, F., Miglioli, L., Baracat, E. C., Madureira, P. R., Capitani, E. M., and Vieira, R. J. [Acute dapsone exposure and methemoglobinemia in children: treatment with multiple doses of activated charcoal with or without the administration of methylene blue]. J. Pediatr. (Rio J). **76(4)** (2000) 290–294.

OBJECTIVE: To study the changes in methemoglobinemia of 17 children admitted with acute exposure to dapsone complicated by a methemoglobin concentration greater than 20% of the total hemoglobin. The children were treated with multiple doses of activated charcoal with or without the administration of methylene blue. PATIENTS AND METHODS: Seventeen patients (ages 1–13 yrs, median 3 yrs), were admitted 1–72 hr after the ingestion of 100–1200 mg (median 350 mg, 10 patients) or an unknown amount of dapsone

(7 patients). The methemoglobin blood concentrations upon admission ranged from 23.5%–49.7% (median 37.8%), and the main clinical features were cyanosis (17), tachycardia (17), vomiting (11) and tachypnea (8). All of the children received multiple doses of activated charcoal orally or via nasogastric tube (1g/kg, 10% solution, 4–6 times/day, 3–16 doses with a median of 8 doses). Twelve of the 14 patients with methemoglobin levels greater than 30% were also treated with a single dose of methylene blue (1–2% solution, 1–2 mg/kg) infused IV over 5 min. RESULTS: There was a progressive decrease in the methemoglobin levels after the beginning of both treatments (multiple doses of activated charcoal alone or associated with methylene blue), and only one dose of methylene blue was necessary. There were no significant statistical differences between the results of the two treatments according to the time-course decrease in methemoglobinemia ($p = 0.49$ Wilcoxon test). CONCLUSIONS: Multiple doses of activated charcoal given when methemoglobin levels were greater than 20% can be considered as a possible treatment for pediatric patients, with or without the administration of methylene blue, after acute dapsone exposure.—Authors' Abstract

De Carsalade, G. Y., Achirafi, A., and Flageul, B. Pentoxifylline in the treatment of Erythema Nodosum Leprosum: Results of an open study. Acta Leprologica **12(3)** 117–122.

Erythema nodosum leprosum (ENL) is a well-known immunological serious complication affecting lepromatous multibacillary leprosy patients. For a long time, ENL has been regarded as an immune complex-mediated disease or Arthus phenomenon. Recently, it has been reported that ENL was associated with high serum tumor necrosis factor-alpha (TNFα) levels, suggesting that this cytokine could also play a central role in the manifestations of ENL. Thalidomide (TH) and systemic steroids (S), both TNFα production inhibitors, are the two current effective drugs for the management of ENL. However, TH is rarely available in leprosy endemic countries, and its teratogenicity and neurotoxicity strongly limit its use. Moreover, the morbidity of S and the frequent steroid-dependence of ENL also create real therapeutic problems. Recently, the efficacy of pentoxifylline (PTX), which also inhibits *in vitro* and *in vivo* production of TNFα, has been suggested for ENL treatment. We report our experience on its use for the treatment of 15 leprosy patients suffering from a first ENL attack (11 cases), a chronic steroid-dependent ENL (3 cases) or chronic steroid- and thalidomide-dependent ENL (1 case). PTX has been given at 800 mg t.i.d. (2 cases), or 400 mg t.i.d. (13 cases) doses. The patients received PTX at the initiating dosage until complete clinical cure. At the end of ENL attacks, PTX was either abruptly stopped or tapered down over the next 4 months. In ten of 11 patients who developed ENL for the first time, the systemic symptoms and neuritic pains disappeared within one week; at three weeks, half of the patients were cured and the other half had striking clinical improvement; complete cure was obtained within 7 to 35 days (mean: 27 days). A relapse occurred within 2–3 months in the 5 patients in which PTX was abruptly stopped. In contrast, no relapse occurred in the patients who benefited from decreasing doses of PTX. Recurrent ENL episodes also responded well to PTX. The 3 patients who had chronic steroid-dependent ENL failed to show any improvement after 3 to 6 weeks of PTX. In contrast, steroid therapy could be stopped in the steroid- and thalidomide-dependent patient. Our results confirm the action of PTX if it is slowly tapered down (4 months seem sufficient) and not abruptly to avoid relapses. As it is safe use, PTX could constitute the first line of ENL attack treatment.—Acta Leprologica

Eriksson, T., Hoglund, P., Turesson, I., Waage, A., Don, B. R., Vu, J., Scheffler, M., and Kaysen, G. A. Pharmacokinetics of thalidomide in patients with impaired renal function and while on and off dialysis. J. Pharm. Pharmacol. **55(12)** (2003) 1701–1706.

There is a renewed interest in thalidomide for use in malignancies and systemic inflammatory diseases. Reduced renal function is not uncommon among patients with these disease states but the pharmacokinetics has not been fully investigated. The aim

of this study was to investigate the pharmacokinetics of thalidomide in haemodialysis patients while on and off dialysis and in myeloma patients with varying degrees of renal function. Two studies were performed. To establish the pharmacokinetics of thalidomide in patients with mild to moderate renal failure, blood samples were taken over 12 weeks from 40 patients with multiple myeloma. A second study was performed in six patients with end-stage renal disease both on a non-dialysis day and before and during a haemodialysis session. Thalidomide concentration was determined by HPLC. A one-compartment open model with first-order absorption and elimination was used to fit total thalidomide concentration to population pharmacokinetics and statistical models using the NONMEM program. Clearance and volumes were slightly below 10 L h-1 and 1 L kg-1, respectively, in both patient groups. The inter- and intrapatient variability was low. Clearance was doubled during dialysis. There was no correlation between thalidomide clearance and renal function. In conclusion, the pharmacokinetics of thalidomide in patients with renal failure are very similar to values reported by others for patients with normal renal function. Although clearance during dialysis is doubled, thalidomide dose need not be changed for patients with decreased kidney function. There is also no need for a supplementary dose due to haemodialysis.—Authors' Abstract

Foroumadi, A., and Soltani, F. Antituberculosis agents. IX. *In vitro* anti-mycobacterial activity of N-(2-phenyl-2-oxoethyl) and N-[2-(4-fluorophenyl)-2-oxoethyl]-ciprofloxacin derivatives against some drug-resistant strains of *Mycobacterium tuberculosis* and *Mycobacterium avium* isolates. Boll. Chim. Farm. **142(6)** (2003) 248–250.

See Current Literature, Other Mycobacterial Diseases, p. 247.

Garcia-Garcia, A., Galvez, J., de Julian-Ortiz, J. V., Garcia-Domenech, R., Munoz, C., Guna, R., and Borras, R. New agents active against *Mycobacterium avium* complex selected by molecular topology: a virtual screening method. J. Antimicrob. Chemother. **53(1)** (2004) 65–73.

OBJECTIVES: In order to select new drugs and to predict their *in vitro* activity against *Mycobacterium avium* complex (MAC), new quantitative structure-activity relationship (QSAR) models were developed. METHODS: The activities against MAC of 29 structurally heterogeneous drugs were examined by means of linear discriminant analysis (LDA) and multilinear regression analysis (MLRA) by using topological indices (TI) as structural descriptors. *In vitro* antimycobacterial activities were determined by a broth microdilution method with 7H9 medium. RESULTS: The topological model obtained successfully classifies over 80% of compounds as active or inactive; consequently, it was applied in the search for new molecules active against MAC. From among the selected candidates demonstrating *in vitro* activity, aflatoxin B1, benzalkonium chloride and pentamidine stand out, with MIC50s between 4 and 32 mg/L. CONCLUSION: The method described in this work is able to select molecules active against MAC.—Authors' Abstract

Hansen, J. M., and Harris, C. A novel hypothesis for thalidomide-induced limb teratogenesis: redox misregulation of the NF-kappaB pathway. Antioxid. Redox Signal **6(1)** (2004) 1–14.

Several hypotheses have been proposed to explain the mechanisms of thalidomide teratogenesis, although none adequately accounts for the observed malformations and explains the basis for species specificity. Recent observations that thalidomide increases the production of free radicals and elicits oxidative stress, coupled with new insights into the redox regulation of nuclear transcription factors, lead to the suggestion that thalidomide may act through redox misregulation of the limb outgrowth pathways. Oxidative stress, as marked by glutathione depletion/oxidation and a shift in intracellular redox potential toward the positive, occurs preferentially in limbs of thalidomide-

sensitive rabbits, but not in resistant rats. DNA binding of nuclear factor kappa-B (NF-kappaB), a redox-sensitive transcription factor and key regulator of limb outgrowth, was shown to be significantly attenuated in rabbit limb cells and could be restored following the addition of a free radical spin-trapping agent, phenyl N-tert-butyl nitrone. The inability of NF-kappaB to bind to its DNA promoter results in the failure of limb cells to express fibroblast growth factor (FGF)-10 and twist in the limb progress zone (PZ) mesenchyme, which in turn attenuates expression of FGF-8 in the apical ectodermal ridge (AER). Failure to establish an FGF-10/FGF-8 feedback loop between the PZ and AER results in the truncation of limb outgrowth. We hypothesize that species-selective alterations in redox microenvironment caused by free radical production from thalidomide results in attenuation of the NF-kappaB-mediated gene expression that is responsible for limb outgrowth.—Authors' Abstract

Li Li, and Xue-jun Zhu. Dapsone Hypersensitivity Syndrome. J. Clin. Derm. **32(Suppl.)** (2003) s115–s117.

A 23-year-old woman with linear IgA dermatosis developed dapsone hypersensitivity syndrome (DHS) after initiation of dapsone therapy. She had fever, jaundice with hepatic dysfunction, lymphadenopathy, anemia and dermatitis. The symptoms disappeared with methylprednisolone treatment 40 mg/day.—Journal of Clinical Dermatology

Lu, K. Q., Brenneman, S., Burns, R. Jr., Vink, A., Gaines, E., Haake, A., and Gaspari, A. Thalidomide inhibits UVB-induced mouse keratinocyte apoptosis by both TNF-alpha-dependent and TNF-alpha-independent pathways. Photodermatol Photoimmunol Photomed. **19(6)** (2003) 272–280.

BACKGROUND: Thalidomide is an anti-inflammatory pharmacologic agent that has been utilized as a therapy for a number of dermatologic diseases. Its anti-inflammatory properties have been attributed to its ability to antagonize tumor necrosis factor-alpha (TNF-alpha) production by monocytes. However, its mechanism of action in the skin is not known. PURPOSE: To test our hypothesis that thalidomide may antagonize TNF-alpha production in the skin, we used a mouse model for acute ultraviolet-B (UVB) exposure, a known stimulus for inducing this cytokine. RESULTS: A single bolus dose of thalidomide (either 100 or 400 mg/kg) given immediately before UVB exposure (40–120 mJ/cm^2) inhibited, in a dose-dependent manner, sunburn cell formation (i.e., keratinocyte (KC) apoptosis as defined by histologic appearance and confirmed by terminal transferase mediated biotinylated dUTP nick end labelling staining) in mouse skin biopsy specimens. However, this agent did not affect the formation of cyclobutane pyrimidine dimers, a measure of UVB-induced DNA damage, which is an early event associated with apoptosis. RNase protection assays confirmed that high (400 mg/kg), but not low (100 mg/kg), doses of thalidomide inhibited the UVB-induced increase in steady-state TNF-alpha mRNA. Additionally, our *in vitro* data using neonatal mouse KCs showed that thalidomide prevented UVB-induced cell death (JAM assay). The anti-apoptotic effects of thalidomide can be reversed by the addition of exogenous recombinant mouse TNF-alpha and hence reconstituting UVB-induced programmed cell death. The inhibition of sunburn cell formation by low-dose thalidomide in the absence of TNF-alpha inhibition suggests that other, unidentified mechanisms of apoptosis inhibition are active. CONCLUSIONS: These data suggest that the anti-inflammatory effects of thalidomide can affect UVB injury, and may, in part, explain its action in photosensitivity diseases such as cutaneous lupus erythematosus.—Authors' Abstract

Lu, T., and Drlica, K. *In vitro* activity of C-8-methoxy fluoroquinolones against mycobacteria when combined with anti-tuberculosis agents. J. Antimicrob. Chemother. **52(6)** (2003) 1025–1028.

OBJECTIVES: To examine the effect of first-line and second-line anti-tuberculosis agents on the ability of fluoroquinolones to

kill mycobacteria. METHODS: A clinical isolate of *Mycobacterium tuberculosis* and a laboratory strain of *Mycobacterium smegmatis* were grown in liquid medium and treated with a fluoroquinolone in the presence or absence of anti-tuberculosis agents. Bacterial survival was determined by viable colony counts on agar medium. RESULTS: When moxifloxacin activity was examined in two-drug combinations containing traditional anti-tuberculosis agents, activity was greater than either compound alone with isoniazid, capreomycin and low, but not high, concentrations of rifampicin. Cycloserine contributed no additional activity, and ethambutol interfered with the lethal action of moxifloxacin and gatifloxacin. Experiments with *M. smegmatis* confirmed that both rifampicin and ethambutol reduce fluoroquinolone lethality. Moreover, ethambutol increased the recovery of fluoroquinolone-resistant mutants newly created by ethyl methanesulphonate treatment. CONCLUSIONS: The intrinsic bactericidal activity of C-8-methoxy fluoroquinolones can be adversely affected by some agents currently used for treatment of tuberculosis.—Authors' Abstract

Pandey, R., Zahoor, A., Sharma, S., and Khuller, G. K. Nanoparticle encapsulated antitubercular drugs as a potential oral drug delivery system against murine tuberculosis. Tuberculosis (Edinb). **83(6)** (2003) 373–378.

Patient non-compliance is the major drawback associated with the long-duration chemotherapy of tuberculosis (TB); hence, reduction in dosing frequency forms an important therapeutic strategy. The present study reports the formulation of three frontline antitubercular drugs (ATD), i.e., rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA) encapsulated in poly (DL-lactide-co-glycolide) (PLG) nanoparticles. Drug encapsulation efficiencies were 56.9 ± 2.7% for RIF, 66.3 ± 5.8% for INH and 68 ± 5.6% for PZA. Following a single oral administration of these preparations to mice, the drugs could be detected in the circulation for 6 days (RIF) and 9 days (INH/PZA), whereas therapeutic concentrations in the tissues were maintained for 9–11 days. Further, on oral administration of drug-loaded nanoparticles to *Mycobacterium tuberculosis*-infected mice at every 10th day, no tubercle bacilli could be detected in the tissues after 5 oral doses of treatment. Therefore, nanoparticle-based ATD therapy forms a sound basis for reduction in dosing frequency for better management of TB.—Authors' Abstract

Selvaraj, P., Chandra, G., Kurian, S. M., Reetha, A. M., and Narayanan, P. R. Association of Vitamin D receptor gene variants of *Bsm*I, *Apa*I, and *Fok*I polymorphisms with susceptability or resistance to pulmonary tuberculosis. Curr. Sci. **84(12)** (2003) 1564–1568.

Zhu, X., Giordano, T., Yu, Q. S., Holloway, H. W., Perry, T. A., Lahiri, D. K., Brossi, A., and Greig, N. H. Thiothalidomides: novel isosteric analogues of thalidomide with enhanced TNF-alpha inhibitory activity. J. Med.Chem. **46(24)** (2003) 5222–5229.

Thalidomide is being increasingly used in the clinical management of a wide spectrum of immunologically-mediated and infectious diseases, and cancers. However, the mechanisms underlying its pharmacological action are still under investigation. In this regard, oral thalidomide is clinically valuable in the treatment of erythema nodosum leprosum (ENL) and multiple myeloma and effectively reduces tumor necrosis factor-alpha (TNF-alpha) levels and angiogenesis *in vivo*. This contrasts with its relatively weak effects on TNF-alpha and angiogenesis in *in vitro* studies and implies that active metabolites contribute to its *in vivo* pharmacologic action and that specific analogues would be endowed with potent activity. Our focus in the structural modification of thalidomide is toward the discovery of novel isosteric active analogues. In this regard, a series of thiothalidomides and analogues were synthesized and evaluated for their TNF-alpha inhibitory activity against lipopolysacharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC), This was combined with a PBMC viability assay to differentiate reductions in TNF-alpha secretion from cellular toxicity. Two isosteric

analogues of thalidomide, compounds 15 and 16, that mostly reflect the parent compound, together with the simple structure, dithioglutarimide 19, potently inhibited TNF-alpha secretion, compared to thalidomide, 1. The mechanism underpinning this most likely is posttranscriptional, as each of these compounds decreased TNF-alpha mRNA stability via its 3′-UTR. The potency of 19 warrants further study and suggests that replacement of the amide carbonyl with a thiocarbonyl may be beneficial for increased TNF-alpha inhibitory action. In addition, an intact phthalimido moiety appeared to be requisite for TNF-alpha inhibitory activity.—Authors' Abstract

Clinical Sciences

Alioua, Z., Sbai, M., Elhaouri, M., Boudi, O., Ghfir, M., Benomar, S., and Sedrati, O. Histoid leprosy with erythema nodosum leprosum. Acta Leprologica **12(3)** (2004) 107–111.

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

Bartt, R. E. Leprosy (Hansen's Disease). Curr. Treat Options Neurol. **6(2)** (2004) 95–103.

Leprosy (Hansen's disease) causes the most common treatable form of neuropathy in the world. Several endemic countries account for the majority of the world's cases and most of the cases seen in the US are amongst immigrants. However, endemic cases of leprosy occur in the US. The pathogen is *Mycobacterium leprae*, a slow-growing, obligate intracellular pathogen that consistently infects skin and peripheral nerves. The clinical appearance of the skin and neurologic deficits develop months to years after infection and are determined by the host's response to the infection. An individual's disease classification can change over time based on the immune status of the individual. Immune-mediated "reactional states" may also occur that require additional recognition and treatment. Varied in its manifestations, a successful treatment approach relies on proper recognition and classification of disease.—Author's Abstract

Beyene, D., Aseffa, A., Harboe, M., Kidane, D., Macdonald, M., Klatser, P. R., Bjune, G. A., and Smith, W. C. Nasal carriage of *Mycobacterium leprae* DNA in healthy individuals in Lega Robi village, Ethiopia. Epidemiol. Infect. **131(2)** (2003) 841–848.

The number of registered leprosy patients world-wide has decreased dramatically after extensive application of WHO recommended Multiple Drug Therapy (MDT). The annual number of new cases has, however, been almost unchanged in several populations, indicating that the infection is still present at community level. Nasal carriage of *Mycobacterium leprae* DNA was studied in Lega Robi village in Ethiopia. MDT had been applied for more than ten years, and 718 residents over 5 years old were eligible for the study. During the first survey nasal swab samples were collected from 664 (92.5%) individuals. The results of a Peptide Nucleic Acid-ELISA test for *M. leprae* DNA interpreted by stringent statistical criteria were available for 589 (88.7%) subjects. Thirty-five (5.9%) individuals without clinical signs of leprosy were positive for *M. leprae* DNA. Seven PCR positive individuals lived in a household where one or two other members were also positive for *M. leprae* DNA. During a second survey 8 (46%) of 175 interpretable PNA-ELISA tests were positive. Of 137 individuals tested twice, only two were positive on both occasions whereas 10 were

PCR positive only once. The study confirms the widespread distribution of *M. leprae* DNA in healthy individuals. The feasibility of curbing possible transmission of subclinical infection needs further consideration.

Cortes, S. L., and Rodriguez, 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 transmission in the community and the efficiency of control programmes. 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 percent). Forty-seven percent of children were 10 years old or more; 28.5 percent shared their dwellings with leprosy patients; 35 percent had only one lesion, and 43 percent were multibacillary cases. Agreement between clinical and histopathological classification was 36 percent; hypochromic chronic eczema and post-inflammatory incontinence of melanin pigment were the clinical lesions most frequently mistaken with leprosy. Leprosy among children represents 7 percent 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.—Authors' Abstract

Doffinger, R., Helbert, M. R., Barcenas-Morales, G., Yang, K., Dupuis, S., Ceron-Gutierrez, L., Espitia-Pinzon, C., Barnes, N., Bothamley, G., Casanova, J. L., Longhurst, H. J., and Kumararatne, D. S. Autoantibodies to interferon-gamma in a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity. Clin. Infect. Dis. 3**8(1)** (2004) e10–4.

We evaluated a patient with disseminated *Mycobacterium tuberculosis* and *Mycobacterium chelonae* infection, of which he died. He also developed autoimmune (type I) diabetes and primary hypothyroidism. His serum contained a high titer of immunoglobulin G autoantibody to interferon-gamma (IFN-gamma) capable of blocking *in vitro* responses to this cytokine by peripheral blood mononuclear cells from normal donors. These results suggest that autoantibodies to IFN-gamma can induce susceptibility to disseminated mycobacterial infection, which may be refractory to chemotherapy.—Authors' Abstract

Fenniche, S., Ben Jennet, S., Marrak, H., Khayat, O., Zghal, M., Ben Ayed, M., and Mokhtar, I. [Cutaneous tuberculosis: anatomoclinical features and clinical course (26 cases)]. Ann. Dermatol. Venereol. **130(11)** (2003) 1021–1024. [Article in French]

INTRODUCTION: Despite prevention programs, tuberculosis is still progressing endemically in developing countries. The prevalence of cutaneous tuberculosis is estimated as 2.1 p. 100 and represents a rare localization among the extra-pulmonary forms. In order to study the epidemiology, the most frequent anatomoclinical forms and the progressive features of cutaneous tuberculosis, we conducted a study in the area of Tunis over a 20-year period. PATIENTS AND METHODS: All cases of cutaneous tuberculosis observed between 1981 and 2000 in the dermatology department of the Habib Thameur hospital were included in a retrospective study. Diagnosis of cutaneous tuberculosis was challenging and required the correlation of clinical, biological and progressive features. RESULTS: Twenty-six patients were observed in the study. There were 12 men and 14 women with a mean age of 30.4 years (range: 6 to 74) and 20 p. 100 of infantile cases. Of the various patterns of cutaneous tuberculosis seen, 11 (42 p. 100) had lupus tuberculosis, 10 (38 p. 100) had scrofuloderma, 4 (15 p. 100) had tuberculosis verrucosa cutis and 1 child had a perianal tubercular ulcer. The Mantoux test was positive in 20/24 patients. Histological tuberculoid granuloma was seen in 25 cases

(96 p. 100) associated with caseating necrosis in 10 cases (38 p. 100). All patients were treated successfully with triple or quadruple anti-tubercular drugs for 6 to 10 months. One patient exhibited a squamous cell carcinoma on a lupus tuberculosis scar four years later. DISCUSSION: The progression of cutaneous tuberculosis remains stable, ranging from 1.4 cases/year between 1981 and 1990 to 1.2 cases/year between 1991 and 2000. In our study, females were slightly more affected than men with a M/F sex ration of 0.86. Before 1984, scrofuloderma was the most frequent form among the cutaneous tuberculoses. Now the frequency of lupus tuberculosis has reached that of scrofuloderma, demonstrating the increase in the incidence of clinical pattern of cutaneous tuberculosis with strong immunity probably related to the improvement in health conditions and generalization of vaccination programs.—Authors' Abstract

Flageul, B. *Prise en charge médicale actuelle de la maladie de Hansen.* Bull. Soc. Pathol. Exot. **96(5)** (2003) 357–360. [Article in French]

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

Jardim, M. R., Antunes, S. L. G., Santos, A. R., Nascimento, O. J. M., Nery, J. A. C., Sales, A. M., Illarramendi, X., Duppre, N., Chimelli, L., Sampio, E. P., Sarno, E. P. N. Criteria for diagnosis of pure neural leprosy. J. Neurol. **250(7)** (2003) 806–809.—Tropical Disease Bulletin

The clinical diagnosis of pure neural leprosy (PNL) remains a public health care problem mainly because skin lesions—the cardinal features of leprosy—are always absent. Moreover, the identification of the leprosy bacillus is not easily achieved even when a nerve biopsy can be performed. In an attempt to reach a reliable PNL diagnosis in patients referred to our Leprosy Outpatient Clinic, this study employed a variety of criteria. The nerve biopsies performed on the 67 individuals whose clinical, neurological, and electrophysiological examination findings strongly suggested peripheral neuropathy were submitted to *M. leprae* identification via a polymerase chain reaction (PCR). Mononeuropathy multiplex was the most frequent clinical and electrophysiological pattern of nerve dysfunction, while sensory impairment occurred in 89% of all cases and motor dysfunction in 81%. Axonal neuropathy was the most prominent electrophysiological finding, while the histopathological nerve study showed epithelioid granuloma in 14% of the patients, acid fast bacilli in 16%, and nonspecific inflammatory infiltrate and/or fibrosis in 39%. PCR for *M. leprae* was positive in 47% of the nerve biopsy samples (n =23). PCR, in conjunction with clinical and neurological examination results, can be a powerful tool in attempting to identify and confirm a PNL diagnosis.—Tropical Disease Bulletin

Matsuo, E. [The sequelae of Hansen's disease. (Pathologic viewpoint of etiologies,

morphologies and countermeasures)]. Nihon Hansenbyo Gakkai Zasshi **72(3)** (2003) 251–257. [Article in Japanese]

The proportion of glomerulonephritis, often a sequence of arteriolitis, among the sequelae of Hansen's disease after the introduction of chemotherapy increased markedly in Japan and nullified that of once prevalent tuberculosis after 1960s. However, most significant aftermath of the disease for numbers of years in the past have been peripheral nerve injuries worldwide for which effective countermeasures are yet to be developed. In this brief autopsy cases study from 1960s to 1990s, we confirmed the presence of cases in which arteriolitis and resulted infarction of peripheral nerves and not *M. leprae* itself were shown to be the major cause of axonal damages. There were also cases in which the accumulation of the bacilli without vascular changes did not damage the axons. The cases as these could not be solitary but should be rather common in this time of chemotherapy. If so, the methods to reconstruct nerves and blood vessels by promoting those regeneration should be developed to cope with the situation for surgeon, assisted by pathologists.—Authors' Abstract

Mert, A., Ozaras, R., Tabak, F., and Ozturk, R. Primary tuberculosis cases presenting with erythema nodosum. J. Dermatol. **31(1)** (2004) 66–68.

Erythema nodosum (EN) is seen only in the primary tuberculosis (TB) form of tuberculous diseases. Among the etiologies of EN, TB is the most frequent disorder in developing countries. We aimed to assess our patients with EN in reference to primary TB. We evaluated 335 patients with the diagnosis of TB during last 20 years; retrospectively 61 (18%) of these cases had pulmonary and 274 (82%) had extrapulmonary TB. Ten (16%) of the pulmonary TB cases were primary. All 10 patients with primary TB presented with EN. Among 50 patients with EN diagnosed and followed during the last 10 years, the etiology was determined in 56%, and primary TB was the most frequent: 20%.—Authors' Abstract

Modi, K., Mancini, M., and Joyce, M. P. Lepromatous leprosy in a heart transplant recipient. Am. J. Transplant **3(12)** (2003) 1600–1603.

Northern Louisiana is not an area for indigenous cases of leprosy. Limited data are available on the occurrence of leprosy in organ transplant recipients. No cases have been reported in heart transplant recipients. Mr J.R. is a 68-year-old man from Shreveport, Louisiana. He underwent orthotopic heart transplantation in March 1996. He presented in March 2000 with a maculopapular skin rash and intermittent hand swelling for 5 months. He also complained of intermittent burning of his feet for a year. The skin lesions were of two types—a fine red migratory, intermittent maculopapular rash over the upper torso and a raised, larger, violaceaous lesion on his hands. Neurological examination revealed complete loss of protective sensation in the right foot by filamentous test and some loss in the left foot. Punch skin biopsies from his right arm and right chest lesion revealed abundant acid-fast bacilli (AFB). Histopathologic examination revealed perivascular, interstitial and perineural granulomatous inflammation and a large number of AFB organisms within histiocytes. Culture of the skin biopsy specimen was negative for *Mycobacterium tuberculosis* or atypical mycobacterium. Polymerase chain reaction (PCR) performed for *Mycobacterium leprae* was positive. The patient was treated with a modified regimen consisting of dapsone 100 mg qd, ethionamide 250 mg qd, and minocycline 100 mg qd. His skin rash and neurological symptoms have resolved.—Authors' Abstract

Moses, A. E., Adelowo, K. A., and Ajayi, B. B. Prevalence of HIV-1 infection among patients with leprosy and pulmonary tuberculosis in a semi-arid region, Nigeria. J. R. Soc. Promotion Health **123(2)** (2003) 117–119.

Much evidence exists on pulmonary tuberculosis (PTB) as a presenting feature of HIV infection or AIDS-related complex, while few reports exist of a direct association between HIV infection and leprosy.

This study was carried out to see whether or not an association between leprosy and HIV infection existed, similar to that of PTB in the region of Maiduguri, Nigeria. Of 105 patients with leprosy, 11 (10.5%) were positive for HIV antibody. Of 58 patients with suspected PTB, 11 (19%) were positive for HIV antibody. Twenty-seven (47%) of the 58 had active PTB, with results of sputum smear and culture positive for mycobacterium, and six of these (22.2%) were also positive for HIV antibody. Odds ratios (OR) obtained by conditional logistic regression (matched) analysis were 3.52 (95%, CI 1.03–12.07) and 2.53 (95%, CI 1.04–6.15) for association between HIV-1 and PTB and leprosy, respectively. HIV infection was more prevalent among leprosy patients aged under 30 years, OR = 4.25 (95%, CI 1.25–14.42). The prevalence of HIV-1 infection was at borderline significance, higher in PTB and leprosy patients than in blood donors, Fisher's exact test (two-tailed) p = 0.07 and p = 0.05, respectively.

Pimentel, M. I. F., Borges, E., da Costa Nery, J. A., and Gonçalves, R. R. Initial neurological exam of multibacillary leprosy: correlation between the presence of affected nerves and the disability present at diagnosis and with the occurrence of overt neuritis. An. bras. Dermatol., Rio de Janeiro, **78(5)** (2003) 561–568.

BACKGROUND: Disabilities constitute the main problem of leprosy. It is important to identify risk factors involved, so it can be possible the prone patients be followed-up more carefully.

OBJECTIVES: To determine if the presence of thick and/or painful peripheral nerves at diagnosis correlates with disabilities already present at the initial examination, as well as with subsequent development of neuritis, during and after multidrug therapy.

METHODS: One hundred and three patients with multibacillary forms of leprosy were studied and we noted the presence of compromised peripheral nerves at diagnosis, the disability grade before treatment (DGBT), and the occurrence of neuritis episodes during and after multibacillary multidrug therapy.

RESULTS: The detection of affected peripheral nerves at diagnosis, correlated statistically ($p < 0.005$) with the occurrence of disabilities (DGBT >0). It also correlated sigificantly with the development of neuritis in the follow-up (average of 64.6 months from diagnosis, during and after multidrug therapy).

CONCLUSIONS: We emphasize the need of a good examination of peripheral nerve trunks in multibacillary patients at the diagnosis, in order to improve the detection of diabilities already present, and specially to prevent further disabilities. Healthy professionals who deal with leprosy patients must be aware to the initial neurological impairments because those patients are more susceptible to the occurrence of neuritis and neurological sequelae.—Anais Braseileiros de Dermatologia

Rodriguez, G. [Generalized adenopathy as a manifestation of type 2 reactional leprosy]. Biomedica **23(4)** (2003) 373–387. [Article in Spanish]

Generalized adenopathy as a manifestation of type 2 reactional leprosy Leprosy patient's reactions are severe clinical manifestations of acute inflammation of chronic lesions, capable of producing irreversible and invalidating damage. We studied a 46 year-old man with a type 2 leprosy reaction, who presented fever, cutaneous nodules, nasal obstruction and generalized adenopathy. The hemogram showed leucocytosis with neutrophilia. None of the initial diagnoses included leprosy. A lymph node biopsy revealed extensive necrotic areas infiltrated with polymorphonuclear lymphocytes, and foamy macrophages. Eosinophylic necrosis and thrombosis of venules with lymphoid nodule depletion was also in evidence. Ziehl Neelsen stain was not done, but the Gomori stain clearly showed Hansen's bacilli. These were were not detected by the pathologist and therefore a final diagnosis was not provided. Twenty months later, the patient presented similar symptoms, but with more generalized lymphadenopathy and presence of cutaneous nodules. Nodule biopsy showed lepromatous leprosy with erythema nodusum leprosum or type 2 reaction. Polychemotherapy treatment and anti-reaction treatment with

thalidomide cured the patient. No sequelae were noted in 3 years following the treatment. A literature review of the type 2 reaction in leprosy is provided, including discussion of risk factors, histopathology, differential diagnosis for leprosy adenopathy, pathogenesis, prognosis, and treatment. Type 2 leprosy must be treated immediately upon diagnosis as it can cause serious and permanent tissue damage. As had occurred in the above patient, the disease can proceed with generalized and symptomatic lymphadenopathy.—Authors' Abstract

Thompson, A. M., Lynn, A. A., Robson, K., Joyce. M, P., Fivenson, D. P., and Scollard, D. Lepromatous phlebitis of the external jugular vein. J. Am. Acad. Dermatol. **49(6)** (2003) 1180–1182.

Mycobacterium leprae (*M. leprae*), the causative agent of Hansen's disease, is endemic in many areas of Asia, sub-Saharan Africa, South and Central America, the Pacific Islands, and the Philippines. The spectrum of clinical disease is dependent on the patient's cell-mediated immunity and might range from localized anesthetic patches or plaques to disseminated disease. If undiagnosed, progression with damage to the involved sensory and motor nerves might occur. Lepromatous vasculitis occurs most commonly in patients with severe disseminated disease. Vascular disease, as the initial presenting sign of tuberculoid leprosy, is, however, rare. We present one patient in whom the development of Hansen's disease was associated with involvement of the external jugular vein and was initially seen as external jugular vein fibrosis.—Authors' Abstract

Warren, R. M., Victor, T. C., Streicher, E. M., Richardson, M., Beyers, N., van Pittius, N. C., and van Helden, P. D. Patients with active tuberculosis often have different strains in the same sputum specimen. Am. J. Respir. Crit. Care Med. **169(5)** (2004) 610–614.

It is generally accepted that tuberculosis results from a single infection with a single *Mycobacterium tuberculosis* strain. Such infections are thought to confer protective immunity against exogenous reinfection. In this study, a novel polymerase chain reaction method was developed to specifically identify *M. tuberculosis* strains belonging to the Beijing and non-Beijing evolutionary lineages in sputum specimens collected from tuberculosis patients resident in an epidemiologic field site in Cape Town, South Africa. The sensitivity and specificity of the polymerase chain reaction-based strain classification method were 100% (95% confidence interval, 85–100%) when compared with DNA fingerprinting and spacer oligotyping (spoligotyping). Application of this method showed that 19% of all patients were simultaneously infected with Beijing and non-Beijing strains, and 57% of patients infected with a Beijing strain were also infected with a non-Beijing strain. Multiple infections were more frequent in retreatment cases (23%) as compared with new cases (17%), but were not associated with sex, age, or smear grading. These results suggest that multiple infections are frequent, implying high reinfection rates and the absence of efficient protective immunity conferred by the initial infection. This finding could influence our understanding of the epidemiology of disease in high-incidence regions and our understanding for vaccine development.—Authors' Abstract

Yan, L., Zhang, G., Zheng, Z., Li, W., Zheng, T., Watson, J. M., and Piefer, A. Comprehensive treatment of complicated plantar ulcers in leprosy. Chin. Med. J. (Engl). **116(12)** (2003) 1946–1948.

OBJECTIVE: To investigate feasible treatment methods for plantar ulcers in leprosy patients according to the agreement between the Ministry of Health (MOH) of China and the Leprosy Mission International (LMI). METHODS: A total of 2599 complicated foot ulcers in 1804 leprosy cases underwent surgic treatment. Plastic fixation and supports were used, dressings were changed regularly, and protective footwear and modified insoles were provided. RESULTS: Of the 2599 foot ulcers 1446 (55.64%) healed. The cure rate of the patients treated in leprosy hospitals was 71.31%, with 219 (15.15%) recurrences of foot ulcers. The recurrence rate of those who

lived at home was 18.35%. CONCLUSIONS: Comprehensive treatment of foot ulcers has a high cure rate and a low recurrence rate. Reduction of workload, avoidance of long distance walking, intensification of education on foot self-care and provision of financial support are the main measures for preventing a recurrence of foot ulcers.—Authors' Abstract

Immuno Pathology

Alvarez, G. R., Zwilling, B. S., and Lafuse, W. P. *Mycobacterium avium* inhibition of IFN-gamma signaling in mouse macrophages: Toll-like receptor 2 stimulation increases expression of dominant-negative STAT1 beta by mRNA stabilization. J. Immunol. 171(12) 2003 6766–6773.

Mycobacterial infections of macrophages have been shown to inhibit the ability of the macrophage to respond to IFN-gamma. We previously reported that *Mycobacterium avium* infection of mouse macrophages decreases IFN-gamma-induced STAT1 tyrosine phosphorylation and STAT1 DNA binding. Because macrophages respond to *M. avium* through Toll-like receptor 2 (TLR2), we determined whether TLR2 stimulation inhibits the response to IFN-gamma. Treatment of mouse RAW264.7 macrophages with TLR2 agonists inhibited the induction of IFN-gamma-inducible genes by IFN-gamma. In contrast to *M. avium* infection, TLR2 agonists did not inhibit the IFN-gamma induction of DNA-binding activity of STAT1 and the tyrosine phosphorylation of STAT1alpha. Instead, IFN-gamma induction of RAW264.7 cells treated with TLR2 agonists resulted in an increase in the tyrosine phosphorylation of the dominant-negative STAT1beta. TLR2 stimulation of RAW264.7 cells increased both STAT1beta protein and mRNA expression, suggesting that the increased STAT1beta phosphoylation results from increased STAT1beta expression. Because STAT1alpha and STAT1beta mRNA have different 3′ untranslated regions, and 3′ untranslated regions can regulate mRNA stability, we examined the effects of TLR2 stimulation on mRNA stability. TLR2 stimulation of RAW264.7 cells increased the stability of STAT1beta mRNA, while not affecting the stability of STAT1alpha mRNA. The ability of STAT1beta to function as a dominant negative was confirmed by overexpression of STAT1beta in RAW264.7 macrophages by transient transfection, which inhibited IFN-gamma-induced gene expression. These findings suggest that *M. avium* infection of mouse macrophages inhibits IFN-gamma signaling through a TLR2-dependent increase in STAT1beta expression by mRNA stablization and a TLR2-independent inhibition of STAT1 tyrosine phosphorylation.—Authors' Abstract

Bisen, P. S., Garg, S. K., Tiwari, R. P., Tagore, P. R., Chandra, R., Karnik, R., Thaker, N., Desai, N., Ghosh, P. K., Fraziano, M., and Colizzi, V. Analysis of the shotgun expression library of the *Mycobacterium tuberculosis* genome for immunodominant polypeptides: potential use in serodiagnosis. Clin. Diagn. Lab. Immunol. **10(6)** (2003) 1051–1058.

See Current Literature, Molecular and Genetic Studies, p. 255.

Chen, K., Lu, J., Wang, L., and Gan, Y. H. Mycobacterial heat shock protein 65 enhances antigen cross-presentation in dendritic cells independent of Toll-like receptor 4 signaling. J. Leukoc. Biol. **75(2)** (2004) 260–266.

Heat shock proteins (HSP) have been shown to enhance antigen processing and presentation through their association with antigenic peptides and delivery of these moieties into major histocompatibility complex class I pathways. In this study, mycobacterial Hsp65 is demonstrated to have the ability to help cross-present an exogenous protein by dendritic cells (DC) to CD8 T cells without the need for complex formation between Hsp65 and the protein. This ability of Hsp65 to enhance cross-presentation is independent of its weak stimulatory effect on DC, the latter seen only after prolonged incubation. When the effect of lipopolysaccharide contamination is abrogated, Hsp65 is unable to activate Toll-like receptor (TLR)4 in the

presence of CD14 and MD2. This accounts for the inability of Hsp65 to drive maturation of DC and shows that Hsp65 is not a potent stimulator of DC. Thus, Hsp65 enhances the cross-presentation of a soluble, free antigen by DC, independent of TLR4 signaling and up-regulation of costimulatory molecules.—Authors' Abstract

Curto, M., Reali, C., Palmieri, G., Scintu, F., Schivo, M. L., Sogos, V., Marcialis, M. A., Ennas, M. G., Schwarz, H., Pozzi, G., and Gremo, F. Inhibition of cytokines expression in human microglia infected by virulent and non-virulent mycobacteria. Neurochem. Int. **44(6)** (2004) 381–392.

The pathogenesis of tuberculosis (TBC) meningitis is still unknown. As shown by previous studies, human microglia can be the target of mycobacteria, but no data are available about their cellular response to infection. Consequently, we studied the expression of tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) and IL-10 in human microglia pure cultures infected with the two variants of *Mycobacterium avium* (domed-opaque (SmD) and transparent (SmT)) and with *Mycobacterium tuberculosis*. Results showed that microglia was productively infected by mycobacteria which could grow inside the cells. Mycobacteria internalization was more rapid for *M. avium*, but *M. tuberculosis* infection turned out to be more efficient due to the incorporation of densely packed bacteria. TNF-alpha expression was not affected by *M. avium*, whereas an increase followed by a decrease was observed in *M. tuberculosis*. Both IL-1 and IL-10 cytokine expression was rapidly inhibited by infection with the more virulent bacteria, whereas the non-pathogenic one had almost no effect. Also, the expression of the co-stimulatory molecule CD137, a member of tumor necrosis factor receptor family, was affected by infection with virulent mycobacteria. Our results show that microglia response to mycobacterial infection is modulated in correlation with virulence, mainly toward inhibition of inflammatory response. This observation might be one of the mechanisms by which non-pathogenic mycobacteria are quickly eliminated, explaining one of the bases of virulence.—Authors' Abstract

Deng, L., Ding, W., and Granstein, R. D. Thalidomide inhibits tumor necrosis factor-alpha production and antigen presentation by Langerhans cells. J. Invest. Dermatol. **121(5)** (2003) 1060–1065.

Thalidomide is an effective treatment for several inflammatory and autoimmune disorders including erythema nodosum leprosum, Behcet's syndrome, discoid lupus erythematosus, and Crohn's disease. Thalidomide is believed to exert its anti-inflammatory effects, at least in part, by inhibiting tumor necrosis factor-alpha (TNF-alpha) production by monocytes. We studied the effects of thalidomide on epidermal Langerhans cells (LC). LCs are epidermal antigen-presenting dendritic cells that play important roles in skin immune responses. Using the murine epidermis-derived dendritic cell lines, XS106A from A/J mice and XS52 from BALB/c mice as surrogates for LC, we found that thalidomide inhibited TNF-alpha production in a concentration-dependent manner. Northern blot analysis revealed that thalidomide significantly decreased the peak-induced mRNA level of TNF-alpha in XS106A cells and XS52 cells. We then examined the effect of thalidomide on fresh LC enriched to approximately 98% using positive selection of Ia+ cells with antibodies conjugated to magnetic microspheres. TNF-alpha production was reduced by 67.7% at a thalidomide concentration of 200 microg per mL. Thalidomide also had a profound inhibitory effect on the ability of LC to present antigen to a responsive TH1 clone. Thalidomide inhibits TNF-alpha production and the antigen-presenting ability of epidermal LCs. These mechanisms may contribute to the therapeutic effects observed with this agent.—Authors' Abstract

Dugue, C., Perraut, R., Youinou, P., and Renaudineau, Y. Effects of anti-endothelial cell antibodies in leprosy and malaria. Infect. Immun. **72(1)** (2004) 301–309.

As a result of damaging endothelial cells (ECs), *Mycobacterium leprae* triggers the production of antibodies (Abs). These anti-EC Abs (AECAs) can be divided into two types. The first type nonspecifically reacts with components of the cytosol (CY) and can be detected by enzyme-linked immunosorbent assay (ELISA). The second specifically reacts with the EC membrane (MB) and requires fluorescence-activated cell sorter (FACS) analysis to be detected. The presence of both types of AECAs was determined in 68 leprosy patients. The ELISA was positive for 35 of them but also for 30 of 34 malaria patients and 17 of 50 healthy African controls. However, whereas FACS analysis showed MB reactivity in only three malaria patients and four controls, this reactivity was found in 27 leprosy patients, more of those having the lepromatous than the tuberculoid form. Specificity for MB, which we failed to absorb by incubation with CY lysates, predominated over that for CY in leprosy, unlike malaria, where the EC reactivity was restricted to the CY. Western blot analysis and two-dimensional electrophoresis revealed that calreticulin, vimentin, tubulin, and heat shock protein 70 were targeted by AECAs from leprosy patients, but other proteins remained unidentified. These auto-Abs, but not those from malaria patients, did activate ECs, as indicated by the E-selectin and intercellular adhesion molecule 1 upregulation, and/or induced them into apoptosis, as documented by four different methods. Our findings suggest that, in some but not all leprosy patients, AECAs may play a role in pathogenesis.—Authors' Abstract

Gaede, K. I., Mamat, U., and Muller-Quernheim, J. Differential gene expression pattern in alveolar macrophages of patients with sarcoidosis and tuberculosis. J. Mol. Med. **82(3)** (2004) 206–210.

Sarcoidosis is a multisystem granulomatous disorder of unknown origin characterized by the presence of epithelioid granulomata in the affected organs. Histological and clinical similarities between sarcoidosis and tuberculosis caused *by M. tuberculosis* suggest a shared underlying pathophysiology. However, specific markers are needed. This study examined the differential gene expression pattern in alveolar macrophages of patients with granulomatous disorders. The differential mRNA regulation pattern of alveolar macrophages in the bronchoalveolar lavage of healthy controls was compared to that of patients with sarcoidosis and tuberculosis by means of differential display reverse transcription PCR. Comparative analysis of 2498 PCR products in controls, sarcoidosis, and tuberculosis revealed a differential regulation of expressed sequence tags in only 6.5%. 1.8% showed a shared expression pattern between sarcoidosis and tuberculosis in contrast to the control. It can be assumed that these alterations are associated with common granulomatous features. In contrast, 3.0% of the amplified sequence tags showed specific up- or downregulation in sarcoidosis and 1.6% in tuberculosis. These data indicate a significant proportion of common granuloma-associated features, independent of the origin of the granulomatous disorder.—Authors' Abstract

Geluk, A., van Meijgaarden, K. E., Franken, K. L., Wieles, B., Arend, S. M., Faber, W. R., Naafs, B., and Ottenhoff, T. H. Immunological crossreactivity of the *Mycobacterium leprae* CFP-10 with its homologue in *Mycobacterium tuberculosis*. Scand. J. Immunol. **59(1)** (2004) 66–70.

Mycobacterium tuberculosis culture filtrate protein-10 (CFP-10) (Rv3874) is considered a promising antigen for the immunodiagnosis of tuberculosis (TB) together with early secreted antigens of *M. tuberculosis* (ESAT-6). Both ESAT-6 and CFP-10 are encoded by the RD1 region that is deleted from all tested *M. bovis* bacille Calmette-Guerin (BCG) strains but present in *M. leprae*, *M. tuberculosis*, *M. bovis*, *M. kansasii*, *M. africanum* and *M. marinum*. In this study, the homologue of CFP-10 in *M. leprae* (ML0050) is identified and characterized. Interferon-gamma production in response to this homologue by T cells from leprosy patients, TB patients and unexposed controls shows that CFP-10 of M. leprae is a potent antigen that crossreacts with CFP-10 of *M. tuberculosis* at the T-cell level. This crossreactivity has implications for the use

of CFP-10 of these mycobacterial species as diagnostic tool in areas endemic for both the diseases.—Authors' Abstract

Imai, K., Kurita-Ochiai, T., and Ochiai, K. *Mycobacterium bovis* bacillus Calmette-Guerin infection promotes SOCS induction and inhibits IFN-gamma-stimulated JAK/STAT signaling in J774 macrophages. FEMS Immunol. Med. Microbiol. **39(2)** (2003) 173–180.

The resurgence in mycobacterial infection worldwide has led to renewed attention to the pathogenesis of Mycobacterium species. Although interferon-gamma (IFN-gamma) is a principal mediator of macrophage activation, macrophages infected with Mycobacterium are poor in response at the cytokine. However, the molecular mechanisms underlying mycobacterial infection remain unclear. The purpose of this study was to elucidate the mechanism of the poor response to IFN-gamma in mycobacterial infection. Our data clearly demonstrate that this is due to induction of suppressor of cytokine signal (SOCS) negative regulators of IFN-gamma signal transduction that closely correlates with the inhibition of JAK/STAT signaling and gene expression stimulated by IFN-gamma. *Mycobacterium bovis* bacillus Calmette-Guerin infection induces the production of SOCS-1 and SOCS-3 in murine J774 macrophages. The level of SOCS-1 mRNA increased 1 h and reached a maximum 3 h after the addition of the bacteria. SOCS-3 mRNA expression appeared as early as 1 h after the infection. We also observed that trehalose 6,6′-dimycolate/cord factor, a major component of the *Mycobacterium tuberculosis* cell wall, induces expression of SOCS and inhibits IFN-gamma-stimulated phosphorylation of STAT1 extensively in the cells. The results in this study suggest that a molecular mechanism of mycobacterial infection affects the unresponsiveness to IFN-gamma in the subsequent growth and spread of macrophages.—Authors' Abstract

Kang, S.-J., and Cresswell, P. Saposins facilitate CD1d-restricted presentation of an exogenous lipid antigen to T cells. Nature Immunol. **5(2)** (2004) 175–181.

Members of the CD1 family present antigenic lipids to T lymphocytes. CD1 molecules survey endocytic compartments for lipid antigens that are sorted into these vesicles after incorporation into the membrane bilayer, and extraction from the bilayer is likely to be a critical step for lipid association. We hypothesized that lysosomal saposins, which are cofactors required for sphingolipid degradation, might be involved in this process. Here we show that saposins, although not required for the autoreactive recognition of CD1d by natural killer T cells, are indispensable for the binding of an exogenous lipid antigen, α-galactosylceramide, to CD1d in the endocytic pathway. We suggest that saposins mobilize monomeric lipids from lysosomal membranes and facilitate their association with CD1d.—Nature Immunology

Mendez-Samperio, P., Ayala, H., Trejo, A., and Ramirez, F. A. Differential induction of TNF-alpha and NOS2 by mitogen-activated protein kinase signaling pathways during *Mycobacterium bovis* infection. J. Infect. **48(1)** (2004) 66–73.

The role of mitogen-activated protein kinase (MAPK) signaling pathways in the regulation of TNF-alpha and NOS2 production by human monocytes infected with *Mycobacterium bovis* BCG was examined. Inhibition studies showed that ERK1/2 and p38 MAPK activation were necessary for the monocyte response to *M. bovis* infection. Analysis of MAPK activation showed rapid phosphorylation of ERK1/2 and p38 in response to *M. bovis* BCG. Phosphorylation was not due to an autocrine effect of TNF-alpha secretion, since an anti-TNF-alpha antibody had no significant effect on the levels of p38 phosphorylation. The inhibitor PD98059 significantly reduced *M. bovis* BCG-induced TNF-alpha production and almost completely abrogated phosphorylation of ERK1/2; in addition the potent MEK inhibitor U0126 also abrogated phosphorylation. In contrast, studies using inhibitors selective for ERK1/2 and p38 showed that p38

plays an essential role in the induction of NOS2, whereas the role of ERK1/2 was minor. These results suggest that ERK1/2 and p38 kinases differentially regulate the *M. bovis* BCG-mediated induction of TNF-alpha and NOS2 in human monocytes.—Authors' Abstract

Penido, C., Vieira-de-Abreu, A., Bozza, M. T., Castro-Faria-Neto, H. C., and Bozza, P. T. Role of monocyte chemotactic protein-1/CC chemokine ligand 2 on gamma delta T lymphocyte trafficking during inflammation induced by lipopolysaccharide or *Mycobacterium bovis* bacille Calmette-Guerin. J. Immunol. **171(12)** (2003) 6788–6794

Gammadelta T lymphocytes are involved in a great variety of inflammatory and infectious responses. However, the mechanisms by which gammadelta T lymphocytes migrate to inflamed sites are poorly understood. In this study we investigate the role of monocyte chemotactic protein (MCP)-1 in regulating gammadelta T cell migration after LPS or *Mycobacterium bovis* bacille Calmette-Guerin (BCG) challenge. LPS-induced gammadelta T cell influx was significantly inhibited by either pretreatment with dexamethasone or vaccinia virus Lister 35-kDa chemokine binding protein, vCKBP, a CC chemokine neutralizing protein, suggesting a role for CC chemokines in this phenomenon. LPS stimulation increased the expression of MCP-1 mRNA and protein at the inflammation site within 6 hr. It is noteworthy that LPS was unable to increase MCP-1 production or gammadelta T cell recruitment in C3H/HeJ, indicative of the involvement of Toll-like receptor 4. Gammadelta T cells express MCP-1 receptor CCR2. Pretreatment with anti-MCP-1 mAb drastically inhibited LPS-induced *in vivo* gammadelta T cell mobilization. Indeed, MCP-1 knockout mice were unable to recruit gammadelta T cells to the pleural cavity after LPS stimulation, effect that could be restored by coadministration of MCP-1. In addition, BCG-induced gammadelta lymphocyte accumulation was significantly reduced in MCP-1 knockout mice when compared with wild-type mice. In conclusion, our results indicate that LPS-induced gammadelta T lymphocyte migration is dependent on Toll-like receptor 4 and sensitive to both dexamethasone and CC chemokine-binding protein inhibition. Moreover, by using MCP-1 neutralizing Abs and genetically deficient mice we show that LPS- and BCG-induced gammadelta T lymphocyte influx to the pleural cavity of mice is mainly orchestrated by the CC chemokine MCP-1.—Authors' Abstract

Ranjbar, S., Ly, N., Thim, S., Reynes, J. M., and Goldfeld, A. E. *Mycobacterium tuberculosis* recall antigens suppress HIV-1 replication in anergic donor cells via CD8+ T cell expansion and increased IL-10 levels. J. Immunol. 172(3) (2004) 1953–1959.

Mycobacterium tuberculosis (MTb) is the leading cause of death in the setting of AIDS. MTb enhances the pathogenicity and accelerates the course of HIV disease and, furthermore, infection with HIV-1 increases the risk of reactivation or reinfection with MTb. In this study, we show that host-specific recall responses to one pathogen, MTb, has a direct effect upon the regulation of a second pathogen, HIV-1. Using cells from immunocompetent former tuberculosis (TB) patients who displayed either a persistently positive (responsive) or negative (anergic), delayed-type hypersensitivity (DTH) reaction to intradermal injection of purified protein derivative (PPD), we investigated the effect of recall Ags to MTb upon the replication of HIV-1 primary isolates *in vitro*. We show that HIV-1 replication of a T cell-tropic isolate was significantly impaired in MTb-stimulated PBMC from PPD-anergic donors. Furthermore, these donors displayed a significant increase in CD8(+) T cells and IL-10 levels and lower levels of IL-2 and TNF-alpha relative to PPD-responsive donors in response to PPD stimulation. Strikingly, CD8(+) T cell depletion and blocking of IL-10 significantly increased HIV-1 replication in these PPD-anergic donors, indicating that an immunosuppressive response to MTb recall Ags inhibits HIV-1 replication in PPD-anergic individuals. Therefore, immunotherapeutic approaches aimed at recapitulating Ag-specific MTb anergy *in vivo* could result in novel and effective approaches to inhibit HIV-1 disease

progression in MTb/HIV-1 coinfection.—Authors' Abstract

Reddy, V. M., and Suleman, F. G. *Mycobacterium avium*-superoxide dismutase binds to epithelial cell aldolase, glyceraldehyde-3-phosphate dehydrogenase and cyclophilin A. Microb. Pathog. **36(2)** (2004) 67–74.

Mycobacterium avium complex (MAC) adheres, invades and multiplies inside epithelial cells. Earlier, we demonstrated two MAC protein adhesins, 25 and 31 kDa, binding with HEp-2 cells. The 25 kDa MAC adhesin was found to be superoxide dismutase (SOD). In this study, epithelial cell (HEp-2 and A549) ligands for MAC-SOD were identified by probing two-dimensional western blots of epithelial extracts with MAC proteins followed by monoclonal anti-MAC-SOD antibodies. Three epithelial cell proteins with molecular masses 43, 40 and 18 kDa, present in both membrane and cytosolic fractions, were found to bind with MAC-SOD. Based on the N-terminal amino acid sequences, the 43, 40 and 18 kDa epithelial proteins were identified as aldolase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and cyclophilin A (CypA), respectively. Furthermore, MAC-SOD was found to bind to purified rabbit muscle aldolase, GAPDH and recombinant CypA in western blotting.—Authors' Abstract

Remus, N., Alcais, A., and Abel, L. Human genetics of common mycobacterial infections. Immunol. Res. **28(2)** (2003) 109–129.

See Current Literature, Molecular and Genetic Studies, p. 260.

Saunders, B. M., Fernando, S. L., Sluyter, R., Britton, W. J., and Wiley, J. S. A loss-of-function polymorphism in the human P2X7 receptor abolishes ATP-mediated killing of mycobacteria. J. Immunol. **171(10)** (2003) 5442–5446.

Protective immunity to mycobacterial infections requires activation of the antibacterial mechanisms of infected macrophages. It has previously been reported that ATP treatment of mycobacteria-infected macrophages induces apoptosis mediated via the P2X(7) pathway and that this leads to the death of both the host cell and the internalized bacilli. We have recently identified a single nucleotide polymorphism in the P2X7 gene (1513A→C), with 1–2% prevalence in the homozygous state, which codes for a non-functional receptor. IFN-gamma-primed, mycobacteria-infected macrophages from wild-type individuals were incubated with ATP and this induced apoptosis and reduced mycobacterial viability by 90%. Similar treatment of macrophages from individuals homozygous for the 1513C polymorphism failed to induce apoptosis and did not lead to mycobacterial killing via the P2X(7)-mediated pathway. These data demonstrate that a single nucleotide polymorphism in the P2X7 gene can allow survival of mycobacteria within infected host cells.—Authors' Abstract

Sen Gupta, R., Hillemann, D., Kubica, T., Zissel, G., Muller-Quernheim, J., Galle, J., Vollmer, E., and Goldmann, T. HOPE-fixation enables improved PCR-based detection and differentiation of *Mycobacterium tuberculosis* complex in paraffin-embedded tissues. Pathol. Res. Pract. **199(9)** (2003) 619–623.

Standard PCR-based detection of mycobacterial DNA in paraffin-embedded specimens may lack sufficient sensitivity because of the degradation of nucleic acids caused by routinely used formalin fixation. Therefore, we set up an approach that aimed at improving the results by applying the novel HOPE-fixative in PCR-detection of mycobacteria in paraffin-embedded tissues. Comparison of PCR-results using DNA extracted from either HOPE- or formalin-fixed specimens in BCG-infected SCID-mice revealed a more than 100fold enhanced sensitivity for the HOPE-fixed material. Owing to the preservation of DNA from degradation in HOPE-fixed tissues, even differentiation within the *M. tuberculosis* complex was possible by spoligotyping. We therefore conclude that the HOPE-fixative is a useful tool for molecular pathology that enhances

the sensitivity of PCR-based methods for the detection of pathogens in paraffin-embedded tissues compared to formalin-fixation. Owing to the better preserved DNA, improved differentiation of mycobacteria from archived materials is possible. These results promise new and a substantially wider range of possibilities in the field of molecular pathology.—Authors' Abstract

Siemion, I. Z., Gawlowska, M., Krajewski, K., Strug, I., and Wieczorek, Z. Analogs of RGDVY and GRGD peptides inhibit *Mycobacterium kansasii* phagocytosis. Peptides **24(8)** (2003) 1109–1115.

Continuing our research on *Mycobacteria kansasii* phagocytosis inhibition, we have examined in that context three series of peptides derived from the RGDVY and GRGD sequences. It was found that the levels of the inhibitory activity depend on the amino acid composition as well as on the particular peptide sequence. Distinct inhibitory activity was found in the case of thymopentin (RKDVY), the active fragment of thymopoietin. In this case the *Mycobacterium phagocytosis* inhibition should be combined with general immunostimulatory activity of RKDVY peptide. Our examination of a series of GRGDV analogs with a successively prolonged oligo-Gly linker inserted into the peptide chain showed that the distance between the Arg and Asp residues required for such an activity should be about 9A.—Authors' Abstract

Stamm, L. M., Morisaki, J. H., Gao, L. Y., Jeng, R. L., McDonald, K. L., Roth, R., Takeshita, S., Heuser, J., Welch, M. D., and Brown, E. J. *Mycobacterium marinum* escapes from phagosomes and is propelled by actin-based motility. J. Exp. Med. **198(9)** (2003) 1361–1368.

Mycobacteria are responsible for a number of human and animal diseases and are classical intracellular pathogens, living inside macrophages rather than as free-living organisms during infection. Numerous intracellular pathogens, including *Listeria* monocytogenes, *Shigella flexneri,* and *Rickettsia rickettsii,* exploit the host cytoskeleton by using actin-based motility for cell to cell spread during infection. Here we show that *Mycobacterium marinum*, a natural pathogen of fish and frogs and an occasional pathogen of humans, is capable of actively inducing actin polymerization within macrophages. *M. marinum* that polymerized actin were free in the cytoplasm and propelled by actin-based motility into adjacent cells. Immunofluorescence demonstrated the presence of host cytoskeletal proteins, including the Arp2/3 complex and vasodilator-stimulated phosphoprotein, throughout the actin tails. In contrast, Wiskott-Aldrich syndrome protein localized exclusively at the actin-polymerizing pole of *M. marinum*. These findings show that *M. marinum* can escape into the cytoplasm of infected macrophages, where it can recruit host cell cytoskeletal factors to induce actin polymerization leading to direct cell to cell spread.—Authors' Abstract

Weir, R. E., Black, G. F., Dockrell, H. M., Floyd, S., Fine, P. E., Chaguluka, S. D., Stenson, S., King, E., Nazareth, B., Warndorff, D. K., Ngwira, B., Crampin, A. C., Mwaungulu, L., Sichali, L., Jarman, E., Donovan, L., and Blackwell, J. M. Mycobacterial purified protein derivatives stimulate innate immunity: Malawians show enhanced tumor necrosis factor alpha, interleukin-1beta (IL-1beta), and IL-10 responses compared to those of adolescents in the United Kingdom. Infect. Immun. **72(3)** (2004) 1807–1811.

To investigate the role of innate immunity in variable efficacy *of Mycobacterium bovis* BCG vaccination in Malawi and the United Kingdom, we examined 24-hr tumor necrosis factor alpha, interleukin-1beta (IL-1beta), and IL-10 responses to mycobacterial purified protein derivatives (PPDs). The rank order in stimulatory potency for different PPDs was the same for all three cytokines. Before vaccination Malawians made higher pro- and anti-inflammatory responses than did United Kingdom subjects. Fewer than 5% of United Kingdom subjects made IL-10 in response to any PPD, compared to 19 to 57% responders among Malawians. Priming for regulatory IL-10 may contribute to the smaller increase in gamma interferon responses in Malawians

compared to United Kingdom subjects following BCG vaccination.—Authors' Abstract

Zhong, J., Gilbertson, B., and Cheers, C. Apoptosis of CD4+ and CD8+ T cells during experimental infection with *Mycobacterium avium* is controlled by Fas/FasL and Bcl-2-sensitive pathways, respectively. Immunol. Cell. Biol. **81(6)** (2003) 480–486.

Both CD4+ and CD8+ T cells from mice infected with *Mycobacterium avium* suffered a high rate of apoptosis, beginning with the onset of the immune response and culminating in the loss of T cells from the tissues and loss of IFN-gamma production. Fas expression increased over the course of infection on both T cell populations, as did their susceptibility to the induction of apoptosis *in vitro* by anti-Fas mAb. Nevertheless, although the rate of apoptosis among CD4+ T cells from infected mice was reduced to normal levels in lpr mice with a defective Fas, CD8+ T cells were unaffected, implying that Fas/FasL interaction was not important in these cells *in vivo*. Conversely, over-expression of B-cell lymphoma-2 (Bcl-2), which is known to protect T cells from apoptosis signalled through the TNF receptor or due to the withdrawal of cytokines, totally protected CD8+ T cells from infected mice but had no effect on CD4+. It is of interest that these two contrasting pathways of T-cell apoptosis operate at the same time during a single infection.—Authors' Abstract

Immuno Pathology (Leprosy)

de la Barrera, S., Finiasz, M., Fink, S., Ilarregui, J., Aleman, M., Olivares, L., Franco, M. C., Pizzariello, G., and del Carmen Sasiain, M. NK cells modulate the cytotoxic activity generated by *Mycobacterium leprae*-hsp65 in leprosy patients: role of IL-18 and IL-13. Clin. Exp. Immunol. **135(1)** (2004) 105–113.

Protection against intracellular pathogens such as *Mycobacterium leprae* is critically dependent on the function of NK cells at early stages of the immune response and on Th1 cells at later stages. In the present report we evaluated the role of IL-18 and IL-13, two cytokines that can influence NK cell activity, in the generation of *M. leprae*-derived hsp65-cytotoxic T lymphocytes (CTL) from peripheral blood mononuclear cells (PBMC) of leprosy patients. We demonstrated that IL-18 modulates hsp65-induced CTL generation and collaborates with IL-12 for this effect. In paucibacillary (PB) patients and normal controls (N) depletion of NK cells reduces the cytolytic activity. Under these conditions, IL-12 cannot up-regulate this CTL generation, while, in contrast, IL-18 increases the cytotoxic activity both in the presence or absence of NK cells. IL-13 down-regulates the hsp65-induced CTL generation and counteracts the positive effect of IL-18. The negative effect of IL-13 is observed in the early stages of the response, suggesting that this cytokine affects IFNgamma production by NK cells. mRNA coding for IFNgamma is induced by IL-18 and reduced in the presence of IL-13, when PBMC from N or PB patients are stimulated with hsp65. Neutralization of IL-13 in PBMC from multibacillary (MB) leprosy patients induces the production of IFNgamma protein by lymphocytes. A modulatory role on the generation of hsp65 induced CTL is demonstrated for IL-18 and IL-13 and this effect takes place through the production of IFNgamma.—Authors' Abstract

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

In this presentation an attempt has been made to describe the nine-banded armadillo as an animal model, probably the only one in which lepromatous leprosy similar to that found in humans can be experimentally produced. Some unique features of the physiology of the animal are mentioned. The pathology and the microbiology of leprosy in the armadillo are described in detail. The discovery of lepromatous leprosy in the wild armadillos in the southern parts of the United States, the transmission of disease among

them through trauma and thorn pricks and the pathogenesis of the disease are presented. The impact of leprosy in the wild animals may have on human leprosy is discussed.—Indian Journal of Pathology and Microbiology

Johansen, P., Raynaud, C., Yang, M., Colston, M. J., Tascon, R. E., and Lowrie, D. B. Anti-mycobacterial immunity induced by a single injection of *M. leprae* Hsp65-encoding plasmid DNA in biodegradable microparticles. Immunol. Lett. **90(2–3)** (2003) 81–85.

See Current Literature, Experimental Infections, p. 235.

Kirkaldy, A. A., Musonda, A. C., Khanolkhar-Young, S., Suneetha, S., and Lockwood, D. N. Expression of CC and CXC chemokines and chemokine receptors in human leprosy skin lesions. Clin. Exp. Immunol. **134(3)** (2003) 447–453.

We have investigated the expression of chemokines and their receptors in leprosy skin lesions using immunohistochemistry. Skin biopsies from 25 leprosy patients across the leprosy spectrum, 11 patients undergoing type I reversal reactions and four normal donors were immunostained by ABC peroxidase method using antibodies against CC and CXC chemokines and their receptors. Using an *in situ* hybridization technique we have also studied the expression of monocyte chemoattractant protein 1 (MCP-1), RANTES and interleukin (IL)-8 chemokines mRNA in leprosy skin lesions. Chemokines and receptor expression was detected in all leprosy skin biopsies. Expression of CC chemokines MCP-1 ($p < 0.01$) and RANTES ($p < 0.01$) were elevated significantly in borderline tuberculoid leprosy in reversal reaction compared to non-reactional borderline tuberculoid leprosy, but there was no difference in the expression of IL-8 chemokine. Surprisingly, there was no significant difference in the expression of CC (CCR2 and CCR5) and CXC (CXCR2) chemokine receptors across the leprosy spectrum. Similarly, there was no significant difference in the expression of mRNA for MCP-1, regulated upon activation normal T cell expressed and secreted (RANTES) and IL-8 chemokines. Here, the presence of a neutrophil chemoattractant IL-8 in leprosy lesions, which do not contain neutrophils, suggests strongly a role of IL-8 as a monocyte and lymphocyte recruiter in leprosy lesions. These results suggest that the chemokines and their receptors, which are known to chemoattract T lymphocytes and macrophages, are involved in assembling the cellular infiltrate found in lesions across the leprosy spectrum.—Authors' Abstract

Leal, A. M., Magalhaes, P. K., Souza, C. S., and Foss, N. T. Adrenocortical hormones and interleukin patterns in leprosy. Parasite Immunol. **25(8–9)** (2003) 457–461.

The functional status of adrenocortical hormones and their relationship to the pattern of inflammatory cytokines in the lepromatous and tuberculoid poles of leprosy were investigated. Interleukin (IL)-1beta, IL-6 and tumour necrosis factor (TNF)-alpha plasma levels, C-reactive protein (CRP) concentrations and erythrocyte sedimentation rates (ESR) were significantly higher in LL/BL (lepromatous) leprosy patients than in control subjects. There was a significant positive correlation between IL-6 and TNF-alpha plasma levels and ESR and CRP concentrations. IL-1beta was positively correlated with ESR but not with CRP. Both baseline and stimulated adrenocorticotropic hormone and cortisol plasma levels were not different between patients and control subjects. In contrast, adrenal androgen dehydroepiandrosterone sulphate (DHEA-S) plasma levels were significantly lower in leprosy patients than in sex-matched control subjects. There was a significant inverse correlation between DHEA-S and IL-6, TNF-alpha, and CRP concentrations. This finding may be of pathogenetic significance in this disease and in other inflammatory states.—Authors' Abstract

Sridevi, K., Neena, K., Chitralekha, K. T., Arif, A. K., Tomar, D., and Rao, D. N. Expression of costimulatory molecules (CD80, CD86, CD28, CD152), accessory

molecules (TCR alphabeta, TCR gammadelta) and T cell lineage molecules (CD4+, CD8+) in PBMC of leprosy patients using *Mycobacterium leprae* antigen (MLCWA) with murabutide and T cell peptide of Trat protein. Int. Immunopharmacol. **4(1)** (2004) 1–14.

In leprosy, cell-mediated immunity (CMI) is more significant than humoral response to eliminate intracellular pathogen. T cell defect is a common feature in lepromatous leprosy (LL) patients as compared to tuberculoid type (TT) patients. For efficient initiation of CD4+, T cell response requires T cell receptor (TCR) activation and costimulation provided by molecules on antigen-presenting cells (APC) and their counter receptors on T cells. In our previous study, the defective T cell function in LL patients was restored to a proliferating state with the release of TH1 type cytokines using mycobacterial antigen(s) with two immunomodulators (Murabutide (MDP-BE) and T cell epitope of Trat protein of *Escherichia coli*) by presenting the antigen in particulate form *in vitro* to PBMC derived from leprosy patients. This observation prompted us to study the expression of the costimulatory molecules (CD80, CD86, CD28, CD152), other accessory molecules (TCR alphabeta/gammadelta) and T cell lineage molecules (CD4+ and CD8+) during constitutive and activated state of peripheral blood mononuclear cells (PBMC) derived from normal and leprosy individuals using different formulations of *Mycobacterium leprae* total cell wall antigen (MLCWA), Trat and MDP-BE using flow cytometric analysis. An increased surface expression of CD80, CD86 and CD28 but decreased CD152 expression was observed when PBMC of normal, BT/TT (tuberculoid) and BL/LL (lepromatous) patients were stimulated *in vitro* with MLCWA+ MDP-BE+Trat peptide using liposomal mode of antigen delivery, while opposite results were obtained with the antigen alone. Antibody inhibition study using antihuman CD80 or CD86 completely abolished the T cell lymphoproliferation, thereby reconfirming the importance of these costimulatory molecules during T cell activation/differentiation. Though the liposome-entrapped antigen formulation has no effect on expression of alphabeta/gammadelta T cell receptor, the constitutive levels of TCR gammadelta were high in lepromatous patients. Thus, TCR bearing gammadelta appears to have a negligible regulatory role in peripheral blood of leprosy patients. The percentage of cells positive for CD4+ are increased in inducible state in all the three groups, while CD8+-positive cells were decreased in LL patients, thereby reconfirming the fact that priming of CD4+ cells are necessary for producing final effector functions. Lastly, intracellular cytokine staining experiment indicated that CD4+ cells are the major producers of IFN-gamma but not NK cells. The study highlights the reversal of T cell anergy especially in lepromatous patients through the modulation of costimulatory molecule expression under the influence of Th1 cytokines, i.e., IL-2 and IFNgamma.—Authors' Abstract

Immuno Pathology (Tuberculosis)

Aagaard, C., Brock, I., Olsen, A., Ottenhoff, T. H., Weldingh, K., and Andersen, P. Mapping immune reactivity toward Rv2653 and Rv2654: two novel low-molecular-mass antigens found specifically in the *Mycobacterium tuberculosis* complex. J. Infect. Dis. **189(5)** 2004 812–819.

New tools are urgently needed for the detection of latent tuberculosis (TB). We evaluated the diagnostic potential of 2 novel *Mycobacterium tuberculosis* complex-specific candidate antigens (Rv2653 and Rv2654) and investigated T cell recognition during natural infection in humans and experimental infection in guinea pigs. Peripheral blood mononuclear cells stimulated with peptide pools covering the full length of Rv2654 induced interferon-gamma release in 10 of 19 patients with TB. Neither Rv2654 single peptides nor Rv2654 pools were recognized by bacille Calmette-Guerin-vaccinated donors. However, peptides from Rv2653 were recognized by both patients group. The cross-reactive epitope(s) in Rv2653 were located in

a 36-amino acid stretch in the center of the molecule. Rv2654 also induced *M. tuberculosis*-specific skin-test responses in 3 of 4 aerosol-infected guinea pigs. Rv2654 is a strongly recognized T cell antigen that is highly specific for TB and has potential as a novel cell-mediated immunity-based TB diagnostic agent.—Authors' Abstract

Agger, E. M., Brock, I., Okkels, L. M., Arend, S. M., Aagaard, C. S., Weldingh, K. N., and Andersen, P. Human T-cell responses to the RD1-encoded protein TB27.4 (Rv3878) from *Mycobacterium tuberculosis*. Immunology **110(4)** 2003 507–512.

In recent years, there has been considerable focus on the discovery and characterization of proteins derived from *Mycobacterium tuberculosis* leading to the identification of a number of candidate antigens for use in vaccine development or for diagnostic purposes. Previous experiments have demonstrated an important immunological role for proteins encoded by the RD1 region, which is absent from all strains of bacillus Calmette-Guerin (BCG) but present in the genomes of virulent *M. bovis* and *M. tuberculosis*. Herein, we have studied human T-cell responses to the antigen encoded by the putative open reading frame (rv3878) of the RD1 region. Immunoblot analysis revealed that rv3878 was expressed and the native protein was designated TB27.4. Immunological evaluations demonstrate that TB27.4 elicits a prominent immune response in human tuberculosis patients with a dominant region in the C-terminal part of the molecule. In contrast, very limited responses were seen in *M. bovis* BCG-vaccinated donors. This study therefore emphasizes the diagnostic potential of proteins encoded by the RD1 region.—Authors' Abstract

Allen, S. S., Cassone, L., Lasco, T. M., and McMurray, D. N. Effect of neutralizing transforming growth factor beta1 on the immune response against *Mycobacterium tuberculosis* in guinea pigs. Infect. Immun. **72(3)** 2004 1358–1363.

Transforming growth factor beta (TGF-beta) is a cytokine which has been shown to suppress the antimycobacterial immune responses of humans and experimental animals. In this study, the contributions of TGF-beta to cytokine production *in vivo* were investigated by using the established guinea pig model of tuberculous pleurisy. *Mycobacterium bovis* BCG-vaccinated guinea pigs were injected intrapleurally with heat-killed virulent *Mycobacterium tuberculosis*. Eight days following induction of an antigen-specific pleural effusion, guinea pigs were injected intrapleurally with anti-TGF-beta1 or isotype control antibody. The following day, pleural exudates were removed, and the fluid volume and characteristics of the infiltrating cells were determined. Pleural fluid was analyzed for total interferon (IFN) and tumor necrosis factor (TNF) protein levels by using appropriate bioassays. RNA from pleural effusion cells was examined to determine TGF-beta1, TNF-alpha, IFN-gamma, and interleukin-8 mRNA levels by using real-time PCR. Proliferative responses of pleural effusion lymphocytes were examined in response to concanavalin A and purified protein derivative (PPD) in vitro. Treatment with anti-TGF-beta1 resulted in decreased pleural fluid volume and decreased cell numbers in the pleural space along with an increased percentage of lymphocytes and a decreased percentage of neutrophils. The bioactive TNF protein levels in pleural fluid were increased in guinea pigs treated with anti-TGF-beta1, while the bioactive IFN protein concentrations were not altered. Expression of TGF-beta1 and TNF-alpha mRNA was significantly increased following TGF-beta1 neutralization. Finally, PPD-induced proliferative responses of pleural cells from anti-TGF-beta1-treated animals were significantly enhanced. Thus, TGF-beta1 may be involved in the resolution of this local, mycobacterial antigen-specific inflammatory response.—Authors' Abstract

Ando, M., Yoshimatsu, T., Ko, C., Converse, P. J., and Bishai, W. R. Deletion of *Mycobacterium tuberculosis* sigma factor E results in delayed time to death with bacterial persistence in the lungs of aerosol-infected mice. Infect. Immun. **71(12)** (2003) 7170–7172.

See Current Literature, Experimental Infections, p. 230.

Brookes, R. H., Pathan, A. A., McShane, H., Hensmann, M., Price, D. A., and Hill, A. V. CD8+ T cell-mediated suppression of intracellular *Mycobacterium tuberculosis* growth in activated human macrophages. Eur. J. Immunol. **33(12)** (2003) 3293–3302.

Animal models of tuberculosis point to a protective role for MHC class I-restricted CD8(+) T cells, yet it is unclear how these cells protect or whether such findings extend to humans. Here we report that macrophages infected with *Mycobacterium tuberculosis*, rapidly process and present an early secreted antigenic target (ESAT-6)-specific HLA class I-restricted CD8(+) T cell epitope. When cocultured with CD8(+) T cells restricted through classical HLA class I molecules the growth of bacilli within macrophages is significantly impaired after 7 days. This slow antimycobacterial activity did not correlate with macrophage lysis but required cell contact. We also found that inhibitors of apoptosis either had no effect or augmented the CD8-mediated suppressive activity, suggesting that an activation signal might be involved. Indeed we show that CD8(+) T cells were able to activate macrophages through receptors that include CD95 (Fas). Consistent with these findings the CD8-mediated suppression of mycobacterial growth was partially reversed by Fas blockade. These data identify a previously unrecognized CD8(+) T cell-mediated mechanism used to control an intracellular infection of macrophages.—Authors' Abstract

Drennan, M. B., Nicolle, D., Quesniaux, V. J., Jacobs, M., Allie, N., Mpagi, J., Fremond, C., Wagner, H., Kirschning, C., and Ryffel, B. Toll-like receptor 2-deficient mice succumb to *Mycobacterium tuberculosis* infection. Am. J. Pathol. **164(1)** (2004) 49–57.

Recognition of *Mycobacterium tuberculosis* by the innate immune system is essential in the development of an adaptive immune response. Mycobacterial cell wall components activate macrophages through Toll-like receptor (TLR) 2, suggesting that this innate immune receptor plays a role in the host response to M. tuberculosis infection. After aerosol infection with either 100 or 500 live mycobacteria, TLR2-deficient mice display reduced bacterial clearance, a defective granulomatous response, and develop chronic pneumonia. Analysis of pulmonary immune responses in TLR2-deficient mice after 500 mycobacterial aerosol challenge showed increased levels of interferon-gamma, tumor necrosis factor-alpha, and interleukin-12p40 as well as increased numbers of CD4(+) and CD8(+) cells. Furthermore, TLR2-deficient mice mounted elevated Ag-specific type 1 T-cell responses that were not protective because all deficient mice succumb to infection within 5 months. Taken together, the data suggests that TLR2 may function as a regulator of inflammation, and in its absence an exaggerated immune inflammatory response develops.—Authors' Abstract

Geiman, D. E., Kaushal, D., Ko, C., Tyagi, S., Manabe, Y. C., Schroeder, B. G., Fleischmann, R. D., Morrison, N. E., Converse, P. J., Chen, P., and Bishai, W. R. Attenuation of late-stage disease in mice infected by the *Mycobacterium tuberculosis* mutant lacking the SigF alternate sigma factor and identification of SigF-dependent genes by microarray analysis. Infect. Immun. **72(3)** (2004) 1733–1745.

The *Mycobacterium tuberculosis* alternate sigma factor, SigF, is expressed during stationary growth phase and under stress conditions *in vitro*. To better understand the function of SigF we studied the phenotype of the *M. tuberculosis* DeltasigF *mutant in vivo* during mouse infection, tested the mutant as a vaccine in rabbits, and evaluated the mutant's microarray expression profile in comparison with the wild type. In mice the growth rates of the DeltasigF mutant and wild-type strains were nearly identical during the first 8 weeks after infection. At 8 weeks, the DeltasigF mutant persisted in the lung, while the wild type continued growing through 20 weeks. Histopathological analy-

sis showed that both wild-type and mutant strains had similar degrees of interstitial and granulomatous inflammation during the first 12 weeks of infection. However, from 12 to 20 weeks the mutant strain showed smaller and fewer lesions and less inflammation in the lungs and spleen. Intradermal vaccination of rabbits with the *M. tuberculosis* DeltasigF strain, followed by aerosol challenge, resulted in fewer tubercles than did intradermal *M. bovis* BCG vaccination. Complete genomic microarray analysis revealed that 187 genes were relatively underexpressed in the absence of SigF in early stationary phase, 277 in late stationary phase, and only 38 genes in exponential growth phase. Numerous regulatory genes and those involved in cell envelope synthesis were down-regulated in the absence of SigF; moreover, the DeltasigF mutant strain lacked neutral red staining, suggesting a reduction in the expression of envelope-associated sulfolipids. Examination of 5′-untranslated sequences among the down-regulated genes revealed multiple instances of a putative SigF consensus recognition sequence: GGTTTCX(18)GGGTAT. These results indicate that in the mouse the *M. tuberculosis* DeltasigF mutant strain persists in the lung but at lower bacterial burdens than wild type and is attenuated by histopathologic assessment. Microarray analysis has identified SigF-dependent genes and a putative SigF consensus recognition site.

Gilleron, M., Stenger, S., Mazorra, Z., Wittke, F., Mariotti, S., Bohmer, G., Prandi, J., Mori, L., Puzo, G., and De Libero, G. Diacylated Sulfoglycolipids Are Novel Mycobacterial Antigens Stimulating CD1-restricted T Cells during Infection with *Mycobacterium tuberculosis*. J. Exp. Med. **199(5)** (2004) 649–659.

Mycobacterial lipids comprise a heterogeneous group of molecules capable of inducing T cell responses in humans. To identify novel antigenic lipids and increase our understanding of lipid-mediated immune responses, we established a panel of T cell clones with different lipid specificities. Using this approach we characterized a novel lipid antigen belonging to the group of diacylated sulfoglycolipids purified from *Mycobacterium tuberculosis*. The structure of this sulfoglycolipid was identified as 2-palmitoyl or 2-stearoyl-3-hydroxyphthioceranoyl-2′-sulfate-alpha-alpha′-d-trehalose (Ac(2)SGL). Its immunogenicity is dependent on the presence of the sulfate group and of the two fatty acids. Ac(2)SGL is mainly presented by CD1b molecules after internalization in a cellular compartment with low pH. Ac(2)SGL-specific T cells release interferon gamma, efficiently recognize *M. tuberculosis*-infected cells, and kill intracellular bacteria. The presence of Ac(2)SGL-responsive T cells *in vivo* is strictly dependent on previous contact with *M. tuberculosis*, but independent from the development of clinically overt disease. These properties identify Ac(2)SGL as a promising candidate to be tested in novel vaccines against tuberculosis.—Authors' Abstract

Gold, J. A., Hoshino, Y., Tanaka, N., Rom, W. N., Raju, B., Condos, R., and Weiden, M. D. Surfactant protein A modulates the inflammatory response in macrophages during tuberculosis. Infect. Immun. **72(2)** (2004) 645–650.

Tuberculosis leads to immune activation and increased human immunodeficiency virus type 1 (HIV-1) replication in the lung. However, *in vitro* models of mycobacterial infection of human macrophages do not fully reproduce these *in vivo* observations, suggesting that there are additional host factors. Surfactant protein A (SP-A) is an important mediator of innate immunity in the lung. SP-A levels were assayed in the human lung by using bronchoalveolar lavage (BAL). There was a threefold reduction in SP-A levels during tuberculosis only in the radiographically involved lung segments, and the levels returned to normal after 1 month of treatment. The SP-A levels were inversely correlated with the percentage of neutrophils in BAL fluid, suggesting that low SP-A levels were associated with increased inflammation in the lung. Differentiated THP-1 macrophages were used to test the effect of decreasing SP-A levels on immune function. In the absence of infection with *Mycobacterium tuberculosis*, SP-A at doses ranging from 5 to 0.01 micro g/ml

inhibited both interleukin-6 (IL-6) production and HIV-1 long terminal repeat (LTR) activity. In macrophages infected with *M. tuberculosis*, SP-A augmented both IL-6 production and HIV-1 LTR activity. To better understand the effect of SP-A, we measured expression of CAAT/enhancer binding protein beta (C/EBPbeta), a transcription factor central to the regulation of IL-6 and the HIV-1 LTR. In macrophages infected with *M. tuberculosis*, SP-A reduced expression of a dominant negative isoform of C/EBPbeta. These data suggest that SP-A has pleiotropic effects even at the low concentrations found in tuberculosis patients. This protein augments inflammation in the presence of infection and inhibits inflammation in uninfected macrophages, protecting uninvolved lung segments from the deleterious effects of inflammation.—Authors' Abstract

Goletti, D., Carrara, S., Vincenti, D., Giacomini, E., Fattorini, L., Garbuglia, A. R., Capobianchi, M. R., Alonzi, T., Fimia, G. M., Federico, M., Poli, G., and Coccia, E. Inhibition of HIV-1 replication in monocyte-derived macrophages *by Mycobacterium tuberculosis*. J. Infect. Dis. **189(4)** (2004) 624–633.

Controversial results have been obtained in studies of the effect of *Mycobacterium tuberculosis* on human immunodeficiency virus type 1 (HIV-1) replication in cells of the macrophage lineage. In the present study, monocyte-derived macrophages (MDMs), previously incubated for 2 days with heat-inactivated *M. tuberculosis*, were infected with HIV-1. *M. tuberculosis* consistently inhibited viral replication, and a similar result also was observed in the presence of supernatants from *M. tuberculosis*-stimulated MDMs, which indicates that this effect was mediated by soluble factors. Although CCR5-binding chemokines were induced by *M. tuberculosis* stimulation, the results of neutralization experiments indicated that it is unlikely that they were responsible for viral suppression. Inhibition occurred mainly after viral entry (demonstrated by use of a vesicular stomatitis virus G-pseudotyped HIV-1 and by analysis of HIV-1 early and late reverse-transcription products). Therefore, *M. tuberculosis*-induced factors may inhibit *in vitro* HIV-1 replication in macrophages by affecting an early postentry step in the HIV-1 cycle.—Authors' Abstract

Guinn, K. M., Hickey, M. J., Mathur, S. K., Zakel, K. L., Grotzke, J. E., Lewinsohn, D. M., Smith, S., and Sherman, D. R. Individual RD1-region genes are required for export of ESAT-6/CFP-10 and for virulence of *Mycobacterium tuberculosis*. Mol. Microbiol. **51(2)** (2004) 359–370.

The RD1 genomic region is present in virulent strains *of Mycobacterium tuberculosis* (MTB), missing from the vaccine strain *M. bovis* BCG, and its importance to virulence has been established experimentally. Based on *in silico* analysis, it has been suggested that RD1 may encode a novel secretion system, but the mechanism by which this region affects virulence is unknown. Here we examined mutants disrupted in five individual RD1 genes. Both *in vitro* and *in vivo*, each mutant displayed an attenuated phenotype very similar to a mutant missing the entire RD1 region. Genetic complementation of individual genes restored virulence. Attenuated mutants could multiply within THP-1 cells, but they were unable to spread to uninfected macrophages. We also examined export of two immunodominant RD1 proteins, CFP-10 and ESAT-6. Export of these proteins was greatly reduced or abolished in each attenuated mutant. Again, genetic complementation restored a wild-type phenotype. Our results indicate that RD1 genes work together to form a single virulence determinant, and argue that RD1 encodes a novel specialized secretion system that is required for pathogenesis of MTB.—Authors' Abstract

Harboe, M., Das, A. K., Mitra, D., Ulvund, G., Ahmad, S., Harkness, R. E., Das, D., Mustafa, A. S., and Wiker, H. G. Immunodominant B-cell epitope in the Mce1A mammalian cell entry protein of *Mycobacterium tuberculosis* cross-reacting with glutathione S-transferase. Scand. J. Immunol. **59(2)** (2004) 190–197.

The TB1-5 76C monoclonal antibody raised against a synthetic 60-mer peptide in the N-terminal part of the Mce1A mammalian cell entry protein of *Mycobacterium tuberculosis* has previously been shown to react with a linear epitope in the KRRITPKD region, residues 131–138 in Mce1A, and to cross-react with Mce1F. Six additional monoclonal antibodies raised against the same peptide were also shown to cross-react with Mce1F. Four of them reacted with a linear epitope in the same area, indicating that this area is immunodominant but showed distinct differrences in fine specificity. Two monoclonal antibodies did not react with synthetic peptides from this region on the solid phase in enzyme-linked immunosorbent assay, indicating greater influence of conformation on reactivity. None of the monoclonal antibodies reacted with 14-mer synthetic peptides from the corresponding area in Mce2A, Mce3A, Mce4A, *M. avium*, *M. smegmatis* or *M. leprae*. The reaction pattern of the monoclonal antibodies was analysed in relation to our model of the Mce1A molecule (AK Das, *et al.* Biochem Biophys Res Commun 2003;302:442–7). The epitope is located on the surface of Mce1A, at the distal beta-strand-loop region in the beta-domain supporting its potential role in promoting uptake of *M. tuberculosis* in host cells. Monoclonal antibody TB1-5 19C cross-reacted with glutathione S-transferase of Schistosoma japonicum containing a PKE triplet. Monoclonal antibody TB1-5 76C gave a major band at about 44 kDa in Western blotting of *M. tuberculosis* sonicate, whereas polyclonal rabbit anti-Mce1A peptide antibodies reacting with the extended TTPKNPTKRRITPKDVI area of Mce1A showed a distinct band above the 160 kDa molecular mass standard.—Authors' Abstract

Higuchi, K., Sekiya, Y., and Harada, N. Characterization of *M. Tuberculosis*-derived IL-12-inducing material by alveolar macrophages. Vaccine **22(5–6)** (2004) 724–734.

We have investigated the substance derived from *Mycobacterium tuberculosis* (Mtb) that induces interleukin (IL)-12 production by alveolar macrophages (AMs) *in vitro*. The cytosol fraction of live Mtb H37Rv induced IL-12 production by AMs in a dose-dependent manner. The addition of interferon-gamma (IFN-gamma) augmented IL-12 production. IL-12-inducing activity by AMs (termed as surely active keeping rescue antigen, SAKRA) was purified by gel filtration and ion exchange column chromatography, and the molecular weight of SAKRA was estimated by gel filtration to be more than 700 kDa. SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting of SAKRA using rabbit anti-SAKRA antibody suggested that SAKRA is composed with several low molecular weight proteins. Amino acids sequence analysis of several bands after SDS-PAGE suggested that SAKRA is a part of ribosomes. RT-PCR showed that SAKRA induced not only expression of IL-12 p40 mRNA, but expression of tumor necrosis factor (TNF)-alpha and inducible nitric oxide synthase (iNOS) mRNA at least 6 hr after stimulation, suggesting that SAKRA activates the bactericidal activity of macrophages. To investigate the potential use of SAKRA as a vaccine against tuberculosis, SAKRA was administered to BALB/c mouse that had been immunized with BCG for 18 months, and mouse were infected with Mtb H37Rv via a respiratory route. Replication of Mtb in lungs and spleens was examined 6 weeks after infection. Administration of SAKRA to BCG-vaccinated mice significantly reduced the numbers of Mtb in lungs and spleens as compared with BCG-vaccinated control mice. Taken together, these results suggest that SAKRA is one of the Mtb-derived immunomodulatory substances which induce IL-12 production during infection and also increases mycobactericidal activities of macrophages, and that SAKRA may be a promising new vaccine candidate against tuberculosis.

Junqueira-Kipnis, A. P., Kipnis, A., Jamieson, A., Juarrero, M. G., Diefenbach, A., Raulet, D. H., Turner, J., and Orme, I. M. NK cells respond to pulmonary infection with *Mycobacterium tuberculosis*, but play a minimal role in protection. J. Immunol. **171(11)** (2003) 6039–6045.

Both innate and adaptive immune systems contribute to host defense against infection with *Mycobacterium tuberculosis*. NK cells have been associated with early resistance against intracellular pathogens and are known to be potent producers of the cytokine IFN-gamma. In C57BL/6 mice infected by aerosol exposure *with M. tuberculosis*, NK cells increased in the lungs over the first 21 days of infection. Expansion of the NK cell subset was associated with increased expression of activation and maturation markers. In addition, NK cells isolated from the infected lungs were capable of producing IFN-gamma and became positive for perforin. *In vivo* depletion of NK cells using a lytic Ab had no influence on bacterial load within the lungs. These findings indicate that NK cells can become activated during the early response to pulmonary tuberculosis in the mouse model and are a source of IFN-gamma, but their removal does not substantially alter the expression of host resistance.—Authors' Abstract

Kanaujia, G. V., Motzel, S., Garcia, M. A., Andersen, P., and Gennaro, M. L. Recognition of ESAT-6 sequences by antibodies in sera of tuberculous nonhuman primates. Clin. Diagn. Lab. Immunol. **11(1)** (2004) 222–226.

See Current Literature, Experimental Infections, p. 235.

Kanaujia, G. V., Garcia, M. A., Bouley, D. M., Peters, R., and Gennaro, M. L. Detection of early secretory antigenic target-6 antibody for diagnosis of tuberculosis in non-human primates. Comp. Med. **53(6)** (2003) 602–606.

See Current Literature, Experimental Infections, p. 235.

Kaplan, G., Post, F. A., Moreira, A. L., Wainwright, H., Kreiswirth, B. N., Tanverdi, M., Mathema, B., Ramaswamy, S. V., Walther, G., Steyn, L. M., Barry, C. E. 3rd, and Bekker, L. G. *Mycobacterium tuberculosis* growth at the cavity surface: a microenvironment with failed immunity. Infect. Immun. **71(12)** (2003) 7099–7108.

Protective immunity against pulmonary tuberculosis (TB) is characterized by the formation in the lungs of granulomas consisting of macrophages and activated T cells producing tumor necrosis factor alpha and gamma interferon, both required for the activation of the phagocytes. In 90% of immunocompetent humans, this response controls the infection. To understand why immunity fails in the other 10%, we studied the lungs of six patients who underwent surgery for incurable TB. Histologic examination of different lung lesions revealed heterogeneous morphology and distribution of acid-fast bacilli; only at the surface of cavities, i.e., in granulomas with a patent connection to the airways, were there numerous bacilli. The mutation profile of the isolates suggested that a single founder strain of *Mycobacterium tuberculosis* may undergo genetic changes during treatment, leading to acquisition of additional drug resistance independently in discrete physical locales. Additional drug resistance was preferentially observed at the cavity surface. Cytokine gene expression revealed that failure to control the bacilli was not associated with a generalized suppression of cellular immunity, since cytokine mRNA was up regulated in all lesions tested. Rather, a selective absence of CD4(+) and CD8(+) T cells was noted at the luminal surface of the cavity, preventing direct T-cell-macrophage interactions at this site, probably allowing luminal phagocytes to remain permissive for bacillary growth. In contrast, in the perinecrotic zone of the granulomas, the two cell types colocalized and bacillary numbers were substantially lower, suggesting that in this microenvironment an efficient bacteriostatic or bactericidal phagocyte population was generated.—Authors' Abstract

Lazarevic, V., Myers, A. J., Scanga, C. A., and Flynn, J. L. CD40, but not CD40L, is required for the optimal priming of T cells and control of aerosol *M. tuberculosis* infection. Immunity. **19(6)** (2003) 823–835.

CD40(–/–) mice succumbed to low-dose aerosol infection with *M. tuberculosis* due to deficient IL-12 production leading to impaired priming of IFN-gamma T cell responses. In contrast, CD40L(–/–) mice were resistant to *M. tuberculosis*. This asymmetry in outcome of infection between the two knockout strains is likely due to the existence of an alternative ligand for CD40. Both *in vitro M. tuberculosis* infection and recombinant *M. tuberculosis* Hsp70 elicited IL-12 production from WT dendritic cells. This response was absent in both CD40(–/–) dendritic cells and CD40(–/–) mice, suggesting that *M. tuberculosis* Hsp70 serves as an alternative ligand for CD40 *in vivo*.—Authors' Abstract

Majlessi, L., Rojas, M. J., Brodin, P., and Leclerc, C. CD8+-T-cell responses of Mycobacterium-infected mice to a newly identified major histocompatibility complex class I-restricted epitope shared by proteins of the ESAT-6 family. Infect. Immun. **71(12)** (2003) 7173–7177.

Here we describe the identification of a new CD8(+)-T-cell epitope, the GYAGTLQSL nonamer, shared by the TB10.3 and TB10.4 proteins of the *Mycobacterium tuberculosis* ESAT-6 family. Cytotoxic T cells from mycobacterium-infected mice efficiently recognized this epitope. GYAGTLQSL-specific T-cell hybridomas, which were able to recognize *Mycobacterium bovis* BCG-infected macrophages, were generated and now allow investigation of mycobacterial-antigen processing through the major histocompatibility complex class I pathway.—Authors' Abstract

Malhotra, V., Sharma, D., Ramanathan, V. D., Shakila, H., Saini, D. K., Chakravorty, S., Das, T. K., Li, Q., Silver, R. F., Narayanan, P. R., Tyagi, J. S. Disruption of response regulator gene, devR, leads to attenuation in virulence of *Mycobacterium tuberculosis*. FEMS Microbiol Lett. **20231(2)** (2004) 237–245.

The devR-devS two-component system of *Mycobacterium tuberculosis* was identified earlier and partially characterized in our laboratory. A devR::kan mutant *of M. tuberculosis* was constructed by allelic exchange. The devR mutant strain showed reduced cell-to-cell adherence in comparison to the parental strain in laboratory culture media. This phenotype was reversed on complementation with a wild-type copy of devR. The devR mutant and parental strains grew at equivalent rates within human monocytes either in the absence or in the presence of lymphocytic cells. The expression of DevR was not modulated upon entry of *M. tuberculosis* into human monocytes. However, guinea pigs infected with the mutant strain showed a significant decrease in gross lesions in lung, liver and spleen; only mild pathological changes in liver and lung; and a nearly 3 log lower bacterial burden in spleen compared to guinea pigs infected with the parental strain. Our results suggest that DevR is required for virulence in guinea pigs but is not essential for entry, survival and multiplication of *M. tuberculosis* within human monocytes *in vitro*. The attenuation in virulence of the devR mutant in guinea pigs together with DevR-DevS being a bona fide signal transduction system indicates that DevR plays a critical and regulatory role in the adaptation and survival of *M. tuberculosis* within tissues.—Authors' Abstract

Mattow, J., Schaible, U. E., Schmidt, F., Hagens, K., Siejak, F., Brestrich, G., Haeselbarth, G., Muller, E. C., Jungblut, P. R., and Kaufmann, S. H. Comparative proteome analysis of culture supernatant proteins from virulent *Mycobacterium tuberculosis* H37Rv and attenuated *M. bovis* BCG Copenhagen. Electrophoresis **24(19–20)** (2004) 3405–3420.

A comprehensive analysis of culture supernatant (CSN) proteins of *Mycobacterium tuberculosis* H37Rv was accomplished by combination of two-dimensional electrophoresis (2-DE), mass spectrometry, and N-terminal sequencing by Edman degradation. Analytical 2-DE gels resolved approximately 1250 protein spots from CSN of *M. tuberculosis* H37Rv, 381 of which were identified by mass spectrometry and/or Edman degradation. This study revealed 137 different proteins, 42 of which had previously been described as secreted. Compar-

ative proteome analysis of CSN from virulent *M. tuberculosis* H37Rv and attenuated *Mycobacterium bovis* BCG Copenhagen identified 39 *M. tuberculosis*-specific spots containing 27 different proteins, representing candidate antigens for novel vaccines and diagnostics in tuberculosis. These included five proteins encoded by open reading frames absent from *M. bovis* BCG, e.g., early secretory antigen target (Esat6), as well as 22 novel differential proteins, such as acetyl-CoA C-acetyltransferase (Rv0243) and two putative Esat6-like proteins (Rv1198, Rv1793).—Authors' Abstract

McCarthy, A. A., Knijff, R., Peterson, N. A., and Baker, E. N. Crystallization and preliminary X-ray analysis of N-acetyl-1-D-myo-inosityl-2-deoxy-alpha-D-glucopyranoside deacetylase (MshB) from *Mycobacterium tuberculosis*. Acta. Crystallogr. D. Biol. Crystallogr. 59(Pt 12) (2003) 2316–2318.

Mycobacteria synthesize mycothiol (MSH) as a low-molecular-weight thiol that protects against oxidative stress in a similar role to that of glutathione in many other species. The absence of MSH in mammals suggests that enzymes from its biosynthetic pathway in *Mycobacterium tuberculosis* could be useful targets for drug design. The gene for MshB (Rv1170), the enzyme that catalyses the second step in MSH biosynthesis in *M. tuberculosis*, has been cloned and the protein has been expressed in Escherichia coli both in native and SeMet-substituted forms and crystallized in two crystal forms. One of these, prepared in the presence of beta-octylglucoside as a key additive, is suitable for high-resolution X-ray structural analysis. The crystals are orthorhombic, with unit-cell parameters a = 71.69, b = 83.74, c = 95.65 A, space group P2(1)2(1)2(1) and two molecules in the asymmetric unit. X-ray diffraction data to 1.9 A resolution have been collected.—Authors' Abstract

Pasquinelli, V., Quiroga, M. F., Martinez, G. J., Zorrilla, L. C., Musella, R. M., Bracco, M. M., Belmonte, L., Malbran, A., Fainboim, L., Sieling, P. A., and Garcia, V. E. Expression of signaling lymphocytic activation molecule-associated protein interrupts IFN-gamma production in human tuberculosis. J. Immunol. **172(2)** (2004) 1177–1185.

Production of the Th1 cytokine IFN-gamma by T cells is considered crucial for immunity against *Mycobacterium tuberculosis* infection. We evaluated IFN-gamma production in tuberculosis in the context of signaling molecules known to regulate Th1 cytokines. Two populations of patients who have active tuberculosis were identified, based on their T cell responses to the bacterium. High responder tuberculosis patients displayed significant *M. tuberculosis*-dependent T cell proliferation and IFN-gamma production, whereas low responder tuberculosis patients displayed weak or no T cell responses to *M. tuberculosis*. The expression of the signaling lymphocytic activation molecule (SLAM)-associated protein (SAP) on cells from tuberculosis patients was inversely correlated with IFN-gamma production in those individuals. Moreover, patients with a nonfunctional SAP gene displayed immune responses to *M. tuberculosis* similar to those of high responder tuberculosis patients. In contrast to SAP, T cell expression of SLAM was directly correlated with responsiveness to *M. tuberculosis* Ag. Our data suggest that expression of SAP interferes with Th1 responses whereas SLAM expression contributes to Th1 cytokine responses in tuberculosis. The study further suggests that SAP and SLAM might be focal points for therapeutic modulation of T cell cytokine responses in tuberculosis.—Authors' Abstract

Pereira, C. B., Palaci, M., Leite, O. H., Duarte, A. J., and Benard, G. Monocyte cytokine secretion in patients with pulmonary tuberculosis differs from that of healthy infected subjects and correlates with clinical manifestations. Microbes Infect. **6(1)** (2004) 25–33.

Cell-mediated immunity, leading to *Mycobacterium tuberculosis* (Mtb)-constraining granuloma formation, is the major component of host defense against tuberculosis and is regulated by the balance of cytokines se-

creted mostly by mononuclear phagocytes and lymphocytes. To better understand the role of monocytes in the regulation of the immune response against pulmonary tuberculosis, we examined IL-10, IL-12 and TNF-alpha release by monocytes from healthy purified protein derivative (PPD) reactors and pulmonary tuberculosis patients with or without systemic reactions (e.g., fever, weight loss, asthenia). Our study shows that, probably as a result of *in vivo* priming by circulating antigens, monocytes from patients, especially those with systemic manifestations, have a biased *ex vivo* cytokine secretion, with high IL-10 and TNF-alpha but low IL-12, in contrast with PPD reactors. Higher spontaneous IL-10 and TNF-alpha release persisted when monocytes were co-cultured with autologous lymphocytes. Challenge of patients' monocytes with a virulent Mtb strain led to a further enhancement of IL-10 and TNF-alpha, but not of IL-12. When lymphocytes were added to these cultures, IL-10 and TNF-alpha elevation persisted and, in the patients with a systemic reaction, both IL-12 and IFN-gamma were significantly reduced compared to PPD reactors. Intragroup comparisons revealed that in the patients with systemic reactions, the lymphocyte-monocyte interaction resulted in a positive feedback for IL-10 secretion, while in the patients without systemic reaction and PPD reactors, the feedback was positive for IL-12 secretion. Thus, in tuberculosis, there appears to exist a relationship between the immunological findings and the distinct clinical manifestations.—Authors' Abstract

Qiao, Y., Prabhakar, S., Canova, A., Hoshino, Y., Weiden, M., and Pine, R. Posttranscriptional inhibition of gene expression by *Mycobacterium tuberculosis* offsets transcriptional synergism with IFN-gamma and posttranscriptional upregulation by IFN-gamma. J. Immunol. **172(5)** (2004) 2935–2943.

Host defense against *Mycobacterium tuberculosis* requires the cytokine IFN-gamma and IFN regulatory factor 1 (IRF-1), a transcription factor that is induced to high levels by IFN-gamma. Therefore, we chose to study regulation of IRF-1 expression as a model for effects of *M. tuberculosis* on response to IFN-gamma. We found that IRF-1 mRNA abundance increased far more than transcription rate in human monocytic THP-1 cells stimulated by IFN-gamma, but less than transcription rate in cells infected by *M. tuberculosis*. IFN-gamma stimulation of infected cells caused a synergistic increase in IRF-1 transcription, yet IRF-1 mRNA abundance was similar in uninfected and infected cells stimulated by IFN-gamma, as was the IRF-1 protein level. Comparable infection by *Mycobacterium bovis* bacillus Calmette-Guerin failed to induce IRF-1 expression and had no effect on the response to IFN-gamma. We also examined the kinetics of transcription, the mRNA t(1/2), and the distribution of IRF-1 transcripts among total nuclear RNA, poly(A) nuclear RNA, and poly(A) cytoplasmic RNA pools in cells that were infected by *M. tuberculosis* and/or stimulated by IFN-gamma. Our data suggest that infection *by M. tuberculosis* inhibits RNA export from the nucleus. Moreover, the results indicate that regulated entry of nascent transcripts into the pool of total nuclear RNA affects IRF-1 expression and that this process is stimulated by IFN-gamma and inhibited by *M. tuberculosis*. The ability of infection by *M. tuberculosis* to limit the increase in IRF-1 mRNA expression that typically follows transcriptional synergism may contribute to the pathogenicity of *M. tuberculosis*.—Authors' Abstract

Rousseau, C., Winter, N., Pivert, E., Bordat, Y., Neyrolles, O., Ave, P., Huerre, M., Gicquel, B., and Jackson, M. Production of phthiocerol dimycocerosates protects *Mycobacterium tuberculosis* from the cidal activity of reactive nitrogen intermediates produced by macrophages and modulates the early immune response to infection. Cell Microbiol. **6(3)** (2004) 277–287.

The growth of *Mycobacterium tuberculosis* mutants unable to synthesize phthiocerol dimycocerosates (DIMs) was recently shown to be impaired in mouse lungs. However, the precise role of these molecules in the course of infection remained to

be determined. Here, we provide evidence that the attenuation of a DIM-deficient strain takes place during the acute phase of infection in both lungs and spleen of mice, and that this attenuation results in part from the increased sensitivity of the mutant to the cidal activity of reactive nitrogen intermediates released by activated macrophages. We also show that the DIM-deficient mutant, the growth and survival of which were not impaired within resting macrophages and dendritic cells, induced these cells to secrete more tumour necrosis factor (TNF)-alpha and interleukin (IL)-6 than the wild-type strain. Although purified DIM molecules by themselves had no effect on the activation of macrophages and dendritic cells *in vitro*, we found that the proper localization of DIMs in the cell envelope of *M. tuberculosis* is critical to their biological effects. Thus, our findings suggest that DIM production contributes to the initial growth of *M. tuberculosis* by protecting it from the nitric oxide-dependent killing of macrophages and modulating the early immune response to infection.—Authors' Abstract

Shanmugalakshmi, S., Dheenadhayalan, V., Muthuveeralakshmi, P., Arivarignan, G., and Pitchappan, R. M. *Mycobacterium bovis* BCG scar status and HLA class II alleles influence purified protein derivative-specific T-cell receptor V? expression in pulmonary tuberculosis patients from Southern India. Infect. Immunity **71(8)** (2003) 4544–4553.

Purified protein derivative (PPD) RT23-recalled T-cell receptor (TCR) Vβ expression was studied in the peripheral blood of 42 pulmonary tuberculosis patients and 44 healthy controls from southern India, a region where tuberculosis is endemic. Forty-eight-hour whole-blood cultures in the presence or absence of PPD-RT23 were set up, and at the end of the culture period total RNA was extracted and cDNA was synthesized. Expression of various TCR Vβ families was assessed by using family-specific primers. PPD-specific expression (usage) of TCR Vβ families 4, 6, 8 to 12, and 14 was found in more controls than patients. Among the responders (individuals who showed PPD-specific expression), endemic controls had significantly higher responses than the patients had for TCR Vβ families 2, 3, 7, 13, and 17. The majority of the patients did not show usage of most of the TCR Vβ families, and this was attributed to T-cell downregulation. A four-way nested classification analysis revealed that TCR Vβ family 1, 5, 9, 12, and 13 usage in the context of HLA class II high-risk alleles) (DRB1*1501, DRB1*08, and DQB1*0601) and *Mycobacterium bovis* BCG scar status were the determining factors in susceptibility and resistance to tuberculosis. The healthier status of controls was attributed to the wider usage of many TCR Vβ families readily recalled by PPD, while the disease status of the patients was attributed to TCR Vβ downregulation and the resultant T-cell (memory cell?) unresponsiveness. Host genetics (HLA status) and BCG vaccination (scar status) seem to play important roles in skewing the immune response in adult susceptibility to pulmonary tuberculosis through TCR Vβ usage.—Tropical Disease Bulletin

Stanton, L. A., Fenhalls, G., Lucas, A., Gough, P., Greaves, D. R., Mahoney, J. A., Helden, P., and Gordon, S. Immunophenotyping of macrophages in human pulmonary tuberculosis and sarcoidosis. Int. J. Exp. Pathol. **84(6)** (2003) 289–304.

Classic studies of tuberculosis (TB) revealed morphologic evidence of considerable heterogeneity of macrophages (MOs), but the functional significance of this heterogeneity remains unknown. We have used newly available specific antibodies for selected membrane and secretory molecules to examine the phenotype of MOs *in situ* in a range of South African patients with TB, compared with sarcoidosis. Patients were human immunodeficiency virus-negative adults and children, and the examined biopsy specimens included lung and lymph nodes. Mature pulmonary MOs (alveolar, interstitial, epithelioid and multinucleated giant cells) selectively expressed scavenger receptor type A and a novel carboxypeptidase-like antigen called carboxypeptidase-related vitellogenin-like MO molecule (CPVL). CPVL did not display enhanced expression

in sarcoidosis, vs. TB patients, as observed with angiotensin-converting enzyme (ACE), a related molecule. Immunocytochemical studies with surfactant proteins (SP)-A and -D showed that type II alveolar cells expressed these collectins, as did MOs, possibly after binding of secreted proteins. Studies with an antibody specific for the C-terminus of fractalkine, a tethered CX3C chemokine, confirmed synthesis of this molecule by bronchiolar epithelial cells and occasional endothelial cells. These studies provide new marker antigens and extend previous studies on MO differentiation, activation and local interactions in chronic human granulomatous inflammation in the lung.—Authors' Abstract

Turner, J., and Orme, I. M. The expression of early resistance to an infection with *Mycobacterium tuberculosis* by old mice is dependent on IFN type II (IFN-gamma) but not IFN type I. Mech. Ageing Dev. **125(1)** (2004) 1–9.

Old mice can express a transient early resistance to infection with *M. tuberculosis* that requires the presence of CD8 T cells within the lungs. Further characterization of those CD8 T cells within the aged lung established that the majority of CD8 T cells from old mice expressed the IL-15 receptor (CD122) in combination with bright expression of CD44 (CD44(hi)), and were capable of producing IFN-gamma after T cell receptor cross-linking. It has been previously described that CD8 CD44(hi) T cells proliferate in response to IFN-I, acting via IL-15, and therefore we determined whether IFN-I signaling could be a participant in the response of CD8 T cells within the lungs of old mice infected with *M. tuberculosis*. We demonstrate here that IFN-I signaling was required for the expansion of CD8 T cells within the aging lung in response to infection with *M. tuberculosis*, but that IFN-I signaling had no influence on the capacity of old mice to express early resistance to an infection with *M. tuberculosis*. Resident CD8 T cells were still however capable of producing IFN-gamma, which we demonstrate here to be critical in the expression of early resistance, suggesting that the expression of early resistance requires the participation, but not expansion, of the CD8 T cell pool within the aging lung.—Authors' Abstract

Vergne, I., Fratti, R. A., Hill, P. J., Chua, J., Belisle, J., and Deretic, V. *Mycobacterium tuberculosis* phagosome maturation arrest: mycobacterial phosphatidylinositol analog phosphatidylinositol mannoside stimulates early endosomal fusion. Mol. Biol. Cell. **15(2)** (2004) 751–760.

Mycobacterium tuberculosis is a facultative intracellular pathogen that parasitizes macrophages by modulating properties of the Mycobacterium-containing phagosome. Mycobacterial phagosomes do not fuse with late endosomal/lysosomal organelles but retain access to early endosomal contents by an unknown mechanism. We have previously reported that mycobacterial phosphatidylinositol analog lipoarabinomannan (LAM) blocks a trans-Golgi network-to-phagosome phosphatidylinositol 3-kinase-dependent pathway. In this work, we extend our investigations of the effects of mycobacterial phosphoinositides on host membrane trafficking. We present data demonstrating that phosphatidylinositol mannoside (PIM) specifically stimulated homotypic fusion of early endosomes in an ATP-, cytosol-, and N-ethylmaleimide sensitive factor-dependent manner. The fusion showed absolute requirement for small Rab GTPases, and the stimulatory effect of PIM increased upon partial depletion of membrane Rabs with RabGDI. We found that stimulation of early endosomal fusion by PIM was higher when phosphatidylinositol 3-kinase was inhibited by wortmannin. PIM also stimulated *in vitro* fusion between model phagosomes and early endosomes. Finally, PIM displayed *in vivo* effects in macrophages by increasing accumulation of plasma membrane-endosomal syntaxin 4 and transferrin receptor on PIM-coated latex bead phagosomes. In addition, inhibition of phagosomal acidification was detected with PIM-coated beads. The effects of PIM, along with the previously reported action of LAM, suggest that *M. tuberculosis* has evolved a two-prong strategy to modify its intracellular niche: its products block acquisition of late endosomal/lysosomal constituents, while facilitating fusion with early

endosomal compartments.—Authors' Abstract

Wang, J. P., Rought, S. E., Corbeil, J., and Guiney, D. G. Gene expression profiling detects patterns of human macrophage responses following *Mycobacterium tuberculosis* infection. FEMS Immunol. Med. Microbiol. **39(2)** (2003) 163–172.

See Current Literature, Molecular and Genetic Studies, p. 262.

Microbiology

Adekambi, T., Colson, P., and Drancourt, M. rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J. Clin. Microbiol. **41(12)** 2003 5699–5708.

Nonpigmented and late-pigmenting rapidly growing mycobacteria (RGM) are increasingly isolated in clinical microbiology laboratories. Their accurate identification remains problematic because classification is labor intensive work and because new taxa are not often incorporated into classification databases. Also, 16S rRNA gene sequence analysis underestimates RGM diversity and does not distinguish between all taxa. We determined the complete nucleotide sequence of the rpoB gene, which encodes the bacterial beta subunit of the RNA polymerase, for 20 RGM type strains. After using in-house software which analyzes and graphically represents variability stretches of 60 bp along the nucleotide sequence, our analysis focused on a 723-bp variable region exhibiting 83.9 to 97% interspecies similarity and 0 to 1.7% intraspecific divergence. Primer pair Myco-F-Myco-R was designed as a tool for both PCR amplification and sequencing of this region for molecular identification of RGM. This tool was used for identification of 63 RGM clinical isolates previously identified at the species level on the basis of phenotypic characteristics and by 16S rRNA gene sequence analysis. Of 63 clinical isolates, 59 (94%) exhibited <2% partial rpoB gene sequence divergence from 1 of 20 species under study and were regarded as correctly identified at the species level. Mycobacterium abscessus *and Mycobacterium mucogenicum* isolates were clearly distinguished from *Mycobacterium chelonae*; *Mycobacterium mageritense* isolates were clearly distinguished from "*Mycobacterium houstonense*." Four isolates were not identified at the species level because they exhibited >3% partial rpoB gene sequence divergence from the corresponding type strain; they belonged to three taxa related to *M. mucogenicum, Mycobacterium smegmatis*, and *Mycobacterium porcinum*. For *M. abscessus* and *M. mucogenicum*, this partial sequence yielded a high genetic heterogeneity within the clinical isolates. We conclude that molecular identification by analysis of the 723-bp rpoB sequence is a rapid and accurate tool for identification of RGM.—Authors' Abstract

Chattopadhyay, C., Sau, S., and Mandal, N. C. Cloning and characterization of the promoters of temperate mycobacteriophage L1. J. Biochem. Mol. Biol. **36(6)** (2003) 586–592.

See Current Literature, Molecular and Genetic Studies, p. 256.

Chui, L. W., King, R., Lu, P., Manninen, K., and Sim, J. Evaluation of four DNA extraction methods for the detection of *Mycobacterium avium* subsp. paratuberculosis by polymerase chain reaction. Diagn. Microbiol. Infect. Dis. **48(1)** (2004) 39–45.

Polymerase chain reaction (PCR) has been widely used due to its high specificity, sensitivity, and rapid turn-around time. However, inhibitory factors may be coextracted with the target nucleic acid that will hinder the performance of PCR. In this study, DNA extraction methods for *Mycobacterium avium* subsp. paratuberculosis were evaluated including rapid lysis, organic extraction, silica-based and magnetic particle-based (MagaZorb) technologies on bacterial cells, and spiked bovine feces. Efficiency of the extraction was determined by PCR end point titration with primers targeting the insertion sequence, IS900. Results of

the end point titrations are identical for bacterial cells and spiked feces. Inhibition was observed in PCR with DNA isolated from spiked feces, and a 1/100 dilution was able to alleviate this problem with DNA extracted by MagaZorb. A 1/1000 dilution was required for the other three methods. MagaZorb proved to be more efficient at removing inhibitory factors and required the least labor and completion time. Further evaluation is required for its utilization in other clinical specimens.—Authors' Abstract

Cociorva, O. M., and Lowary, T. L. Synthesis of oligosaccharides as potential inhibitors of mycobacterial arabinosyltransferases. Di- and trisaccharides containing C-5 modified arabinofuranosyl residues. Carbohydr. Res. **339(4)** (2004) 853–865.

The synthesis of a panel of oligosaccharides containing C-5 arabinofuranosyl residues (9–20) is described. These compounds are of interest as potential inhibitors of the alpha-(1→5)-arabinosyltransferase involved in the assembly of mycobacterial cell-wall arabinan. In the series of compounds prepared, the 5-OH group on the nonreducing residue(s) is replaced, independently, with an amino, azido, fluoro, or methoxy functionality. The synthesis of the target compounds involved the preparation of a series of C-5 modified arabinofuranosyl thioglycosides (24–26) and their subsequent coupling to the appropriate acceptor species (21–23). Deprotection of the glycosylation products afforded the azido, fluoro, or methoxy analogs directly. The amino derivatives were obtained in one additional step by reduction of the azido compounds.—Authors' Abstract

Daniel, A. K., Lee, R. E., Portaels, F., and Small, P. L. Analysis of Mycobacterium species for the presence of a macrolide toxin, mycolactone. Infect. Immun. **72(1)** (2004) 123–132.

Mycobacterium ulcerans is an environmental organism which is responsible for the disease Buruli ulcer, a necrotizing skin disease emerging in west Africa. *M. ulcerans* produces the polyketide-derived macrolide mycolactone, which is required for the immunosuppression and tissue damage which characterizes Buruli ulcer. We have extracted lipids from the cell envelope and culture filtrate from 52 isolates of Mycobacterium species, analyzed them with thin-layer chromatography, and tested them in a murine fibroblast cell line (L929) cytotoxicity assay to investigate whether these mycobacterial species produce mycolactone. For these studies chloroform-methanol (2:1, vol/vol) extracts were prepared from representative fast- and slow-growing mycobacterial species. Isolates tested included 16 uncharacterized, slow-growing, environmental mycobacterial species isolated from areas in which *M. ulcerans* infection is endemic. Although several strains of mycobacteria studied produced cytopathic lipids, none of these produced a phenotype on cultured cells consistent with that produced by mycolactone. Two mycobacterial species, *M. scrofulaceum* and *M. kansasii*, and eight of the environmental mycobacterial isolates contained cell-associated lipids cytopathic to fibroblasts at concentrations of 33 to 1000 microg/ml. In contrast, mycolactone produces cytotoxicity at less than 2 ng/ml. Analysis of 16S rRNA sequences from the eight environmental isolates suggests that these are novel mycobacterial species. Results from these studies suggest that, although production of cytopathic lipids is relatively common among mycobacterial species, the production of mycolactone as a cell-associated or secreted molecule appears so far to be restricted to *M. ulcerans*.—Author's Abstract

Ergin, A., and Hascelik, G. Non-tuberculous mycobacteria (NTM) in patients with underlying diseases: results obtained by using polymerase chain reaction-restriction enzyme analysis between 1997–2002. New Microbiol. **27(1)** (2004) 49–53.

In this study, we aimed to evaluate the frequency of non-tuberculous mycobacteria (NTM) isolated from clinical specimens using Polymerase Chain Reaction-Restriction Enzyme Analysis (PCR-REA) and to investigate the patients who had clinically signif-

icant NTM infections in our hospital through the five year period from May 1997 to June 2002. A total of 364 mycobacterial strains isolated from clinical specimens which gave positive growth index in the BACTEC 460 radiometric system in Hacettepe University Hospital Clinical Microbiology Laboratory were evaluated by PCR-REA and clinical data were obtained from the patient records. Three hundred and one of the strains (82.7%) were identified as *Mycobacterium tuberculosis* and 63 (17.3%) were identified as nontuberculous mycobacteria. Seven (11.1%) of 63 NTM patients were regarded as having clinical mycobacteriosis. Chronic obstructive pulmonary disease and other preexisting lung diseases were seen in 39 (61.9%) of the patients, 11 (17.5%) of the patients had chronic renal failure. Four (6.3%) and 9 (14.3%) of them had AIDS and carcinomas, respectively. PCR-REA was found to be a reliable method for typing of our mycobacterial isolates to the species level. These data may shed light on the epidemiology of the mycobacterial species and help to select a proper treatment regimen.—Authors' Abstract

Harmsen, D., Dostal, S., Roth, A., Niemann, S., Rothganger, J., Sammeth, M., Albert, J., Frosch, M., and Richter, E. RIDOM: Comprehensive and public sequence database for identification of Mycobacterium species. BMC Infect. Dis. **3(1)** (2003) 26.

BACKGROUND: Molecular identification of Mycobacterium species has two primary advantages when compared to phenotypic identification: rapid turn-around time and improved accuracy. The information content of the 5′ end of the 16S ribosomal RNA gene (16S rDNA) is sufficient for identification of most bacterial species. However, reliable sequence-based identification is hampered by many faulty and some missing sequence entries in publicly accessible databases. METHODS: In order to establish an improved 16S rDNA sequence database for the identification of clinical and environmental isolates, we sequenced both strands of the 5′ end of 16S rDNA (*Escherichia coli* positions 54 to 510) from 199 mycobacterial culture collection isolates. All validly described species (n = 89; up to March 21, 2000) and nearly all published sequevar variants were included. If the 16S rDNA sequences were not discriminatory, the internal transcribed spacer (ITS) region sequences (n = 84) were also determined. RESULTS: Using 5′-16S rDNA sequencing a total of 64 different mycobacterial species (71.9%) could be identified. With the additional input of the ITS sequence, a further 16 species or subspecies could be differentiated. Only *Mycobacterium tuberculosis* complex species, *M. marinum*/*M. ulcerans* and the *M. avium* subspecies could not be differentiated using 5′-16S rDNA or ITS sequencing. A total of 77 culture collection strain sequences, exhibiting an overlap of at least 80% and identical by strain number to the isolates used in this study, were found in the GenBank. Comparing these with our sequences revealed that an average of 4.31 nucleotide differences (S.D. ± 0.57) were present. CONCLUSIONS: The data from this analysis show that it is possible to differentiate most mycobacterial species by sequence analysis of partial 16S rDNA. The high-quality sequences reported here, together with ancillary information (e.g., taxonomic, medical), are available in a public database, which is currently being expanded in the RIDOM project http://www.ridom-rdna.de), for similarity searches.—Authors' Abstract

Hirano, K., Aono, A., Takahashi, M., and Abe, C. Mutations including IS6110 insertion in the gene encoding the MPB64 protein of Capilia TB-negative *Mycobacterium tuberculosis* isolates. J. Clin. Microbiol. **42(1)** (2004) 390–392.

A simple immunochromatographic assay, Capilia TB, using anti-MPB64 monoclonal antibodies, is a kit for discriminating between the *Mycobacterium tuberculosis* complex and mycobacteria other than tubercle bacilli. The sensitivity of the kit was estimated to be 99.2% (381 of 384 samples). The sequencing analysis revealed that all of the Capilia TB-negative isolates had mutations within the mpb64 gene, leading to the production of an incomplete protein as a result of a deletion of the C-terminal region of the protein.—Authors' Abstract

Jenkin, G. A., Stinear, T. P., Johnson, P. D., and Davies, J. K. Subtractive hybridization reveals a type I polyketide synthase locus specific to *Mycobacterium ulcerans*. J. Bacteriol. **185(23)** (2003) 6870–6882.

Mycobacterium ulcerans causes Buruli ulcer, the third most prevalent mycobacterial infection of immunocompetent humans after tuberculosis and leprosy. Recent work has shown that the production by *M. ulcerans* of mycolactone, a novel polyketide, may partly explain the pathogenesis of Buruli ulcer. To search for the genetic basis of virulence in *M. ulcerans*, we took advantage of the close genetic relationship between *M. ulcerans* and *Mycobacterium marinum* by performing genomic suppressive subtractive hybridization of *M. ulcerans* with *M. marinum*. We identified several DNA fragments specific to *M. ulcerans*, in particular, a type I polyketide synthase locus with a highly repetitive modular arrangement. We postulate that this locus is responsible for the synthesis of mycolactone in *M. ulcerans*.—Authors' Abstract

Marsollier, L., Stinear, T., Aubry, J., Saint Andre, J. P., Robert, R., Legras, P., Manceau, A. L., Audrain, C., Bourdon, S., Kouakou, H., and Carbonnelle, B. Aquatic plants stimulate the growth of and biofilm formation by *Mycobacterium ulcerans* in axenic culture and harbor these bacteria in the environment. Appl. Environ. Microbiol. **70(2)** (2004) 1097–1103.

Mycobacterium ulcerans is the causative agent of Buruli ulcer, one of the most common mycobacterial diseases of humans. Recent studies have implicated aquatic insects in the transmission of this pathogen, but the contributions of other elements of the environment remain largely unknown. We report here that crude extracts from two green algae added to the BACTEC 7H12B culture medium halved the doubling time of *M. ulcerans* and promoted biofilm formation. Using the 7H12B medium, modified by the addition of the algal extract, and immunomagnetic separation, we also demonstrate that *M. ulcerans* is associated with aquatic plants in an area of the Ivory Coast where Buruli ulcer is endemic. Genotype analysis showed that plant-associated *M. ulcerans* had the same profile as isolates recovered in the same region from both aquatic insects and clinical specimens. These observations implicate aquatic plants as a reservoir *of M. ulcerans* and add a new potential link in the chain of transmission of *M. ulcerans* to humans.—Authors' Abstract

Morita, Y. S., Patterson, J. H., Billman-Jacobe, H., and McConville, M. J. Biosynthesis of mycobacterial phosphatidylinositol mannosides. Biochem. J. 378(Pt 2) (2004) 589–597.

All mycobacterial species, including pathogenic *Mycobacterium tuberculosis*, synthesize an abundant class of phosphatidylinositol mannosides (PIMs) that are essential for normal growth and viability. These glycolipids are important cell-wall and/or plasma-membrane components in their own right and can also be hyperglycosylated to form other wall components, such as lipomannan and lipoarabinomannan. We have investigated the steps involved in the biosynthesis of the major PIM species in a new *M. smegmatis* cell-free system. A number of apolar and polar PIM intermediates were labelled when this system was continuously labelled or pulse-chase-labelled with GDP-[3H]Man, and the glycan head groups and the acylation states of these species were determined by chemical and enzymic treatments and octyl-Sepharose chromatography respectively. These analyses showed that (1) the major apolar PIM species, acyl-PIM2, can be synthesized by at least two pathways that differ in the timing of the first acylation step, (2) early PIM intermediates containing a single mannose residue can be modified with two fatty acid residues, (3) formation of polar PIM species from acyl-PIM2 is amphomycin-sensitive, indicating that polyprenol phosphate-Man, rather than GDP-Man, is the donor for these reactions, (4) modification of acylated PIM4 with alpha1-2- or alpha1-6-linked mannose residues is probably the branch point in the biosyntheses of polar PIM and lipoarabinomannan respectively and (5) GDP strongly

inhibits the synthesis of early PIM intermediates and increases the turnover of polyprenol phosphate-Man. These findings are incorporated into a revised pathway for mycobacterial PIM biosynthesis.—Authors' Abstract

Pina-Vaz, C., Costa-Oliveira, S., Rodrigues, A. G., and Salvador, A. Novel method using a laser scanning cytometer for detection of mycobacteria in clinical samples. J. Clin. Microbiol. **42(2)** (2004) 906–908.

In order to evaluate the capacity of laser scanning cytometry (LSC) to detect acid-fast bacilli directly on clinical samples, a comparison between Kinyoun-stained smears analyzed under light microscopy and propidium iodide-auramine-stained smears analyzed by LSC was performed. The results were compared with those for culture on BACTEC MGIT 960. LSC is a new, reliable methodology to detect MYCOBACTERIA.—Authors' Abstract

Portevin, D., De Sousa-D'Auria, C., Houssin, C., Grimaldi, C., Chami, M., Daffe, M., and Guilhot, C. A polyketide synthase catalyzes the last condensation step of mycolic acid biosynthesis in mycobacteria and related organisms. Proc. Natl. Acad. Sci. U.S.A. **101(1)** (2004) 314–319.

Mycolic acids are major and specific constituents of the cell envelope of Corynebacterineae, a suborder of bacterial species including several important human pathogens such as *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Corynebacterium diphtheriae*. These long-chain fatty acids are involved in the unusual architecture and impermeability of the cell envelope of these bacteria. The condensase, the enzyme responsible for the final condensation step in mycolic acid biosynthesis, has remained an enigma for decades. By in silico analysis of various mycobacterial genomes, we identified a candidate enzyme, Pks13, that contains the four catalytic domains required for the condensation reaction. Orthologs of this enzyme were found in other Corynebacterineae species. A Corynebacterium glutamicum strain with a deletion in the pks13 gene was shown to be deficient in mycolic acid production whereas it was able to produce the fatty acids precursors. This mutant strain displayed an altered cell envelope structure. We showed that the pks13 gene was essential for the survival of *Mycobacterium smegmatis*. A conditional *M. smegmatis* mutant carrying its only copy of pks13 on a thermosensitive plasmid exhibited mycolic acid biosynthesis defect if grown at nonpermissive temperature. These results indicate that Pks13 is the condensase, a promising target for the development of new antimicrobial drugs against Corynebacterineae.—Authors' Abstract

Rindi, L., Bonanni, D., Lari, N., and Garzelli, C. Most human isolates of *Mycobacterium avium* Mav-A and Mav-B are strong producers of hemolysin, a putative virulence factor. J. Clin. Microbiol. **41(12)** (2003) 5738–5740.

Hemolysin was quantified in 58 isolates of *Mycobacterium avium* from human, animal, and environmental sources. Human Mav-A and Mav-B isolates were the strongest producers; in contrast, animal and environmental Mav-A isolates and human, animal, and environmental Mav-C organisms were low-level producers. Hemolysin production was not restricted to isolates causing invasive infections.—Authors' Abstract

Song, T., Dove, S. L., Lee, K. H., and Husson, R. N. RshA, an anti-sigma factor that regulates the activity of the mycobacterial stress response sigma factor SigH. Mol. Microbiol. **50(3)** (2003) 949–959.

See Current Literature, Molecular and Genetic Studies, p. 261.

Tortoli, E. *Mycobacterium kansasii*, species or complex? Biomolecular and epidemiological insights. Kekkaku. **78(11)** (2003) 705–709.

Mycobacterium kansasii is one of the best known nontuberculous mycobacteria and large awareness exists about its involvement

in diseases both of immunocompetent and immunocompromised patients. Two phenotypic variants within this species, which differ for the virulence in guinea pig too, have been detected since 1962. It was however following recent progress in genetic studies that a large variability emerged. Major contributions to the disclosure of such findings came from the DNA probes hybridization, the nucleotide sequencing of 16 rDNA and internal transcribed spacer (ITS), and from the analyses of repetitive DNA sequences polymorphism. At present five subtypes *of M. kansasii* are recognized, defined by the ITS sequence and by the polymorphism revealed by different restriction enzyme technologies. Such variants differ from the epidemiological point of view too, with type i being isolated from humans, type ii both from humans and environment, and types iii, iv and v, from the environment only. A revision of the present taxonomic status of *M. kansasii* and its splitting into different species or subspecies seems nowadays necessary. –Authors Abstract

Wade, M. M., and Zhang, Y. Mechanisms of drug resistance *in Mycobacterium tuberculosis*. Front Biosci. **9** (2004) 975–994.

Tuberculosis is a worldwide health problem posing increasing threat with the spread of HIV infection and drug resistant *Mycobacterium tuberculosis* strains. Consequently, control of this disease has become a significant challenge despite the availability of chemotherapy and BCG vaccine. Drug resistance for all first-line anti-tuberculosis agents and some second-line agents has been observed. Moreover, the occurrence of strains of *M. tuberculosis* resistant to multiple anti-tuberculosis drugs is increasing. Mechanisms of action and resistance of major anti-tuberculosis drugs are reviewed. In addition, the phenotypic drug resistance such as dormant or persistent tubercle bacilli and its importance are also emphasized. In order to combat the threat of drug resistant tuberculosis and to more effectively control the disease, an understanding of the mechanisms underlying drug resistance is necessary. This knowledge could be used for the development of molecular tests for rapid detection of drug resistant bacilli and future anti-tuberculosis drugs.—Authors' Abstract

Xu, Z. Q., Barrow, W. W., Suling, W. J., Westbrook, L., Barrow, E., Lin, Y. M., and Flavin, M. T. Anti-HIV natural product (+)-calanolide A is active against both drug-susceptible and drug-resistant strains of *Mycobacterium tuberculosis*. Bioorg. Med. Chem. **12(5)** (2004) 1199–1207.

Naturally occurring anti-HIV-1 agent (+)-calanolide A was found to be active against all of the strains of *Mycobacterium tuberculosis* tested, including those resistant to the standard antitubercular drugs. Efficacy evaluations in macrophages revealed that (+)-calanolide A significantly inhibited intracellular replication of *M. tuberculosis* H37Rv at concentrations below the MIC observed *in vitro*. Preliminary mechanistic studies indicated that (+)-calanolide A rapidly inhibits RNA and DNA synthesis followed by an inhibition of protein synthesis. Compared with known inhibitors, this scenario is more similar to effects observed with rifampin, an inhibitor of RNA synthesis. Since (+)-calanolide A was active against a rifampin-resistant strain, it is believed that these two agents may involve different targets. (+)-Calanolide A and its related pyranocoumarins are the first class of compounds identified to possess antimycobacterial and antiretroviral activities, representing a new pharmacophore for anti-TB activity.—Authors' Abstract

Microbiology (Leprosy)

Matsuoka, M., Zhang, L., Budiawan, T., Saeki, K., and Izumi, S. Genotyping of *Mycobacterium leprae* on the basis of the polymorphism of TTC repeats for analysis of leprosy transmission. J. Clin. Microbiol. **42(2)** (2004) 741–745.

The polymorphism of TTC repeats in *Mycobacterium leprae* was examined using the bacilli obtained from residents in villages at North Maluku where *M. leprae* infections are highly endemic (as well as from patients at North Sulawesi of Indonesia) to elucidate

the possible mode of leprosy transmission. TTC genotypes are stable for several generations of passages in nude mice footpads and, hence, are feasible for the genotyping of isolates and epidemiological analysis of leprosy transmission. It was found that bacilli with different TTC genotypes were distributed among residents at the same dwelling in villages in which leprosy is endemic and that some household contacts harbored bacilli with a different genotype from that harbored by the patient. Investigations of a father-and-son pair of patients indicated that infections of bacilli with 10 and 18 copies, respectively, had occurred. Genotypes of TTC repeats were found to differ between a son under treatment and two brothers. These results reveal the possibility that in addition to exposure via the presence of a leprosy patient with a multibacillary infection who was living with family members, there might have been some infectious sources to which the residents had been commonly exposed outside the dwellings. A limited discriminative capacity of the TTC polymorphism in the epidemiological analysis implies the need of searching other useful polymorphic loci for detailed subdivision of clinical isolates.—Authors' Abstract

Microbiology (Tuberculosis)

Cheng, A. F., Yew, W. W., Chan, E. W., Chin, M. L., Hui, M. M., and Chan, R. C. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. Antimicrob. Agents Chemother. **48(2)** (2004) 596–601.

See Current Literature, Molecular and Genetic Studies, p. 256.

Darwin, K. H., Ehrt, S., Gutierrez-Ramos, J. C., Weich, N., and Nathan, C. F. The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. Science. **302(5652)** (2003) 1963–1996.

The production of nitric oxide and other reactive nitrogen intermediates (RNI) by macrophages helps to control infection by *Mycobacterium tuberculosis* (Mtb). However, the protection is imperfect and infection persists. To identify genes that Mtb requires to resist RNI, we screened 10,100 Mtb transposon mutants for hypersusceptibility to acidified nitrite. We found 12 mutants with insertions in seven genes representing six pathways, including the repair of DNA (uvrB) and the synthesis of a flavin cofactor (fbiC). Five mutants had insertions in proteasome-associated genes. An Mtb mutant deficient in a presumptive proteasomal adenosine triphosphatase was attenuated in mice, and exposure to proteasomal protease inhibitors markedly sensitized wild-type Mtb to RNI. Thus, the mycobacterial proteasome serves as a defense against oxidative or nitrosative stress.—Authors' Abstract

Ewann, F., Locht, C., and Supply, P. Intracellular autoregulation of the *Mycobacterium tuberculosis* PrrA response regulator. Microbiology **150(Pt 1)** (2004) 241–246.

Two-component systems are major regulatory systems for bacterial adaptation to environmental changes. During the infectious cycle of *Mycobacterium tuberculosis*, adaptation to an intracellular environment is critical for multiplication and survival of the micro-organism within the host. The *M. tuberculosis* prrA gene, encoding the regulator of the two-component system PrrA-PrrB, has been shown to be induced upon macrophage phagocytosis and to be transiently required for the early stages of macrophage infection. In order to study the mechanisms of regulation of the PrrA-PrrB two-component system, PrrA and the cytoplasmic part of the PrrB histidine kinase were produced and purified as hexahistidine-tagged recombinant proteins. Electrophoretic mobility shift assays indicated that PrrA specifically binds to the promoter of its own operon, with in-

creased affinity upon phosphorylation. Moreover, induction of fluorescence was observed after phagocytosis of a wild-type *M. tuberculosis* strain containing the gfp reporter gene under the control of the prrA-prrB promoter, while this induction was not seen in a prrA/B mutant strain containing the same construct. These results indicate that the early intracellular induction of prrA depends on the autoregulation of this two-component system.—Authors' Abstract

Goulding, C. W., Apostol, M. I., Gleiter, S., Parseghian, A., Bardwell, J., Gennaro, M., and Eisenberg, D. Gram-positive DsbE proteins function differently from Gram-negative DsbE homologs. A structure to function analysis of DsbE from *Mycobacterium tuberculosis*. J. Biol. Chem. **279(5)** (2004) 3516–3524.

Mycobacterium tuberculosis, a Gram-positive bacterium, encodes a secreted Dsb-like protein annotated as Mtb DsbE (Rv2878c, also known as MPT53). Because Dsb proteins in Escherichia coli and other bacteria seem to catalyze proper folding during protein secretion and because folding of secreted proteins is thought to be coupled to disulfide oxidoreduction, the function of Mtb DsbE may be to ensure that secreted proteins are in their correctly folded states. We have determined the crystal structure of Mtb DsbE to 1.1 A resolution, which reveals a thioredoxin-like domain with a typical CXXC active site. These cysteines are in their reduced state. Biochemical characterization of Mtb DsbE reveals that this disulfide oxidoreductase is an oxidant, unlike Gram-negative bacteria DsbE proteins, which have been shown to be weak reductants. In addition, the pK(a) value of the active site, solvent-exposed cysteine is approximately 2 pH units lower than that of Gram-negative DsbE homologs. Finally, the reduced form of Mtb DsbE is more stable than the oxidized form, and Mtb DsbE is able to oxidatively fold hirudin. Structural and biochemical analysis implies that Mtb DsbE functions differently from Gram-negative DsbE homologs, and we discuss its possible functional role in the bacterium.—Authors' Abstract

Huard, R. C., Chitale, S., Leung, M., Lazzarini, L. C., Zhu, H., Shashkina, E., Laal, S., Conde, M. B., Kritski, A. L., Belisle, J. T., Kreiswirth, B. N., Lapa e Silva, J. R., and Ho, J. L. The *Mycobacterium tuberculosis* complex-restricted gene cfp32 encodes an expressed protein that is detectable in tuberculosis patients and is positively correlated with pulmonary interleukin-10. Infect. Immun. **71(12)** (2003) 6871–6883.

Human tuberculosis (TB) is caused by the bacillus *Mycobacterium tuberculosis*, a subspecies of the *M. tuberculosis* complex (MTC) of mycobacteria. Postgenomic dissection of the *M. tuberculosis* proteome is ongoing and critical to furthering our understanding of factors mediating *M. tuberculosis* pathobiology. Towards this end, a 32-kDa putative glyoxalase in the culture filtrate (CF) of growing *M. tuberculosis* (originally annotated as Rv0577 and hereafter designated CFP32) was identified, cloned, and characterized. The cfp32 gene is MTC restricted, and the gene product is expressed *ex vivo* as determined by the respective Southern and Western blot testing of an assortment of mycobacteria. Moreover, the cfp32 gene sequence is conserved within the MTC, as no polymorphisms were found in the tested cfp32 PCR products upon sequence analysis. Western blotting of *M. tuberculosis* subcellular fractions localized CFP32 predominantly to the CF and cytosolic compartments. Data to support the *in vivo* expression of CFP32 were provided by the serum recognition of recombinant CFP32 in 32% of TB patients by enzyme-linked immunosorbent assay (ELISA) as well as the direct detection of CFP32 by ELISA in the induced sputum samples from 56% of pulmonary TB patients. Of greatest interest was the observation that, per sample, sputum CFP32 levels (a potential indicator of increasing bacterial burden) correlated with levels of expression in sputum of interleukin-10 (an immunosuppressive cytokine and a putative contributing factor to disease progression) but not levels of gamma interferon (a key cytokine in the protective immune response in TB), as measured by ELISA. Combined, these data suggest that CFP32 serves a necessary biological function(s) in tubercle bacilli and may

contribute to the *M. tuberculosis* pathogenic mechanism. Overall, CFP32 is an attractive target for drug and vaccine design as well as new diagnostic strategies.—Authors' Abstract

Jaeger, T., Budde, H., Flohe, L., Menge, U., Singh, M., Trujillo, M., and Radi, R. Multiple thioredoxin-mediated routes to detoxify hydroperoxides in *Mycobacterium tuberculosis*. Arch. Biochem. Biophys. **423(1)** (2004) 182–191.

Drug resistance and virulence of *Mycobacterium tuberculosis* are in part related to the pathogen's antioxidant defense systems. KatG(–) strains are resistant to the first line tuberculostatic isoniazid but need to compensate their catalase deficiency by alternative peroxidase systems to stay virulent. So far, only NADH-driven and AhpD-mediated hydroperoxide reduction by AhpC has been implicated as such virulence-determining mechanism. We here report on two novel pathways which underscore the importance of the thioredoxin system for antioxidant defense in *M. tuberculosis*: (i) NADPH-driven hydroperoxide reduction by AhpC that is mediated by thioredoxin reductase and thioredoxin C and (ii) hydroperoxide reduction by the atypical peroxiredoxin TPx that equally depends on thioredoxin reductase but can use both, thioredoxin B and C. Kinetic analyses with different hydroperoxides including peroxynitrite qualify the redox cascade comprising thioredoxin reductase, thioredoxin C, and TPx as the most efficient system to protect *M. tuberculosis* against oxidative and nitrosative stress *in situ*.—Authors' Abstract

Kocincova, D., Sonden, B., de Mendonca-Lima, L., Gicquel, B., and Reyrat, J. M. The Erp protein is anchored at the surface by a carboxy-terminal hydrophobic domain and is important for cell-wall structure in *Mycobacterium smegmatis*. FEMS Microbiol. Lett. **231(2)** (2004) 191–196.

Erp (Exported Repetitive Protein), also known as P36, Pirg and Rv3810, is a member of a mycobacteria-specific family of extracellular proteins. In pathogenic species, the erp gene has been described as a virulence factor. The Erp proteins comprise three domains. The N- and C-terminal domains are similar in all mycobacterial species, while the central domain consists of a repeated module that differs considerably between species. Here we show that the Erp protein is loosely attached to the surface and that the carboxy-terminal domain, which displays hydrophobic features, anchors Erp at the surface of the bacillus. The hydrophobic region is not necessary for the complementation of the altered colony morphology of a *Mycobacterium smegmatis* erp- mutant but proved to be necessary to achieve resistance to detergent at wild-type levels.—Authors' Abstract

Kotlowski, R., Shamputa, I. C., El Aila, N. A., Sajduda, A., Rigouts, L., van Deun, A., and Portaels, F. PCR-based genotyping of *Mycobacterium tuberculosis* with new GC-rich repeated sequences and IS6110 inverted repeats used as primers. J. Clin. Microbiol. **42(1)** (2004) 372–377.

In the present study we attempted to develop a PCR-based epidemiological tool for the differentiation of *Mycobacterium tuberculosis* isolates. Use of the designed primers Mtb1 (5′-CCG-GCG-GGG-CCG-GCG-G) and Mtb2 (5′-CGG-CGG-CAA-CGG-CGG-C) targeting frequently repeated 16-bp sequences in combination with primers sited at the inverted repeats flanking IS6110 allowed differentiation of *M. tuberculosis* isolates.—Authors' Abstract

Morlock, G. P., Metchock, B., Sikes, D., Crawford, J. T., and Cooksey, R. C. ethA, inhA, and katG loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates. Antimicrob. Agents Chemother. **47(12)** (2003) 3799–3805.

Ethionamide (ETH) is a structural analog of the antituberculosis drug isoniazid (INH). Both of these drugs target InhA, an enzyme involved in mycolic acid biosynthesis. INH requires catalase-peroxidase (KatG) activation, and mutations in katG are a major INH

resistance mechanism. Recently an enzyme (EthA) capable of activating ETH has been identified. We sequenced the entire ethA structural gene of 41 ETH-resistant *Mycobacterium tuberculosis* isolates. We also sequenced two regions of inhA and all or part of katG. The MICs of ETH and INH were determined in order to associate the mutations identified with a resistance phenotype. Fifteen isolates were found to possess ethA mutations, for all of which the ETH MICs were ≥50 microg/ml. The ethA mutations were all different, previously unreported, and distributed throughout the gene. In eight of the isolates, a missense mutation in the inhA structural gene occurred. The ETH MICs for seven of the InhA mutants were ≥100 microg/ml, and these isolates were also resistant to ≥8 microg of INH per ml. Only a single point mutation in the inhA promoter was identified in 14 isolates. A katG mutation occurred in 15 isolates, for which the INH MICs for all but 1 were ≥32 microg/ml. As expected, we found no association between katG mutation and the level of ETH resistance. Mutations within the ethA and inhA structural genes were associated with relatively high levels of ETH resistance. Approximately 76% of isolates resistant to ≥50 microg of ETH per ml had such mutations.—Authors' Abstract

Movahedzadeh, F., Smith, D. A., Norman, R. A., Dinadayala, P., Murray-Rust, J., Russell, D. G., Kendall, S. L., Rison, S. C., McAlister, M. S., Bancroft, G. J., McDonald, N. Q., Daffe, M., Av-Gay, Y., and Stoker, N. G. The *Mycobacterium tuberculosis* ino1 gene is essential for growth and virulence. Mol. Microbiol. **51(4)** (2004) 1003–1014.

Inositol is utilized by *Mycobacterium tuberculosis* in the production of its major thiol and of essential cell wall lipoglycans. We have constructed a mutant lacking the gene encoding inositol-1-phosphate synthase (ino1), which catalyses the first committed step in inositol synthesis. This mutant is only viable in the presence of extremely high levels of inositol. Mutant bacteria cultured in inositol-free medium for four weeks showed a reduction in levels of mycothiol, but phosphatidylinositol mannoside, lipomannan and lipoarabinomannan levels were not altered. The ino1 mutant was attenuated in resting macrophages and in SCID mice. We used site-directed mutagenesis to alter four putative active site residues; all four alterations resulted in a loss of activity, and we demonstrated that a D310N mutation caused loss of the active site Zn2+ ion and a conformational change in the NAD+ cofactor.—Authors' Abstract

Parish, T. Starvation survival response of *Mycobacterium tuberculosis*. J. Bacteriol. **185(22)** (2003) 6702–6706.

The ability of *Mycobacterium tuberculosis* auxotrophs to survive long-term starvation was measured. Tryptophan and histidine auxotrophs did not survive single-amino-acid starvation, whereas a proline auxotroph did. All three auxotrophs survived complete starvation. THP-1 cells were also able to restrict the growth of the tryptophan and histidine auxotrophs.—Author's Abstract

Pashley, C. A., and Parish, T. Efficient switching of mycobacteriophage L5-based integrating plasmids in *Mycobacterium tuberculosis*. FEMS Microbiol. Lett. **229(2)** (2003) 211–215.

We previously used a mycobacteriophage L5-derived integrating vector to demonstrate that glnE and aroK are essential genes in *Mycobacterium tuberculosis* by showing that we were unable to excise the integrated vector when it carried the only functional copy of these genes. We tested three systems to replace the integrated copy with alternative alleles. The most efficient method was to transform the strain with a second copy of the integrating vector. Excision of the resident vector and integration of the incoming vector occurred at an extremely high efficiency. This technique will allow us to study the role and functionality of essential genes in this important human pathogen.—Authors' Abstract

Shimono, N., Morici, L., Casali, N., Cantrell, S., Sidders, B., Ehrt, S., and Riley, L. W. Hypervirulent mutant of

Mycobacterium tuberculosis resulting from disruption of the mce1 operon. Proc. Natl. Acad. Sci. U.S.A. **100(26)** (2003) 15918–15923.

An estimated one-third of the world's population is latently infected with *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis. Here, we demonstrate that, unlike wild-type *M. tuberculosis*, a strain of *M. tuberculosis* disrupted in the mce1 operon was unable to enter a stable persistent state of infection in mouse lungs. Instead, the mutant continued to replicate and killed the mice more rapidly than did the wild-type strain. Histological examination of mouse lungs infected with the mutant strain revealed diffusely organized granulomas with aberrant inflammatory cell migration. Murine macrophages infected *ex vivo* with the mutant strain were reduced in their ability to produce tumor necrosis factor alpha, IL-6, monocyte chemoattractant protein 1, and nitric oxide (NO), but not IL-4. The mce1 mutant strain complemented with the mce1 genes stimulated tumor necrosis factor alpha and NO production by murine macrophages at levels stimulated by the wild-type strain. These observations indicate that the mce1 operon mutant is unable to stimulate T helper 1-type immunity in mice. The hypervirulence of the mutant strain may have resulted from its inability to stimulate a proinflammatory response that would otherwise induce organized granuloma formation and control the infection without killing the organism. The mce1 operon of *M. tuberculosis* may be involved in modulating the host inflammatory response in such a way that the bacterium can enter a persistent state without being eliminated or causing disease in the host.—Authors' Abstract

Theus, S. A., Cave, M. D., and Eisenach, K. D. Activated THP-1 cells: an attractive model for the assessment of intracellular growth rates of *Mycobacterium tuberculosis* isolates. Infect. Immun. **72(2)** (2004) 1169–1173.

Capacity of certain *Mycobacterium tuberculosis* isolates to grow more rapidly in human macrophages may be indicative of increased virulence. Significant differences were observed in intracellular growth of two isolates from sites of tuberculosis transmission, with an outbreak-associated strain growing faster than a strain causing disease in only one person. Activated THP-1 cells are a suitable alternative to peripheral blood monocyte models.—Authors' Abstract

Timm, J., Post, F. A., Bekker, L. G., Walther, G. B., Wainwright, H. C., Manganelli, R., Chan, W. T., Tsenova, L., Gold, B., Smith, I., Kaplan, G., and McKinney, J. D. Differential expression of iron-, carbon-, and oxygen-responsive mycobacterial genes in the lungs of chronically infected mice and tuberculosis patients. Proc. Natl. Acad. Sci. U.S.A. **100(24)** (2003) 14321–14326.

Pathogenetic processes that facilitate the entry, replication, and persistence of *Mycobacterium tuberculosis* (MTB) in the mammalian host likely include the regulated expression of specific sets of genes at different stages of infection. Identification of genes that are differentially expressed *in vivo* would provide insights into host-pathogen interactions in tuberculosis (TB); this approach might be particularly valuable for the study of human TB, where experimental opportunities are limited. In this study, the levels of selected MTB mRNAs were quantified *in vitro* in axenic culture, *in vivo* in the lungs of mice, and in lung specimens obtained from TB patients with active disease. We report the differential expression of MTB mRNAs associated with iron limitation, alternative carbon metabolism, and cellular hypoxia, conditions that are thought to exist within the granulomatous lesions of TB, in the lungs of wild-type C57BL/6 mice as compared with bacteria grown *in vitro*. Analysis of the same set of mRNAs in lung specimens obtained from TB patients revealed differences in MTB gene expression in humans as compared with mice.—Authors' Abstract

Tufariello, J. M., Jacobs, W. R. Jr., and Chan, J. Individual *Mycobacterium tuberculosis* resuscitation-promoting factor homologues are dispensable for growth *in vitro* and *in vivo*. Infect. Immun. **72(1)** (2004) 515–526.

Mycobacterium tuberculosis possesses five genes with significant homology to the resuscitation-promoting factor (Rpf) of *Micrococcus luteus*. The *M. luteus* Rpf is a secreted approximately 16-kDa protein which restores active growth to cultures *of M. luteus* rendered dormant by prolonged incubation in stationary phase. More recently, the Rpf-like proteins of *M. tuberculosis* have been shown to stimulate the growth of extended-stationary-phase cultures of *Mycobacterium bovis* BCG. These data suggest that the Rpf proteins can influence the growth of mycobacteria; however, the studies do not demonstrate specific functions for the various members of this protein family, nor do they assess the function of *M. tuberculosis* Rpf homologues *in vivo*. To address these questions, we have disrupted each of the five rpf-like genes in *M. tuberculosis* Erdman, and analyzed the mutants for their growth *in vitro* and *in vivo*. In contrast to *M. luteus*, for which rpf is an essential gene, we find that all of the *M. tuberculosis* rpf deletion mutant strains are viable; in addition, all show growth kinetics similar to Erdman wild type both *in vitro* and in mouse organs following aerosol infection. Analysis of rpf expression in *M. tuberculosis* cultures from early log phase through late stationary phase indicates that expression of the rpf-like genes is growth phase-dependent, and that the expression patterns of the five *M. tuberculosis* rpf genes, while overlapping to various degrees, are not uniform. We also provide evidence that mycobacterial rpf genes are expressed *in vivo* in the lungs of mice acutely infected with virulent *M. tuberculosis*.—Authors' Abstract

Zahrt, T. C., Wozniak, C., Jones, D., and Trevett, A. Functional analysis of the *Mycobacterium tuberculosis* MprAB two-component signal transduction system. Infect. Immun. **71(12)** (2003) 6962–6970.

The mechanisms utilized by *Mycobacterium tuberculosis* to establish, maintain, or reactivate from latent infection in the host are largely unknown but likely include genes that mediate adaptation to conditions encountered during persistence. Previously, a two-component signal transduction system, mprAB, was found to be required in *M. tuberculosis* for establishment and maintenance of persistent infection in a tissue- and stage-specific fashion. To begin to characterize the role of this system in *M. tuberculosis* physiology and virulence, a functional analysis of the mprA and mprB gene products was initiated. Here, evidence is presented demonstrating that sensor kinase MprB and response regulator MprA function as an intact signal-transducing pair *in vitro* and *in vivo*. Sensor kinase MprB can be autophosphorylated, can donate phosphate to MprA, and can act as a phospho-MprA phosphatase *in vitro*. Correspondingly, response regulator MprA can accept phosphate from MprB or from small phosphodonors including acetyl phosphate. Mutagenesis of residues His249 in MprB and Asp48 in MprA abolished the ability of these proteins to be phosphorylated *in vitro*. Introduction of these alleles into *Mycobacterium bovis* BCG attenuated virulence in macrophages *in vivo*. Together, these results support a role for the mprAB two-component system in *M. tuberculosis* physiology and pathogenesis. Characterization of two-component signal transduction systems will enhance our understanding of processes regulated by *M. tuberculosis* during acute and/or persistent infection in the host.—Authors' Abstract

Experimental Infections and Prevention

Ando, M., Yoshimatsu, T., Ko, C., Converse, P. J., and Bishai, W. R. Deletion of *Mycobacterium tuberculosis* sigma factor E results in delayed time to death with bacterial persistence in the lungs of aerosol-infected mice. Infect. Immun. **71(12)** (2003) 7170–7172.

The stress-induced extracytoplasmic sigma factor E (SigE) of *Mycobacterium tuberculosis* shows increased expression after heat shock, sodium dodecyl sulfate treatment, and oxidative stress, as well as after phagocytosis in macrophages. We report that deletion of sigE results in delayed

lethality in mice without a significant reduction of bacterial numbers in lungs.—Authors' Abstract

Arevalo, M. I., Escribano, E., Calpena, A., Domenech, J., and Queralt, J. Thermal hyperalgesia and light touch allodynia after intradermal *Mycobacterium butyricum* administration in rat. Inflammation **27(5)** (2003) 293–299.

We examined the time course (7 weeks) of thermal hyperalgesia and light touch allodynia in rats after intradermal administration of *Mycobacterium butyricum*. Nociceptive thresholds to heat and light touch were assessed. Paw edema and temperature, motor function, body weight, and propioception were also tested. Some rats developed arthritis (named AA rats) but others did not (named non-AA rats). Both groups were compared with healthy animals. Persistent hyperalgesia was found in both groups; in AA rats it appeared before clinical evidence of arthritis. Transient allodynia ocurred only after edema development and fell when edema decreased. Motor function was impaired only in AA rats. The results of this study demonstrate that hyperalgesia, but not allodynia, appeared after *Mycobacterium butyricum* in both groups, suggesting that changes in sensitivity were not merely the result of local hypersensitivity of the inflamed tissue, but may also be due to alterations in nociception in the central nervous system.—Authors' Abstract

Bastos, R. G., Dellagostin, O. A., Barletta, R. G., Doster, A. R., Nelson, E., Zuckermann, F., and Osorio, F. A. Immune response of pigs inoculated with *Mycobacterium bovis* BCG expressing a truncated form of GP5 and M protein of porcine reproductive and respiratory syndrome virus. Vaccine **22(3–4)** (2004) 467–474.

Pigs were immunised with recombinant BCG (rBCG) expressing a truncated form of GP5 (lacking the first 30 NH(2)-terminal residues) (rBCGGP5) and M protein (rBCGM) of porcine reproductive and respiratory syndrome virus (PRRSV). At 30 days postinoculation (dpi), pigs inoculated with rBCGGP5 and rBCGM developed a specific humoral immune response against the viral proteins, as detected by commercial ELISA and Western blot tests, and at 60 dpi, three out of five animals developed neutralizing antibodies with titers ranging from 1:4 to 1:8. At 67 dpi, an IFN-gamma response against BCG antigens, but not against the viral proteins, was detected by ELISPOT in inoculated pigs. Following challenge with a pathogenic strain of PRRSV, pigs inoculated with rBCG showed lower ($p < 0.05$) temperature, viremia and virus load in bronchial lymph nodes than control animals, suggesting the establishment of partial protection against PRRSV infection.—Authors' Abstract

Chen, L., Wang, J., Zganiacz, A., and Xing, Z. Single intranasal mucosal *Mycobacterium bovis* BCG vaccination confers improved protection compared to subcutaneous vaccination against pulmonary tuberculosis. Infect. Immun. **72(1)** (2004) 238–246.

Whether the intranasal (i.n.) route of *Mycobacterium bovis* BCG vaccination provides better protection against pulmonary tuberculosis than subcutaneous (s.c.) vaccination remains an incompletely solved issue. In the present study, we compared both immune responses and protection elicited by single BCG vaccinations via the i.n. or s.c. route in BALB/c mice. While both i.n. and s.c. vaccination triggered comparable levels of primary immune activation in the spleen and draining lymph nodes, i.n. vaccination led to a greater antigen-specific gamma interferon recall response in splenocytes than s.c. vaccination upon secondary respiratory mycobacterial challenge, accompanied by an increased frequency of antigen-specific lymphocytes. There was also a quicker cellular response in the lungs of i.n. vaccinated mice upon mycobacterial challenge. Mice vaccinated i.n. were found to be much better protected, particularly in the lung, than s.c. vaccinated counterparts against pulmonary tuberculosis at both 3 and 6 months postvaccination. These results suggest that the i.n. route of vaccination improves the protective effect of the current BCG vaccine.—Authors' Abstract

Chung, S. W., Choi, S. H., and Kim, T. S. Induction of persistent *in vivo* resistance to *Mycobacterium avium* infection in BALB/c mice injected with interleukin-18-secreting fibroblasts. Vaccine **22(3–4)** (2004) 398–406

Interferon-gamma (IFN-gamma) is closely associated with the generation of cell-mediated immunity and resistance to intracellular parasites. Interleukin-18 (IL-18) is known to strongly induce IFN-gamma production by T cells and natural killer (NK) cells. To determine whether the paracrine secretion of IL-18 can efficiently stimulate the resistance to *Mycobacterium avium* complex (MAC) infection, 3T3 fibroblasts were stably transfected to secrete bioactive IL-18 and their effects on MAC infection were investigated in genetically susceptible BALB/c mice, compared with that of free recombinant IL-18. Immunization with IL-18-secreting fibroblasts (3T3/IL-18) during intranasal infection with MAC resulted in a significant decrease in bacterial load of lung during the entire 8-week observation period, while rIL-18 reduced the bacterial load at initial 1 week but not by 8 weeks postinfection. Immunization with the 3T3/IL-18 cells induced and maintained significantly higher levels of cytotoxic activity and nitric oxide production by lung cells than those of rIL-18 immunization. Furthermore, lung cells in mice injected with the 3T3/IL-18 cells showed persistent production of IFN-gamma throughout the 8-week period, suggesting that the 3T3/IL-18 cells induced the resistance to MAC infection via IFN-gamma production. This work suggests that IL-18-secreting fibroblasts may serve as a vehicle for paracrine secretion of IL-18 in immunotherapy of MAC infection.—Authors' Abstract

Collins, D. M., Kawakami, R. P., Buddle, B. M., Wards, B. J., and de Lisle, G. W. Different susceptibility of two animal species infected with isogenic mutants of *Mycobacterium bovis* identifies phoT as having roles in tuberculosis virulence and phosphate transport. Microbiology **149(Pt 11)** (2003) 3203–3212.

The *Mycobacterium tuberculosis* complex includes *Mycobacterium bovis,* which causes tuberculosis in most mammals, including humans. In previous work, it was shown that *M. bovis* ATCC 35721 has a mutation in its principal sigma factor gene, sigA, causing a single amino acid change affecting binding of SigA with the accessory transcription factor WhiB3. ATCC 35721 is avirulent when inoculated subcutaneously into guinea pigs but can be restored to virulence by integration of wild-type sigA to produce *M. bovis* WAg320. Subsequently, it was surprising to discover that WAg320 was not virulent when inoculated intratracheally into the Australian brushtail possum (*Trichosurus vulpecula*), a marsupial that is normally very susceptible to infection with *M. bovis*. In this study, an *in vivo* complementation approach was used with ATCC 35721 to produce *M. bovis* WAg322, which was virulent in possums, and to identify the virulence-restoring gene, phoT. There are two point deletions in the phoT gene of ATCC 35721 causing frameshift inactivation, one of which is also in the phoT of BCG. Knockout of phoT from ATCC 35723, a virulent strain of *M. bovis,* produced *M. bovis* WAg758, which was avirulent in both guinea pigs and possums, confirming that phoT is a virulence gene. The effect on virulence of mode of infection versus animal species susceptibility was investigated by inoculating all the above strains by aerosol into guinea pigs and mice and comparing these to the earlier results. Characterization of PhoT indicated that it plays a role in phosphate uptake at low phosphate concentrations. At least *in vitro*, this role requires the presence of a wild-type sigA gene and appears separate from the ability of phoT to restore virulence to ATCC 35721. This study shows the advantages of using different animal models as tools for the molecular biological investigation of tuberculosis virulence.—Authors' Abstract

Collins, D. M., Kawakami, R. P., Wards, B. J., Campbell, S., and de Lisle, G. W. Vaccine and skin testing properties of two avirulent *Mycobacterium bovis* mutants with and without an additional esat-6 mutation. Tuberculosis (Edinb). **83(6)** (2003) 361–366.

SETTING: Molecular techniques are now available to develop new live tuberculosis

vaccines by producing avirulent strains of the *Mycobacterium tuberculosis* complex with known genes deleted. OBJECTIVES: Determine if removal of esat-6 from new live tuberculosis vaccines with known attenuating mutations affects their vaccine efficacy and if it could enable the development of discriminating diagnostic tests. DESIGN: Remove the esat-6 gene by allelic exchange from two illegitimate mutants of *Mycobacterium bovis* that had previously been shown to have similar vaccine efficacy to BCG in a guinea pig vaccination model. Determine the effect this removal has on virulence, vaccine efficacy and skin test reactivity in guinea pigs. RESULTS: Two double knockout strains of *M. bovis* were produced and their virulence and vaccine efficacy were compared to their parent strains. Removal of the esat-6 gene had no significant effect on vaccine efficacy. In skin tests, animals inoculated with the double knockout strains reacted to PPD but not ESAT-6, whereas those inoculated with the parent strains had similar skin test reactivity to both PPD and esat-6. CONCLUSION: Removal of esat-6 from new live tuberculosis vaccine candidates has no significant effect on vaccine properties but does enable the use of skin tests to distinguish between vaccination and infection.—Authors' Abstract

Derrick, S. C., Repique, C., Snoy, P., Yang, A. L., and Morris, S. Immunization with a DNA vaccine cocktail protects mice lacking CD4 cells against an aerogenic infection with *Mycobacterium tuberculosis*. Infect. Immun. **72(3)** (2004) 1685–1692.

Tuberculosis (TB) is the most common opportunistic disease and a potentially fatal complication among immunocompromised individuals infected with human immunodeficiency virus (HIV). Effective vaccination against TB in persons with HIV has been considered unlikely because of the central role that CD4 cells play in controlling tuberculous infections. Here we show that the vaccination of CD8(–/–) mice with a TB DNA vaccine cocktail did not significantly enhance protective responses to a *Mycobacterium tuberculosis* infection. In contrast, immunization with a DNA vaccine cocktail or with the current TB vaccine, *Mycobacterium bovis* BCG, induced considerable antituberculosis protective immunity in immune-deficient mice lacking CD4 cells. In vaccinated CD4(–/–) animals, substantially reduced bacterial burdens in organs and much improved lung pathology were seen 1 month after an aerogenic *M. tuberculosis* challenge. Importantly, the postchallenge mean times to death of vaccinated CD4(–/–) mice were significantly extended (mean with DNA cocktail, 172 ± 7 days; mean with BCG, 156 ± 22 days) compared to that of naive CD4(–/–) mice (33 ± 6 days). Furthermore, the treatment of DNA-vaccinated CD4(–/–) mice with an anti-CD8 or anti-gamma interferon (IFN-gamma) antibody significantly reduced the effect of immunization, and neither IFN-gamma(–/–) nor tumor necrosis factor receptor-deficient mice were protected by DNA immunization; therefore, the primary vaccine-induced protective mechanism in these immune-deficient mice likely involves the secretion of cytokines from activated CD8 cells. The substantial CD8-mediated protective immunity that was generated in the absence of CD4 cells suggests that it may be possible to develop effective TB vaccines for use in HIV-infected populations.—Authors' Abstract

Gorodezky, C., Alaez, C., Munguia, A., Cruz, R., Vazquez, A., Camacho, A., Flores, O., Rodriguez, M., and Rodriguez, O. Molecular mechanisms of MHC linked susceptibility in leprosy: towards the development of synthetic vaccines. Tuberculosis (Edinb). **84(1–2)** (2004) 82–92.

Tuberculoid (TT) and lepromatous leprosy (LL) develop in the human host depending on his ability to trigger a specific cellular immune response(CIR). Different genes have been demonstrated in susceptibility/protection and may explain the forms of leprosy. The major histocompatibility complex (MHC) play an important role. The aim of the study was to explore the contribution of human leukocyte antigen (HLA) DRB1, DQA1, DQB1 and DQ promoter genes in LL Mexican patients. Six families (26 LL, three TT patients and 27 controls)

were analyzed; 114 unrelated patients were compared with 204 controls. Class I typing was done by the standard microlymphocytotoxicity and class II typing using PCR-SSOP. Haplotype segregation correlated with specific CIR in vivo and in vitro using lepromin. Haplotype sharing was significantly deviated in the affected sibs ($p = 0.01$). Six healthy sibs were non-responders to lepromin and four of them were DQ1 homozgotes. DQ1 was significantly associated with LL and with non-responders. We set up macrophage activation experiments after infecting these cells with $5 \times 10(6)$ bacilli to demonstrate if elimination occurred in the context or DQ1. When DQ1 was present on macrophages and on T cells, bacteria were poorly eliminated from the cell (32%) while when absent, 76% of the individuals were able to eliminate the bacilli ($p = 0.03$). DRB1*1501 DQA1*0102-DQB1*0602 (DQ1 subtype) was significantly increased in the patients, indicating its participation in susceptibility. QBP 5.11/5.12 promoter present in the mentioned haplotype, and QAP 1.4, linked to DRB1*1301/02 haplotypes were also associated. Two mechanisms are suggested: the promoter polymorphisms may influence allele expression and thus the amount of peptides presented to the T-cell receptor, leading to a deficient CIR: HLA restriction is important for vaccine design; the way peptides anchor the DRB1*1501 groove may be relevant to the activation of TH1 cells, which contribute to an efficient presentation of peptides inducing a protective T-cell response.—Authors' Abstract

Holten-Andersen, L., Doherty, T. M., Korsholm, K. S., and Andersen, P. Combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and synthetic mycobacterial cord factor as an efficient adjuvant for tuberculosis subunit vaccines. Infect. Immun. **72(3)** (2004) 1608–1617.

Recombinant, immunodominant antigens derived from *Mycobacterium tuberculosis* can be used to effectively vaccinate against subsequent infection. However, the efficacy of these recombinant proteins is dependent on the adjuvant used for their delivery. This problem affects many potential vaccines, not just those for tuberculosis, so the discovery of adjuvants that can promote the development of cell-mediated immunity is of great interest. We have previously shown that the combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and the immunomodulator modified lipid A synergistically potentiates Th1 T-cell responses. Here we report a screening program for other adjuvants with reported Th1-promoting activity and identify a second novel adjuvant formulation that drives the development of Th1 responses with an extremely high efficacy. The combination of dimethyl dioctadecyl ammonium bromide and the synthetic cord factor trehalose dibehenate promotes strong protective immune responses, without overt toxicity, against *M. tuberculosis* infection in a vaccination model and thus appears to be a very promising candidate for the development of human vaccines.—Authors' Abstract

Hsieh, M. J., Junqueira-Kipnis, A. P., Hoeffer, A., Turner, O. C., and Orme, I. M. Incorporation of CpG oligodeoxynucleotide fails to enhance the protective efficacy of a subunit vaccine against *Mycobacterium tuberculosis*.Vaccine **22(5–6)** (2004) 655–659.

Vaccines which offer better protection than BCG are now badly needed for controlling tuberculosis infection throughout the world. Immunological adjuvants capable of inducing a TH1 type of protective response are necessary to augment the immune response, particularly in the case of subunit vaccines. It is now well established that oligodeoxynucleotides (ODN) containing cytidine phosphate guanosine (CpG) motifs enhance cell-mediated responses *in vivo* by increasing the production of the TH1 cytokines IL-12 and interferon gamma (IFNgamma). To determine if this would improve subunit vaccination of mice CpG ODN were added to a subunit vaccine consisting of the culture filtrate proteins (CFP) of *Mycobacterium tuberculosis* H37Rv. It was observed that although adding CpG ODN to the vaccines promoted substantially increased IFNgamma production by lymph node cells draining sites of inoculation, this failed to translate after aerosol challenge

into any degree of enhancement of bacterial clearance in the lungs, influx of IFN-positive T cells, or changes in histopathology. These data suggest that the vaccine enhancing effects of CpG ODN are relatively transient.—Authors' Abstract

Johansen, P., Raynaud, C., Yang, M., Colston, M. J., Tascon, R. E., and Lowrie D. B. Anti-mycobacterial immunity induced by a single injection of *M. leprae* Hsp65-encoding plasmid DNA in biodegradable microparticles. Immunol. Lett. **90(2–3)** (2003) 81–85.

A single sub-cutaneous injection of a plasmid DNA encoding a mycobacterial heat shock protein 65 (Hsp65) entrapped in biodegradable poly(lactic-co-glycolic acid) microspheres produced high titers of antibodies, measured 5 months after the injection in BALB/c mice. Splenocytes secreted IFN-gamma and exerted an anti-bacterial effect on macrophages infected *in vitro* with *Mycobacterium tuberculosis*. The results are encouraging with regard to obtaining good compliance and vaccination coverage with candidate plasmid DNA vaccines, especially in developing countries.—Authors' Abstract

Junqueira-Kipnis, A. P., Turner, J., Gonzalez-Juarrero, M., Turner, O. C., and Orme, I. M. Stable T-cell population expressing an effector cell surface phenotype in the lungs of mice chronically infected with *Mycobacterium tuberculosis*. Infect. Immun. **72(1)** (2004) 570–575.

Analysis of T-cell subsets accumulating in the lungs of C57BL/6 mice chronically infected with *Mycobacterium tuberculosis* revealed that both CD4 and CD8 T-cell populations expressed a cell surface phenotype consistent with that of effector T cells and that a significant proportion of these cells were in the process of secreting gamma interferon.—Authors' Abstract

Kanaujia, G. V., Motzel, S., Garcia, M. A., Andersen, P., and Gennaro, M. L. Recognition of ESAT-6 sequences by antibodies in sera of tuberculous nonhuman primates. Clin. Diagn. Lab. Immunol. **11(1)** (2004) 222–226.

Previous work in our laboratory showed that the ESAT-6 protein of *Mycobacterium tuberculosis* and *Mycobacterium bovis* induces strong antibody responses in a large proportion (approximately 90%) of experimentally or naturally infected nonhuman primates. Here, the antibody response to ESAT-6 in tuberculous monkeys was characterized at the epitope level by measuring antibodies to overlapping, synthetic peptides spanning the ESAT-6 sequence. The antibody response against the COOH-terminal portion of the protein was the strongest in both experimentally and naturally infected animals. Moreover, these antibodies became detectable the earliest during experimental infection, suggesting an ordered expansion of ESAT-6-specific B-cell clones in the course of infection. The data support use of synthetic peptides in lieu of the full-length ESAT-6 protein in diagnostic antibody detection assays.—Authors' Abstract

Kanaujia, G. V., Garcia, M. A., Bouley, D. M., Peters, R., and Gennaro, M. L. Detection of early secretory antigenic target-6 antibody for diagnosis of tuberculosis in non-human primates. Comp. Med. **53(6)** (2003) 602–606.

Tuberculosis is one of the most economically devastating, zoonotic infections of captive non-human primates. The limitations of the tuberculin skin test, which is currently used to diagnose tuberculosis in living non-human primates, make it necessary to find new, simple, and economical diagnostic methods. We describe use of an enzyme-linked immunoassay to detect IgG antibodies against early secretory antigenic target (ESAT)-6, a small protein secreted by virulent tubercle bacilli, in paired (pre- and post-outbreak) sera from 57 non-human primates involved in an outbreak *of Mycobacterium bovis* infection in a research colony. Of 25 animals with tuberculosis lesions at necropsy, 22 (88%) had high serum levels of the ESAT-6 antibody. The ESAT-6 antibody was found in 16% (5/32) of post-outbreak sera from animals in which tuberculosis could not be confirmed at necropsy. The

strong association between the ESAT-6 antibody and tuberculosis in non-human primates documented in this study, together with the robustness of the serologic assay, make the ESAT-6 ELISA a valuable tool for diagnosis of tuberculosis in captive non-human primates.—Authors' Abstract

Kumar, H., Malhotra, D., Goswami, S., and Bamezai, R. N. How far have we reached in tuberculosis vaccine development? Crit. Rev. Microbiol. **29(4)** (2003) 297–312.

Tuberculosis, a bacterial disease prevalent since ancient times, continues to cause the most deaths globally compared with all other diseases. The causative agent *Mycobacterium tuberculosis* is responsible for different types of tuberculosis in humans; however, pulmonary tuberculosis is the most common and causes the most deaths. *Mycobacterium tuberculosis* is an intracellular pathogenic bacterium, which has developed sophisticated mechanisms to survive inside host mononuclear phagocytes and thus evade the host immune system. This is attributed primarily to an inadequate immune response toward infecting bacteria, which results in temporary growth inhibition rather than death and subsequently allows the bacteria to multiply immensely, leading to full-blown disease in an individual. This disease has become a challenge due to poor diagnosis, a low-efficiency tuberculosis vaccine (*Mycobacterium bovis* Bacillus Calmette-Guerin [BCG]), a long-term antibacterial chemotherapy regimen (approximately 6 months), and an emergence of multiple drug resistant strains of *Mycobacterium tuberculosis* especially in people with human immune deficiency virus (HIV) infection, for whom researchers worldwide must develop effective short-term chemotherapy and an effective vaccine. In this review different aspects of vaccines in tuberculosis are discussed, and these include the traditional BCG vaccine, the modern auxotrophic vaccine, the subunit or acellular vaccine; and a DNA vaccine. We discuss also the potential of mycobacterial lipids as a vaccine or as an adjuvant in the future. Since complete genome information of *Mycobacterium tuberculosis* H37Rv and bioinformatics tools are available, it is possible to develop new strategies for a better and effective tuberculosis vaccine, which can replace the traditional BCG vaccine.—Authors' Abstract

Lasco, T. M., Yamamoto, T., Yoshimura, T., Allen, S. S., Cassone, L., and McMurray, D. N. Effect of *Mycobacterium bovis* BCG vaccination on Mycobacterium-specific cellular proliferation and tumor necrosis factor alpha production from distinct guinea pig leukocyte populations. Infect. Immun. **71(12)** (2003) 7035–7042.

In this study, we focused on three leukocyte-rich guinea pig cell populations, bronchoalveolar lavage (BAL) cells, resident peritoneal cells (PC), and splenocytes (SPC). BAL cells, SPC, and PC were stimulated either with live attenuated *Mycobacterium tuberculosis* H37Ra or with live or heat-killed virulent *M. tuberculosis* H37Rv (multiplicity of infection of 1:100). Each cell population was determined to proliferate in response to heat-killed virulent H37Rv, whereas no measurable proliferative response could be detected upon stimulation with live mycobacteria. Additionally, this proliferative capacity (in SPC and PC populations) was significantly enhanced upon prior vaccination with *Mycobacterium bovis* BCG. Accordingly, in a parallel set of experiments we found a strong positive correlation between production of antigen-specific bioactive tumor necrosis factor alpha (TNF-alpha) and prior vaccination with BCG. A nonspecific stimulus, lipopolysaccharide, failed to induce this effect on BAL cells, SPC, and PC. These results showed that production of bioactive TNF-alpha from mycobacterium-stimulated guinea pig cell cultures positively correlates with the vaccination status of the host and with the virulence of the mycobacterial strain.—Authors' Abstract

Lasco, T. M., Turner, O. C., Cassone, L., Sugawara, I., Yamada, H., McMurray, D. N., and Orme, I. M. Rapid accumulation of eosinophils in lung lesions in guinea pigs infected with *Mycobacterium tuberculosis*. Infect. Immun. **72(2)** (2004) 1147–1149.

Guinea pig eosinophils were positively identified in bronchoalveolar lavage populations and in the lung granulomas of *Mycobacterium tuberculosis*-infected guinea pigs. It is possible that the rapid influx of these cells, and their subsequent degranulation during acute pulmonary tuberculosis, may play a key role in the susceptibility of this animal model.—Authors' Abstract

Lee, J., Choi, K., Olin, M. R., Cho, S. N., and Molitor, T. W. Gammadelta T cells in immunity induced by *Mycobacterium bovis* bacillus Calmette-Guerin vaccination. Infect. Immun. **72(3)** (2004) 1504–1511.

Mycobacterium bovis bacillus Calmette-Guerin (BCG) vaccination is efficacious for newborns or adults with no previous exposure to environmental mycobacteria. To determine the relative contribution and the nature of gammadelta T-cell receptor-positive T cells in newborns, compared to CD4(+) T cells, in immunity induced by *M. bovis* BCG vaccination, 4-week-old specific-pathogen-free pigs were vaccinated with *M. bovis* BCG and monitored by following the gammadelta T-cell immune responses. A flow cytometry-based proliferation assay and intracellular staining for gamma interferon (IFN-gamma) were used to examine gammadelta T-cell responses. Pigs were found to mount Th1-like responses to *M. bovis* BCG vaccination as determined by immunoproliferation and IFN-gamma production. The gammadelta T-cell lymphoproliferation and IFN-gamma production to stimulation with mycobacterial antigens were significantly enhanced by *M. bovis* BCG vaccination. The relative number of proliferating gammadelta T cells after stimulating peripheral blood mononuclear cells with *Mycobacterium tuberculosis* H37Rv culture filtrate protein was higher than that of CD4(+) T cells at an early time point after *M. bovis* BCG vaccination, but CD4(+) T cells were found to be more abundant at a later time point. Although the gammadelta T-cell responses were dependent on the presence of CD4(+) T cells for the cytokine interleukin-2, the enhanced gammadelta T cells were due to the intrinsic changes of gammadelta T cells caused by *M. bovis* BCG vaccination rather than being due solely to help from CD4(+) T cells. Our study shows that gammadelta T cells from pigs at early ages are functionally enhanced by *M. bovis* BCG vaccination and suggests an important role for this T-cell subset in acquired immunity conferred by *M. bovis* BCG vaccination.—Authors' Abstract

Lima, K. M., dos Santos, S. A., Santos, R. R., Brandao, I. T., Rodrigues, J. M. Jr., and Silva, C. L. Efficacy of DNA-hsp65 vaccination for tuberculosis varies with method of DNA introduction *in vivo*. Vaccine **22(1)** (2003) 49–56.

A DNA vaccine codifying the mycobacterial hsp65 can prevent infection with *Mycobacterium tuberculosis* in a prophylactic setting and also therapeutically reduce the number of bacteria in infected mice. The protective mechanism is thought to be related to Th1-mediated events that result in bacterial killing. To determine the best method of hsp65 introduction for vaccination efficacy against tuberculosis (TB), we evaluated the immunogenicity and protection of DNA-hsp65 administered by gene gun bombardment or intramuscular (i.m.) injection of naked DNA. Immunization by gene gun induced immune response with plasmid doses 100-fold lower than those required for intramuscular immunization. However, in contrast to intramuscular immunization, which was protective in these studies, gene gun immunization did not protect BALB/c mice against challenge infection.—Authors' Abstract

Nuermberger, E. L., Yoshimatsu, T., Tyagi, S., Bishai, W. R., and Grosset, J. H. Paucibacillary tuberculosis in mice after prior aerosol immunization with *Mycobacterium bovis* BCG. Infect. Immun. **72(2)** (2004) 1065–1071.

To develop a murine model of paucibacillary tuberculosis for experimental chemotherapy of latent tuberculosis infection, mice were immunized with viable *Mycobacterium bovis* BCG by the aerosol or intravenous route and then challenged six weeks later with virulent *Mycobacterium tu-*

berculosis. The day after immunization, the counts were 3.71 ± 0.10 log(10) CFU in the lungs of aerosol-immunized mice and 3.65 ± 0.11 and 4.93 ± 0.07 log(10) CFU in the lungs and spleens of intravenously immunized mice, respectively. Six weeks later, the lungs of all BCG-immunized mice had many gross lung lesions and splenomegaly; the counts were 5.97 ± 0.14 and 3.54 ± 0.07 log(10) CFU in the lungs and spleens of aerosol-immunized mice, respectively, and 4.36 ± 0.28 and 5.12 ± 0.23 log(10) CFU in the lungs and spleens of intravenously immunized mice, respectively. Mice were then aerosol challenged with *M. tuberculosis* by implanting 2.37 ± 0.13 log(10) CFU in the lungs. Six weeks after challenge, *M. tuberculosis* had multiplied so that the counts were 6.41 ± 0.27 and 4.44 ± 0.14 log(10) CFU in the lungs and spleens of control mice, respectively. Multiplication of *M. tuberculosis* was greatly limited in BCG-immunized mice. Six weeks after challenge, the counts were 4.76 ± 0.24 and 3.73 ± 0.34 log(10) CFU in the lungs of intravenously immunized and aerosol-immunized mice, respectively. In contrast to intravenously immunized mice, there was no detectable dissemination to the spleen in aerosol-immunized mice. Therefore, immunization of mice with BCG by the aerosol route prior to challenge with a low dose of *M. tuberculosis* resulted in improved containment of infection and a stable paucibacillary infection. This model may prove to be useful for evaluation of new treatments for latent tuberculosis infection in humans.

Pinto, R., Saunders, B. M., Camacho, L. R., Britton, W. J., Gicquel, B., and Triccas, J. A. *Mycobacterium tuberculosis* defective in phthiocerol dimycocerosate translocation provides greater protective immunity against tuberculosis than the existing bacille Calmette-Guerin vaccine. J. Infect. Dis. **189(1)** (2004) 105–112.

We demonstrate that *Mycobacterium tuberculosis* that is unable to export the complex lipid phthiocerol dimycocerosate has a decreased capacity to replicate in mice and affords sustained protective immunity against *M. tuberculosis* infection Protection was significantly better than that provided by the existing vaccine, *Mycobacterium bovis* bacille Calmette-Guerin (BCG), and this improved protective efficacy was maintained for at least 24 weeks after vaccination. Protection afforded by this attenuated strain coincided with a number of factors that were not associated with BCG vaccination: long-term persistence of the strain within the host, sustained and potent induction of antimycobacterial interferon-gamma-secreting cells equal to that induced by virulent *M. tuberculosis*, and elicitation of T cells recognizing dominant *M. tuberculosis* antigens absent from BCG. These results suggest that the BCG vaccine may be too attenuated to afford effective protective immunity against tuberculosis, and vaccine strains that can provide sustained delivery of mycobacterial antigens are promising antituberculosis vaccine candidates.—Authors' Abstract

Portaels, F., Aguiar, J., Debacker, M., Guedenon, A., Steunou, C., Zinsou, C., and Meyers, W. M. *Mycobacterium bovis* BCG vaccination as prophylaxis against *Mycobacterium ulcerans* osteomyelitis in Buruli ulcer disease. Infect. Immun. **72(1)** (2004) 62–65.

Mycobacterium ulcerans disease, or Buruli ulcer (BU), causes significant morbidity in West Africa. Clinically, the disease presents in the skin as either nonulcerative or ulcerative forms and often invades bones either subjacent to the skin lesion (contiguous osteomyelitis) or remote from the skin lesion (metastatic osteomyelitis). Osteomyelitis represents a severe form of the disease that often requires numerous surgical interventions, even amputations. Surgery is accepted as the present definitive treatment for BU. In the absence of an effective drug treatment, the need for the development of preventive and control strategies becomes paramount. No specific vaccine, however, is presently available for BU. Of 372 consecutive patients in Benin presenting with BU (confirmed by microbiological and histopathological analyses) whose *Mycobacterium bovis* BCG scar statuses were known, 196 children (<15 years old) and 108 adults had neonatal BCG vaccination scars. Of 196 children with BCG scars, 17 (8.7%) had os-

teomyelitis, while 7 of 28 children without BCG scars (25.0%) had osteomyelitis. Of 108 adults with BCG scars, 17 (15.7%) had osteomyelitis, while 14 of 40 adults without BCG scars (35.0%) had osteomyelitis. Our results show that effective BCG vaccination at birth provides significant protection against the development of *M. ulcerans* osteomyelitis in children and adults. Therefore, health authorities should give attention to the enhancement of neonatal BCG vaccination coverage in all countries of Africa where BU is endemic. Protection against severe forms of BU and childhood tuberculosis would likewise be improved by this intervention.—Authors' Abstract

Prem Raj, P., Srivastava, S., Jain, S. K., Srivastava, B. S., and Srivastava, R. Protection by live *Mycobacterium habana* vaccine against *Mycobacterium tuberculosis* H37Rv challenge in mice. Indian J. Med. Res. **117** (2003) 139–145

BACKGROUND & OBJECTIVES: In recent years the efficacy of BCG vaccine against tuberculosis has been questioned and there is no alternative vaccine available. Several strategies are being applied to get a satisfactory vaccine. Two approaches are generally considered: the subunit vaccines and the whole cell vaccines. The objective of this investigation was to evaluate an avirulent mycobacteria, *Mycobacterium habana*, as a whole cell vaccine to protect mice from infection of *M. tuberculosis* H37Rv. METHODS: AKR and immunocompromised SJL/J mice were immunized with live *M. habana* vaccine. These mice were challenged with *M. tuberculosis* H37Rv eight weeks later along with unimmunized control mice. Protection by *M. habana* vaccine was measured through several parameters, which included survival of challenged mice, dissemination of challenge strain and histopathology of lung tissues. RESULTS: *M. habana* vaccinated animals were healthier than the unvaccinated mice after challenge with *M. tuberculosis* and survived with significant increase in mean survival time. The viable count of challenge strain was at least 100-fold less in vaccinated mice than the control mice. The lung tissues in unvaccinated mice showed marked bronchopneumonia with clusters of acid fast bacilli, whereas vaccinated mice showed small areas of damage and evidence of protection subsequently. INTERPRETATION & CONCLUSION: It may be concluded from the evidence presented here that mice vaccinated with *M. habana* were protected from challenge with *M. tuberculosis* in both normal and immunocompromised states.—Authors' Abstract

Price, N. M., Gilman, R. H., Uddin, J., Recavarren, S., and Friedland, J. S. Unopposed matrix metalloproteinase-9 expression in human tuberculous granuloma and the role of TNF-alpha-dependent monocyte networks. J. Immunol. **171(10)** (2003) 5579–5586.

Tuberculosis is characterized by granuloma formation and caseous necrosis, but the factors causing tissue destruction are poorly understood. Matrix metalloproteinase (MMP)-9 (92-kDa gelatinase) secretion from monocytes is stimulated by *Mycobacterium tuberculosis* (*M. tb*) and associated with local tissue injury in tuberculosis patients. We demonstrate strong immunohistochemical MMP-9 staining in monocytic cells at the center of granuloma and adjacent to caseous necrosis in *M. tb*-infected patient lymph nodes. Minimal tissue inhibitor of MMPs-1 staining indicated that MMP-9 activity is unopposed. Because granulomas characteristically contain few mycobacteria, we investigated whether monocyte-monocyte cytokine networks amplify MMP-9 secretion. Conditioned medium from *M. tb*-infected primary human monocytes or THP-1 cells (CoMTB) stimulated MMP-9 gene expression and a >10-fold increase in MMP-9 secretion by monocytes at 3–4 days ($p < 0.009$, vs controls). Although CoMTB stimulated dose-dependent MMP-9 secretion, MMP-1 (52-kDa collagenase) was not induced. Anti-TNF-alpha Ab but not IL-1R antagonist pretreatment decreased CoMTB-induced MMP-9 secretion by 50% ($p = 0.0001$). Anti-TNF-alpha Ab also inhibited MMP-9 secretion from monocytic cells by 50%, 24 hr after direct M. tb infection ($p = 0.0002$). Conversely, TNF-alpha directly stimulated dose-dependent MMP-9 secretion. Pertussis toxin inhibited CoMTB-

induced MMP-9 secretion and enhanced the inhibitory effect of anti-TNF-alpha Ab (p = 0.05). Although chemokines bind to G protein-linked receptors, CXCL8, CXCL10, CCL2, and CCL5 did not stimulate monocyte MMP-9 secretion. However, the response to cholera toxin confirmed that G protein signaling pathways were intact. In summary, MMP-9 within tuberculous granuloma is associated with tissue destruction, and TNF-alpha, critical for antimycobacterial granuloma formation, is a key autocrine and paracrine regulator of MMP-9 secretion.—Authors' Abstract

Rickman, L., Saldanha, J. W., Hunt, D. M., Hoar, D. N., Colston, M. J., Millar, J. B., and Buxton, R. S. A two-component signal transduction system with a PAS domain-containing sensor is required for virulence of *Mycobacterium tuberculosis* in mice. Biochem. Biophys. Res. Commun. **314(1)** (2004) 259–267.

Mycobacterium tuberculosis, the causative organism of tuberculosis, encounters oxidative stress during phagocytosis by the macrophage and following macrophage activation during an acquired immune response, and also from internally generated sources of radical oxygen intermediates through intermediary metabolism. We have identified the SenX3 protein, a sensor in 1 of the 11 complete pairs of two-component signal transduction systems in *M. tuberculosis*, as a possible orthologue of the Mak2p protein from the fission yeast Schizosaccharomyces pombe that is known to sense peroxide stress. Moreover, the SenX3-RegX3 two-component system was the top scoring hit in a homology search with the *Escherichia coli* ArcB-ArcA global control system of aerobic genes. Using structural modelling techniques we have determined that SenX3 contains a PAS-like domain found in a variety of prokaryotic and eukaryotic sensors of oxygen and redox. Mutants with knockouts of senX3 or of the accompanying transcriptional regulator regX3 were constructed and found to have reduced virulence in a mouse model of tuberculosis infection, the mutant bacteria persisting for up to 4 months post-infection; complemented mutants had regained virulence confirming that it was mutations of this two-component system that were responsible for the avirulent phenotype. This work identifies the PAS domain as a possible drug target for tuberculosis and mutations in the senX3-regX signal transduction system as potentially useful components of live vaccine strains.—Authors' Abstract

Shim, T. S., Turner, O. C., and Orme, I. M. Toll-like receptor 4 plays no role in susceptibility of mice to *Mycobacterium tuberculosis* infection. Tuberculosis (Edinb). **83(6)** (2003) 367–371

Although various members of the pattern recognition Toll-like receptor (TLR) family have been implicated in host resistance to *Mycobacterium tuberculosis* infection, it remains unclear if the TLR4 receptor plays an important role. We demonstrate here that infection of TRL4-competent and TLR4-deficient mice on the C3H inbred mouse strain background had similar outcomes, measured in terms of the course of the disease, cell accumulation patterns in the lungs, and lung histopathology. These data argue against a significant role for TLR4 in immunity to tuberculosis in the mouse model.—Authors' Abstract

Shirtcliffe, P. M., Goldkorn, A., Weatherall, M., Tan, P. L., and Beasley, R. Pilot study of the safety and effect of intranasal delipidated acid-treated *Mycobacterium vaccae* in adult asthma. Respirology **8(4)** (2003) 497–503.

OBJECTIVE: There is epidemiological and experimental evidence that exposure to mycobacteria has the potential to suppress the development of atopy and/or asthma. Delipidated, deglycolipidated and arabinogalactan-depleted *autoclaved Mycobacterium vaccae* (delipidated acid-treated *M. vaccae*) has been shown to suppress allergen-induced airway eosinophilia in mice. METHODOLOGY: Thirty-seven adults with stable moderately severe asthma who were skin prick test-positive to house dust mite were randomized to receive two doses 2 weeks apart of delipidated acid-treated *M. vaccae* (first dose 0.4 mg and second dose 0.8 mg) or

phosphate buffered saline, given as drops intranasally. Safety, tolerability and markers of asthma severity (including peak flow, FEV1, major and minor exacerbations, symptom scores and beta-agonist use), and nasal symptom scores, blood eosinophil and IgE levels were monitored for 8 weeks. RESULTS: Delipidated acid-treated *M. vaccae* was safe and well tolerated although there was an occasional mild local reaction. There were no statistically significant differences between the treatment group and placebo for any of the outcome variables. CONCLUSIONS: There is a requirement to elucidate the reasons why mycobacterial-based vaccines have not shown equivalent efficacy in human trials compared with animal models. The role of factors such as duration of disease, route of administration and the active component of mycobacteria need to be addressed.—Authors' Abstract

Singh, R., Rao, V., Shakila, H., Gupta, R., Khera, A., Dhar, N., Singh, A., Koul, A., Singh, Y., Naseema, M., Narayanan, P. R., Paramasivan, C. N., Ramanathan, V. D., and Tyagi, A. K. Disruption of mptpB impairs the ability of *Mycobacterium tuberculosis* to survive in guinea pigs. Mol. Microbiol. **50(3)** (2003) 751–762.

Protein tyrosine kinases and tyrosine phosphatases from several bacterial pathogens have been shown to act as virulence factors by modulating the phosphorylation and dephosphorylation of host proteins. The identification and characterization of two tyrosine phosphatases namely MptpA and MptpB from *Mycobacterium tuberculosis* has been reported earlier. MptpB is secreted by *M. tuberculosis* into extracellular mileu and exhibits a pH optimum of 5.6, similar to the pH of the lysosomal compartment of the cell. To determine the role of MptpB in the pathogenesis of *M. tuberculosis*, we constructed a mptpB mutant strain by homologous recombination and compared the ability of parent and the mutant strain to survive intracellularly. We show that disruption of the mptpB gene impairs the ability of the mutant strain to survive in activated macrophages and guinea pigs but not in resting macrophages suggesting the importance of its role in the host-pathogen interaction. Infection of guinea pigs with the mutant strain resulted in a 70-fold reduction in the bacillary load of spleens in infected animals as compared with the bacillary load in animals infected with the parental strain. Upon reintroduction of the mptpB gene into the mutant strain, the complemented strain was able to establish infection and survive in guinea pigs at rates comparable to the parental strain. These observations demonstrate a role of MptpB in the pathogenesis of *M. tuberculosis*.—Authors' Abstract

Stanford, J., Stanford, C., and Grange, J. Immunotherapy with *Mycobacterium vaccae* in the treatment of tuberculosis. Front Biosci. **9** (2004) 1701–1719.

All the trials of immunotherapy of tuberculosis with killed *Mycobacterium vaccae*, published or not, that are known to the authors are reviewed here. Following an introduction giving a brief account of some earlier immunotherapies for tuberculosis, the origins of the concept of immunotherapy with *M. vaccae* are considered. Progress is traced from the early work with irradiation-killed organisms in leprosy to the study in London of modulation of tuberculin skin-test responses, and the first comparative trials in The Gambia and Kuwait. In the last of these studies, dosages and different preparations were compared. As a result of this subsequent studies have used 109 heat-killed organisms, equivalent to 1mg wet-weight of bacilli, as a standard dose. A series of small trials in Argentina, India, Nigeria, Romania, South Africa and Vietnam have pioneered the way forward, disclosing geographic variability, with South Africa as the only country where almost no effects were recorded. Together the studies have shown that a single dose may not be sufficient. These studies have confirmed the mode of action of *M. vaccae* to be regulation of cell-mediated immunity with enhancement of Th1 and down-regulation of Th2, and they have shown benefits in faster bacteriological conversion, reduction in ESR, recovery of body weight and resolution of radiological opacities, leading to better recovery from the disease even when given to patients receiving directly observed therapy, short-

course (DOTS). Three major randomised, placebo-controlled and partly blinded trials have been carried out in Africa. The first, in South Africa showed no *M. vaccae*-related effects. The second trial, in Uganda, confirmed the observations made in the earlier studies of faster sputum conversion and better radiological clearance. The third trial, in Zambia and Malawi, showed a trend towards benefits in the treatment of HIV seronegative patients but failed to show beneficial effects in HIV seropositive patients. Studies in patients with multi-drug-resistant tuberculosis have shown that multiple doses of immunotherapy are required in most cases, and that these markedly improve cure-rates for these patients. This is especially so when they are also treated with chemotherapy tailored to the resistance pattern of their infecting organisms. A small study has just commenced in which repeated doses of *M. vaccae* are being administered to a group of patients who have failed treatment with DOTS-Plus (directly observed therapy with drugs selected on the basis of drug susceptibility profiles). Late in the investigation came publications from China supporting and confirming the data in both drug-sensitive and drug-resistant disease, by the use of multiple injections of their own different preparation of *M. vaccae*. The trial that is now beginning in Vietnam of 3 doses of *M. vaccae* in the treatment of newly diagnosed pulmonary tuberculosis, is accompanied by a chemotherapeutic regimen with a shortened continuation phase. If this important study is successful, immunotherapy with killed *M. vaccae* should be introduced into the treatment regimens for tuberculosis worldwide.

Sugawara, I., Yamada, H., Mizuno, S., Takeda, K., and Akira, S. Mycobacterial infection in MyD88-deficient mice. Microbiol. Immunol. **47(11)** (2003) 841–847.

MyD88 is an adaptor protein that plays a major role in TLR/IL-1 receptor family signaling. To understand the role of MyD88 in the development of murine tuberculosis *in vivo*, MyD88 knockout (KO) mice aerially were infected with *Mycobacterium tuberculosis*. Infected MyD88 mice were not highly susceptible to *M. tuberculosis* infection, but they developed granulomatous pulmonary lesions with neutrophil infiltration which were larger than those in wild-type (WT) mice ($p < 0.01$). The pulmonary tissue levels of mRNA for iNOS and IL-18 were slightly lower, but levels of mRNA for IL-1 beta, IL-2, IL-4, IL-6, IL-10, IFN-gamma, and TGF-beta were higher in MyD88 KO mice. IFN-gamma, TNF-alpha, IL-1 beta, and IL-12 also were high in the sera of MyD88 KO mice. There were no statistically significant differences in the expression of TNF-alpha, IL-12, and ICAM-1 mRNA between MyD88 KO and WT mice. Thus, MyD88 deficiency did not influence the development of murine tuberculosis. NF-kappa B activity was similar in the alveolar macrophages from the lung tissues of MyD88 KO and WT mice. Also, there may be a TLR2-specific, MyD88-independent IL-1 receptor/TLR-mediated pathway to activate NF-kappa B in the host defense against mycobacterial infection.—Author's Abstract

Tomioka, H., Shimizu, T., Sato, K., Sano, C., Kamei, T., Emori, M., and Saito, H. Comparative roles of macrophages and NK cells in the host innate resistance of mice to *Mycobacterium fortuitum* infection. J. Infect. **48(1)** (2004) 74–80.

OBJECTIVES: Profiles of host innate resistance to *Mycobacterium fortuitum* (MFT) infection in mice and the roles of macrophages (Mphis) and NK cells in host resistance to MFT infection were studied. METHODS: MFT-infected mice with or without the treatments to reduce Mphis and NK cells were examined for survival and the bacterial loads in the kidneys during the course of infection. RESULTS: A unique profile of strain difference was found in the innate resistance of mice to MFT. A/J, C3H/He and DBA/2 mice were susceptible, while BALB/c, B10A and C57BL/6 mice were resistant, in terms of survival after MFT infection. Such profiles of host resistance to MFT were essentially correlated with the ability of individual strain mice to prevent the bacterial growth in the early periods after infection. These profiles were different from the strain difference controlled by Bcg gene. Studies using carrageenan, anti-asialo GM1 antibody, and NK cell-

deficient beige mice indicated the important roles of Mphis and NK cells in the host innate defense against MFT. CONCLUSIONS: These findings suggest that Bcg gene does not control the host resistance to MFT and that both Mphis and NK cells play crucial roles in the host innate resistance to MFT infection.—Authors' Abstract

Walker, C., Sawicka, E., and Rook, G. A. Immunotherapy with mycobacteria. Curr. Opin. Allergy Clin. Immunol. **3(6)** (2003) 481–486.

SUMMARY: PURPOSE OF REVIEW: To summarize and evaluate critically recent progress with mycobacteria as a potential novel disease modifying treatment strategy in asthma. RECENT FINDINGS: The link between exposure to pathogenic or saprophytic mycobacteria and protection from allergic diseases is still controversial, and recent epidemiological studies, which addressed only exposure to *Mycobacterium tuberculosis* or bacillus Calmette-Guerin, did not help to clarify this issue. Moreover, the clear efficacy of mycobacterial treatment seen in animal models has not been reproduced in human asthma, and a recent small study testing the hypothesis that heat-killed *Mycobacterium vaccae* attenuates asthmatic reactions after allergen challenge did not provide convincing results. However, it has been shown that treatment of mice with *M. vaccae* induces the generation of allergen-specific T regulatory cells capable of suppressing allergen-mediated eosinophilic lung inflammation, suggesting that a general deficiency of T regulatory cell activity might be responsible for the increased prevalence of asthma. This hypothesis is supported by findings that a lack of T regulatory cells, as found in genetic disorders of man and mouse attributable to a mutation of Foxp3, a transcription factor specifically expressed by T regulatory cells, is associated with manifestations of severe atopy and autoimmunity, precisely the spectrum of diseases linked to the hygiene hypothesis. SUMMARY: Further studies on the relationship between mycobacteria and atopic disorders are needed, but there is reason to believe that the novel findings and molecular mechanisms associated with mycobacterial infections will further strengthen the currently unproved therapeutic value of immunotherapy with mycobacteria.—Authors' Abstract

Waters, W. R., Palmer, M. V., Nonnecke, B. J., Whipple, D. L., and Horst, R. L. *Mycobacterium bovis* infection of vitamin D-deficient NOS2–/– mice. Microb. Pathog. **36(1)** (2004) 11–17.

Vitamin D deficiency is associated with an increased risk for tuberculosis infection. Studies using *in vitro* systems indicate that 1,25-dihydroxyvitamin D(3) [i.e., 1,25(OH)(2)D(3)], the most active form of the vitamin, enhances mycobacterial killing by increasing nitric oxide (NO) production. To evaluate concurrently the role of 1,25(OH)(2)D(3) and NO on the host response to tuberculosis infection, mice deficient in NO synthase 2 (NOS2(–/–)) and/or vitamin D were aerosol-challenged with *Mycobacterium bovis* and subsequently evaluated for mycobacterial colonization and lesion formation. Infected NOS2(–/–) mice developed severe necrotizing pyogranulomatous inflammation of the lungs with heavy *M. bovis* colonization and systemic dissemination of the bacillus. Colonization and lung lesion area of NOS2(–/–) mice exceeded that of NOS2(+/+) mice. Additionally, disease progression was more rapid in NOS2(–/–) mice than in NOS2(+/+) mice. Lung colonization and lesion area of vitamin D deficient mice exceeded that of vitamin D replete mice, regardless of NOS2 phenotype. However, effects of vitamin D on colonization, but not lesion area, were more pronounced in NOS2(+/+) mice than in NOS2(–/–) mice. These findings are consistent with the current hypothesis that 1,25(OH)(2)D(3) enhances mycobacterial killing through a NO-dependent mechanism. As responses of NOS2(–/–) mice were affected by 1,25(OH)(2)D(3) deficiency, albeit to a lesser extent than were those of NOS2(+/+) mice, NO-independent actions of 1,25(OH)(2)D(3) also likely exist.—Authors' Abstract

Epidemiology and Prevention

Bierrenbach, A. L., Cunha, S. S., Barreto, M. L., Pereira, S. M., Dourado, I., Ichihara, M. Y., Brito, S. C., and Rodrigues, L. C. Tuberculin reactivity in a population of school children with high BCG vaccination coverage. Pan American J. Pub. Health **13(5)** (2003) 285–293.

A study was conducted to investigate the influence of BCG vaccination or revaccination on tuberculin skin test reactivity, in order to guide the correct interpretation of this test in a setting of high neonatal BCG vaccination coverage and an increasing BCG revaccination coverage at school age. In 1997, we conducted tuberculin skin testing and BCG scar reading in 1148 children aged 7–14 years old in the city of Salvador, Bahia, Brazil. We measured the positive effect of the presence of one or two BCG scars on the proportion of tuberculin skin test results above different cut-off levels (induration sizes of ≥5 mm, ≥10 mm, and ≥15 mm) and also using several ranges of induration size (0, 1–4, 5–9, 10–14, and ≥15 mm). We also measured the effects that age, gender, and the school where the child was enrolled had on these proportions. The proportion of tuberculin results ≥10 mm was 14.2% (95% confidence interval (CI) = 8.0%–20.3%) for children with no BCG scar, 21.3% (95% CI = 18.5%–24.1%) for children with one BCG scar, and 45.0% (95% CI = 32.0%–58.0%) for children with two BCG scars. There was evidence for an increasing positive effect of the presence of one and two BCG scars on the proportion of results ≥5 mm and ≥10 mm. Similarly, there was evidence for an increasing positive effect of the presence of one and two scars on the proportion of tuberculin skin test results in the ranges of 5–9 mm and of 10–14 mm. The BCG scar effect on the proportion of results ≥5 mm and ≥10 mm did not vary with age. There was no evidence for BCG effect on the results ≥15 mm. In Brazilian school children, BCG-induced tuberculin reactivity is indistinguishable, for results under 15 mm, from reactivity induced by *Mycobacterium tuberculosis* infection. BCG revaccination at school age increases the degree of BCG-induced tuberculin reactivity found among school children. This information should be taken into account in tuberculin skin test surveys intended to estimate *M. tuberculosis* prevalence or to assess transmission patterns as well as in tuberculin skin testing of individuals used as an auxiliary tool in diagnosing tuberculosis. Taking this information into consideration is especially important when there is increasing BCG revaccination coverage.

Dony, J. F., Ahmad, J., and Khen Tiong, Y. Epidemiology of tuberculosis and leprosy, Sabah, Malaysia. Tuberculosis (Edinb). **84(1–2)** (2004) 8–18.

The objectives in this epidemiology review are to measure and report the extent of morbidity and mortality due to tuberculosis (TB), the proportion of new sputum smear positive cases in districts and the status of cohort analysis as of 1999. As for leprosy, the main objective is to determine morbidity and the treatment outcomes of Multiple Drug Therapy (MDT). Based on the results obtained, a comprehensive action plan for prevention, control and monitoring of tuberculosis and leprosy cases and patients is being produced and implemented throughout the state. The analysis concentrated on patients diagnosed at all out-patient units and admitted in all of the state's hospitals. The patient particulars were recorded using a standardized format based on TB and Leprosy Health Management Information System (TB HMIS). TB was the second highest by notification of communicable diseases in Malaysia in 2001. 29% or about one-third of the national TB cases are from Sabah. However, it has been noted that there was an average decline of 2.6% in annual notification since 10 years ago to date. There was also a reduction of 11.4% in 2001 as compared to annual notification in 2000. Immigrants contribute more than 24% in detection of new cases since 1990. Treatment success rate in term of completion of treatment to date is 82%. Mortality rate has steadily declined from 14 deaths to 7 deaths per 100,000 population. Leprosy in Sabah also contributes to 30% of the yearly total caseload

of Malaysia and has the highest notification rate of 2 per every 100,000 population as compared to other states. The average registered leprosy cases over the past 5 years are 239 cases and the prevalence rate is 0.7/10,000 population. The state has successfully achieved its goal to decrease leprosy as per the World Health Organization (WHO) goal of yearly overall prevalence rate of less than 1 case for every 10,000 population. However, the districts of Kudat, Tawau, Lahad Datu, Kota Kinabalu and Semporna are still within the prevalence rate of more than one per 10,000 population. This review highlights some interesting findings which can be incorporated into the State and Districts action plans and strategies. It is also noted that in order to translate National Plans and Strategies into effective action at the community level, health workers need relevant up-to-date knowledge of the pattern of health and disease, and of their determinants, in each district. The Sabah Health Department continues to organize and support programs related to management and control of tuberculosis and leprosy to progressively reduce the incidence of these diseases in the community by breaking the chain of transmission of *Mycobacterium tuberculosis* and *M. leprae*, respectively.—Authors' Abstract

Other Mycobacterial Diseases

Arkwright, P. D., and David, T. J. Effect of *Mycobacterium vaccae* on atopic dermatitis in children of different ages. Br. J. Dermatol. **149(5)** (2003) 1029–1034.

BACKGROUND: Exposure to environmental microorganisms is associated with variations in the prevalence and severity of atopic diseases. We have previously shown that administration of a *Mycobacterium vaccae* suspension significantly reduced the severity of atopic dermatitis (AD) in children aged 5–18 years. OBJECTIVES: This study aimed to extend these observations to younger children. METHODS: Fifty-six children aged 2–6 years with moderate to severe AD were enrolled in a randomized, double-blind, placebo-controlled trial and given one intradermal injection of either killed *M. vaccae* suspension or buffer solution (placebo). Skin surface area affected and dermatitis severity score were assessed before and 1, 3 and 6 months after treatment. RESULTS: Although a 38–54% reduction in surface area affected by dermatitis was noted at all time points after *M. vaccae* administration ($p = 0.005$), this improvement was not significantly different from that observed in the placebo group. Meta-analysis of this and our previous cohort (97 children aged 2–18 years) showed that *M. vaccae* was associated with a significant improvement in clinical severity at all ages, whereas within the placebo group, younger but not older children showed a similar improvement. CONCLUSIONS: Despite a reduction in clinical severity associated with *M. vaccae* at all ages, no benefit could be found after administering *M. vaccae* to children with AD aged 2–6 years when compared with placebo. *M. vaccae* may offer greater benefit in children over 5 years old, whose AD appears less likely to regress spontaneously.—Authors' Abstract

Coussens, P. M., Jeffers, A., and Colvin, C. Rapid and transient activation of gene expression in peripheral blood mononuclear cells from Johne's disease positive cows exposed to *Mycobacterium paratuberculosis in vitro*. Microb. Pathog. 36(2) (2004) 93–108.

Mycobacterium avium subspecies paratuberculosis (*M. paratuberculosis*) is the causative agent of Johne's disease in ruminants. *M. paratuberculosis* is a slow-growing intracellular bacterium and infections with *M. paratuberculosis* can persist in a subclinical state for several years. An early and appropriate T cell-mediated cytotoxic response (Th1-like) to *M. paratuberculosis* infection is often replaced with an antibody or Th 2-like response as infected animals move toward a progressively more clinical state. The reasons for this shift in immune response are unknown. Recent studies suggest that *in vitro* exposure of peripheral blood mononuclear cells (PBMCs) from

Johne's disease positive cows *to M. paratuberculosis* for 18–24 hr results in suppressed expression of numerous immune cell genes. This effect appears at odds with the notion that immune cells from infected cows would respond to *M. paratuberculosis*-specific antigens in a vigorous and positive manner. In this report, we detail experiments designed to test the hypothesis that many positive changes in PBMC gene expression induced by *M. paratuberculosis in vitro* are transient, being rapidly suppressed by as yet unknown mechanisms. Our results demonstrate that, indeed, *in vitro* stimulation with *M. paratuberculosis* induces rapid changes in infected cow PBMC gene expression (within 2–4 hr of exposure) and that many of these changes are no longer evident by 8–16 hr of exposure to *M. paratuberculosis*. Although precise mechanisms responsible for rapid *M. paratuberculosis*-mediated activation of PBMC gene expression and the loss thereof remain to be determined, our novel results suggest that PBMCs from Johne's disease positive cows are indeed capable of vigorously responding to *M. paratuberculosis* and that, for many genes, this response is tempered within 8 hr of exposure.—Authors' Abstract

Darie, H. Infection par *Mycobacterium ulcerans*: aspects epidemiologiques, cliniques et thérapeutiques. Bull. Soc. Pathol. Exot. **96(5)** (2003) 368–371.

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

den Broeder, A. A., Vervoort, G., van Assen, S., Verduyn Lunel, F., de Lange, W. C., and de Sevaux, R. G. Disseminated *Mycobacterium gordonae* infection in a renal transplant recipient. Transpl. Infect. Dis. **5(3)** (2003) 151–155.

The use of more intensive immunosuppressive regimens and the increasing number of patients that are exposed to immunosuppressive strategies in transplantation medicine have changed the spectrum of infections that is encountered by the clinician. We describe a 62-year-old female renal transplant recipient receiving immunosuppressive therapy who developed complaints of weight loss, diarrhoea, cough, and fever. Increased C-reactive protein and pancytopenia were found. The presence of *Mycobacterium gordonae*, a non-tuberculous mycobacterium, was eventually demonstrated in bronchoalveolar lavage fluid, bone marrow, spleen, and liver. Determination of the pathogen was accelerated using a Line Probe Assay, a reverse hybridisation technique using an RNA fragment specific for different mycobacterium species. Treatment was initiated using a combination of clarithromycin, ethambutol, and rifampicin. The initial response to treatment was good, but splenectomy and change of immunosuppressive and antimycobacterial therapy were necessary for long-term control of the infection. Problems in the diagnosis and treatment of this uncommon pathogen are discussed.—Authors' Abstract

Ferrara, E., Lemire, J., Grimm, P. C., Reznik, V. M., Mendoza, S. A., Leake, J. A., and Benador, N. M. Mycobacterial peritonitis in pediatric peritoneal dialysis patients. Pediatr. Nephrol. **19(1)** (2004) 114–117.

Peritonitis is the most common complication and the leading cause of death in pediatric peritoneal dialysis (PD) patients. According to the most recent data available from the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS), approximately 25% of pediatric PD patients who die succumb to infection. There are no reported cases of *Mycobacterium tuberculosis* (MTB) or *Mycobacterium avium*-intracellulare peritonitis in the NAPRTCS registry. With an increasing incidence of MTB worldwide and the impairment of cellular immunity in chronic renal failure patients, it is not surprising that mycobacterium peritonitis can occur in PD patients. We report two pediatric PD patients with mycobacterial peritoneal infection diagnosed over an 11-year period at our institution. One patient presented with a malfunctioning Tenckhoff catheter and again 3 years later with hyponatremia and ascites. The other presented with recurrent culture-negative peritonitis. These cases illustrate the importance of more extensive evaluation of PD complications, to include evaluation for mycobacterium with special media or peritoneal biopsy, in the above clinical settings if the routine work-up is unrevealing.—Authors' Abstract

Fogla, R., Rao, S. K., and Padmanabhan, P. Interface keratitis due to *Mycobacterium fortuitum* following laser in situ keratomileusis. Indian J. Ophthalmol. **51(3)** (2003) 263–265

A case of unilateral interface keratitis due to *Mycobacterium fortuitum* following simultaneous bilateral LASIK procedure for low myopia is reported. Excimer phototherapeutic keratectomy was performed to the stromal bed to reduce the infective load. Intensive topical therapy with topical amikacin and ciprofloxacin resulted in resolution of the keratitis.—Authors' Abstract

Foroumadi, A., and Soltani, F. Antituberculosis agents. IX. *In vitro* antimycobacterial activity of N-(2-phenyl-2-oxoethyl) and N-[2-(4-fluorophenyl)-2- oxoethyl]-ciprofloxacin derivatives against some drug-resistant strains of *Mycobacterium tuberculosis* and *Mycobacterium avium* isolates. Boll. Chim. Farm. **142(6)** (2003) 248–250.

The *in vitro* antimycobacterial activity of two ciprofloxacin analogues (2a and 2b) containing 2-phenyl-2-axoethyl and 2-(4-fluorophenyl)-2-axoethyl groups attached to N-4 position of piperazin ring, was evaluated against *M. tuberculosis* strains resistant to Isoniazid (MIC 2a and 2b = 3.13 micrograms/ml), Ethambutol (MIC 2a and 2b = 1.56 micrograms/ml), Rifampin (MIC 2a and 2b = 1.56 micrograms/ml), Kanamycin (MIC 2a and 2b = 1.56 micrograms/ml) and ciprofloxacin (MIC 2a and 2b>25 micrograms/ml). Furthermore, the minimum bactericidal concentration (MBC) of 2a and 2b was determined against *M. tuberculosis* H37Rv (MBC2a = 6.25 and 2b = 25 micrograms/ml) and strains resistant to isoniazid (MBCs >25 micrograms/ml) and rifampin (MBC2a = 3.13 and 2b = 6.25 micrograms/ml). Also, in this study the activity of 2a and 2b was determined against a strain of *M. avium* (%Inhibition 2a = 89 and 2b = 95%). Expanded primary screening was conducted for 2b (having MIC <6.25 micrograms/ml) against five clinical isolates of *M. avium* using the MABA and BACTEC 460 systems (MIC = 2–4 micrograms/ml). The significant anti-*Mycobacterium avium* activity of 2b suggested further *M. avium* assays and so, 2b was then retested at lower concentrations against 30 strains of *M. avium* including five strains resistant to clarithromycin (MIC = 2–16 micrograms/ml).—Authors' Abstract

Fox, L. P., Geyer, A. S., Husain, S., Della-Latta, P., and Grossman, M. E. *Mycobacterium abscessus* cellulitis and multifocal abscesses of the breasts in a transsexual from illicit intramammary injections of silicone. J. Am. Acad. Dermatol. **50(3)** (2004) 450–454.

We report the case of a 29-year-old transsexual who developed *Mycobacterium abscessus* infection after receiving intramammary liquid silicone injections in the nonphysician office setting. Our patient represents 1 of 14 confirmed and 11 suspected

cases in New York City of *M. abscessus* infection after illicit cosmetic procedures. As injectable cosmetic procedures are becoming increasingly popular, dermatologists should be aware of both the common and unusual complications. Furthermore, all physicians should be alerted to the current cluster of *M. abscessus* infections after injections for cosmetic purposes by nonmedical practitioners in New York City.—Authors' Abstracts

Hong, H., Gates, P. J., Staunton, J., Stinear, T., Cole, S. T., Leadlay, P. F., and Spencer, J. B. Identification using LC-MSn of co-metabolites in the biosynthesis of the polyketide toxin mycolactone by a clinical isolate of *Mycobacterium ulcerans*. Chem. Commun. (Camb). **22** (2003) 2822–2823.

LC-MSn analysis of mycolactone toxin from extracts of *Mycobacterium ulcerans* has shown that minor co-metabolites, including two previously unreported, differ structurally from mycolactone only in a small portion of the polyketide side-chain.—Authors' Abstract

Hong, T., Butler, W. R., Hollis, F., Floyd, M. M., Toney, S. R., Tang, Y. W., Steele, C., and Leggiadro, R. J. Characterization of a novel rapidly growing Mycobacterium species associated with sepsis. J. Clin. Microbiol. **41(12)** (2003) 5650–5653.

A rapidly growing mycobacterium was isolated five times from blood cultures from a 6-year-old female patient with relapsed pre-B-cell acute lymphocytic leukemia. All five isolates had identical nucleotide sequences for the first 500 bp of the 16S rRNA gene, indicative of a single species. High-performance liquid chromatography analysis of mycolic acids indicated that the species was similar to *Mycobacterium smegmatis*. Sequence analysis of the 16S rRNA gene (1,455 bp) for one isolate demonstrated that the species was closely related to *Mycobacterium diernhoferi*. Based on the phenotypic features and phylogenetic analysis, it was concluded that the isolates represented a novel rapidly growing Mycobacterium species. The name "Mycobacterium hackensackense" is proposed for this unique strain, 147-0552(T), which was deposited in the American Type Culture Collection as ATCC BAA-823(T).—Authors' Abstract

Jenkin, G. A., Stinear, T. P., Johnson, P. D., and Davies, J. K. Subtractive hybridization reveals a type I polyketide synthase locus specific to *Mycobacterium ulcerans*. J. Bacteriol. **185(23)** (2003) 6870–6882.

See Current Literature, Microbiology, p. 222.

Koranyi, K. I., and Ranalli, M. A. *Mycobacterium aurum* bacteremia in an immunocompromised child. Pediatr. Infect. Dis. J. **22(12)** (2003) 1108–1109.

Mycobacterium aurum was cultured from the Broviac catheter of a 5-year-old child with metastatic Wilms tumor. Removal of the catheter resulted in prompt resolution of the fever and sterilization of the blood culture. This rapidly growing mycobacterium, previously believed to be a commensal, can cause disease in the immunocompromised host.—Authors' Abstract

Marie, I., Heron, F., Lecomte, F., Jarlier, V., Truffot-Pernot, C., Laquerriere, A., Huerre, M., Levesque, H., and Courtois, H. Multiple cerebral abscesses as a complication of *Mycobacterium fortuitum* infection. Eur. J. Intern. Med. **14(6)** (2003) 386–389.

Mycobacterium fortuitum is a rapidly growing, nontuberculous mycobacteria that has rarely been associated with central nervous system impairment. We describe the case of a patient who developed multiple cerebral abscesses revealing *Mycobacterium fortuitum* infection. Brain biopsy specimens showed suppurative, noncaseating, granulomatous inflammation consisting of epithelioid histiocytes and multinucleated giant cells. All clinical signs and CT scan cerebral

lesions disappeared after institution of appropriate antimycobacterial therapy.—Authors' Abstract

Marsollier, L., Prevot, G., Honore, N., Legras, P., Manceau, A. L., Payan, C., Kouakou, H., and Carbonnelle, B. Susceptibility of *Mycobacterium ulcerans* to a combination of amikacin/rifampicin. Int J. Antimicrob. Agents. **22(6)** (2003) 562–566.

The effectiveness of rifampicin (RIF), amikacin (AMK) and their combination were estimated in the treatment of mice experimentally infected by *Mycobacterium ulcerans* and the risk of relapse after the treatment was evaluated. After 7 weeks of treatment with RIF or with the combination of AMK/RIF and 8 weeks with AMK alone, no viable bacilli were found in the infected tissues and these remained uninfected during the following 6 months. Among the mice treated with AMK alone, three mice relapsed, but the minimal inhibitory concentration of AMK for these isolates remained unchanged. With RIF alone, two mice relapsed and the minimal inhibitory concentration of these isolated strains was higher. However, with all the mice treated with both RIF and AMK, no relapse was observed.—Authors' Abstract

Martinelli, C., Farese, A., Carocci, A., Giorgini, S., Tortoli, E., and Leoncini, F. First case of *Mycobacterium haemophilum* infection in an AIDS patient in Italy. J. Eur. Acad. Dermatol. Venereol. **18(1)** (2004) 83–85.

Mycobacterium haemophilum, a strongly acid- and alcohol-fast bacillus belonging to the group of non-tuberculous mycobacteria was first described in 1978 as the cause of cutaneous ulcerating lesions in a woman with Hodgkin's disease. Infection due to *M. haemophilum* is rare but increasing in prevalence in immnunosuppressed subjects, particularly in patients with acquired immunodeficiency syndrome (AIDS) patients. The skin is the most common site of infection with erythematous or violaceous papules and/or nodules that are usually painless at first, but some elements develop into abscesses or ulcers that can become very painful. The incidence of *M. haemophilum* is unknown, but cases of infection have been reported in Australia, Canada, the United States, France, Israel, the United Kingdom and Taiwan; to date no cases have been reported in Italy, thus the case reported here is apparently the first one observed in our country.—Authors' Abstract

Menard, A., Couppié, P., Sainte-Marie, D., Pradinaud, R. Diagnostic par PCR de l'infection due à *Mycobacerium ulcerans:* à propos de trois cas observés en Guyane Française. Bull. Soc. Pathol. Exot. **96(5)** 403–405.

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

Nolt, D., Michaels, M. G., and Wald, E. R. Intrathoracic disease from nontuberculous mycobacteria in children: two cases and a review of the literature. Pediatrics **112(5)** (2003) e434.

Pulmonary disease caused by nontuberculous mycobacteria (NTM) is a relatively rare occurrence in immunocompetent children. Two cases of endobronchial NTM infection in immunocompetent children are described. In addition, 41 other children

with NTM pulmonary disease reported in the English literature between 1930 and 2003 are reviewed. Clinical manifestations are either purely respiratory or respiratory with more widespread systemic symptoms. Compared with children with only respiratory complaints, children with constitutional symptoms from NTM pulmonary disease 1) had symptoms for a shorter period before presentation (10 vs 28 days), 2) had more radiographic evidence of pulmonary disease, and 3) were treated longer with antimycobacterial agents (11.5 months vs 6 months). The most common causative organism was *Mycobacterium avium* complex. Pediatricians should be increasingly aware of NTM in the differential diagnosis of persistent pulmonary disease in previously healthy children.—Authors' Abstract

Olivier, K. N.; NTM in CF Study Group. The natural history of nontuberculous mycobacteria in patients with cystic. Pediatr. Respir. Rev. **5 Suppl** (2004) A:S213–A:S216.

With the increasing lifespan of persons with cystic fibrosis (CF), the emergence of a variety of previously seldom seen pathogens, including the nontuberculous mycobacteria (NTM), has been seen. Determining the impact of these indolent organisms on the natural history of cystic fibrosis lung disease has been difficult. We initiated a two-stage study in 1993 to assess the prevalence and clinical impact of these organisms among persons with CF in US CF Centers. These organisms were frequently recovered from older patients with relatively mild disease. While over the short, 15-month course of follow-up no significant differences in the rate of decline of lung function attributable to NTM were seen, concerning changes and progression of high-resolution computed tomography findings were seen in patients from whom these organisms were repeatedly recovered.—Authors' Abstract

Portaels, F., Aguiar, J., Debacker, M., Guedenon, A., Steunou, C., Zinsou, C., and Meyers, W. M. *Mycobacterium bovis* BCG vaccination as prophylaxis against *Mycobacterium ulcerans* osteomyelitis in Buruli ulcer disease. Infect. Immun. **72(1)** (2004) 62–65.

See Current Literature, Experimental Infections, p. 238.

Pumberger, W., Hallwirth, U., Pawlowsky, J., and Pomberger, G. Cervicofacial lymphadenitis due to atypical mycobacteria: a surgical disease. Pediatr. Dermatol. **21(1)** (2004) 24–29.

Despite the increasing prevalence of cervicofacial lymphadenitis due to atypical mycobacteria (AMB) in children, the true nature of AMB infection in clinical practice is poorly understood. The purpose of our study was to define the most common signs and symptoms, and to establish a workable scheme of diagnosis and treatment. Patients fulfilling the criteria of AMB infection (i.e., clinical signs, positive cultures or polymerase chain reaction, histologic features) were included in the study. All children underwent a standard surgical procedure, depending on pretreatment and the course of the disease. Sixteen infants presented with characteristic unilateral lymphadenopathy predominantly involving the submandibular area (13/16). Eight children had been initially treated at various institutions by fine-needle puncture or incision, and 7 of the 16 patients had received antituberculous multidrug treatment for a varying length of time. Complete excision of the affected lymph nodes was the definitive treatment in all patients. Three children had transient marginal mandibular nerve paralysis that resolved within a few months in all cases. Recognition of the characteristic features of AMB adenitis may permit early diagnosis and appropriate surgical treatment.—Authors' Abstract

Redaelli de Zinis, L. O., Tironi, A., Nassif, N., and Ghizzardi, D. Temporal bone infection caused by atypical mycobacterium: case report and review of the literature. Otol. Neurotol. **24(6)** (2003) 843–849.

OBJECTIVE: To discuss the clinical aspects and management of nontuberculous mycobacteriosis of the temporal bone.

STUDY DESIGN: Case report and review of the literature. SETTING: University hospital, tertiary referral center. PATIENT, INTERVENTION, AND RESULTS: The authors describe an uncommon case of nontuberculous mycobacteriosis of the temporal bone in an immunosuppressed 62-year-old woman with facial nerve paralysis caused by disease complication. The case was cured with radical tympanomastoidectomy and prolonged multiple antibiotic therapy. CONCLUSIONS: Nontuberculous mycobacteriosis should be suspected in immunosuppressed patients with intractable middle ear granulations. Cultural and histologic examinations are the mainstay for diagnosis. Long-standing multiantibiotic therapy together with aggressive surgery should be considered as appropriate management.—Authors' Abstract

Roupe, G. [Buruli ulcer—Africa's latest mycobacterial scourge]. Lakartidningen. **100(45)** (2003) 3596–3597. [Article in Swedish]

Buruliulcer is an extensive ulceration usually on the extremities. The ulcer can spread to subcutaneous fat, muscle and even bone causing osteomyelitis and death. It is the the third most common mycobacterial disease in humans after tuberculosis and leprosy. The bacterium grows in still standing water and infects children through small ulcerations in their skin. *Mycobacterium ulcerans* may also be transmitted by the bite of aquatic bugs (Naucordiae), which harbor the bacterium in their salivary glands. The disease affects poor people in rural, tropical areas where deforestation has led to flooding rivers, stagnant bodies of water and marsh. Benin, Cote d'Ivoire and Ghana in West Africa are seriously hit. Skin transplantation is the treatment of choice. Treatment with antibiotics has been disappointing.—Author's Abstract

Saeki, S., Matsuse, H., Shimoda, T., Soejima, Y., Ohno, H., and Kohno, S. [A case of pulmonary *Mycobacterium gordonae* infection with pleural effusion]. Nihon Kokyuki Gakkai Zasshi. **42(1)** (2004) 103–107. [Article in Japanese].

A 65-year-old woman, treated with prednisolone (5 mg daily) for rheumatoid arthritis, visited our hospital because of right chest pain. Chest CT showed small nodular shadows in the right lung accompanied with right pleural effusion. A pulmonary *Mycobacterium gordonae* infection was diagnosed, since *M. gordonae* was identified twice from her sputum. She was treated with rifampicin, ethambutol and streptomycin for two months, and then streptomycin was replaced with clarithromycin. Three months after the initial treatment, *M. gordonae* was eradicated from her sputum. Pleural puncture revealed bloody, exudative, lymphocytotic pleural effusion, but no malignant cells were identified. Although pathological diagnosis by thoracoscopic pleural biopsy could not be performed, it is likely that the pleural effusion was associated with the pulmonary *M. gordonae* infection in the present case.—Authors' Abstract

Saiman, L. The mycobacteriology of nontuberculous mycobacteria. Paediatr. Respir. Rev. **5 Suppl.** (2004) A:S221– A:S223.

The genus *Mycobacterium* consists of >50 species that have been associated with human disease. Mycobacterium are categorised into *M. tuberculosis* and NTM that are also subdivided into rapid growers and non-rapid growers. Five major clinical syndromes have been described that are attributable to mycobacterium. These include: pulmonary disease; lymphadenitis; skin, soft tissue, and skeletal infections; catheter-related blood-stream infections in immunocompromised hosts; and disseminated disease in persons with AIDS. There is very limited documentation of person-to-person transmission of NTM. Nosocomial infections and outbreaks caused by inadequate disinfection/sterilization of medical devices or environmental contamination of medications or medical devices are well described. Staining for AFB, culture, histopathologic, or genetic amplification technologies are used to detect and identify mycobacterium. Pulsed-field gel electrophoresis is the method of choice to determine strain relatedness. At present, susceptibility testing for non-tuberculous mycobacteria is not fully standardized and has not been correlated with clinical outcomes.—Authors' Abstract

Sechi, L. A., Mura, M., Tanda, E., Lissia, A., Fadda, G., and Zanetti, S. *Mycobacterium avium* sub. paratuberculosis in tissue samples of Crohn's disease patients. New Microbiol. **27(1)** (2004) 75–77.

Crohn's disease is a non-specific chronic transmural inflammatory disease. The disease was associated with a frameshift mutation in the NOD2 gene. Nevertheless, other researchers associated the presence of *M. paratuberculosis* within the intestinal tissues of patients with the disease. An adapted "*in situ* hybridization" technique was used to detect IS900 *M. paratuberculosis* DNA in paraffin embedded tissue from Crohns tissue disease samples. We were able to identify *M. paratuberculosis* DNA in around 69% of the paraffine embedded intestinal samples of Crohn's disease patients analysed. The presence of *M. paratuberculosis* DNA in the intestinal samples analysed does not necessarily mean that *M. paratuberculosis* is responsible for Crohn's disease. Our results support the hypothesis that infection may be caused by cell wall defective *M. paratuberculosis* since no bacteria were detected by Ziehl Neelsen stain.—Authors' Abstract

Sermet-Gaudelus, I., Le Bourgeouis, M., Pierre-Audigier, C., Offredo, C., Guillemot, D., Halley, S., Akoua-Koffi, C., Vincent, V., Sivadon-Tardy, V., Ferroni, A., Berche, P., Scheinmann, P., Lenoir, G., and Gaillard, J.-L. Mycobacterium abcessus with Cystic Fibrosis. Emerging Infect. Dis. **9(12)** (2003) 1587–1591.

We prospectively studied 298 patients with cystic fibrosis (mean age 11.3 years; range 2 months to 32 years; sex ratio, 0.47) for nontuberculous mycobacteria in respiratory samples from January 1, 1996, to December 31, 1999. *Mycobacterium abscessus* was by far the most prevalent nontuberculous mycobacterium: 15 patients (6 male, 9 female; mean age 11.9 years; range 2.5–22 years) had at least one positive sample for this microorganism (versus 6 patients positive for *M. avium* complex), including 10 with >3 positive samples (versus 3 patients for *M. avium* complex). The *M. abscessus* isolates from 14 patients were typed by pulsed-field gel electrophoresis: each of the 14 patients harbored a unique strain, ruling out a common environmental reservoir or person-to-person transmission. Water samples collected in the cystic fibrosis center were negative for *M. abscessus.* This major mycobacterial pathogen in children and teenagers with cystic fibrosis does not appear to be acquired nosocomially.—Emerging Infectious Diseases

Shiratsuch, H., and Basson, M. D. Caspase activation may be associated with *Mycobacterium avium* pathogenicity. Am. J. Surg. **186(5)** (2003) 547–551.

BACKGROUND: *Mycobacterium avium* causes disseminated infection in immunocompromised patients and triggers a process resembling Crohn's disease in goats. Colony morphotypes predict pathogenicity. Smooth-transparent (SmT) morphotypes are more virulent and induce less interleukin (IL)-1beta and IL-18 production than avirulent smooth-domed (SmD) morphotypes. Caspases are essential for IL-1beta and IL-18 production. METHODS: Caspase activation was examined in human monocytes after *M. avium* infection. RESULTS: Fresh monocytes constitutively expressed caspase-1 mRNA and pro-caspase-1. The *M. avium* infection increased monocyte caspase-1 mRNA expression. Furthermore, SmD-infected monocytes expressed 2.3-fold higher levels ($p < 0.05$, $N = 3$) of activated caspases than SmT-infected monocytes. Caspase-1 inhibition significantly reduced IL-1beta production by SmT- and SmD-infected monocytes ($p < 0.05$, $N = 4$). Caspase-3 inhibition inhibited IL-1beta production 43.5% ± 8.0% ($p < 0.02$, $N = 4$) by SmD-infected but not SmT-infected monocytes. CONCLUSIONS: Decreased mature IL-1beta release by SmT-infected monocytes may reflect selective induction of caspase-1 activity but not caspase-3. Differential caspase expression in monocytes after infection may contribute to *M. avium* pathogenicity in humans.—Authors' Abstract

Sugihara, E., Hirota, N., Niizeki, T., Tanaka, R., Nagafuchi, M., Koyanagi,

T., Ono, N., Rikimaru, T., and Aizawa, H. Usefulness of bronchial lavage for the diagnosis of pulmonary disease caused by *Mycobacterium avium*-intracellulare complex (MAC) infection. J. Infect. Chemother. **9(4)** (2003) 328–332.

To evaluate the usefulness of bronchial lavage for the diagnosis of pulmonary disease due to *Mycobacterium avium*-intracellulare complex (MAC) infection, we examined the clinical records and bacteriologic findings of patients admitted to our hospital between 1999 and 2002 who fulfilled the 1997 American Thoracic Society (ATS) criteria for MAC pulmonary infection. Bronchoscopic examinations were performed in those patients with MAC pulmonary disease who showed negative sputum smears for mycobacteria on 3 consecutive days (N = 14) or who could not expectorate sputum (n = 2). The bronchial lavage sample was smear-positive for acid-fast bacilli in 8 of the 16 patients (50.0%), polymerase chain reaction (PCR)-positive for MAC in 10 of 15 (66.7%), and culture-positive for MAC in 15 of 16 (93.7%). The brushing sample was positive for MAC in 5 of 14 patients (35.7%), and transbronchial lung biopsy (TBLB)-positive for MAC in 2 of 5 (40.0%). MAC was isolated by culture of bronchial lavage samples in a higher percentage of patients than that in whom MAC was isolated by sputum culture, and we could make an early diagnosis of MAC pulmonary disease based on the smear and PCR results for bronchial lavage samples. Bronchial lavage is useful to screen sputum smear-negative patients suspected of having MAC pulmonary disease.—Authors' Abstract

Teelken, M. A., Stienstra, Y., Ellen, D. E., Quarshie, E., Klutse, E., van der Graaf, W. T. A., van der Werf, T. S. Buruuli ulcer: differences in treatment outcome between two centers in Ghana. Acta Tropica **88(1)** (2003) 51–56.

Objectives: Assess treatment effects by following up patients treated for Buruli ulcer in two hospitals with different treatment aspects, including widely differing surgical practices. Patients/methods: Treated patients were retrospectively identified from hospital records. Between 1994 and July 2000, 136 patients had been admitted for Buruli ulcer in both hospitals, and lived in areas covered in the research period. 78 (57%) Patients were included in the study. Treatment and status of the patient were analyzed. Results: 27 (35%) Patients were not healed. Of the 33 patients treated in hospital A, six (18%) were not healed at follow-up, whereas of the 45 patients treated in hospital B, 21 (47%) were not healed. The length of stay in hospital A was significantly longer ($p = 0.002$), and more operations on average were done per patient ($p = 0.002$). In a univariate analysis, treatment in hospital A; the use of rifampicin ($p = 0.013$); and BCG vaccination status ($p = 0.04$) were all significantly associated with ulcer healing. Using a logistic regression model for multivariate analysis, only treatment as given in hospital A, with standard prctice of wide surgical excision, appeared to predict ulcer healing independently ($p = 0.02$). Conclusions: This study shows large differences in treatment outcome between the two hospitals; the results support the hypothesis that extent of surgical treatment influences the chance of healing of Buruli ulcer.—Tropical Disease Bulletin

Ticca, F., Comparcola, D., Graziani, M. C., Lancella, L., Marsella, P., Nicolosi, L., Pierro, V., Rivosecchi, M. R., Ticca, C., and Tieri, L. [Otomastoiditis caused by *Mycobacterium avium*: a case report]. Infez. Med. 5(2) (1997) 114–117. [Article in Italian]

Non tuberculous Mycobacterial (NTM) infections mainly affect immunocompromised patients, appearing as disseminated or pulmonary disease. In immunocompetent children the most common form of infection with NTM is cervical adenitis. Ear infection seems to be a rare disease. We present a case of otomastoiditis caused by *Mycobacterium avium* in a 15 months old child, immunologically normal. Patient was referred for persistent right otitis unresponsive to routine medical therapy. TC scan of the ear and temporal bones revealed: soft tissue in external auditory canal, Eustachian canal, and middle ear overlying ossicles with erosion of tegmen tympani. Tuberculin skin test was positive

with 5 units PPD and culture yielded *M. avium*. The patient undergo timpanomastoidectomy and medical therapy with antituberculous drugs and steroids, subsequently he was given Clarithromycin and Rifabutin. *M. avium* is an ubiquitous low grade pathogen found in soil, water, dust and food. There is no evidence of direct transmission. Only a few cases of otomastoiditis due to *M. avium* have previously been reported. The case presented underlines the importance of microbiological investigations. When a NTM infection is suspected surgeons and infectious diseases specialists should cooperate to find an optimal treatment regimen of this unusual disease.—Authors' Abstract

Toy, B. R. Foreign-body reaction with *Mycobacterium abscessus* superinfection. Dermatol. Online J. 9(4) (2003) 29.

Mycobacterium abscessus is a rare cause of skin and soft tissue infections that often results from inoculation with contaminated foreign material. A 41-year-old woman is described regarding an outbreak of *M. abscessus* following soft tissue augmentation. Clinical features and treatment options are reviewed.—Author's Abstract

Winthrop, K. L., Albridge, K., South, D., Albrecht, P., Abrams, M., Samuel, M. C., Leonard, W., Wagner, J., and Vugia, D. J. The clinical management and outcome of nail salon-acquired *Mycobacterium fortuitum* skin infection. Clin. Infect. Dis. **38(1)** (2004) 38–44.

Nontuberculous mycobacterial infections are becoming more common. Recently, *Mycobacterium fortuitum* and other rapidly growing mycobacteria have been found to cause severe skin and soft-tissue infections in association with nail salon whirlpool footbaths. We recently investigated a large outbreak of *M. fortuitum* furunculosis among women who received pedicures at a single nail salon. To better define the clinical course of such infections, we collected clinical details from physicians who were treating outbreak patients. We constructed multivariable linear models to evaluate the effect of antibiotic treatment on disease duration. Sixty-one patients were included in the investigation. The mean disease duration was 170 days (range, 41–336 days). Forty-eight persons received antibiotic therapy for a median period of 4 months (range, 1–6 months), and 13 persons were untreated. Isolates were most susceptible to ciprofloxacin and minocycline. Early administration of therapy was associated with shorter duration of disease only in persons with multiple boils ($p < .01$). One untreated, healthy patient had lymphatic disease dissemination.—Authors' Abstract

Molecular and Genetic Studies

Adekambi, T., Colson, P., and Drancourt, M. rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J. Clin. Microbiol. **41(2)** 2003 5699–5708.

See Current Literature, Microbiology, p. 219.

Al-Attiyah, R., and Mustafa, A. S. Computer-assisted prediction of HLA-DR binding and experimental analysis for human promiscuous Th1-cell peptides in the 24 kDa secreted lipoprotein (LppX) of *Mycobacterium tuberculosis*. Scand. J. Immunol. **59(1)** 2004 16–24.

The secreted 24 kDa lipoprotein (LppX) is an antigen that is specific for *Mycobacterium tuberculosis* complex and *M. leprae*. The present study was carried out to identify the promiscuous T helper 1 (Th1)-cell epitopes of the *M. tuberculosis* LppX (MT24, Rv2945c) antigen by using 15 overlapping synthetic peptides (25 mers overlapping by 10 residues) covering the sequence of the complete protein. The analysis of Rv2945c sequence for binding to 51 alleles of nine serologically defined HLA-DR molecules,

by using a virtual matrix-based prediction program (propred), showed that eight of the 15 peptides of Rv2945c were predicted to bind promiscuously to ≥10 alleles from more than or equal to three serologically defined HLA-DR molecules. The Th1-cell reactivity of all the peptides was assessed in antigen-induced proliferation and interferon-gamma (IFN-gamma)-secretion assays with peripheral blood mononuclear cells (PBMCs) from 37 bacille Calmette-Guerin (BCG)-vaccinated healthy subjects. The results showed that 17 of the 37 donors, which represented an HLA-DR-heterogeneous group, responded to one or more peptides of Rv2945c in the Th1-cell assays. Although each peptide stimulated PBMCs from one or more donors in the above assays, the best positive responses (12/17 (71%) responders) were observed with the peptide p14 (aa 196–220). This suggested a highly promiscuous presentation of p14 to Th1 cells. In addition, the sequence of p14 is completely identical among the LppX of *M. tuberculosis*, *M. bovis* and *M. leprae*, which further supports the usefulness of Rv2945c and p14 in the subunit vaccine design against both tuberculosis and leprosy.—Authors' Abstract

Beyene, D., Aseffa, A., Harboe, M., Kidane, D., Macdonald, M., Klatser, P. R., Bjune, G. A., and Smith, W. C. Nasal carriage of *Mycobacterium leprae* DNA in healthy individuals in Lega Robi village, Ethiopia. Epidemiol. Infect. **131(2)** (2003) 841–848.

See Current Literature, Clinical Sciences, p. 192.

Bhanu, N. V., van Soolingen, D., van Embden, J. D., and Seth, P. Two *Mycobacterium fortuitum* strains isolated from pulmonary tuberculosis patients in Delhi harbor IS6110 homologue. Diagn. Microbiol. Infect. Dis. **48(2)** (2004) 107–110.

We report 2 isolates of *Mycobacterium fortuitum* from patients with pulmonary tuberculosis lesions hybridizing to IS6110 probe in restriction fragment length polymorphism (RFLP) typing. Results of polymerase chain reaction-hybridization formats using the non-specific region of IS6110 for the molecular detection of mycobacteria in clinical material should be interpreted with caution.—Authors' Abstract

Bisen, P. S., Garg, S. K., Tiwari, R. P., Tagore, P. R., Chandra, R., Karnik, R., Thaker, N., Desai, N., Ghosh, P. K., Fraziano, M., and Colizzi, V. Analysis of the shotgun expression library of the *Mycobacterium tuberculosis* genome for immunodominant polypeptides: potential use in serodiagnosis. Clin. Diagn. Lab. Immunol. **10(6)** (2003) 1051–1058.

A recombinant DNA strategy was applied to analyze and screen the shotgun expression library from a clinically confirmed local virulent isolate of *Mycobacterium tuberculosis* with sera from tuberculosis patients, which led to expression and purification of highly immunoreactive and specific mycobacterial antigens expressed during the course of active disease which could be of diagnostic significance. An enzyme-linked immunoassay for diagnosis of tuberculosis was devised by using a shotgun immunoexpression library in the lambdagt11 vector. DNA from a virulent *M. tuberculosis* patient isolate (TBW-33) confirmed with the BACTEC 460 system was sheared and expressed to generate shotgun polypeptides. beta-Galactosidase fusion proteins capable of demarcating active tuberculosis infections from *Mycobacterium bovis* BCG-vaccinated healthy subjects or people harboring environmental mycobacteria were selected by comparative immunoreactivity studies. Promising mycobacterial DNA cassettes were subcloned and expressed into the glutathione S-transferase (GST) fusion vector pGEX-5X-1 with a strong tac promoter and were expressed in *Escherichia coli* BL21. These fusion proteins were severed at a built-in factor Xa recognition site to separate the GST tags and were utilized in an indirect enzyme-linked immunoassay for serodiagnosis of patients with active tuberculosis. The system offered a clear demarcation between BCG-vaccinated healthy subjects and patients with active tuberculosis and proved to be effective in detecting

pulmonary as well as extrapulmonary tuberculosis, with an overall sensitivity of 84.33% and an overall specificity of 93.62%.—Authors' Abstract

Chattopadhyay, C., Sau, S., and Mandal, N. C. Cloning and characterization of the promoters of temperate mycobacteriophage L1. J. Biochem. Mol. Biol. **36(6)** (2003) 586–592.

Four putative promoters of the temperate mycobacteriophage L1 were cloned by detecting the beta-galactosidase reporter expression in *E. coli* transformants that carried L1 specific operon-fusion library. All of the four L1 promoters were also found to express differentially in the homologous environment of mycobacteria. Of the four promoters, two were suggested to be the putative early promoters of L1 since they express within 0 to 10 min of the initiation of the lytic growth of L1. One of the putative early promoters showed a relatively better and almost identical activity in both *E. coli* and *M. smegmatis*. By a sequence analysis, we suggest that the L1 insert that contained the stronger early promoter possibly carries two convergent *E. coli* sigma70-like L1 promoters, which are separated from each other by about 300 nucleotides. One of them is the early promoter of L1 as it showed a 100% similarity with the early Pleft promoter of the homoimmune phage L5. The second promoter, designated P4, was suggested for its appreciable level of reporter activity in the absence of the -10 element of the Pleft equivalent of L1. By analyzing most of the best characterized mycobacteriophages-specific promoters, including the L1 promoter P4, we suggest that both the -10 and -35 hexamers of the mycobacteriophage promoters are highly conserved and almost similar to the consensus -10 and -35 hexamers of the *E. coli* sigma70 promoters.—Authors' Abstract

Cheng, A. F., Yew, W. W., Chan, E. W., Chin, M. L., Hui, M. M., and Chan, R. C. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. Antimicrob. Agents Chemother. **48(2)** (2004) 596–601.

A new strategy known as multiplex PCR amplimer conformation was developed for detection of mutation in the gyrA gene of 138 clinical isolates *of Mycobacterium tuberculosis*. The method generated a single-stranded and heteroduplex DNA banding pattern of multiplex PCR amplimers of the region of interest that was extremely sensitive to specific mutations, thus enabling much more sensitive and reliable mutation analysis compared to the standard single-stranded conformation polymorphism technique. The genetic profiles of the gyrA gene of the 138 isolates as detected by MPAC were confirmed by nucleotide sequencing and were found to correlate strongly with the in vitro susceptibilities of the mutant strains to six fluoroquinolones (ofloxacin, levofloxacin, sparfloxacin, moxifloxacin, gatifloxacin, and sitafloxacin). All 32 isolates that contained gyrA mutations exhibited cross-resistance to the six fluoroquinolones (ofloxacin MIC for 90% of strains >16 mg/liter), although moxifloxacin, gatifloxacin, and sitafloxacin (MIC for 90% of strains ≤4 mg/liter) were apparently more active than ofloxacin, levofloxacin, and sparfloxacin (MIC for 90% of strains >/=="" BORDER="0" >16 mg/liter). All gyrA mutations were clustered in codons 90, 91, and 94, and aspartic acid 94 was most frequently mutated. Twenty-three isolates without gyrA mutations were also found to exhibit reduced susceptibility to ofloxacin (MIC for 90% of strains = 4 mg/liter), but largely remained susceptible to other drugs (MIC for 90% of strains ≤1 mg/liter). Another 83 isolates without mutations were fully susceptible to all six fluoroquinolones (ofloxacin MIC for 90% of strains = 1 mg/liter). In conclusion, high-level phenotypic resistance to fluoroquinolones among *M. tuberculosis* clinical isolates, which appears to be predominantly due to gyrA mutations, may be readily detected by genotyping techniques such as multiplex PCR amplimer conformation.—Authors' Abstract

Jamieson, S. E., Miller, E. N., Black, G. F., Peacock, C. S., Cordell, H. J., Howson,

J. M., Shaw, M. A., Burgner, D., Xu, W., Lins-Lainson, Z., Shaw, J. J., Ramos, F., Silveira, F., and Blackwell, J. M. Evidence for a cluster of genes on chromosome 17q11-q21 controlling susceptibility to tuberculosis and leprosy in Brazilians. Genes Immun. **5(1)** (2004) 46–57.

The region of conserved synteny on mouse chromosome 11/human 17q11-q21 is known to carry a susceptibility gene(s) for intramacrophage pathogens. The region is rich in candidates including NOS2A, CCL2/MCP-1, CCL3/MIP-1alpha, CCL4/MIP-1beta,CCL5/RANTES, CCR7, STAT3 and STAT5A/5B. To examine the region in man, we studied 92 multicase tuberculosis (627 individuals) and 72 multicase leprosy (372 individuals) families from Brazil. Multipoint nonparametric analysis (ALLEGRO) using 16 microsatellites shows two peaks of linkage for leprosy at D17S250 (Z(lr) score 2.34; p = 0.01) and D17S1795 (Z(lr) 2.67; p = 0.004) and a single peak for tuberculosis at D17S250 (Z(lr) 2.04; p = 0.02). Combined analysis shows significant linkage (peak Z(lr) 3.38) at D17S250, equivalent to an allele sharing LOD score 2.48 (p = 0.0004). To determine whether one or multiple genes contribute, 49 informative single nucleotide polymorphisms were typed in candidate genes. Family-based allelic association testing that was robust to family clustering demonstrated significant associations with tuberculosis susceptibility at four loci separated by intervals (NOS2A-8.4 Mb-CCL18-32.3 kb-CCL4-6.04 Mb-STAT5B) up to several Mb. Stepwise conditional logistic regression analysis using a case/pseudocontrol data set showed that the four genes contributed separate main effects, consistent with a cluster of susceptibility genes across 17q11.2.—Authors' Abstract

Kalate, R. N., Tambe, S. S., and Kulkarni, B. D. Artificial neural networks for prediction of mycobacterial promoter sequences. Comput. Biol. Chem. **27(6)** (2003) 555–564.

A multilayered feed-forward ANN architecture trained using the error-back-propagation (EBP) algorithm has been developed for predicting whether a given nucleotide sequence is a mycobacterial promoter sequence. Owing to the high prediction capability (congruent with 97%) of the developed network model, it has been further used in conjunction with the caliper randomization (CR) approach for determining the structurally/functionally important regions in the promoter sequences. The results obtained thereby indicate that: (i) upstream region of -35 box, (ii) -35 region, (iii) spacer region and, (iv) -10 box, are important for mycobacterial promoters. The CR approach also suggests that the -38 to -29 region plays a significant role in determining whether a given sequence is a mycobacterial promoter. In essence, the present study establishes ANNs as a tool for predicting mycobacterial promoter sequences and determining structurally/functionally important sub-regions therein.—Authors' Abstract

Lee, C. K., Gi, H. M., Cho, Y., Kim, Y. K., Lee, K. N., Song, K. J., Song, J. W., Park, K. S., Park, E. M., Lee, H., and Bai, G. H. The genomic heterogeneity among *Mycobacterium terrae* complex displayed by sequencing of 16S rRNA and hsp 65 genes. Microbiol. Immunol. **48(2)** (2004) 83–90.

The species identification within *Mycobacterium terrae* complex has been known to be very difficult. In this study, the genomic diversity of *M. terrae* complex with eighteen clinical isolates, which were initially identified as *M. terrae* complex by phenotypic method, was investigated, including that of three type strains (*M. terrae, M. nonchromogenicum*, and *M. triviale*). 16S rRNA and 65-kDa heat shock protein (hsp 65) gene sequences of mycobacteria were determined and aligned with eleven other references for the comparison using similarity search against the GenBank and Ribosomal Database Project II (RDP) databases. 16S rRNA and hsp 65 genes of *M. terrae* complex showed genomic heterogeneity. Amongst the eighteen clinical isolates, nine were identified as *M. nonchromogenicum*, eight as *M. terrae*, one as *M. mucogenicum* with the molecular characteristic of rapid growth. *M. nonchromogenicum* could be subdivided into three subgroups,

while *M. terrae* could be subdivided into two subgroups using a 5 bp criterion (>1% difference). Seven isolates in two subgroups of *M. nonchromogenicum* were *Mycobacterium* sp. strain MCRO 6, which was closely related to *M. nonchromogenicum*. The hsp 65 gene could not differentiate one *M. nonchromogenicum* from *M. avium* or one *M. terrae* from *M. intracellulare*. The nucleotide sequence analysis of 16S rRNA and hsp 65 genes was shown to be useful in identifying the *M. terrae* complex, but hsp 65 was less discriminating than 16S rRNA.—Authors' Abstract

Liu, W., Zhang, C. Y., Tian, L., Li, C. Z., Wu, X. M., Zhao, Q. M., Zhang, P. H., Yang, S. M., Yang, H., and Cao, W. C. [A case-control study on natural-resistance-associated macrophage protein 1 gene polymorphisms and susceptibility to pulmonary tuberculosis]. Zhonghua Yu Fang Yi Xue Za Zhi. 37(6) (2003) 408–411. [Article in Chinese]

OBJECTIVE: To investigate association between the natural-resistance-associated macrophage protein 1 (NRAMP1) gene polymorphisms and susceptibility to pulmonary tuberculosis (TB) in Chinese Han population. METHODS: Hospital-based case-control study design was adopted. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique were used to type three NRAMP1 polymorphisms (INT4, D543N and 3′UTR). Information on related factors of tuberculosis was collected using a pretested standard questionnaire. Univariate and multivariate unconditional logistic analyses were conducted using SPSS for window software package. Totally, 110 cases of TB were selected during April 2001 to June 2002, with an average age of (27.7 ± 12.7) years. Also, 180 cases of healthy control were selected, aged (27.3 ± 9.2) years in average. Locus of NRAMP1 polymorphism was analysed with univariate method. RESULTS: Univariate analysis demonstrated that the D543N G/A and 3′UTR TGTG+/del genotype occurred more frequently in the cases than in the controls, with crude odds ratios (OR) (95% CI) of 2.22 (1.03–4.78) and 1.93 (1.14–3.26), respectively. No significant association was observed between TB and INT4 polymorphisms. In multivariate analysis, associations of TB and D543N G/A and 3′UTR TGTG+/del genotypes remained, adjusted for exposure history and bacille Camette-Guerin immunization. Adjusted OR (95% CI) was 3.04 (1.12–8.27) and 2.36 (1.20–4.64), respectively. Still, no significant association between INT4 polymorphisms and TB was found. CONCLUSION: Polymorphisms of D543N and 3′UTR locus in NRAMP1 gene might affect their susceptibility to TB in Chinese Han population.—Authors' Abstract

Majeed, A. A., Ahmed, N., Rao, K. R., Ghousunnissa, S., Kauser, F., Bose, B., Nagarajaram, H. A., Katoch, V. M., Cousins, D. V., Sechi, L. A., Gilman, R. H., and Hasnain, S. E. AmpliBASE MTTM: a *Mycobacterium tuberculosis* diversity knowledgebase. Bioinformatics **20(6)** (2004) 989–992.

SUMMARY: AmpliBASE MT trade mark is an online databank of high-resolution DNA fingerprints representing fluorescent amplified fragment length polymorphism (FAFLP) profiles or amplitypes developed for the *Mycobacterium tuberculosis* complex strains from 48 different countries. AmpliBASE MT trade mark is based on a relational database management system that is hyperlinked to visualize genotyping results in the form of DNA fingerprint images for individual strains. A flexible search system based on systematic comparisons of fragment sizes in base pairs allows inter-laboratory comparison of FAFLP profiles. Besides this, the database also displays previously published data on IS6110 profiles, spoligotypes, MIRU-VNTRs and large sequence polymorphisms along with the FAFLP records that will give the overall comparisons. Being the first of its kind, AmpliBASE MT trade mark is expected to be a very helpful tool in strengthening the concept of 'geographic genomics' and will be very helpful to molecular epidemiologists and those interested in diagnostic development for tuberculosis.—Authors' Abstract

Marmiesse, M., Brodin, P., Buchrieser, C, Gutierrez, C., Simoes, N., Vincent, V.,

Glaser, P., Cole, S. T., and Brosch, R. Macro-array and bioinformatic analyses reveal mycobacterial 'core' genes, variation in the ESAT-6 gene family and new phylogenetic markers for the *Mycobacterium tuberculosis* complex. Microbiology. **150(Pt 2)** (2004) 483–496.

To better understand the biology and the virulence determinants of the two major mycobacterial human pathogens *Mycobacterium tuberculosis* and *Mycobacterium lepra*e, their genome sequences have been determined recently. *In silico* comparisons revealed that among the 1439 genes common to both *M. tuberculosis* and *M. leprae*, 219 genes code for proteins that show no similarity with proteins from other organisms. Therefore, the latter 'core' genes could be specific for mycobacteria or even for the intracellular mycobacterial pathogens. To obtain more information as to whether these genes really were mycobacteria-specific, they were included in a focused macro-array, which also contained genes from previously defined regions of difference (RD) known to be absent from *Mycobacterium bovis* BCG relative to *M. tuberculosis*. Hybridization of DNA from 40 strains of the *M. tuberculosis* complex and *in silico* comparison of these genes with the near-complete genome sequences from *Mycobacterium avium, Mycobacterium marinum* and *Mycobacterium smegmatis* were undertaken to answer this question. The results showed that among the 219 conserved genes, very few were not present in all the strains tested. Some of these missing genes code for proteins of the ESAT-6 family, a group of highly immunogenic small proteins whose presence and number is variable among the genomically highly conserved members of the *M. tuberculosis* complex. Indeed, the results suggest that, with few exceptions, the 'core' genes conserved among *M. tuberculosis* H37Rv and *M. leprae* are also highly conserved among other mycobacterial strains, which makes them interesting potential targets for developing new specific anti-mycobacterial drugs. In contrast, the genes from RD regions showed great variability among certain members of the *M. tuberculosis* complex, and some new specific deletions in *Mycobacterium canettii*, *Mycobacterium microti* and seal isolates were identified and further characterized during this study. Together with the distribution of a particular 6 or 7 bp micro-deletion in the gene encoding the polyketide synthase pks15/1, these results confirm and further extend the revised phylogenetic model for the *M. tuberculosis* complex recently presented.—Authors' Abstract

Matsuoka, M., Zhang, L., Budiawan, T., Saeki, K., and Izumi, S. Genotyping of *Mycobacterium leprae* on the basis of the polymorphism of TTC repeats for analysis of leprosy transmission. J. Clin. Microbiol. **42(2)** (2004) 741–745.

See Current Literature, Microbiology, Leprosy, p. 224.

Miller, E. N., Jamieson, S. E., Joberty, C., Fakiola, M., Hudson, D., Peacock, C. S., Cordell, H. J., Shaw, M. A., Lins-Lainson, Z., Shaw, J. J., Ramos, F., Silveira, F., and Blackwell, J. M. Genome-wide scans for leprosy and tuberculosis susceptibility genes in Brazilians. Genes Immun. **5(1)** (2004) 63–67.

Genome-wide scans were conducted for tuberculosis and leprosy per se in Brazil. At stage 1, 405 markers (10 cM map) were typed in 16 (178 individuals) tuberculosis and 21 (173 individuals) leprosy families. Nonparametric multipoint analysis detected 8 and 9 chromosomal regions respectively with provisional evidence ($p < 0.05$) for linkage. At stage 2, 58 markers from positive regions were typed in a second set of 22 (176 individuals) tuberculosis families, with 22 additional markers typed in all families; 42 positive markers in 50 (192 individuals) new leprosy families, and 30 additional markers in all families. Three regions (10q26.13, 11q12.3, 20p12.1) retained suggestive evidence (peak LOD scores 1.31, 1.85, 1.78; $p = 0.007, 0.0018, 0.0021$) for linkage to tuberculosis, 3 regions (6p21.32, 17q22, 20p13) to leprosy (HLA-DQA, 3.23, $p = 5.8 \times 10(-5)$; D17S1868, 2.38, $p = 0.0005$; D20S889, 1.51, $p = 0.004$). The peak at D20S889 for leprosy is 3.5 Mb distal to that reported at D20S115 for leprosy in India. (151 words).—Authors' Abstract

Mostowy, S., Cousins, D., and Behr, M. A. Genomic interrogation of the dassie bacillus reveals it as a unique RD1 mutant within the *Mycobacterium tuberculosis* complex. J. Bacteriol. **186(1)** (2004) 104–149.

Despite their remarkable genetic homology, members of the *Mycobacterium tuberculosis* complex express very different phenotypes, most notably in their spectra of clinical presentation. For example, *M. tuberculosis* is regarded as pathogenic to humans, whereas members having deleted RD1, such as *Mycobacterium microti* and *Mycobacterium bovis* BCG, are not. The dassie bacillus, an infrequent variant of the *M. tuberculosis* complex characterized as being most similar to *M. microti*, is the causative agent of tuberculosis (TB) in the dassie (*Procavia capensis*). Intriguingly, the dassie bacillus is not pathogenic to rabbits or guinea pigs and has never been documented to infect humans. Although it was identified more than a half-century ago, the reasons behind its attenuation are unknown. Because large sequence polymorphisms have presented themselves as the most obvious genomic distinction among members of the *M. tuberculosis* complex, the DNA content of the dassie bacillus was interrogated by Affymetrix GeneChip to identify regions that are absent from it but present in *M. tuberculosis* H37Rv. Comparison has led to the identification of nine regions of difference (RD), five of which are shared with *M. microti* (RDs 3, 7, 8, 9, and 10). Although the dassie bacillus does not share the other documented deletions in *M. microti* (RD1(mic), RD5(mic), MID1, MID2, and MID3), it has endured unique deletions in the regions of RD1, RD5, N-RD25, and Rv3081-Rv3082c (virS). RD1(das), affecting only Rv3874-Rv3877, is the smallest natural deletion of the RD1 region uncovered and points to genes within this region that are likely implicated in virulence. Newfound deletions from the dassie bacillus are discussed in relation to their evolutionary and biological significance.—Authors' Abstract

Remus, N., Alcais, A., and Abel, L. Human genetics of common mycobacterial infections. Immunol. Res. **28(2)** (2003) 109–129.

There is increasing interest in and understanding of the role of human genetic factors controlling susceptibility/resistance to infectious diseases. This is of particular importance for the two most common mycobacterial infections, tuberculosis and leprosy, because this will allow a genetic dissection of antimycobacterial immunity and should open new fields of preventive and therapeutic measures. In this review we will initially discuss various methods of genetic epidemiology that have been and are being developed to identify human genes controlling infectious diseases, and then illustrate the findings obtained in the numerous studies performed in tuberculosis and leprosy. Although the most convincing results were observed for HLA-DR2 and NRAMP1 (or a closely linked gene) in pulmonary tuberculosis and leprosy subtypes and for a 10p13 locus in paucibacillary leprosy, the molecular basis of their effects remains to be established.—Authors' Abstract

Sampson, S. L., Richardson, M., Van Helden, P. D., and Warren, R. M. IS6110-mediated deletion polymorphism in isogenic strains of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **42(2)** (2004) 895–898.

Previous studies have described IS6110-mediated polymorphism as an important driving force in *Mycobacterium tuberculosis* genome evolution and have provided indirect evidence for IS6110-driven deletion events. This study provides the first description of an IS6110-mediated deletion event in truly isogenic strains. We also provide further support for the hypothesis that the region from Rv1754 to Rv1765 is a hot spot for IS6110 insertion and deletion events.—Authors' Abstract

Sen Gupta, R., Hillemann, D., Kubica, T., Zissel, G., Muller-Quernheim, J., Galle, J., Vollmer, E., and Goldmann, T. HOPE-fixation enables improved PCR-based detection and differentiation of *Mycobacterium tuberculosis* complex in paraffin-embedded tissues. Pathol. Res. Pract. **199(9)** (2003) 619–623.

See Current Literature, Immunopathology, p. 203.

Song, T., Dove, S. L., Lee, K. H., and Husson, R. N. RshA, an anti-sigma factor that regulates the activity of the mycobacterial stress response sigma factor SigH. Mol. Microbiol. **50(3)** (2003) 949–959.

SigH, an alternative sigma factor of *Mycobacterium tuberculosis*, is a central regulator of the response to oxidative and heat stress. Exposure to these stresses results in increased expression of sigH itself, and of genes encoding additional regulators and effectors of the bacterial response to these stresses. In this work we show that RshA, a protein encoded by a gene in the sigH operon, is an anti-sigma factor of SigH. We demonstrate that RshA binds to SigH *in vitro* and *in vivo*. This protein-protein interaction, as well as the ability of RshA to inhibit SigH-dependent transcription, is redox-dependent, with RshA functioning as a negative regulator of SigH activity only under reducing conditions. The interaction of SigH and RshA is also disrupted *in vitro* by elevated temperature. RshA, a protein of 101 amino acids, contains five conserved cysteine residues of which two appear to be essential for RshA to inhibit SigH activity, suggesting that these cysteines may be important for the redox state dependence of RshA function. Our results indicate that RshA is a sensor that responds to oxidative stress, and also to heat stress, resulting in activation of SigH and expression of the SigH-dependent genes that allow *M. tuberculosis* to adapt to these stresses.—Authors' Abstract

Stratmann, J., Strommenger, B., Goethe, R., Dohmann, K., Gerlach, G. F., Stevenson, K., Li, L. L., Zhang, Q., Kapur, V., and Bull, T. J. A 38-kilobase pathogenicity island specific for *Mycobacterium avium* subsp. paratuberculosis encodes cell surface proteins expressed in the host. Infect. Immun. **72(3)** (2004) 1265–1274.

We have used representational difference analysis to identify a novel *Mycobacterium avium* subsp. paratuberculosis-specific ABC transporter operon (mpt), which comprises six open reading frames designated mptA to -F and is immediately preceded by two putative Fur boxes. Functional genomics revealed that the mpt operon is flanked on one end by a fep cluster encoding proteins involved in the uptake of Fe(3+) and on the other end by a sid cluster encoding nonribosome-dependent heterocyclic siderophore synthases. Together these genes form a 38-kb *M. avium* subsp. paratuberculosis-specific locus flanked by an insertion sequence similar to IS1110. Expression studies using Western blot analyses showed that MptC is present in the envelope fraction of *M. avium* subsp. paratuberculosis. The MptD protein was shown to be surface exposed, using a specific phage (fMptD) isolated from a phage-peptide library, by differential screening of *Mycobacterium smegmatis* transformants. The phage fMptD-derived peptide could be used in a peptide-mediated capture PCR with milk from infected dairy herds, thereby showing surface-exposed expression of the MptD protein in the host. Together, these data suggest that the 38-kb locus constitutes an *M. avium* subsp. paratuberculosis pathogenicity island.—Authors' Abstract

van den Braak, N., Simons, G., Gorkink, R., Reijans, M., Eadie, K., Kremers, K., van Soolingen, D., Savelkoul, P., Verbrugh, H., and van Belkum, A. A new high-throughput AFLP approach for identification of new genetic polymorphism in the genome of the clonal microorganism *Mycobacterium tuberculosis*. J. Microbiol. Methods **56(1)** (2004) 49–62.

We have here applied high-throughput amplified fragment length polymorphism (htAFLP) analysis to strains belonging to the five classical species of the *Mycobacterium tuberculosis* complex. Using 20 strains, three enzyme combinations and eight selective amplification primer pairs, 24 AFLP reactions were performed per strain. Overall, this resulted in 480 DNA fingerprints and more than 1200 htAFLP-amplified PCR fragments were visualised per strain. The cumulative dendrogram correctly clustered strains from the various species, albeit

within a distance of 6.5% for most of them. The single isolate of *Mycobacterium canettii* presented separately at 19% distance. All over, 169 fragments (14%) appeared to be polymorphic. Sixty-eight were specific for *M. canetti* and forty-five for *Mycobacterium bovis*. For the 10 different *M. tuberculosis* strains included in the present analysis, 56 polymorphic markers were identified. Upon sequencing 20 of these marker regions and comparisons with the H37Rv genome sequence, 25% appeared to share homology to members of the antigenically variable PE/PPE surface protein encoding gene family confirming previous findings on the genetic heterogeneity within these genes. In addition, homologues for phage genes and insertion element-encoded genes were detected. Forty-five percent of the sequences derived from ORFs with a currently unknown function, which was corroborated by genome sequence comparison for the clinical *M. tuberculosis* CD 1551 isolate. Sequence variation in *M. tuberculosis* was assessed in more detail for a subset of these loci by newly designed PCR restriction fragment length polymorphism (RFLP) tests and direct sequencing. Fourteen novel PCR RFLP tests were developed and twelve novel single nucleotide polymorphisms (SNPs) were identified, all suited for epidemiological analysis of *M. tuberculosis*. The tests allowed for identification of the major Mycobacterium species and *M. tuberculosis* variants and clones.—Authors' Abstract

Wang, J. P., Rought, S. E., Corbeil, J., and Guiney, D. G. Gene expression profiling detects patterns of human macrophage responses following *Mycobacterium tuberculosis* infection. FEMS Immunol. Med. Microbiol. **39(2)** (2003) 163–172.

High-density oligonucleotide microarrays allow simultaneous monitoring of the expression of a large number of cellular genes. Microarrays were used to screen the global human monocyte-derived macrophage transcriptional response to infection with the intracellular pathogen *Mycobacterium tuberculosis*. The microarray detected reproducible patterns of regulated gene expression. Analysis of the expression data showed induction of cytokines and chemokines, ribosomal proteins, and the interferon-response gene Stat1. Several changes were validated by quantitative reverse transcription polymerase chain reaction and immunoblot assays. Augmentation of the respiratory burst and preservation of the response to interferon-gamma were also demonstrated. These data supplement existing knowledge on macrophage responses to tuberculosis infection.—Authors' Abstract