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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

Araujo, M. G. [Leprosy in Brazil]. Rev. Soc. Bras. Med. Trop. **36(3)** (2003) 373–382. [Article in Portuguese]

Leprosy or Hansen's disease is a chronic infectious disease caused by the *Mycobacterium leprae*. The skin and nervous manifestations of the disease present a singular clinical picture that is easily recognized. After India, Brazil still is the second country with the greatest number of cases in the world. Around 94% of the known cases and 94% of the new cases reported in America, come from Brazil. The disease presents itself in two well-defined stable and opposite poles (lepromatous and tuberculoid) and two unstable groups (indeterminate and dimorphic). The spectrum of presentation of the disease may also be classified as: tuberculoid tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL) and lepromatous lepromatous (LL). The finding of acid fast bacillus in tissue is the most useful method of diagnosis. The effective treatment of leprosy includes the use of specific therapy, suppression of lepra reactions, prevention of physical incapacity, and physical and psychosocial rehabilitation. Chemotherapy with rifampin, dapsone, and clofazimine have produced very good results and the control of the disease in Brazil in the foreseeable future is likely.—Author's Abstract

Dionne, M. S., Ghori, N., and Schneider, D. S. *Drosophila melanogaster* is a genetically tractable model host for *Mycobacterium marinum*. Infect. Immun. **71(6)** (2003) 3540–3550.

Mycobacterium marinum is a pathogenic mycobacterial species that is closely related to *Mycobacterium tuberculosis* and causes tuberculosis-like disease in fish and frogs. We infected the fruit fly *Drosophila melanogaster* with *M. marinum*. This bacterium caused a lethal infection in the fly, with a 50% lethal dose (LD(50)) of 5 CFU. Death was accompanied by widespread tissue damage. *M. marinum* initially prolifer-

ated inside the phagocytes of the fly; later in infection, bacteria were found both inside and outside host cells. Intracellular *M. marinum* blocked vacuolar acidification and failed to colocalize with dead *Escherichia coli*, similar to infections of mouse macrophages. *M. marinum* lacking the mag24 gene were less virulent, as determined both by LD(50) and by death kinetics. Finally, in contrast to all other bacteria examined, mycobacteria failed to elicit the production of antimicrobial peptides in DROSOPHILA: We believe that this system should be a useful genetically tractable model for mycobacterial infection.—Authors' Abstract

Greenstein, R. J. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. Lancet Infect. Dis. **3(8)** (2003) 507–514.

Although Crohn's disease is considered to be autoimmune in origin, there is increasing evidence that it may have an infectious cause. The most plausible candidate is *Mycobacterium avium* subspecies paratuberculosis (MAP). Intriguingly, Koch's postulates may have been fulfilled for MAP and Crohn's disease, even though they still have not been met for *Mycobacterium leprae* and leprosy. In animals MAP causes Johne's disease, a chronic wasting intestinal diarrhoeal disease evocative of Crohn's disease. Johne's disease occurs in wild and domesticated animals, including dairy herds. Viable MAP is found in human and cow milk, and is not reliably killed by standard pasteurisation. MAP is ubiquitous in the environment including in potable water. Since cell-wall-deficient MAP usually cannot be identified by Ziehl-Neelsen staining, identification of MAP in human beings requires culture or detection of MAP DNA or RNA. If infectious in origin, Crohn's disease should be curable with appropriate antibiotics. Many studies that argue against a causative role for MAP in Crohn's disease have used antibiotics that are inactive against MAP. However, trials that include macrolide antibiotics indicate that a cure for Crohn's disease is possible. The necessary length of therapy remains to be determined. Mycobacterial diseases have protean clinical manifestations, as does Crohn's disease. The necessity of stratifying Crohn's disease into two clinical manifestations (perforating and non-perforating) when interpreting the results of antibiotic therapy is discussed. Rational studies to evaluate appropriate therapies to cure Crohn's disease are proposed.—Author's Abstract

Hernandez, A., Martro, E., Matas, L., and Ausina, V. *In-vitro* evaluation of Perasafe compared with 2% alkaline glutaraldehyde against *Mycobacterium* spp. J. Hosp. Infect. **54(1)** (2003) 52–56.

Quantitative suspension and carrier tests were used to compare the activity of Perasafe and Cidex against *Mycobacterium tuberculosis*, *Mycobacterium avium*-intracellulare, *Mycobacterium fortuitum*, and *Mycobacterium chelonae*. The interference of an organic load, and of hard water was also considered. Both agents achieved reductions exceeding 10(5)-fold within 20 and 30 min for all the strains tested. Perasafe is thus mycobactericidal and a viable alternative to Cidex for intermediate or high-level disinfection.—Authors' Abstract

Chemotherapy

Babaoglu, K., Page, M. A., Jones, V. C., McNeil, M. R., Dong, C., Naismith, J. H., and Lee, R. E. Novel inhibitors of an emerging target in *Mycobacterium tuberculosis;* substituted thiazolidinones as inhibitors of dTDP-rhamnose synthesis. Bioorg Med. Chem. Lett. **13(19)** (2003) 3227–3230.

The emergence of multi-drug resistant tuberculosis, coupled with the increasing overlap of the AIDS and tuberculosis pandemics has brought tuberculosis to the forefront as a major worldwide health concern. In an attempt to find new inhibitors of the enzymes in the essential rhamnose biosynthetic pathway, a virtual library of 2,3,5 trisubstituted-

4-thiazolidinones was created. These compounds were then docked into the active site cavity of 6′hydroxyl; dTDP-6-deoxy-D-xylo-4-hexulose 3,5-epimerase (RmlC) from *Mycobacterium tuberculosis*. The resulting docked conformations were consensus scored and the top 5% were slated for synthesis. Thus far, 94 compounds have been successfully synthesized and initially tested. Of those, 30 (32%) have ≥50% inhibitory activity (at 20 microM) in the coupled rhamnose synthetic assay with seven of the 30 also having modest activity against whole-cell *M.tuberculosis*.—Authors' Abstract

Bermudez, L. E., Reynolds, R., Kolonoski, P., Aralar, P., Inderlied, C. B., and Young, L. S. Thiosemicarbazole (thiacetazone-like) compound with activity *against Mycobacterium avium* in mice. Antimicrob. Agents Chemother. **47(8)** (2003) 2685–2687.

In vitro screening of thiacetazone derivatives indicated that two derivatives, SRI-286 and SRI-224, inhibited a panel of 25 *Mycobacterium avium* complex (MAC) isolates at concentrations of 2 micro g/ml or lower. In mice, SRI-224 and thiacetazone had no significant activity against the MAC in livers and spleens, but treatment with SRI-286 resulted in significant reduction of bacterial loads in livers and spleens. A combination of SRI-286 and moxifloxacin was significantly more active than single drug regimens in liver and spleen.—Authors' Abstract

Betts, J. C., McLaren, A., Lennon, M. G., Kelly, F. M., Lukey, P. T., Blakemore, S. J., and Duncan, K. Signature gene expression profiles discriminate between isoniazid-, thiolactomycin-, and triclosan-treated *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **47(9)** (2003) 2903–2913.

Genomic technologies have the potential to greatly increase the efficiency of the drug development process. As part of our tuberculosis drug discovery program, we used DNA microarray technology to profile drug-induced effects in *Mycobacterium tuberculosis*. Expression profiles of *M. tuberculosis* treated with compounds that inhibit key metabolic pathways are required as references for the assessment of novel antimycobacterial agents. We have studied the response of *M. tuberculosis* to treatment with the mycolic acid biosynthesis inhibitors isoniazid, thiolactomycin, and triclosan. Thiolactomycin targets the beta-ketoacyl-acyl carrier protein (ACP) synthases KasA and KasB, while triclosan inhibits the enoyl-ACP reductase InhA. However, controversy surrounds the precise mode of action of isoniazid, with both InhA and KasA having been proposed as the primary target. We have shown that although the global response profiles of isoniazid and thiolactomycin are more closely related to each other than to that of triclosan, there are differences that distinguish the mode of action of these two drugs. In addition, we have identified two groups of genes, possibly forming efflux and detoxification systems, through which *M. tuberculosis* may limit the effects of triclosan. We have developed a mathematical model, based on the expression of 21 genes, which is able to perfectly discriminate between isoniazid-, thiolactomycin-, or triclosan-treated *M. tuberculosis*. This model is likely to prove invaluable as a tool to improve the efficiency of our drug development programs by providing a means to rapidly confirm the mode of action of thiolactomycin analogues or novel InhA inhibitors as well as helping to translate enzyme activity into whole-cell activity.—Authors' Abstract

Ciccone, R., Mariani, F., Cavone, A., Persichini, T., Venturini, G., Ongini, E., Colizzi, V., and Colasanti, M. Inhibitory effect of NO-releasing ciprofloxacin (NCX 976) on *Mycobacterium tuberculosis* survival. Antimicrob. Agents Chemother. **47(7)** (2003) 2299–2302.

Here, we report the antimycobacterial activity of NCX 976, a new molecule obtained adding a NO moiety to the fluoroquinolone ciprofloxacin, on *Mycobacterium tuberculosis* H37Rv strain, both in a cell-free model and in infected human macrophages. Unlike unaltered ciprofloxacin, NCX976 displayed a marked activity also

at low-nanomolar concentrations.—Authors' Abstract

Cynamon, M. H. and Sklaney, M. Gatifloxacin and ethionamide as the foundation for therapy of tuberculosis. Antimicrob. Agents Chemother. **47(8)** (2003) 2442–2444.

The use of gatifloxacin (GAT) in combination with ethionamide (ETA) with or without pyrazinamide (PZA) for a 12-week treatment period followed by an 8-week observation period was evaluated in a model of tuberculosis in mice. Mice treated with GAT at 300 mg/kg of body weight in combination with ETA (25 mg/kg) for 5 days per week had sterile lungs, whereas mice treated with GAT (100 mg/kg) and ETA (25 mg/kg) had about 10 CFU/lung; however, there was regrowth of the organisms in both groups at the end of the observation period. When PZA (450 mg/kg 5 days per week) was added to the high-dose GAT-ETA regimen, no viable mycobacteria were present after the 8-week observation period. GAT in combination with ETA and PZA has great promise for the treatment of tuberculosis.—Authors' Abstract

Dredge, K., Marriott, J. B., and Dalgleish, A. G. Immunological effects of thalidomide and its chemical and functional analogs. Crit. Rev. Immunol. **22(5–6)** (2002) 425–437.

Thalidomide has recently shown considerable promise in the treatment of a number of conditions, such as leprosy and cancer. Its effectiveness in the clinic has been ascribed to wide-ranging properties, including anti-TNF-alpha, T-cell costimulatory and antiangiogenic activity. Novel compounds with improved immunomodulatory activity and side effect profiles are also being evaluated. These include selective cytokine inhibitory drugs (SelCIDs), with greatly improved TNF-alpha inhibitory activity, and immunomodulatory drugs (IMiDs) that are structural analogs of thalidomide, with improved properties. A third group recently identified within the SelCID group, with phosphodiesterase type 4-independent activity, is in the process of being characterized in laboratory studies. This review describes the emerging immunological properties of thalidomide, from a historical context to present-day clinical applications, most notably in multiple myeloma but also in other cancers, inflammatory disease, and HIV. We also describe the laboratory studies that have led to the characterization and development of SelCIDs and IMiDs into potentially clinically relevant drugs. Early trial data suggest that these novel immunomodulatory compounds may supercede thalidomide to become established therapies, particularly in certain cancers. Further evidence is required, however, to correlate the clinical efficacy of these compounds with their known immunomodulatory, antiangiogenic, and antitumor properties.—Authors' Abstract

Forslow, U., Geborek, A., Hjelte, L., Petrini, B., and Heurlin, N. Early chemotherapy for non-tuberculous mycobacterial infections in patients with cystic fibrosis. Acta Paediatr. **92(8)** (2003) 910–915.

AIM: To evaluate the response rate to antimycobacterial drug therapy in patients with cystic fibrosis (CF) suffering from infection by non-tuberculous mycobacteria (NTM). METHODS: Ten patients, aged 10–34 yrs, out of 180 CF patients, were diagnosed with NTM disease. They had been regularly checked and examined for pulmonary symptoms, and had had chest X-rays and sputum cultures (including for mycobacteria) performed. One additional 36-yr-old female received her CF diagnosis soon after the NTM diagnosis. RESULTS: *Mycobacterium avium*-intracellulare complex (MAC) was found in 10 out of 11 patients and *M. kansasii* in 1 patient. Treatment with antimycobacterial drugs resulted in clinical improvement (weight gain or stabilization of weight and/or improved or stabilized lung function in 8 out of 11 patients) and mycobacterial culture turned negative in 10 out of 1. CONCLUSION: Promising results may be associated with early intervention with antimycobacterial therapy in CF patients.—Authors' Abstract

Gomez-Reino, J. J., Carmona, L., Valverde, V. R., Mola, E. M., Montero, M. D., and BIOBADASER Group. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum. **48(8)** (2003) 2122–2127.

OBJECTIVE: The long-term safety of therapeutic agents that neutralize tumor necrosis factor (TNF) is uncertain. Recent evidence based on spontaneous reporting shows an association with active tuberculosis (TB). We undertook this study to determine and describe the long-term safety of 2 of these agents, infliximab and etanercept, in rheumatic diseases based on a national active-surveillance system following the commercialization of the drugs. METHODS: We analyzed the safety data actively collected in the BIOBADASER (Base de Datos de Productos Biologicos de la Sociedad Espanola de Reumatologia) database, which was launched in February 2000 by the Spanish Society of Rheumatology. For the estimation of TB risk, the annual incidence rate in patients treated with these agents was compared with the background rate and with the rate in a cohort of patients with rheumatoid arthritis (RA) assembled before the era of anti-TNF treatment. RESULTS: Seventy-one participating centers sent data on 1578 treatments with infliximab (86%) or etanercept (14%) in 1540 patients. Drug survival rates (reported as the cumulative percentage of patients still receiving medication) for infliximab and etanercept pooled together were 85% and 81% at 1 year and 2 years, respectively. Instances of discontinuation were essentially due to adverse events. Seventeen cases of TB were found in patients treated with infliximab. The estimated incidence of TB associated with infliximab in RA patients was 1893 per 100,000 in the year 2000 and 1113 per 100,000 in the year 2001. These findings represent a significant increased risk compared with background rates. In the first 5 months of 2002, after official guidelines were established for TB prevention in patients treated with biologics, only 1 new TB case was registered (in January). CONCLUSION: Therapy with infliximab is associated with an increased risk of active TB. Proper measures are needed to prevent and manage this adverse event.—Authors' Abstract

Luzzio, F. A., Mayorov, A. V., Ng, S. S., Kruger, E. A., and Figg, W. D. Thalidomide metabolites and analogues. 3. Synthesis and antiangiogenic activity of the teratogenic and TNFalpha-modulatory thalidomide analogue 2-(2,6-dioxopiperidine-3-yl)phthalimidine. J. Med. Chem. **46(18)** (2003) 3793–3799.

Versatile synthesis of the teratogenic, TNFalpha-modulatory, and antiangiogenic thalidomide analogue 2-(2,6-dioxopiperidine-3-yl)phthalimidine (1) and its direct antiangiogenic properties are described. With thalidomide or thalidomide derivatives as precursors, the synthesis involved either carbonyl reduction/thiation-desulfurization or carbonyl reduction/acyliminium ion reduction protocols. Compared to earlier studies with thalidomide, which was only active with microsomal treatment, 1 exhibited marginal inhibitory activity in the rat aortic ring assay, thereby demonstrating the requirement for metabolic activation.—Authors' Abstract

Marriott, J. B., Dredge, K., and Dalgleish, A. G. Thalidomide derived immunomodulatory drugs (IMiDs) as potential therapeutic agents. Curr. Drug Targets Immune Endocr. Metabol. Disord. **3(3)** (2003) 181–186.

Thalidomide is known to be effective in the treatment of a number of conditions, including leprosy and various cancers. The exact mechanisms of action remain unclear although these are known to include anti-tumour necrosis factor (TNF)-alpha, T cell costimulatory, anti-angiogenic and anti-tumour activities. However, thalidomide is being superceded by novel structural derivatives which have been designed to have improved immunomodulatory activity and side effect profiles. These are currently being characterised and some are entering the clinic in phase I/II studies. One novel group of structural analogues are classified as the

Immunomodulatory Drugs (IMiDs). This review describes the emerging immunological, anti-angiogenic and direct anti-tumour properties of thalidomide and the characterisation and clinical application of its IMiD analogues. We describe the laboratory studies which have led to the characterisation and development of IMiDs into potentially clinically relevant drugs. Early trial data suggests that these compounds may themselves become established therapies, particularly in certain cancers. Furthermore, ongoing studies will determine how best to apply these compounds to the appropriate clinical settings. We will describe the various clinical studies of lead compounds that are in progress and speculate as to the potential and future development of these exciting compounds.—Authors' Abstract

Nasca, M. R., Micali, G., Cheigh, N. H., West, L. E., and West, D. P. Dermatologic and nondermatologic uses of thalidomide. Ann. Pharmacother. **37(9)** (2003) 1307–1320.

OBJECTIVE: To review published data on thalidomide, with emphasis on current knowledge about mechanism of action, new and/or potential dermatologic and nondermatologic therapeutic applications, well-known and emerging adverse effects, and current indications for its safe use. DATA SOURCES: Review articles, *in vitro* research studies, references from retrieved articles, case reports, and clinical trials were identified from a computerized literature search using MEDLINE and OVID (1966–January 2003) and on the Cochrane Clinical Trials Register (January 2003). Information available from meetings' abstract books, Internet, or pharmaceutical companies was also considered. STUDY SELECTION AND DATA EXTRACTION: All articles identified as relevant, including those from non-English literature, were considered in an attempt to provide to the reader both the theoretical basis and practical guidelines for thalidomide pharmacotherapy. DATA SYNTHESIS: Thalidomide has hypnosedative, antiangiogenic, antiinflammatory, and immunomodulatory properties. Moreover, it has been shown to selectively inhibit the production of tumor necrosis factor-alpha and reduce the expression of various integrin receptors on the membrane of leukocytes and other cell types in a dose-dependent fashion. Controlled trials demonstrated the efficacy of thalidomide in a number of diseases, including erythema nodosum leprosum, lupus erythematosus, aphthosis, graft-versus-host disease, prurigo nodularis, and actinic prurigo. Single case reports or studies in small series have also suggested a possible role for thalidomide in numerous other dermatologic and nondermatologic disorders. Possibly severe and sometimes irreversible risks related to the clinical use of thalidomide include teratogenicity and neurotoxicity. CONCLUSIONS: Although teratogenicity and neurotoxicity are significant adverse effects requiring cautious use, thalidomide is an effective therapeutic modality in a variety of difficult-to-treat disorders and, providing careful selection of patients, should offer an acceptable risk-to-benefit ratio.—Authors' Abstract

Clinical Sciences

Abad, S., Gyan, E., Moachon, L., Bouscary, D., Sicard, D., Dreyfus, F., and Blanche, P. Tuberculosis due to *Mycobacterium bovis* after alemtuzumab administration. Clin. Infect. Dis. **37(2)** (2003) e27–e28.

We describe a patient with relapsing B chronic lymphocytic leukemia who developed systemic bacille Calmette-Guerin infection (BCGitis) after administration of alemtuzumab (Campath-1H).—Authors' Abstract

Ang, P., Tay, Y. K., Ng, S. K., and Seow, C. S. Fatal Lucio's phenomenon in 2 patients with previously undiagnosed leprosy. J. Am. Acad. Dermatol. **48(6)** (2003) 958–961.

We report 2 cases of Lucio's phenomenon, a rare, aggressive, occasionally fatal type 2 reaction occurring in the diffuse nonnodular type of lepromatous leprosy. The clinical diagnosis of Lucio's phenomenon is difficult, and there are no known predictive or prognostic factors. Despite institution of aggressive treatment after diagnosis, our 2 cases had fatal outcomes.—Authors' Abstract

Baker, B., Evans, M., DeCastro, F., and Schosser, R. Leprosy in a Mexican immigrant. J. Ky. Med. Assoc. **101(7)** (2003) 289–294.

A new diagnosis of borderline lepromatous leprosy was established in a man who had immigrated to Kentucky from Mexico. He was placed on a World Health Organization treatment regimen consisting of dapsone, clofazimine, and rifampin. The biology of leprosy, its diagnosis, treatment, and worldwide impact are reviewed. Because of the potential for highly mobile populations to export endemic diseases, Kentucky physicians must expand their lists of differential diagnoses.—Authors' Abstract

Bandoh, S., Fujita, J., Ueda, Y., Tojo, Y., Ishii, T., Kubo, A., Yamamoto, Y., Nishiyama, Y., and Ishida, T. Uptake of fluorine-18-fluorodeoxyglucose in pulmonary *Mycobacterium avium* complex infection. Intern. Med. **42(8)** (2003) 726–729.

Two patients showing abnormal fluorine-18-fluorodeoxyglucose (FDG) uptake due to *Mycobacterium avium* complex (MAC) infection are presented. Intense focal FDG uptake in the lung field could have been caused by an infectious disease such as MAC. This should be considered as a possibility when FDG whole-body scans of patients with pulmonary nodules are interpreted. To our knowledge, this is the first description of an FDG-positron emission tomography (FDG-PET) image of MAC infection of the lung.—Authors' Abstract

Bhat, R. M. and Radhakrishnan, K. A case report of fatal dapsone-induced agranulocytosis in an Indian mid-borderline leprosy patient. Lepr. Rev. **74(2)** (2003) 167–170.

Fatal agranulocytosis in an Indian male receiving 100 mg of dapsone daily, hospitalized for mid-borderline leprosy in type I reaction with triple nerve paralysis is reported. Various case reports concerning dapsone-induced agranulocytosis are reviewed.—Authors' Abstract

Daines, B. S., Vroman, D. T., Sandoval, H. P., Steed, L. L., and Solomon, K. D. Rapid diagnosis and treatment of mycobacterial keratitis after laser in situ keratomileusis. J. Cataract Refract. Surg. **29(5)** (2003) 1014–1018.

We report the results of laser *in situ* keratomileusis (LASIK) in a 51-year-old woman with subsequent mycobacterial keratitis diagnosed by staining with acid-fast and fluorochrome methods, a technique known to have good sensitivity and specificity for mycobacteria. A rapid diagnosis was made without waiting for cultures, and treatment was instituted, including tapering of topical steroids and appropriate antibiotic therapy. The result was preservation of the LASIK flap and a favorable visual outcome at 6 months.—Authors' Abstract

Daniel, E., Koshy, S., Joseph, G. A., and Rao, P. S. Ocular complications in incident relapsed borderline lepromatous and lepromatous leprosy patients in south India. Indian J. Ophthalmol. **51(2)** (2003) 155–159.

PURPOSE: To determine the magnitude of ocular complications that present in incident cases of relapsed borderline lepromatous (BL) and lepromatous leprosy (LL) patients. METHOD: From 1991 to 1997, all new BL and LL patients who had relapsed from an earlier disease, detected by active case finding in the geographically defined area of Gudiyattam taluk, were invited for ocular examination after their leprosy status was confirmed clinically and histopathologically. RESULTS: Sixty relapsed lepromatous patients, 45 male and

15 females, were examined. Fifty-two patients had relapsed after receiving only dapsone mono-therapy, 4 after receiving paucibacillary multi-drug therapy (PB-MDT) preceded by dapsone mono-therapy and 4 after only PB-MDT. Three (5%) patients had lagophthalmos, 1 (1.6%) patients each had ectropion and trichiasis, 32 (53%) patients had impaired corneal sensation in both eyes, 2 (3.3%) patients each had corneal opacity (associated with reduced vision), corneal nerve beading, punctate keratitis, keratic precipitates, and iris atrophy, 4 (6.6%) patients had cataract associated with decreased vision, 1 (1.6%) patient had blocked naso-lacrimal duct and 13 (21.7%) patients had pterygium. Seven (12%) patients had a visual acuity of 6/18 or less, 4 (6.7%) patients had 6/60 or less and one patients had vision below 3/60. General ocular complications rather than leprosy-related ocular complications were responsible for reduced vision. Lagophthalmos was associated with increased duration of the disease ($p = 0.009$), Grade II deformity ($p = 0.001$), punctate keratitis ($p < 0.001$) and cataract ($p < 0.001$). Beaded corneal nerves were associated with lepromatous leprosy ($p < 0.001$) and high mycobacterial infection ($p = 0.05$). Patients whose initial disease was categorised as BL and LL had greater impairment of vision ($p = 0.037$), more iris atrophy ($p = 0.013$), increased keratic precipitates ($p = 0.013$) and more corneal nerve beading ($p = 0.013$), when compared with the group comprising Tuberculoid-tuberculoid (TT), Borderline-tuberculoid (BT) and Intermediate (IND). CONCLUSION: This first report on ocular complications in relapsed lepromatous patients demonstrates that general and leprosy-related ocular complications occur in these patients. However, they are not in excess of those reported in other leprosy groups. Borderline and lepromatous leprosy patients tend to have had more ocular complications than patients with tuberculoid leprosy.—Authors' Abstract

Delobel, P., Launois, P., Djossou, F., Sainte-Marie, D., and Pradinaud, R. American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis coinfection with downregulation of the T-helper 1 cell response. Clin. Infect. Dis. **37(5)** (2003) 628–633.

Cutaneous leishmaniasis, leprosy, and tuberculosis are caused by intracellular pathogens whose development depends on impaired cell-mediated immunity. We report an exceptional triple association of American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis in a man with no recognized immunodeficiency. Normal immunological assessment of the interferon-gamma pathway does not support the hypothesis of a genetic defect in any of the genes involved in the T helper (Th)-1 cytokine cascade in this patient. Unresponsiveness to interleukin (IL)-12 of his T cells after stimulation with *Leishmania guyanensis*, *Mycobacterium bovis* bacille Calmette-Guerin, and *Mycobacterium leprae* antigens suggested the inability to mount an appropriate Th cell response to upregulate the IL-12 receptor expression.—Authors' Abstract

Holzer, M. P., Solomon, K. D., Sandoval, H. P., and Auffarth. G. U. [Diagnosis and treatment of mycobacterial keratitis following LASIK. Case report and review of the literature.] Ophthalmologe **100(7)** (2003) 550–553. [Article in German].

BACKGROUND: Mycobacterial keratitis is a rare complication following LASIK but can lead to an extremely unfavourable outcome. The diagnosis and treatment is often delayed due to confusion with other entities including diffuse lamellar keratitis and poor clinical outcomes with flap amputation and/or keratoplasty are often the case. PATIENT AND METHODS: We report the results of LASIK in a 51-year-old woman with subsequent early-diagnosed mycobacterial keratitis and compared this case to treatments and outcomes reported in the literature. RESULTS: The patient presented 10 days following LASIK with a white focal infiltrate in the stromal interface. The flap was lifted and cultures from the stromal bed and the reverse of the flap were obtained and the interface irrigated. The patient was treated with topical antibiotics (ciprofloxacin 0.3%, amikacin 2.5%, clarithromycin 40 mg/ml and tobramycin 15

mg/ml) for 8 weeks and at the most recent follow-up she had a visual acuity of 1.25. CONCLUSION: In a large number of published cases in the literature the flap had to be amputated and/or corneal transplants were necessary. Early diagnosis and treatment however, are essential to successfully treat post-LASIK keratitis. Therefore the patients should be followed up carefully in the early postoperative period.—Authors' Abstract

Hong, S. T., Hong, S. J., Lee, S. H., Kim, I. S., and Shin, J. S. [A Study On The Intestinal Helminths Of The Patients In A Leprosarium In Korea.] Kisaengchunghak Chapchi. **21(1)** (1983) 102–104. [Article in Korean]

A total of 2026 leprosy patients of the National Sorokdo Hospital was examined their intestinal parasites by cellophane thick smear method in January 1983. The egg positive cases of *Taenia* spp. were treated with bithionol and the segments of Taenia were collected for species identification. The results were as follows: 1. Total egg positive rate of any kind helminth was 78.2% and cumulative total was 85.2%. The egg positive rate for each helminth was as follow; *Taenia* spp. 3.4%, Ascaris lumbricoides 4.5%, *Trichuris trichiura* 72.l%, *Clonorchis sinensis* 2.8% and other 0.05%. 2. A total of 66 Taenia egg positive cases was treated; out of them proglottids of Taenia were collected from 26 cases. All of the collected worms were identified as *T. saginata*. The results revealed significantly high egg positive rate of *T. trichiura*. However, *A. lumbricoides* was found to be controlled considerably by repeated chemotherapy during past 3 years. If chemotherapeutic agent is replaced with oxantel-pyrantel tablet, better result is expected. No clue was found for prevalence of *T. solium* from both human and the pig in the island.—Authors' Abstract

Nakayama, S., Uesaka, Y., Kunimoto, M., Mikata, T., Shimizu, J., and Ishii, N. [The painful multiple mononeuropathy of acute onset in the left arm which was diagnosed as leprous neuropathy]. Rinsho Shinkeigaku. **43(5) (**2003) 265–269. [Article in Japanese]

A 31-year-old man from Myanmar with leprous neuropathy was reported. The progress of the disease was subacute but the painful symptom at the time of the onset was acute. Multiple mononeuropathy was diagnosed by the biopsy findings of the left superficial radial nerve. He was admitted to our hospital with the complaint of the weakness of his left hand and fingers which were very painful and got worse in several weeks. Motor palsy was observed in his left ulnar, median, and radial nerves, and there was the hypesthesia or anesthesia in his left hand, forearm and the medial side of his left upper arm. On nerve conduction studies, the amplitudes of CMAP and SNAP severely diminished or not detected. The pattern was compatible with multiple mononeuropathy. The biopsy of the left superficial radial nerve was performed. The pathological findings were the destruction of nerve fascicles, replacement of nerve fibers with inflammatory cells, and *Mycobacterium leprae* was found with the specific stain. These findings confirmed the diagnosis of the leprous neuropathy. Leprous neuropathy is one of the commonest causes of infectious neuropathy in the world, especially in Southeast Asia. These days many foreign workers from that area are staying in Japan, and the chances to see the disease are increasing. We have to recognize leprous neuropathy as a candidate for the multiple mononeuropathy of acute onset with painful dysesthesia similar to vascular neuropathy.—Authors' Abstract

Immuno-Pathology

Anes, E., Kuhnel, M. P., Bos, E., Moniz-Pereira, J., Habermann, A., and Griffiths, G. Selected lipids activate phagosome actin assembly and maturation resulting in killing of pathogenic mycobacteria. Nat Cell Biol. **5(9)** (2003) 793–802.

Pathogenic mycobacteria such as *Mycobacterium tuberculosis* and *Mycobacterium avium* facilitate disease by surviving intracellularly within a potentially hostile environment: the macrophage phagosome. They inhibit phagosome maturation processes, including fusion with lysosomes, acidification and, as shown here, membrane actin assembly. An *in vitro* assay developed for latex bead phagosomes (LBPs) provided insights into membrane signalling events that regulate phagosome actin assembly, a process linked to membrane fusion. Different lipids were found to stimulate or inhibit actin assembly by LBPs and mycobacterial phagosomes *in vitro*. In addition, selected lipids activated actin assembly and phagosome maturation in infected macrophages, resulting in a significant killing of *M. tuberculosis* and *M. avium*. In contrast, the polyunsaturated sigma-3 lipids behaved differently and stimulated pathogen growth. Thus, lipids can be involved in both stimulatory and inhibitory signalling networks in the phagosomal membrane.—Authors' Abstract

Bafica, A., Scanga, C. A., Schito, M. L., Hieny, S., and Sher, A. Cutting edge: *in vivo* induction of integrated HIV-1 expression by mycobacteria is critically dependent on Toll-like receptor 2. J. Immunol. **171(3)** (2003) 1123–1127.

Mycobacterial infection has been implicated as a possible factor in AIDS progression in populations where HIV-1 and *Mycobacterium tuberculosis* are coendemic. In support of this concept, we have previously shown that HIV-1-transgenic (Tg) mice infected with mycobacteria display enhanced viral gene and protein expression. In this study, we demonstrate that the induction of HIV-1 observed in this model is dependent on Toll-like receptor 2 (TLR2), a pattern recognition receptor known to be involved in mycobacteria-host interaction. Spleen cells from HIV-1-Tg mice deficient in TLR2 (Tg/TLR2(–/–)) were found to be completely defective in p24 production induced in response to live *M. tuberculosis* or *Mycobacterium avium* as well as certain mycobacterial products. Importantly, following *in vivo* mycobacterial infection, Tg/TLR2(–/–) mice failed to display the enhanced HIV-1 gag/env mRNA and p24 protein synthesis exhibited by wild-type Tg animals. Together, these results argue that TLR2 plays a crucial role in the activation of HIV-1 expression by mycobacterial coinfections.—Authors' Abstract

Black, G. F., Weir, R. E., Chaguluka, S. D., Warndorff, D., Crampin, A. C., Mwaungulu, L., Sichali, L., Floyd, S., Bliss, L., Jarman, E., Donovan, L., Andersen, P., Britton, W., Hewinson, G., Huygen, K., Paulsen, J., Singh, M., Prestidge, R., Fine, P. E., and Dockrell, H. M. Gamma interferon responses induced by a panel of recombinant and purified mycobacterial antigens in healthy, non-*Mycobacterium bovis* BCG-vaccinated Malawian young adults. Clin. Diagn. Lab. Immunol. **10(4)** (2003) 602–611.

We have previously shown that young adults living in a rural area of northern Malawi showed greater gamma interferon (IFN-gamma) responses to purified protein derivatives (PPD) prepared from environmental mycobacteria than to PPD from *Mycobacterium tuberculosis*. In order to define the mycobacterial species to which individuals living in a rural African population have been exposed and sensitized, we tested T-cell recognition of recombinant and purified antigens from *M. tuberculosis* (38 kDa, MPT64, and ESAT-6), *M. bovis* (MPB70), *M. bovis* BCG (Ag85), and *M. leprae* (65 kDa, 35 kDa, and 18 kDa) in >600 non-*M. bovis* BCG-vaccinated young adults in the Karonga District of northern Malawi. IFN-gamma was measured by enzyme-linked immunosorbent assay (ELISA) in day 6 supernatants of diluted whole-blood cultures. The recombinant *M. leprae* 35-kDa and 18-kDa and purified native *M. bovis* BCG Ag85 antigens induced the highest percentages of responders, though both leprosy and bovine tuberculosis are now rare in this population. The *M. tuberculosis* antigens ESAT-6 and MPT64 and the *M. bovis* antigen MPB70 induced the lowest percentages of responders. One of the subjects subsequently developed extrapulmonary tuberculosis; this individual had a 15-mm-diameter reaction to the Mantoux test and responded to *M. tuberculosis*

PPD, Ag85, MPT64, and ESAT-6 but not to any of the leprosy antigens. We conclude that in this rural African population, exposure to *M. tuberculosis* or *M. bovis* is much less frequent than exposure to environmental mycobacteria such as *M. avium*, which have antigens homologous to the *M. leprae* 35-kDa and 18-kDa antigens. M. tuberculosis ESAT-6 showed the strongest association with the size of the Mantoux skin test induration, suggesting that among the three *M. tuberculosis* antigens tested it provided the best indication of exposure to, or infection with, *M. tuberculosis*.—Authors' Abstract

Chua-Intra, B., Wattanapokayakit, S., Srisungngam, S., Srisungngam, T., Mahotarn, K., Brennan, P. J., and Ivanyi, J. T-cell recognition of peptides from the *Mycobacterium leprae* 35 kDa protein in Thai leprosy patients, healthy contacts, and non-contacts. Immunol. Lett. **88(1)** (2003) 71–76.

The objective of the study was to identify *Mycobacterium leprae*-specific immunogenic peptides for the development of a skin test reagent. Such a reagent is required for the detection of *M. leprae* infection and possibly for the diagnosis of patients with active leprosy. For this purpose, we analyzed the *in vitro* responses of human peripheral blood mononuclear cell (PBMCs) to peptides from the 35 kDa protein of *M. leprae*. This protein is of interest since it has no homologue within the *Mycobacterium tuberculosis* complex, although it has a homologue in *Mycobacterium avium*. The subjects enrolled in the study were paucibacillary (PB) and multibacillary (MB) leprosy patients, healthy contacts, and non-contacts. Seventy-three PB and 124 MB leprosy patients were recruited from four leprosy clinics in Thailand. Fifty-seven healthy contacts were household contacts. Twenty non-leprosy contacts had no family history of or exposure to leprosy. PBMCs from individuals were tested for stimulation with 12 overlapping peptides from the *M. leprae* 35 kDa protein using the lymphocyte proliferation assay. These peptides were located in four areas containing three to six residues which were distinct for the *M. leprae* product in comparison to that from *M. avium*. Four peptides (p60–76, p132–151, p206–224 and p267–286), which were the most permissive from each region and recognized by non-contacts with significantly lower frequencies than other subject groups, were identified. From this preliminary result, we conclude that these four peptides were likely to be *M. leprae*-specific.—Authors' Abstract

Dannenberg, A. M. Jr. Macrophage turnover, division and activation within developing, peak and "healed" tuberculous lesions produced in rabbits by BCG. Tuberculosis (Edinb). **83(4)** (2003) 251–260.

This review is a synthesis and analysis of our nine experimental pathology papers on macrophage kinetics in dermal tuberculous lesions produced in rabbits by BCG. It is presented at this time to summarize the macrophage kinetics in both active and essentially healed tuberculous lesions and to suggest that the bacilli frequently multiply and are destroyed in the viable granulation tissue of many small arrested tuberculous lesions.The turnover of mononuclear cells (MN)—which were mostly macrophages with some medium and large lymphocytes—was most rapid in BCG lesions at 2–3 weeks (when tuberculin sensitivity and acquired cellular resistance were at their peaks). At this time, more macrophages entered, more died or left, more remained at the site, and more became activated than before or afterwards. Before this time, the host had no delayed-type hypersensitivity (DTH) and cell-mediated immunity (CMI), so that no antigen-specific enhancement of the inflammatory response occurred. After this time, the bacilli and their antigenic products had decreased, so that the stimuli for cell infiltration and activation were reduced. In "healed" lesions, the MN turnover still occurred, but was decreased.The continuous entry of live non-activated macrophages into the viable parts of tuberculous lesions provides fresh intracellular sites where tubercle bacilli may multiply before they are again inhibited by the DTH and CMI of the host. In tuberculosis, bacillary dormancy of long duration may only be present in caseous necrotic tissue where no live host cells exist.—Author's Abstract

Florido, M., Correia-Neves, M., Cooper, A. M., and Appelberg, R. The cytolytic activity of natural killer cells is not involved in the restriction of *Mycobacterium avium* growth. Int. Immunol. **15(8)** (2003) 895–901.

Severe combined immunodeficiency (SCID) mice were used to analyze the role of NK cells in resistance to *Mycobacterium avium*. The neutralization of IFN-gamma in these animals led to an exacerbation of the infection associated with a reduction in macrophage activation, suggesting a role for NK cells in innate immunity to mycobacteria. In contrast, administration of anti-asialo-GM(1) polyclonal serum or mAb specific for Thy1.2 did not affect mycobacterial growth or macrophage activation despite causing the almost complete abrogation of the natural cytolysis of a tumor cell target. Treatment with anti-asialo-GM(1)-specific serum depleted only two-thirds of the Thy1.2+ spleen cells, and anti-Thy1.2 treatment allowed for the persistence of a small number of cells still exhibiting an NK cell marker recognized by mAb DX5 and able to express IFN-gamma as analyzed by flow cytometry. *In vivo* treatment of B6.SCID mice with anti-NK1.1 mAb again failed to affect resistance to infection and allowed for the persistence of 2–8% of IFN-gamma-producing cells, many of them still expressing the DX5 marker. *In vitro* depletion studies showed that removal of IFN-gamma-expressing cells required the combined action of anti-Thy1.2, anti-Ly49C and DX5 antibodies in the presence of complement. Our data show that resistance to *M. avium* mediated by NK cells is independent of their cytolytic activity, and that there is a marked phenotypic and functional heterogeneity of the NK cell lineage *in vivo* during infection.—Authors' Abstract

Heldwein, K. A., Liang, M. D., Andresen, T. K., Thomas, K. E., Marty, A. M., Cuesta, N., Vogel, S. N., and Fenton, M. J. TLR2 and TLR4 serve distinct roles in the host immune response against *Mycobacterium bovis* BCG. J. Leukoc. Biol. **74(2)** (2003) 277–286.

Toll-like receptor (TLR) proteins mediate cellular activation by microbes and microbial products. To delineate the role of TLR proteins in the development of host immune responses against mycobacteria, wild-type and TLR-deficient mice were infected with nonpathogenic *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). Two weeks after intraperitoneal challenge with BCG, few bacilli were present in the lungs of wild-type and TLR4(–/–) mice, whereas bacterial loads were tenfold higher in the lungs of infected TLR2(–/–) mice. BCG challenge *in vitro* strongly induced proinflammatory cytokine secretion by macrophages from wild-type and TLR4(–/–) mice but not by TLR2(–/–) macrophages. In contrast, intracellular uptake, intracellular bacterial growth, and suppression of intracellular bacterial growth *in vitro* by interferon-gamma (IFN-gamma) were similar in macrophages from all three mouse strains, suggesting that BCG growth in the lungs of TLR2(–/–) mice was a consequence of defective adaptive immunity. Antigenic stimulation of splenocytes from infected wild-type and TLR4(–/–) mice induced T cell proliferation *in vitro*, whereas T cells from TLR2(–/–) mice failed to proliferate. Unexpectedly, activated CD4(+) T cells from both TLR-deficient mouse strains secreted little IFN-gamma *in vitro* compared with control T cells. A role for TLR4 in the control of bacterial growth and IFN-gamma production *in vivo* was observed only when mice were infected with higher numbers of BCG. Thus, TLR2 and TLR4 appear to regulate distinct aspects of the host immune response against BCG.—Authors' Abstract

Lopez, J. P., Clark, E., and Shepherd, V. L. Surfactant protein A enhances *Mycobacterium avium* ingestion but not killing by rat macrophages. J. Leukoc. Biol. **74(4)** (2003) 523–530.

Mycobacterium avium complex (MAC) is a significant cause of opportunistic infection in patients with acquired immunodeficiency syndrome. Although the major route of entry of MAC is via the gastrointestinal tract, MAC can infect humans through the respiratory tract and eventually encounter alveolar macrophages within the lung. Once in the lung, MAC can potentially interact with surfactant protein A (SP-A), an important component of the pulmonary

innate-immune response. Previous work on other pulmonary pathogens including *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) suggests that SP-A participates in promoting efficient clearance of these organisms by alveolar macrophages. In the present study, we investigated the role of SP-A in clearance of MAC by cultured rat macrophages. SP-A bound to MAC organisms and enhanced the ingestion of the mycobacteria by macrophages. Infection of macrophages with SP-A-MAC complexes induced the production of nitric oxide (NO) and tumor necrosis factor-alpha. However, intracellular survival of MAC was not altered by preopsonization with SP-A. In addition, inhibitors of inducible NO synthase did not alter MAC clearance. These results suggest that SP-A can bind to and enhance the uptake of MAC by alveolar macrophages, similar to previous findings with BCG and *Mycobacterium tuberculosis*.However, unlike BCG and other pulmonary pathogens that are cleared effectively in the presence of SP-A via a NO-dependent pathway, macrophage-mediated clearance of MAC is not enhanced by SP-A.—Authors' Abstract

Immuno-Pathology (Leprosy)

Antunes, S. L., Liang, Y., Neri, J. A., Haak-Frendscho, M., and Johansson, O. The expression of NGFr and PGP 9.5 in leprosy reactional cutaneous lesions: an assessment of the nerve fiber status using immunostaining. Arq. Neuropsiquiatr. **61(2B)** (2003) 346–352.

The effects of reactional episodes on the cutaneous nerve fibers of leprosy patients was assessed in six patients (three with reversal reactions and three with erythema nodosum leprosum). Cryosections of cutaneous biopsy of reactional lesions taken during the episode and of another sample during the remission period were immunostained with anti-NGFr and anti-PGP 9.5 (indirect immunofluorescence). We found no significant statistical difference in the number of NGFr- and PGP 9.5-positive fibers between the reactional and post-reactional groups. A significant difference was detected between the number of NGFr and PGP 9.5-stained fibers inside of the reactional group of biopsy cryosections but this difference was ascribed to the distinct aspects of the nerve fibers displayed whether stained with anti-NGFr or with anti-PGP 9.5; NGFr-positive branches looked larger and so interpreted as containing more fibers. In addition, a substantial number NGFr-positive fibers were PGP 9.5-negative. No differences in the number of stained fibers among the distinct cutaneous regions examined (epidermis + upper dermis, mid and deep dermis) was detected. In conclusion, the number of PGP- and NGFr-positive fibers were not significantly different in the reactional and post-reactional biopsies in the present study. NGFr-staining of the nerve fibers is different from their PGP-imunoreactivity and the evaluation of the nerve fiber status on an innervated target organ should be carried out choosing markers for both components of nerve fibers (Schwann cells and axons).—Authors' Abstract

Antunes, S. L., Liang, Y., Neri, J. A., Sarno, E. N., Haak-Frendscho, M., and Johansson, O. Mast cell subsets and neuropeptides in leprosy reactions. Arq Neuropsiquiatr. **61(2A)** (2003) 208–219.

The immunohistochemical identification of neuropeptides (calcitonin gene-related peptide, vasoactive intestinal polypeptide, substance P, alpha-melanocyte stimulating hormone and gamma-melanocyte stimulating hormone) quantification of mast cells and their subsets (tryptase/chymase-immunoreactive mast cells = TCMC and tryptase-immunoreactive mast cells = TMC) were determined in biopsies of six patients with leprosy reactions (three patients with type I reaction and three with type II). Biopsies were compared with those taken from the same body site in the remission stage of the same patient. We found a relative increase of TMC in the inflammatory

infiltrate of the reactional biopsies compared to the post-reactional biopsy. Also, the total number of mast cells and the TMC/TCMC ratio in the inflammatory infiltrate was significantly higher than in the intervening dermis of the biopsies of both periods. No significant difference was found regarding neuroptide expression in the reactional and post-reactional biopsies. The relative increase of TMC in the reactional infiltrates could implicate this mast cell subset in the reported increase of the immune response in leprosy reactions.—Authors' Abstract

Immuno-Pathology (Tuberculosis)

Boitel, B., Ortiz-Lombardia, M., Duran, R., Pompeo, F., Cole, S. T., Cervenansky, C., and Alzari, P. M. PknB kinase activity is regulated by phosphorylation in two Thr residues and dephosphorylation by PstP, the cognate phospho-Ser/Thr phosphatase, in *Mycobacterium tuberculosis*. Mol. Microbiol. **49(6)** (2003) 1493–1508.

See Current Literature, Molecular and Genetic Studies, p. 422

Bose, M., Farnia, P., Sharma, S., Chattopadhya, D., and Saha, K. Nitric oxide dependent killing of *Mycobacterium tuberculosis* by human mononuclear phagocytes from patients with active tuberculosis. Int. J. Immunopathol. Pharmacol. **12(2)** (1999) 69–79.

In this study we have demonstrated that nitric oxide, the product of the arginine dependent pathway of human mononuclear phagocytes effectively kills the *M. tuberculosis in-vitro*. The release of reactive nitrogen intermediates was triggered by incubation with various proinflammatory cytokines namely IFN gamma,TNF-alpha and IL-1R. We have earlier shown that human mononuclear phagocytes can be induced to release nitric, oxide (NO) radicals which can kill tumour cells. In the present communication, by using colony forming assays we demonstrated that human mononuclear phagocytes can effectively kill *M. tuberculosis* by using a NO dependent pathway. Treatment of mononuclear phagocytes with L-arginine resulted in markedly increased killing activity whereas, by using NGMMA, an analogue of L-arginine, the cidal activity could be brought down to the basal level. These results clearly suggest that cytokines, particularly IFN-gamma, induced NO release and its reactive product with oxygen radical, peroxynitrite, could play an important role in the killing of *M. tuberculosis* by human mononuclear phagocytes. A significant production of interleukin-4 and interleukin-10, by the *ex-vivo* matured, untreated macrophages from the active tuberculosis patients indicate that regulation of cytokine network to encourage *in situ*/local production of nitric oxide may be useful in the management of pulmonary tuberculosis.—Authors' Abstract

Botha, T. and Ryffel, B. Reactivation of latent tuberculosis infection in TNF-deficient mice. J. Immunol. **171(6)** (2003) 3110–3118.

TNF-deficient mice are highly susceptible to *Mycobacterium tuberculosis* H37Rv infection. Here we asked whether TNF is required for postinfectious immunity in aerosol-infected mice. Chemotherapy for 4 wk commencing 2 wk postinfection reduced CFU to undetectable levels. While wild-type mice had a slight rise in CFU, but controlled infection upon cessation of chemotherapy, TNF-deficient mice developed reactivation of infection with high bacterial loads in lungs, spleen, and liver, which was fatal within 13–18 wk. The increased susceptibility of TNF-deficient mice was accompanied by diminished recruitment and activation of T cells and macrophages into the lung, with defective granuloma formation and reduced inducible NO synthase expression. Reduced chemokine production in the lung might explain

suboptimal recruitment and activation of T cells and uncontrolled infection. Therefore, despite a massive reduction of the mycobacterial load by chemotherapy, TNF-deficient mice were unable to compensate and mount a protective immune response. In conclusion, endogenous TNF is critical to maintain latent tuberculosis infection, and in its absence no specific immunity is generated.—Authors' Abstract

Caccamo, N., Barera, A., Di Sano, C., Meraviglia, S., Ivanyi, J., Hudecz, F., Bosze, S., Dieli, F., and Salerno, A. Cytokine profile, HLA restriction and TCR sequence analysis of human CD4+ T clones specific for an immunodominant epitope *of Mycobacterium tuberculosis* 16-kDa protein. Clin. Exp. Immunol. **133(2)** (2003) 260–266.

The identification of immunodominant and universal mycobacterial peptides could be applied to vaccine design and have an employment as diagnostic reagents. In this paper we have investigated the fine specificity, clonal composition and HLA class II restriction of CD4+ T cell clones specific for an immunodominant epitope spanning amino acids 91–110 of the 16-kDa protein of *Mycobacterium tuberculosis*. Twenty-one of the tested 28 clones had a Th1 profile, while seven clones had a Th0 profile. None of the clones had a Th2 profile. While the TCR AV gene usage of the clones was heterogeneous, a dominant TCR BV2 gene family was used by 18 of the 28 clones. The CDR3 regions of BV2+ T cell clones showed variation in lengths, but a putative common motif R-L/V-G/S-Y/W-E/D was detected in 13 of the 18 clones. Moreover, the last two to three residues of the putative CDR3 loops, encoded by conserved BJ sequences, could also play a role in peptide recognition. Antibody blockade and fine restriction analysis using HLA-DR homozygous antigen-presenting cells established that 16 of 18 BV2+ peptide-specific clones were DR restricted and two clones were DR-DQ and DR-DP restricted. Additionally, five of the 18 TCRBV2+ clones recognized peptide 91–110 in association with both parental and diverse HLA-DR molecules, indicating their promiscuous recognition pattern. The ability of peptide 91–110 to bind a wide range of HLA-DR molecules, and to stimulate a Th1-type interferon (IFN)-gamma response more readily, encourage the use of this peptide as a subunit vaccine component.—Authors' Abstract

Danelishvili, L., McGarvey, J., Li, Y. J., and Bermudez, L. E. *Mycobacterium tuberculosis* infection causes different levels of apoptosis and necrosis in human macrophages and alveolar epithelial cells. Cell. Microbiol. **5(9)** (2003) 649–660.

Mycobacterium tuberculosis interacts with macrophages and epithelial cells in the alveolar space of the lung, where it is able to invade and replicate in both cell types. *M. tuberculosis*-associated cytotoxicity to these cells has been well documented, but the mechanisms of host cell death are not well understood. We examined the induction of apoptosis and necrosis of human macrophages (U937) and type II alveolar epithelial cells (A549) by virulent (H37Rv) and attenuated (H37Ra) *M. tuberculosis* strains. Apoptosis was determined by both enzyme-linked immunosorbent assay (ELISA) and TdT-mediated dUTP nick end labelling (TUNEL) assay, whereas necrosis was evaluated by the release of lactate dehydrogenase (LDH). Both virulent and attenuated *M. tuberculosis* induced apoptosis in macrophages; however, the attenuated strain resulted in significantly more apoptosis than the virulent strain after 5 days of infection. In contrast, cytotoxicity of alveolar cells was the result of necrosis, but not apoptosis. Although infection with *M. tuberculosis* strains resulted in apoptosis of 14% of the cells on the monolayer, cell death associated with necrosis was observed in 59% of alveolar epithelial cells after 5 days of infection. Infection with *M. tuberculosis* suppressed apoptosis of alveolar epithelial cells induced by the kinase inhibitor, staurosporine. Because our findings suggest that *M. tuberculosis* can modulate the apoptotic response of macrophages and epithelial cells, we carried out an apoptosis pathway-specific cDNA microarray analysis of human macrophages and alveolar epithelial cells. Whereas the inhibitors of

apoptosis, bcl-2 and Rb, were upregulated over 2.5-fold in infected (48 hr) alveolar epithelial cells, the proapoptotic genes, bad and bax, were downregulated. The opposite was observed when U937 macrophages were infected with *M. tuberculosis*. Upon infection of alveolar epithelial cells with *M. tuberculosis*, the generation of apoptosis, as determined by the expression of caspase-1, caspase-3 and caspase-10, was inhibited. Inhibition of replication of intracellular bacteria resulted in an increase in apoptosis in both cell types. Our results showed that the differential induction of apoptosis between macrophages and alveolar epithelial cells represents specific strategies of *M. tuberculosis* for survival in the host.—Authors' Abstract

De La Barrera, S. S., Finiasz, M., Frias, A., Aleman, M., Barrionuevo, P., Fink, S., Franco, M. C., Abbate, E., and del C Sasiain, M. Specific lytic activity against mycobacterial antigens is inversely correlated with the severity of tuberculosis. Clin. Exp. Immunol. **132(3)** (2003) 450–461.

The ability of peripheral blood mononuclear cells (PBMC) from patients with active tuberculosis to display cytotoxic responses against autologous *Mycobacterium tuberculosis* (Mtb)-pulsed macrophages was evaluated. Non-MHC restricted cell-dependent lytic activity was observed in ex vivo effector cells from tuberculosis patients and was mediated mainly by CD3(+)gammadelta TCR(+) T (gammadelta T) cells bearing CD56 and/or CD16 molecules. MHC-restricted and non-MHC restricted cytotoxic T cells (CTL) were differentially expanded upon stimulation with Mtb in tuberculosis patients and normal controls (N). Class-I restricted CD8(+) CTL and class-II restricted CD4(+) CTL were generated in PPD(+)N and to a lesser extent in PPD(–)N. Mtb-stimulated effector cells from tuberculosis patients became progressively non-MHC restricted CD4(–)CD8(–)gammadelta T cells, while lytic activity of CD4(+) and CD8(+)CTL decreased gradually as the disease became more severe. On the other hand, target cells were lysed by *ex vivo* cells from tuberculosis patients through the Fas-FasL and perforin pathways. Mtb-induced CD4(+) CTL from tuberculosis patients and N controls preferentially employed the Fas-FasL mechanism. Mtb-induced CD8(+) CTL effector cells from patients used the perforin-based mechanism while cells from N controls also used the Fas-FasL pathway. While Mtb-induced gammadelta CTL from patients and PPD(–)N employed the latter mechanism cells from PPD(+)N individuals also used the perforin pathway. It can be concluded that shifts in the CTL response and the cytolytic mechanisms take place as the pulmonary involvement becomes more severe.—Authors' Abstract

Dieli, F., Taniguchi, M., Kronenberg, M., Sidobre, S., Ivanyi, J., Fattorini, L., Iona, E., Orefici, G., De Leo, G., Russo, D., Caccamo, N., Sireci, G., Di Sano, C., and Salerno, A. An anti-inflammatory role for Valpha14 NK T cells in *Mycobacterium bovis* bacillus Calmette-Guerin-infected mice. J. Immunol. **171(4)** (2003) 1961–1968.

The possible contribution of NKT cells to resistance to *Mycobacterium tuberculosis* infection remains unclear. In this paper we characterized the Valpha14 NKT cell population following infection with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). BCG infection determined an early expansion of Valpha14 NKT cells in liver, lungs, and spleen, which peaked on day 8 and was sustained until day 30. However, an NK1.1(+) Valpha14 NKT population preferentially producing IFN-gamma predominated at an early stage (day 8), which was substituted by an NK1.1(–) population preferentially producing IL-4 at later stages (day 30). Despite the fact that Valpha14 NKT cell-deficient mice eliminated BCG as did control mice, they had significantly higher numbers of granulomas in liver and lungs. Additionally, while control mice developed organized small granulomas, those in Valpha14 NKT-deficient mice had signs of caseation, large cellular infiltrates, and some multinucleated macrophages, suggesting that Valpha14 NKT cells may actually work as anti-inflammatory cells by limiting excessive lymphocyte influx and tis-

sue pathology. In agreement, we found an increased spontaneous production and mRNA expression of TNF-alpha in liver and lungs of Valpha14 NKT-deficient mice, whose neutralization *in vivo* by anti-TNF-alpha mAbs consistently reduced the number of granulomas in liver and lungs. Together, our results support a regulatory role for Valpha14 NKT cells in the course of BCG infection through their ability to limit the extent of inflammatory response and point to an important role for this cell subset as a regulator of the balance between protective responses and immunopathology.—Authors' Abstract

Dubnau, E. and Smith, I. *Mycobacterium tuberculosis* gene expression in macrophages. Microbes Infect. **5(7)** (2003) 629–637.

This review provides a discussion on the current information about the response of *Mycobacterium tuberculosis* to the environment encountered in the macrophage. We focus on the types of genes shown to be upregulated when the pathogen grows in macrophages and discuss the possible roles of these genes in adaptation to the conditions in the eukaryotic cell, in the context of enhancing the survival of the pathogen during infection.—Authors' Abstract

Flynn, J. L. and Chan, J. Immune evasion by *Mycobacterium tuberculosis*: living with the enemy. Curr. Opin. Immunol. **15(4)** (2003) 450–455.

Mycobacterium tuberculosis is successful as a pathogen because of its ability to persist in an immunocompetent host. This bacterium lives within the macrophage, a cell whose function is the elimination of microbes. Recent advances have improved our understanding of how *M. tuberculosis* evades two major antimicrobial mechanisms of macrophages: phagolysosome fusion and the production of toxic reactive nitrogen intermediates. *M. tuberculosis* also modulates antigen presentation to prevent the detection of infected macrophages by CD4(+) T cells.—Authors' Abstract

Garcia-Perez, B. E., Mondragon-Flores, R., and Luna-Herrera, J. Internalization of *Mycobacterium tuberculosis* by macropinocytosis in non-phagocytic cells. Microb. Pathog. **35(2)** (2003) 49–55.

Mycobacterium tuberculosis (MTB) is an intracellular pathogen that initially invades the alveolar macrophages of infected individuals. MTB is also known to invade respiratory epithelial cells. To understand the mechanism of epithelial invasion, we investigated the interaction of MTB (H37Rv strain) with non-phagocytic type-II (A549) human pneumocytes. The internalization of the organism was analyzed through optical, fluorescent and electron (transmission and scanning) microscopy. Infection of A549 cells with MTB showed intracellular multiplication of the organism. Microscopy revealed the formation of membrane ruffles totally or partially surrounding the surface adherent mycobacteria. Fluorescent microscopy showed that MTB induced changes in the distribution of actin filaments. Since heat killed MTB failed to induce actin mobilization, perhaps, internalization process is mediated by the soluble products of the metabolically active mycobacterium. Overall, these findings suggest that internalization of MTB by non-phagocytic cells might be through a macropinocytosis or induced-phagocytosis processes, and possibly some bacterial secretory product is responsible for triggering this phenomenon.—Authors' Abstract

Gehring, A. J., Rojas, R. E., Canaday, D. H., Lakey, D. L., Harding, C. V., and Boom, W. H. The *Mycobacterium tuberculosis* 19-kilodalton lipoprotein inhibits gamma interferon-regulated HLA-DR and Fc gamma R1 on human macrophages through Toll-like receptor 2. Infect. Immun. **71(8)** (2003) 4487–4497.

Mycobacterium tuberculosis survives in macrophages in the face of acquired CD4(+) T-cell immunity, which controls but does not eliminate the organism. Gamma interferon (IFN-gamma) has a central role in host defenses against *M. tuberculosis* by activating macrophages and regulating major histocompatibility complex

class II (MHC-II) antigen (Ag) processing. *M. tuberculosis* interferes with IFN-gamma receptor (IFN-gamma R) signaling in macrophages, but the molecules responsible for this inhibition are poorly defined. This study determined that the 19-kDa lipoprotein from *M. tuberculosis* inhibits IFN-gamma-regulated HLA-DR protein and mRNA expression in human macrophages. Inhibition of HLA-DR expression was associated with decreased processing and presentation of soluble protein Ags and *M. tuberculosis* bacilli to MHC-II-restricted T cells. Inhibition of HLA-DR required prolonged exposure to 19-kDa lipoprotein and was blocked with a monoclonal antibody specific for Toll-like receptor 2 (TLR-2). The 19-kDa lipoprotein also inhibited IFN-gamma-induced expression of Fc gamma RI. Thus, *M. tuberculosis*, through 19-kDa lipoprotein activation of TLR-2, inhibits IFN-gamma R signaling in human macrophages, resulting in decreased MHC-II Ag processing and recognition by MHC-II-restricted CD4 T cells. These findings provide a mechanism for *M. tuberculosis* persistence in macrophages.—Authors' Abstract

Gil, D., Garcia, L. F., and Rojas, M. Modulation of macrophage apoptosis by antimycobacterial therapy: physiological role of apoptosis in the control of *Mycobacterium tuberculosis*. Toxicol. Appl. Pharmacol. **190(2)** (2003) 111–119.

Apoptosis is a form of cell death that avoids inflammatory responses. We had previously reported that *Mycobacterium tuberculosis* (Mtb) and Purified Protein Derivative (PPD) induce apoptosis in murine macrophages. The production of TNFalpha and IL-10 in response to Mtb infection modulates apoptosis by controlling nitric oxide production and caspase activation. To further explore the role of macrophage apoptosis in tuberculosis, we studied the capacity of standard antimycobacterial drugs to modulate different events associated with the induction of apoptosis. The B10R murine macrophage line was infected or not with Mtb (5:1 bacteria to macrophage ratio) or exposed to PPD (10 microg/ml), in the presence or absence of varying concentrations (1–20 microg/ml) of anti mycobacterial drugs (isoniazid, rifampin, thiacetazone, streptomycin, and ethambutol). Inhibition of the intracellular growth of *M. tuberculosis* by all drugs studied/correlated with inhibition of permeability transition (PT) alterations; TNFalpha, IL-10, and nitric oxide production, and caspase-1 activation. However, these drugs did not affect PPD-induced apoptosis or its associated events, suggesting that the ability of antimycobacterial drugs to block macrophage apoptosis could be explained by their effects on the metabolic activities of Mtb. All drugs, except isoniazid, at higher concentrations, induced PT alterations in noninfected macrophages in a way that appears to be dependent of calcium, since a calcium chelator prevented it. The results presented herein suggest that the pharmacological manipulation of pathways associated with macrophage apoptosis may affect the intracellular growth of Mtb.—Authors' Abstract

Hanekom, W. A., Mendillo, M., Manca, C., Haslett, P. A., Siddiqui, M. R., Barry, C. 3rd, and Kaplan, G. *Mycobacterium tuberculosis* inhibits maturation of human monocyte-derived dendritic cells *in vitro*. J. Infect. Dis. **188(2)** (2003) 257–266.

To induce effector immunity, dendritic cells (DCs) must differentiate into fully mature cells. We show that, after human monocyte-derived DCs were infected with virulent *Mycobacterium tuberculosis*, upregulation of cellular-surface maturation markers was minimal and reversible. In the presence of a potent stimulus for maturation (tumor necrosis factor [TNF]-alpha, interleukin [IL]-1beta, and prostaglandin E2 [PGE2]), *M. tuberculosis* inhibited phenotypic DC maturation. *M. tuberculosis*-infected DCs had an impaired ability to induce allogeneic lymphoproliferation and activated autologous memory CD4+ and CD8+ T cells optimally only in the presence of TNF-alpha, IL-1beta, and PGE2. Thus, virulent *M. tuberculosis* inhibits phenotypic and functional maturation of human monocyte-derived DCs. This mechanism, which has been described elsewhere for various viruses and for the virulent mycobacte-

rium *M. leprae*, may be a novel mechanism that this pathogen uses to evade the host's immune response.—Authors' Abstract

Hohn, H., Kortsik, C., Tully, G., Nilges, K., Necker, A., Freitag, K., Neukirch, C., Galle, P., Lohr, H., and Maeurer, M. J. Longitudinal analysis of *Mycobacterium tub*erculosis 19-kDa antigen-specific T cells in patients with pulmonary tuberculosis: association with disease activity and cross-reactivity to a peptide from HIVenv gp120. Eur. J. Immunol. **33(6)** (2003) 1613–1623.

CD8(+) T cells play a central role in immune protection against infection with *Mycobacterium tuberculosis*. One of the target epitopes for anti-*M. tuberculosis* directed CD8(+) T cells is the HLA-A2-restricted 19-kDa lipoprotein peptide VLTDGNPPEV. T cell clones directed against this epitope recognized not only the nominal peptide ligand, but also a closely related peptide (VPTDPNPPEV) from the HIV envelope gp120 (HIV(env) gp120) protein characterized by IFN-gamma release. This cross-reactivity was confirmed in *ex vivo* in *M. tuberculosis* 19-kDa tetramer-sorted T cells from patients with tuberculosis and in HIVgp120 tetramer-reactive T cells sorted from HIV(+) patients. *M. tuberculosis* 19-kDa antigen-reactive T cells were present in HLA-A2(+) patients (10/10) with HIV infection with no evidence of *M. tuberculosis* infection, but they are absent in peripheral blood lymphocytes from healthy HLA-A2(+) individuals (10/10). *M. tuberculosis* 19-kDa antigen-reactive T cells were elevated in acute pulmonary tuberculosis, declined with response to therapy (7/10 patients) and resided in the terminally differentiated CD8(+) T cell subset. CD8(+) cross-reactive T cells recognizing HIV(env) or *M. tuberculosis* 19-kDa antigens may contribute to pathogenesis in individuals co-infected with both pathogens and may also present a marker for active tuberculosis.—Authors' Abstract

Hovav, A. H., Mullerad, J., Davidovitch, L., Fishman, Y., Bigi, F., Cataldi, A., and Bercovier, H. The *Mycobacterium tuberculosis* recombinant 27-kilodalton lipoprotein induces a strong Th1-type immune response deleterious to protection. Infect. Immun. **71(6)** (2003) 3146–3154.

Th1 immune response is essential in the protection against mycobacterial intracellular pathogens. Lipoproteins trigger both humoral and cellular immune responses and may be candidate protective antigens. We studied in BALB/c mice the immunogenicity and the protection offered by the recombinant 27-*kDa Mycobacterium tuberculosis* lipoprotein and the corresponding DNA vaccine. Immunization with the 27-kDa antigen resulted in high titers of immunoglobulin G1 (IgG1) and IgG2a with a typical Th1 profile and a strong delayed hypersensitivity response. A strong proliferation response was observed in splenocytes, and significant nitric oxide production and gamma interferon secretion but not interleukin 10 secretion were measured. Based on these criteria, the 27-kDa antigen induced a typical Th1-type immune response thought to be necessary for protection. Surprisingly, in 27-kDa-vaccinated mice (protein or DNA vaccines) challenged by *M. tuberculosis* H37Rv or BCG strains, there was a significant increase in the numbers of CFU in the spleen compared to that for control groups. Furthermore, the protection provided by BCG or other mycobacterial antigens was completely abolished once the 27-kDa antigen was added to the vaccine preparations. This study indicates that the 27-kDa antigen has an adverse effect on the protection afforded by recognized vaccines. We are currently studying how the 27-kDa antigen modulates the mouse immune response.—Authors' Abstract

Jo, E. K., Park, J. K., and Dockrell, H. M. Dynamics of cytokine generation in patients with active pulmonary tuberculosis. Curr. Opin. Infect. Dis. **16(3)** (2003) 205–210.

PURPOSE OF REVIEW: Cytokines have been implicated in the protective immunity, pathophysiology and development of tuberculosis. Most people who become infected with *Mycobacterium tuberculosis* mount an effective protective immune re-

sponse, but 5–10% develop disease. Active pulmonary tuberculosis can be considered to reflect an ineffective immune response against mycobacterial infection. A better understanding of how cytokine production contributes to immunity and pathology would aid the development of new vaccines and therapeutic strategies. RECENT FINDINGS: At the time of diagnosis, production of *M. tuberculosis* or mycobacterial antigen-induced interferon-gamma by peripheral blood mononuclear cells from tuberculosis patients is usually depressed, compared with that of healthy control subjects, whereas cytokine production at the site of disease is elevated. In most patients, depressed interferon-gamma production by peripheral blood mononuclear cells seems to be a transient response because it is significantly increased in most active tuberculosis patients during and following successful antituberculous therapy. However, some patients remain anergic *in vivo* and *in vitro* after chemotherapy, and the underlying biochemical mechanisms for T cell anergy in modulating protection or pathology in tuberculosis needs further clarification. Among the cytokines contributing to protective immunity, interleukins 12 and 18, and tumour necrosis factor-alpha are important, the basis of recent studies with tuberculosis patients. SUMMARY: A more complete understanding of cytokine dynamics in individual cells in active pulmonary tuberculosis patients will provide further knowledge about immunopathogenesis and protective immunity in human tuberculosis. This should ultimately enhance development of preventive and therapeutic strategies against this enormously successful intracellular pathogen.—Authors' Abstract

Kamath, A. B., Alt, J., Debbabi, H., and Behar. S. M. Toll-like receptor 4-defective C3H/HeJ mice are not more susceptible than other C3H substrains to infection with *Mycobacterium tuberculosis*. Infect. Immun. **71(7)** (2003) 4112–4118.

Mycobacterium tuberculosis produces a variety of molecules capable of activating Toll-like receptors, a family of pattern recognition receptors expressed by macrophages and a variety of other cells. To determine whether Toll-like receptor 4 (TLR4) was critical in resistance to *M. tuberculosis* infection, we compared the morbidity and mortality of TLR4-defective C3H/HeJ mice to those of TLR4-sufficient C3H mouse substrains. TLR4-defective C3H/HeJ mice and TLR4-sufficient C3H/HeSnJ, C3HeB/FeJ, and C3H/HeOuJ mice were infected by the aerosol route with *M. tuberculosis*. TLR4-defective C3H/HeJ mice had levels of cytokines in their bronchoalveolar lavage fluids and *in vitro* mycobacterial antigen-specific recall responses similar to those of other C3H mouse substrains. In addition, bacterial replication and long-term survival of mice following infection appeared to be independent of TLR4. Interestingly, C3HeB/FeJ mice were significantly more susceptible to *M. tuberculosis* infection, indicating that genetic heterogeneity among inbred C3H mouse substrains modifies resistance to infection. Therefore, cautious interpretation is required when the C3H/HeJ strain is used as a model of a TLR4-defective mouse strain, as there are significant allelic differences between C3H/HeJ and other C3H mouse substrains in response to *M. tuberculosis* infection. With this caveat, our data indicate that TLR4 may not be required for optimal immunity of mice to *M. tuberculosis*.—Authors' Abstract

Mukae, H., Ashitani. J., Tokojima, M., Ihi, T., Kohno, S., and Matsukura, S. Elevated levels of circulating adhesion molecules in patients with active pulmonary tuberculosis. Respirology. **8(3)** (2003) 326–331.

Elevated levels of circulating adhesion molecules in patients with active pulmonary tuberculosis MUKAE H, ASHITANI J-I, TOKOJIMA M, IHI T, KOHNO S, MATSUKURA S. Respirology 2003; 8: 326–331 OBJECTIVE: Recent studies have indicated the importance of cell adhesion molecules in the pathogenesis of various inflammatory lung diseases. Our study was designed to determine whether five soluble adhesion molecules including soluble L-, E- and P-selectin (sL-, sE- and sP-selectin), intercellular adhesion molecule-1 (sICAM-1), and vascular cell adhesion molecule-1 (sVCAM-1) in serum reflect the severity of

active pulmonary tuberculosis (TB), and whether there is a distinct profile of these soluble molecules in this disease. METHODOLOGY: Using enzyme-linked immunosorbent assays, we measured the serum levels of these five soluble adhesion molecules in 31 patients with active TB and 11 healthy volunteers. RESULTS: Serum levels of sE-selectin, sP-selectin and sICAM-1, but not sL-selectin or sVCAM-1, were significantly higher in patients with active TB than in the control subjects ($p < 0.001$, each). Significant correlations were detected only between serum levels of sE-selectin and sP-selectin, sE-selectin and sICAM-1, and sP-selectin and sICAM-1. There was a significant correlation between the Gaffky scale result (a scale assessing the number of mycobacteria bacilli present) and all of the above adhesion molecules, except for sL-selectin. Serum levels of sE-selectin, sL-selectin and sICAM-1 also correlated with the CXR radiological score. Higher levels of sL-selectin and sICAM-1 were detected in the serum of patients with radiological cavity formation compared to those without. The ESR, C-reactive protein and circulating neutrophil counts all correlated significantly with sE-selectin, sP-selectin, sICAM-1 and sVCAM-1. CONCLUSION: The results suggest that there is a distinct profile of soluble adhesion molecules in active pulmonary TB and that sE-selectin, sP-selectin, and especially sICAM-1 appear to be the most sensitive clinical measures of disease severity.—Authors' Abstract

Microbiology

Agarwal, N. and Tyagi, A. K. Role of 5′-TGN-3′ motif in the interaction of mycobacterial RNA polymerase with a promoter of 'extended-10′ class. FEMS Microbiol. Lett. **225(1)** (2003) 75–83.

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Bull, T. J., Sidi-Boumedine, K., McMinn, E. J., Stevenson, K., Pickup, R., and Hermon-Taylor, J. Mycobacterial interspersed repetitive units (MIRU) differentiate *Mycobacterium avium* subspecies paratuberculosis from other species of the *Mycobacterium avium* complex. Mol. Cell. Probes. **17(4)** (2003) 157–164.

Mycobacterial interspersed repetitive units (MIRU) comprise short tandem repeat structures found at multiple loci throughout the *Mycobacterium tuberculosis* genome and have been used for typing these pathogens. We have identified MIRU at 18 conserved loci throughout the common portions of the *Mycobacterium avium* subspecies paratuberculosis (MAP) and *M. avium* subspecies avium (MAA) genomes. Six of these loci were found to differ between MAA and MAP in the number of tandem repeat motifs occurring at each MIRU locus. Locus specific PCR at 4 of these loci segregated MAP into two major groups, which could be differentiated from ovine-pigmented strains of MAP and the MAP vaccine strain 316F. The same PCR differentiated MAA into five MIRU profiles. PCR at either MIRU locus 1 or MIRU locus 4 distinguished between MAP and all other *M. avium* complex (MAC) tested. PCR at both loci 1 and 4 also distinguished MAP from Mycobacterium intracellulare. MIRU typing may provide an additional simple and rapid procedure for the differentiation between MAP and other MAC.—Authors' Abstract

Consaul, S. A., Jacobs, W. R. Jr., and Pavelka, M. S. Jr. Extragenic suppression of the requirement for diaminopimelate in diaminopimelate auxotrophs *of Mycobacterium smegmatis*. FEMS Microbiol. Lett. **225(1)** (2003) 131–135.

Mycobacteria, like many prokaryotes, have a peptidoglycan with peptides composed of L-alanine (or glycine), D-isoglutamine, meso-diaminopimelate, and

D-alanine. We sought to study mycobacterial peptidoglycan biosynthesis by constructing diaminopimelate (DAP) auxotrophs of *Mycobacterium smegmatis* and then isolating spontaneous mutants of these auxotrophs that can grow in the absence of DAP. Here we report the isolation and characterization of seven classes of spontaneous *M. smegmatis* mutants with extragenic mutations that can suppress the DAP requirement of DAP auxotrophs.—Authors' Abstract

Falkinham, J. O. 3rd. Factors influencing the chlorine susceptibility of *Mycobacterium avium, Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*. Appl. Environ. Microbiol. **69(9)** (2003) 5685–5689.

The susceptibility of representative strains of *Mycobacterium avium, Mycobacterium intracellulare,* and *Mycobacterium scrofulaceum* (the MAIS group) to chlorine was studied to identify factors related to culture conditions and growth phase that influenced susceptibility. *M. avium* and *M. intracellulare* strains were more resistant to chlorine than were strains of *M. scrofulaceum*. Transparent and unpigmented colony variants were more resistant to chlorine than were their isogenic opaque and pigmented variants (respectively). Depending on growth stage and growth rate, MAIS strains differed in their chlorine susceptibilities. Cells from strains of all three species growing in early log phase at the highest growth rates were more susceptible than cells in log and stationary phase. Rapidly growing cells were more susceptible to chlorine than slowly growing cells. The chlorine susceptibility of *M. avium* cells grown at 30 degrees C was increased when cells were exposed to chlorine at 40 degrees C compared to susceptibility after exposure at 30 degrees C. Cells of *M. avium* grown in 6% oxygen were significantly more chlorine susceptible than cells grown in air. Chlorine-resistant MAIS strains were more hydrophobic and resistant to Tween 80, para-nitrobenzoate, hydroxylamine, and nitrite than were the chlorine-sensitive strains.—Author's Abstract

Garnier, T., Eiglmeier, K., Camus, J. C., Medina, N., Mansoor, H., Pryor, M., Duthoy, S., Grondin, S., Lacroix, C., Monsempe, C., Simon, S., Harris, B., Atkin, R., Doggett, J., Mayes, R., Keating, L., Wheeler, P. R., Parkhill, J., Barrell, B. G., Cole, S. T., Gordon, S. V., and Hewinson, R. G. The complete genome sequence of *Mycobacterium bovis*. Proc. Natl. Acad. Sci. U.S.A. **100(13)** (2003) 7877–7882.

Mycobacterium bovis is the causative agent of tuberculosis in a range of animal species and man, with worldwide annual losses to agriculture of $3 billion. The human burden of tuberculosis caused by the bovine tubercle bacillus is still largely unknown. *M. bovis* was also the progenitor for the *M. bovis bacillus* Calmette-Guerin vaccine strain, the most widely used human vaccine. Here we describe the 4,345,492-bp genome sequence *of M. bovis* AF2122/97 and its comparison with the genomes of *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Strikingly, the genome sequence of *M. bovis* is >99.95% identical to that of *M. tuberculosis*, but deletion of genetic information has led to a reduced genome size. Comparison with *M. leprae* reveals a number of common gene losses, suggesting the removal of functional redundancy. Cell wall components and secreted proteins show the greatest variation, indicating their potential role in host-bacillus interactions or immune evasion. Furthermore, there are no genes unique to *M. bovis*, implying that differential gene expression may be the key to the host tropisms of human and bovine bacilli. The genome sequence therefore offers major insight on the evolution, host preference, and pathobiology of *M. bovis*.—Authors' Abstract

Guerardel, Y., Maes, E., Briken, V., Chirat, F., Leroy, Y., Locht, C., Strecker, G., and Kremer, L. Lipomannan and lipoarabinomannan from a clinical isolate of *Mycobacterium kansasii:* novel structural features and apoptosis-inducing properties. J. Biol. Chem. **278(38)** (2003) 36637–36651.

Although *Mycobacterium kansasii* has emerged as an important pathogen frequently encountered in immunocompromised patients, little is known about the mechanisms of *M. kansasii* pathogenicity. Lipoarabinomannan (LAM), a major mycobacterial cell wall lipoglycan, is an important virulence factor for many mycobacteria, as it modulates the host immune response. Therefore, the detailed structures of the of *M. kansasii* LAM (KanLAM), as well as of its biosynthetic precursor lipomannan (KanLM), were determined in a clinical strain isolated from a human immunodeficiency virus-positive patient. Structural analyses revealed that these lipoglycans possess important differences as compared with those from other mycobacterial species. KanLAM carries a mannooligosaccharide cap but is devoid of the inositol phosphate cap present in *Mycobacterium smegmatis.* Characterization of the mannan core of KanLM and KanLAM demonstrated the following occurrences: 1) alpha1,2-oligo-mannopyranosyl side chains, contrasting with the single mannopyranosyl residues substituting the mannan core in all the other structures reported so far; and 2) 5-methylthiopentose residues that were described to substitute the arabinan moiety from *Mycobacterium tuberculosis* LAM. With respect to the arabinan domain of KanLAM, succinyl groups were found to substitute the C-3 position on 5-arabinofuranosyl residues, reported to be linked to the C-2 of the 3,5-arabinofuranose in *Mycobacterium bovis* bacillus calmette-guerin LAM. Because *M. kansasii* has been reported to induce apoptosis, we examined the possibility of the *M. kansasii* lipoglycans to induce apoptosis of THP-1 cells. Our results indicate that, in contrast to KanLAM, KanLM was a potent apoptosis-inducing factor. This work underlines the diversity of LAM structures among various pathogenic mycobacterial species and also provides evidence of LM being a potential virulence factor in *M. kansasii* infections by inducing apoptosis.—Authors' Abstract

Microbiology (Leprosy)

Amako, K., Takade, A., Umeda, A., Matsuoka, M., Yoshida, S., and Nakamura, M. Degradation process of *Mycobacterium leprae* cells in infected tissue examined by the freeze-substitution method in electron microscopy. Microbiol Immunol. **47(6)** (2003) 387–394.

Mycobacterium leprae cells (strain Thai-53) harvested from infected mouse foot pads were examined by electron microscopy using the freeze-substitution technique. The population of *M. leprae* cells from the infected tissue consisted of a large number of degraded cells and a few normal cells. These thin sectioned cell profiles could be categorized into four groups depending on the alteration of the membrane structures, and the degradation process is considered to occur in stages, namely from stages 1 to 3. These are the normal cells with an asymmetrical membrane, a seemingly normal cell but with a symmetrical membrane (stage 1), a cell possessing contracted and highly concentrated cytoplasm with a membrane (stage 2), and a cell that has lost its membrane (stage 3). The peptidoglycan layer was found to remain intact in these cell groups.—Authors' Abstract

Kang, T. J., Kim, S. K., Lee, S. B., Chae, G. T., and Kim, J. P. Comparison of two different PCR amplification products (the 18-kDa protein gene vs. RLEP repetitive sequence) in the diagnosis of *Mycobacterium leprae*. Clin. Exp. Dermatol. **28(4)** (2003) 420–424.

To determine the best molecular method for diagnosing leprosy, two sets of *Mycobacterium leprae*-specific primers were compared. Fresh biopsies and slit skin smear samples were obtained from 67 leprosy patients and examined by touchdown (TD) PCR using primers amplifying either a 129-bp fragment of the RLEP repetitive sequence or a 360-bp fragment of the 18-kDa protein gene of *M. leprae*. Seventeen of 30 (56.7%) biopsy specimens and four of

37 (10.8%) slit skin smear specimens were positive using the primer for the 18-kDa protein gene, whereas 24 of 30 (80%) biopsy and 27 of 37 (73%) slit skin smear samples showed detectable PCR products in the RLEP repetitive sequence. Twenty-one of 31 cases (67.7%) with a bacterial index of zero were PCR positive for the primer RLEP repetitive sequence. These results demonstrate that detection of *M. leprae* using PCR with primers to a RLEP sequence is more sensitive and specific than PCR with the 18-kDa protein gene primers and also slit smears with acid fast staining. PCR of RLEP repetitive sequences is therefore a useful means of detecting *M. leprae* DNA even when it is present at very low levels.—Authors' Abstract

Microbiology (Tuberculosis)

Banaiee, N., Bobadilla-del-Valle, M., Riska, P. F., Bardarov, S. Jr., Small, P. M., Ponce-de-Leon, A., Jacobs, W. R. Jr., Hatfull, G. F., and Sifuentes-Osornio, J. Rapid identification and susceptibility testing of *Mycobacterium tuberculosis* from MGIT cultures with luciferase reporter mycobacteriophages. J. Med. Microbiol. **52(Pt 7)** (2003) 557–561.

In a prospective study conducted in a diagnostic laboratory in Mexico City, luciferase reporter mycobacteriophages (LRPs) were evaluated for their utility and performance in identification and antibiotic-susceptibility testing of *Mycobacterium tuberculosis* complex (MTC) isolates from MGIT-960 cultures. Eighty-four consecutive MGIT cultures recovered from 54 patients were included in this study. The LRPs confirmed mycobacterial growth in 79 (94%) of 84 MGIT cultures. Failure to confirm growth was due to low inoculum (N = 1) or growth with non-tuberculous mycobacteria (N = 4). The median time to confirmation of MGIT cultures was 1 day (range 1–55). Confirmed cultures were identified with p-nitro-alpha-acetylamino-beta-hydroxypropiophenone (NAP), a selective inhibitor of MTC species, and results obtained with LRPs were compared with those obtained by BACTEC-460. The sensitivity and specificity of the LRP NAP test were respectively 97 and 100%, and the median turnaround time for identification was 3 days with both methods. The accuracy and speed of the LRPs for susceptibility testing with rifampicin, streptomycin, isoniazid and ethambutol were compared with BACTEC-460 and discrepant results were tested by the conventional agar proportion method. In total, 72 MTC cultures were tested. The overall agreement between the LRPs and BACTEC-460 was 98.6%. Four isolates (5.6%) were falsely identified as ethambutol-resistant. The median turnaround time for susceptibility testing was 3 days (range 3–57) with the LRPs and 9 days (range 7–29) with BACTEC-460. LRPs offer an accurate and rapid approach for identification and susceptibility testing of *M. tuberculosis* from MGIT-960 cultures.—Authors' Abstract

Carvalho, W. S., Spindola de Miranda, S., Costa, K. M., Araujo, J. G., Augusto, C. J., Pesquero, J. B., Pesquero, J. L., and Gomes, M. A. Low-stringency single-specific-primer PCR as a tool for detection of mutations in the rpoB gene of rifampin-resistant *Mycobacterium tuberculosis*. J. Clin. Microbiol. **41(7)** (2003) 3384–3386.

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Dahl, J. L., Kraus, C. N., Boshoff, H. I., Doan, B., Foley, K., Avarbock, D., Kaplan, G., Mizrahi, V., Rubin, H., and Barry, C. E. III. The role of RelMtb-mediated adaptation to stationary phase in long-term persistence of *Mycobacterium tuberculosis* in mice. Proc Natl. Acad. Sci. U. S. A. **100(17)** (2003) 10026–10031.

Long-term survival of nonreplicating *Mycobacterium tuberculosis* (Mtb) is ensured by the coordinated shutdown of active metabolism through a broad transcriptional pro-

gram called the stringent response. In Mtb, this response is initiated by the enzymatic action of RelMtb and deletion of relMtb produces a strain (H37RvDeltarelMtb) severely compromised in the maintenance of long-term viability. Although aerosol inoculation of mice with H37RvDeltarelMtb results in normal initial bacterial growth and containment, the ability of this strain to sustain chronic infection is severely impaired. Significant histopathologic differences were noted in lungs and spleens of mice infected with H37RvDeltarelMtb compared with controls throughout the course of the infection. Microarray analysis revealed that H37RvDeltarelMtb suffers from a generalized alteration of the transcriptional apparatus, as well as specific changes in the expression of virulence factors, cell-wall biosynthetic enzymes, heat shock proteins, and secreted antigens that may alter immune recognition of the recombinant organism. Thus, RelMtb is critical for the successful establishment of persistent infection in mice by altering the expression of antigenic and enzymatic factors that may contribute to successful latent infection.—Authors' Abstract

Florczyk, M. A., McCue, L. A., Purkayastha, A., Currenti, E., Wolin, M. J., and McDonough, K. A. A family of acr-coregulated *Mycobacterium tuberculosis* genes shares a common DNA motif and requires Rv3133c (dosR or devR) for expression. Infect. Immun. **71(9)** (2003) 5332–5343.

Previous work has shown that the divergently transcribed *Mycobacterium tuberculosis* genes acr (hspX, Rv2031c) and acg (Rv2032) are induced under conditions of shallow standing culture and low oxygen and intracellularly within macrophages. We used a combination of computational and experimental methods to identify promoters for eight additional genes that are regulated in a similar manner and that comprise an acr-coregulated promoter (ACP) family. Transcriptional regulation of these ACP family members was evaluated by using a plasmid-based promoter-green fluorescent protein fusion system and flow cytometry. All promoters showed increased expression in shallow standing versus shaking cultures, in low- versus high-oxygen conditions, and intracellularly within macrophages versus extracellularly in tissue culture medium. However, there were quantitative differences in expression among promoters and among conditions for each promoter. A conserved 18-bp palindromic sequence motif was identified in all ACPs by Gibbs sampling-based computational analyses. Two such motifs overlap regions in the acr and acg promoters that were previously shown to be required for their expression. In addition, we found that 5% carbon dioxide was required for growth of *Mycobacterium bovis* BCG under microaerophilic (1.3% O(2)) culture conditions and fully prevented the growth cessation typically associated with rapid removal of oxygen. These findings are likely to be relevant to the *in vivo* environment and will contribute to our understanding of the pathogenesis of tuberculosis infection.—Authors' Abstract

Fossati, G., Izzo, G., Rizzi, E., Gancia, E., Modena, D., Moras, M. L., Niccolai, N., Giannozzi, E., Spiga, O., Bono, L., Marone, P., Leone, E., Mangili, F., Harding, S., Errington, N., Walters, C., Henderson, B., Roberts, M. M., Coates, A. R., Casetta, B., and Mascagni, P. *Mycobacterium tuberculosis* chaperonin 10 is secreted in the macrophage phagosome: is secretion due to dissociation and adoption of a partially helical structure at the membrane? J. Bacteriol. **185(14)** (2003) 4256–4267.

To confirm that *Mycobacterium tuberculosis* chaperonin 10 (Cpn10) is secreted outside the live bacillus, infected macrophages were examined by electron microscopy. This revealed that the mycobacterial protein accumulates both in the wall of the bacterium and in the matrix of the phagosomes in which ingested mycobacteria survive within infected macrophages. To understand the structural implications underlying this secretion, a structural study of *M. tuberculosis* Cpn10 was performed under conditions that are generally believed to mimic the membrane environment. It was found that in buffer-organic solvent mixtures, the mycobacterial protein forms two

main species, namely, a partially helical monomer that prevails in dilute solutions at room temperature and a dimer that folds into a beta-sheet-dominated structure and prevails in either concentrated protein solutions at room temperature or in dilute solutions at low temperature. A partially helical monomer was also found and was completely associated with negatively charged detergents in a micelle-bound state. Remarkably, zwitterionic lipids had no effect on the protein structure. By using N- and C-truncated forms of the protein, the C- and N-terminal sequences were identified as possessing an amphiphilic helical character and as selectively associating with acidic detergent micelles. When the study was extended to other chaperonins, it was found that human Cpn10 is also monomeric and partially helical in dilute organic solvent-buffer mixtures. In contrast, *Escherichia coli* Cpn10 is mostly dimeric and predominately beta-sheet in both dilute and concentrated solutions. Interestingly, human Cpn10 also crosses biological membranes, whereas the E. coli homologue is strictly cytosolic. These results suggest that dissociation to partially helical monomers and interaction with acidic lipids may be two important steps in the mechanism of secretion of *M. tuberculosis* Cpn10 to the external environment.—Authors' Abstract

Gao, L. Y., Laval, F., Lawson, E. H., Groger, R. K., Woodruff, A., Morisaki, J. H., Cox, J. S., Daffe, M., and Brown, E. J. Requirement for kasB in *Mycobacterium mycolic* acid biosynthesis, cell wall impermeability and intracellular survival: implications for therapy. Mol. Microbiol. **49(6)** (2003) 1547–1563.

Mycobacterium tuberculosis infects one-third of the world's population and causes two million deaths annually. The unusually low permeability of its cell wall contributes to the ability of *M. tuberculosis* to grow within host macrophages, a property required for pathogenesis of infection. *Mycobacterium marinum* is an established model for discovering genes involved in mycobacterial infection. *Mycobacterium marinum* mutants with transposon insertions in the beta-ketoacyl-acyl carrier protein synthase B gene (kasB) grew poorly in macrophages, although growth *in vitro* was unaffected. Detailed analyses by thin-layer chromatography, nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, infrared spectroscopy, and chemical degradations showed that the kasB mutants synthesize mycolic acids that are 2–4 carbons shorter than wild type; the defect was localized to the proximal portion of the meromycolate chain. In addition, these mutants showed a significant (approximately 30%) reduction in the abundance of keto-mycolates, with a slight compensatory increase of both alpha- and methoxy-mycolates. Despite these small changes in mycolate length and composition, the kasB mutants exhibited strikingly altered cell wall permeability, leading to a marked increase in susceptibility to lipophilic antibiotics and the host antimicrobial molecules defensin and lysozyme. The abnormalities of the kasB mutants were fully complemented by expressing *M. tuberculosis* kasB, but not by the closely related gene kasA. These studies identify kasB as a novel target for therapeutic intervention in mycobacterial diseases.—Authors' Abstract

Gao, L. Y., Laval, F., Lawson, E. H., Groger, R. K., Woodruff, A., Morisaki, J. H., Cox, J. S., Daffe, M., and Brown, E. J. Requirement for kasB in Mycobacterium mycolic acid biosynthesis, cell wall impermeability and intracellular survival: implications for therapy. Mol. Microbiol. **49(6)** (2003) 1547–1563.

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He, X. Y., Zhuang, Y. H., Zhang, X. G., and Li, G. L. Comparative proteome analysis of culture supernatant proteins of *Mycobacterium tuberculo*sis H37Rv and H37Ra. Microbes Infect. **5(10)** (2003) 851–856.

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Mani, C., Selvakumar, N., Kumar, V., Narayanan, S., and Narayanan, P. R. comparison of DNA sequencing, PCR-SSCP and PhaB assays with indirect sensitivity testing for detection of rifampicin resistance in *Mycobacterium tuberculosis*. Int. J. Tuberc. Lung Dis. **7(7)** (2003) 652–659.

SETTING: Tuberculosis Research Centre, Chennai, India. OBJECTIVE: To rapidly identify multidrug-resistant *Mycobacterium tuberculosis* using phenotypic and genotypic methods. DESIGN: Two genotypic assays, DNA sequencing and polymerase chain reaction single strand conformation polymorphism (PCR-SSCP), and one phenotypic assay, phage amplified biological assay (PhaB) were standardised in-house and performed on coded 101 rifampicin-resistant and 100 rifampicin-sensitive *M. tuberculosis* clinical isolates for the identification of rifampicin resistance. RESULTS AND CONCLUSION: The results obtained using the three assays were compared with those from the conventional indirect sensitivity test. The sensitivities and specificities of DNA sequencing, PCR-SSCP and PhaB were 97% and 100%, 76% and 100%, and 97% and 84%, respectively. DNA sequencing was found to be more sensitive and specific than the other tests.—Authors' Abstract

Murray, S. J., Barrett, A., Magee, J. G., and Freeman, R. Optimisation of acid fast smears for the direct detection of mycobacteria in clinical samples. J. Clin. Pathol. **56(8)** (2003) 613–615.

AIMS: Despite its long history, the acid fast smear remains unstandardised. Technical variations in both the preparation of clinical material and subsequent staining mean that smear sensitivity relative to culture may vary from 50% to over 80%. This study assessed the sensitivity of acid fast microscopy at each of five stages of sample preparation and by both commonly used staining methods. METHODS: Sputum samples thought for varying reasons to be highly likely to be culture positive were used to prepare a series of smears in which the effects of digestion (liquefaction), concentration (centrifugation), and decontamination (sodium hydroxide) could be assessed, together with a comparison of staining by the auramine/phenol and Ziehl-Neelsen techniques. RESULTS: The most effective method for the demonstration of acid fast organisms in sputum was found to be an auramine phenol stain applied to a liquefied, concentrated sample and examined before the decontamination process. CONCLUSIONS: The auramine phenol stain applied to a liquefied, concentrated sample and examined before the decontamination process is the most effective method for the demonstration of acid fast organisms in sputum.—Authors' Abstract

Experimental Infections

Baek, K. M., Ko, S. Y., Lee, M., Lee, J. S., Kim, J. O., Ko, H. J., Lee, J. W., Lee, S. H., Cho, S. N., and Kang, C. Y. Comparative analysis of effects of cytokine gene adjuvants on DNA vaccination against *Mycobacterium tuberculosis* heat shock protein 65. Vaccine **21(25–26)** (2003) 3684–3689.

DNA-based vaccines generate potent cellular immunity as well as humoral immunity. It seems evident that cytokines play a crucial role in generation of effector T cell subsets and in determining the magnitude of the response by DNA vaccines. In this study, we compared the effects of several TH1 cytokine genes as adjuvant in DNA vaccination using mycobacterial Hsp65 as a model antigen. Our results demonstrated that although the overall immune response to Hsp65 was enhanced by co-injection of Hsp65 DNA with cytokine genes, each cytokine gene was shown to affect different immune response elements. Co-injection of Hsp65 DNA with IL-12 or GM-CSF led to an increase in IFN-gamma production and represented potent protections against *Mycobacterium tuberculosis* challenge, while that with Eta-1, IL-12 or IL-18 gene led to an elevated IgG2a/IgG1 ratio. Interestingly, co-administration of Flt3L gene was shown to enhance the Ag-specific CTL response. These results show that the direction and magnitude of immune response in DNA vaccination against Hsp65 of *M. tuberculosis* could be modulated in different ways by co-injection of an appropriate cytokine gene as adjuvant.—Authors' Abstract

Dascher, C. C., Hiromatsu, K., Xiong, X., Morehouse, C., Watts, G., Liu, G., McMurray, D. N., LeClair, K. P., Porcelli, S. A., and Brenner, M. B. Immunization with a mycobacterial lipid vaccine improves pulmonary pathology in the guinea pig model of tuberculosis. Int. Immunol. **15(8)** (2003) 915–925.

Lipids and glycolipid molecules derived from *Mycobacterium tuberculosis* can be presented to T cells by CD1 cell-surface molecules in humans. These lipid-specific T cells are cytolytic, secrete pro-inflammatory cytokines and have bactericidal activity. Here, we describe studies in which lipids from *M. tuberculosis* were incorporated into liposomes with adjuvant and tested as vaccines in a guinea pig aerosol tuberculosis challenge model. Animals vaccinated with mycobacterial lipids showed reduced bacterial burdens in the lung and spleen at 4 weeks after infection. In addition, the lungs of lipid-vaccinated animals also had significantly less pathology, with granulomatous lesions being smaller and more lymphocytic. In contrast, animals receiving only vehicle control immunizations had granulomatous lesions that were larger and often contained caseous necrotic centers. Quantification of histopathology by morphometric analysis revealed that the overall percentage of lung occupied by diseased tissue was significantly smaller in lipid-vaccinated animals as compared to vehicle control animals. In addition, the mean area of individual granulomatous lesions was found to be significantly smaller in both lipid- and bacillus Calmette-Guerin-vaccinated guinea pigs. These data support an important role for lipid antigens in the immune response to *M. tuberculosis* infection, potentially through the generation of CD1-restricted T cells. Immunogenic lipids thus represent a novel class of antigens that might be included to enhance the protective effects of subunit vaccine formulations.—Authors' Abstract

Gonzalez-Juarrero, M., Shim, T. S., Kipnis, A., Junqueira-Kipnis, A. P., and Orme, I. M. Dynamics of macrophage cell populations during murine pulmonary tuberculosis. J. Immunol. **171(6)** (2003) 3128–3135.

The influx of macrophages into the lungs is the major component of the granulomatous response to infection with *Mycobacterium tuberculosis*. In this investigation we used flow cytometric analysis to define macrophage populations entering the airways and lung tissues of infected mice. We

demonstrate that by the judicious use of cell surface markers, especially CD11b and CD11c, several cell populations can be distinguished, allowing cell sorting and morphological definition. Primary populations of CD11b(–)/CD11c(+/high) were defined as alveolar macrophages, CD11b(high)/CD11c(+/high) as dendritic cells, and CD11b(+/mid)/CD11c(+/mid) as small macrophages or monocytes, and changes in the activation phenotype of these populations were followed over the early course of the infection. In further studies, these cell populations were compared with cells harvested during the chronic stage of the disease. During the chronic stage of infection, Ag-presenting class II molecules and activation markers were poorly expressed on dendritic, small macrophage, and monocyte cell populations, which may have important implications for the breakdown of the lesions during reactivation disease. This analytical approach may facilitate the further characterization of macrophage populations entering into the lung tissues and their relative contributions to host resistance to tuberculosis infection.—Authors' Abstract

Goonetilleke, N. P., McShane, H., Hannan, C. M., Anderson, R. J., Brookes, R. H., and Hill, A. V. Enhanced immunogenicity and protective efficacy against *Mycobacterium tuberculosis* of bacille Calmette-Guerin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara. J. Immunol. **171(3)** (2003) 1602–1609.

Heterologous prime-boost immunization strategies can evoke powerful T cell immune responses and may be of value in developing an improved tuberculosis vaccine. We show that recombinant modified vaccinia virus Ankara, expressing *Mycobacterium tuberculosis* Ag 85A (M.85A), strongly boosts bacille Calmette-Guerin (BCG)-induced Ag 85A specific CD4(+) and CD8(+) T cell responses in mice. A comparison of intranasal (i.n.) and parenteral immunization of BCG showed that while both routes elicited comparable T cell responses in the spleen, only i.n. delivery elicited specific T cell responses in the lung lymph nodes, and these responses were further boosted by i.n. delivery of M.85A. Following aerosol challenge with *M. tuberculosis*, i.n. boosting of BCG with either BCG or M.85A afforded unprecedented levels of protection in both the lungs (2.5 log) and spleens (1.5 log) compared with naive controls. Protection in the lung correlated with the induction of Ag 85A-specific, IFN-gamma-secreting T cells in lung lymph nodes. These findings support further evaluation of mucosally targeted prime-boost vaccination approaches for tuberculosis.—Authors' Abstract

Ha, S. J., Jeon, B. Y., Kim, S. .C., Kim, D. J., Song, M. K., Sung, Y. C., and Cho, S. N. Therapeutic effect of DNA vaccines combined with chemotherapy in a latent infection model after aerosol infection of mice with *Mycobacterium tuberculosis*. Gene Ther. **10(18)** (2003) 1592–1599.

The prevention of *Mycobacterium tuberculosis* (*M. tuberculosis*) reactivation would greatly reduce the incidence of the disease, particularly among the elderly. Here, we evaluated the efficacy of DNA vaccine in combination with a conventional TB chemotherapy on the prevention of *M. tuberculosis* reactivation. Mice were treated with isoniazid and pyrazinamide for 3 months from 4 weeks after aerosol infection with *M. tuberculosis* H37Rv. During this period of chemotherapy, DNA immunization was performed three times monthly with an antigen 85A (Ag85A) DNA or an IL-12 mutant (IL-12N220L) DNA, which is known to lead to a reduction in the secretion of the p40 subunit, but not of a bioactive IL-12p70. The reactivation of *M. tuberculosis* was dramatically reduced in mice treated with either Ag85A DNA ($p < 0.01$) or IL-12N220L DNA ($p < 0.05$) in combination with chemotherapy, compared with control mice receiving only chemotherapy. Ag85A DNA vaccine showed higher IFN-gamma responses to Ag85A protein, but a lower response to culture filtrate than IL-12N220L DNA vaccine. In addition, Ag85A DNA vaccine prevented the reactivation of *M. tuberculosis* more efficiently than IL-12N220L DNA vaccine, indicating

that Ag85A-specific IFN-gamma response might correlate with *M. tuberculosis* control. This study suggests that immunotherapy using Ag85A or IL-12N220L DNA vaccine combined with conventional chemotherapy might be effective clinically for the prevention of tuberculosis reactivation and may offer a more effective cure for humans than chemotherapy alone.—Authors' Abstract

Hamasur, B., Haile, M., Pawlowski, A., Schroder, U., Williams, A., Hatch, G., Hall, G., Marsh, P., Kallenius, G., and Svenson, S. B. *Mycobacterium tuberculosis* arabinomannan-protein conjugates protect against tuberculosis. Vaccine **21(25–26)** (2003) 4081–4093.

Lipoarabinomannan (LAM) is a major structural surface component of mycobacteria. Arabinomannan (AM) oligosaccharides derived from LAM of *Mycobacterium tuberculosis* H37Rv were isolated and covalently conjugated to tetanus toxoid (TT) or to short-term culture filtrate proteins (antigen 85B (Ag85B) or a 75kDa protein) from *M. tuberculosis* strain Harlingen. The different AM oligosaccharide (AMOs)-protein conjugate vaccine candidates proved to be highly immunogenic, inducing boosterable IgG responses against the AMOs portion of the conjugates in rabbits and guinea-pigs. Proliferation of T-cells from C57BL/6 mice immunized with the conjugates was seen upon *in vitro* stimulation with PPD. In C57BL/6 mice subcutaneous immunization with the AMOs-antigen 85B conjugate in alum provided significant protection compared to sham (alum only) immunized mice ($p < 0.021$) as estimated by long term survival against intravenous challenge with 10(5) *M. tuberculosis* H37Rv. Subcutaneous immunization followed by nasal boost with an AMOs-TT conjugate in Eurocine L3 adjuvant provided high ($p < 0.025$) protection as determined by long term survival after intranasal challenge with 10(5) virulent *M. tuberculosis* strain Harlingen. This level of protection was comparable to that obtained with the conventional live attenuated BCG vaccine. In guinea-pigs, immunization with AMOs-Ag85B in Eurocine L3 adjuvant followed by aerogenic challenge with *M. tuberculosis* H37Rv resulted in increased survival and reduced pathology in lungs and spleens relative to non-immunized animals.—Authors' Abstract

Lai, X., Shen, Y., Zhou, D., Sehgal, P., Shen, L., Simon, M., Qiu, L., Letvin, N. L., and Chen, Z. W. Immune biology of macaque lymphocyte populations during mycobacterial infection. Clin. Exp. Immunol. **133(2)** (2003) 182–192.

Immune responses of lymphocyte populations during early phases of mycobacterial infection and reinfection have not been well characterized in humans. A non-human primate model of *Mycobacterium bovis* bacille Calmette-Guerin (BCG) infection was employed to characterize optimally the immune responses of mycobacteria-specific T cells. Primary BCG infection induced biphasic immune responses, characterized by initial lymphocytopenia and subsequent expansion of CD4+, CD8+ and gammadelta T cell populations in the blood, lymph nodes and the pulmonary compartment. The potency of detectable T cell immune responses appears to be influenced by the timing and route of infection as well as challenge doses of BCG organisms. Systemic BCG infection introduced by intravenous challenge induced a dose-dependent expansion of circulating CD4+, CD8+ and gammadelta T cells whereas, in the pulmonary compartment, the systemic infection resulted in a predominant increase in numbers of gammadelta T cells. In contrast, pulmonary exposure to BCG through the bronchial route induced detectable expansions of CD4+, CD8+ and gammadelta T cell populations in only the lung but not in the blood. A rapid recall expansion of these T cell populations was seen in the macaques reinfected intravenously and bronchially with BCG. The expanded alphabeta and gammadelta T cell populations exhibited their antigen specificity for mycobacterial peptides and non-peptide phospholigands, respectively. Finally, the major expansion of T cells was associated with a resolution of active BCG infection and reinfection. The patterns and kinetics of CD4+, CD8+ and gammadelta T cell immune responses during BCG infection might con-

tribute to characterizing immune protection against tuberculosis and testing new tuberculosis vaccines in primates.—Authors' Abstract

Mogga, S. J., Mustafa, T., Sviland, L., and Nilsen, R. *In situ* expression of CD40, CD40L (CD154), IL-12, TNF-alpha, IFN-gamma and TGF-beta1 in murine lungs during slowly progressive primary tuberculosis. Scand. J. Immunol. **58(3)** (2003) 327–334.

The distribution and expression of CD40, its ligand CD40L (154) and related cytokines interleukin-12 (IL-12), tumour necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma) and transforming growth factor-beta1 (TGF-beta1) were studied in the lungs of B6D2F1 hybrid mice during slowly progressive primary tuberculosis (TB) by immunohistochemistry. CD40 and CD40L are implicated in cell-mediated immunity (CMI) causing activation or apoptosis of infected cells. The phenomenon of apoptosis is associated with *Mycobacterium tuberculosis* survival. In this study, using frozen lung sections (N = 33), our results showed increased CD40, IL-12 and TGF-beta1 expression in macrophages with progression of disease. High percentages of mycobacterial antigens (M.Ags), CD40L and IFN-gamma expression were maintained throughout infection, and TNF-alpha-expressing cells were decreased. In lymphocytes, the percentage of IFN-gamma-positive cells was increased, but CD40L and IL-12 were maintained with the progression of disease. M.Ags, CD40 and CD40L were expressed in the same areas of the lesions. We conclude that changes in the expression of CD40-CD40L and cytokines associated with *M. tuberculosis* infection favour the hypothesis that *M. tuberculosis* causes resistance of host cells to apoptosis causing perpetuation of infection.—Authors' Abstract

Epidemiology and Prevention

Figueiredo, I. A. and da Silva, A. A. [Increase in leprosy detection rates in Sao Luis, Maranhao, Brazil, from 1993 to 1998: is the endemic expanding?] Cad Saude Publica. **19(2)** (2003) 439–445. [Article in Portuguese]

A descriptive epidemiologic study on the detection of new leprosy cases was conducted in Sao Luis, Maranhao, Brazil, from 1993 to 1998. A database was created for the purpose, covering 2796 reported cases. General detection rates were calculated, as well as specific rates by gender, clinical type, and age group. Linear, exponential, geometric, and log adjustment models were performed to analyze time trends in the disease. An increase in detection was observed, involving mostly female and paucibacillary cases, mainly of tuberculoid leprosy. The increase in detection was most evident in the 15 to 19 year-old population. The percentage of detection under 15 indicated the need for active case search in this group.—Authors' Abstract

Hatta, M. Epidemiology of leprosy. Molecular, biological, and immunological approach. Adv. Exp. Med. Biol. **531** (2003) 269–278.

Leprosy is an infectious disease for which humans are considered the only source of infection. The major hindrance in leprosy control and thus in reaching the elimination goal is that numerous leprosy cases remain undetected for a long time. Many of these patients are a continuous source of infection and, and hence perpetuate transmission. The goal of the World Health Organization (WHO) is to eliminate leprosy as a public problem by the year 2000; that is, to reach as a global prevalence of <1 per 10,000 people. The epidemiological data generated routinely by health services are greatly influenced by their policies and activities. The data do not, however necessarily reflect the true situation in the field. Information on the magnitude of the leprosy problem in any one area is important for the health services with regard to their planning, monitoring

and evaluation of leprosy control activities. Our studies have suggested that the high prevalence of antibodies in children may be indicative of the active transmission of *M. leprae* in their surroundings. The prevalence of these antibodies may also be important for leprosy control programs in order to detect new patients as early as possible and in an effective and sustainable manner. Based on PCR data, it seems that the environment also plays an important role in the transmission of leprosy in endemic areas. The results of our study show that contact with a leprosy patient is the major determinant in the incidence of leprosy and that this concept shows similarities with the "stone-in-the-pond" principle of tuberculosis transmission in concentric circle around patients.—Author's Abstract

Kumar, A., Girdhar, A., Girdhar, B. K. Epidemiology of leprosy in urban Agra. Lepr. Rev. **74(1)** (2003) 31–34.—Tropical Disease Bulletin

During May 2000–June 2001, a survey was carried out in the urban areas of Agra, India, to evaluate the prevalence of leprosy in the area. A total of 60,179 persons from more than 120 smaller localities in both semi-urban and slum areas were examined. Chi-square test (χ^2) was used to determine the prevalence, while logistic regression was used to compute for adjusted odd ratios. The overall prevalence of leprosy was 33.9 per 10,000 population (range, 9.7–40.7), whereas the new case detection rate was 28.2 per 10,000 (range, 9.7–30.7). Among children <15 years, the leprosy prevalence was 4.4 per 10,000. Adult males aged >15 years had a significantly higher prevalence rate than females of similar age group (92.0 versus 41.6 per 10,000). It was noted that the prevalence rates increased with increasing age ($p < 0.0001$). Moreover, workers engaged in manual work were found to have significantly higher prevalence rates than other workers (94.9 versus 21.3, $p < 0.0001$). Of the 204 leprosy cases detected, 84.2% were new cases. Of all the cases, 37.3% were of the multibacillary type. Disabilities of Grade II or higher were observed in 12.7% of all cases, of which 9.4% were new cases.—Tropical Disease Bulletin

Rehabilitation and Social Concerns

Brandsma, J. W. 26th Kellersberger Memorial Lecture. Lessons from leprosy rehabilitation for general rehabilitation. Ethiop. Med. J. **41(1)** (2003) 77–87.

Leprosy is primarily a disease of skin and peripheral nerves. Because of nerve function impairment, leprosy patients may develop primary nerve related impairments such as, loss of sensation and weakness or paralysis. These primary impairments may lead to secondary impairments such as ulceration and contractures. Many other diseases and disorders present with similar impairments as seen in leprosy e.g., diabetes and peripheral nerve injuries. Nerve function assessment and ulcer prevention and treatment are areas that have been researched in leprosy but these research findings are not yet commonly known and adopted in diseases and disorders that 'relate' to leprosy. Rehabilitation is a relatively new field in medicine and not (well) developed in many developing countries. Rehabilitation requires an integrated approach from different disciplines and professionals. As for other medical specialty fields, rehabilitation demands evidence based practice.—Author's Abstract

Kalk, A. [Cooperation between an NGO and "host" states in the control of leprosy in Latin America] Cad Saude Publica. **19(2)** (2003) 663–666. [Article in Portuguese]

The proliferation of nongovernmental organizations (NGOs) can be considered the result of the inability of the current democratic system to perform all the tasks desired by its citizens. Although NGOs often do quite positive work, they tend to diminish governmental power and are capable of

interfering in the internal affairs of other countries. In this context, there are efforts to control their activities, and this control can produce both negative effects (blocking the defense of human rights) and positive ones (correcting the lack of coordination in the work by NGOs). NGOs working with the control of leprosy have a long history of cooperation with "host" states in Latin America. In the worst cases they work in a vacuum left by the state. In a country like Brazil, where the government prioritizes the control of Hansen disease and community participation in the political process—NGOs generally work "in harmony" with national authorities. The most useful contribution to state efforts has been the technical and financial support for training health personnel, supervision, and awareness-raising campaigns. Thus, the NGO becomes "quasi-governmental" in performing its tasks.—Author's Abstract

Other Mycobacterial Diseases

Albright, J. T. and Pransky, S. M. Nontuberculous mycobacterial infections of the head and neck. Pediatr. Clin. North Am. **50(2)** (2003) 503–514.

Nontuberculous mycobacteria are ubiquitous in the environment. Immunocompetent children are commonly infected by these resilient organisms. Cervical lymphadenitis, the most frequent head and neck manifestation of NTM infection, often presents as chronic, unilateral lymphadenopathy with characteristic violaceous overlying skin changes. Diagnosis is ultimately dependent on culture or histopathologic examination of specimen obtained through excisional lymph node biopsy or FNA. The principal treatment of NTM infection remains the surgical excision of diseased tissue. Antibiotics augment surgical therapy and their potential role as a single-modality therapy continues to be investigated.—Authors' Abstract

Amoateng-Adjepong, Y., Salloum, A., St Martin, D., and Coles, M. J. *Mycobacterium marinum* infection in Connecticut: report of four cases. Conn. Med. **67(6)** (2003) 333–335.

Mycobacterium marinum is emerging as an important human pathogen in the United States. We report four cases incidentally diagnosed from culture of biopsy specimens of wrist lesions at a Connecticut inner city hospital between 1996 and 1999. There was no clear association with aquatic exposure and only one patient recalled prior trauma. All were successfully treated with ethambutol and rifampicin. The current literature on the epidemiology, clinical characteristics and management of *Mycobacterium marinum* infections is reviewed.—Authors' Abstract

Amrami, K. K., Sundaram, M., Shin, A. Y., and Bishop, A. T. *Mycobacterium marinum* infections of the distal upper extremities: clinical course and imaging findings in two cases with delayed diagnosis. Skeletal. Radiol. **32(9)** (2003) 546–549.

Mycobacterium marinum infections cause tenosynovitis of the distal upper extremities and develop as a consequence of skin abrasions acquired in contaminated water. We report on two patients whose MR imaging studies showed tenosynovitis of the distal upper extremity secondary to *M. marinum*. In one patient sequential MR imaging showed development of bony erosions. Appropriate treatment was delayed in both patients because the diagnosis was not considered. We report on and discuss the clinical course and MR imaging findings in two patients with *M. marinum* infection.—Authors' Abstract

Ara, M., de Santamaria, C. S., Zaballos, P., Yus, C., and Lezcano, M. A. *Mycobacterium chelonae* infection with multiple cutaneous lesions after treatment with acupuncture. Int. J. Dermatol. **42(8)** (2003) 642–644.

A 58-year-old woman was first seen in November 1999 with a 4-week history of

several tender, deep red or purple, suppurating subcutaneous nodules on the skin of the abdomen, suggestive of a panniculitis. She had no history of systemic immunosuppression. Three months prior to examination, the patient had treated with acupuncture for obesity. Two biopsy specimens of the nodules were taken and sent for culture and histologic examination. Histology showed a pattren of panniculitis with chronic inflammatory cells mixed with areas of polymorphonuclear abscesses and necrosis. Culture of the biopsy specimen grew acid-fast bacilli within 4 days, later identified with biochemical and molecular tests as *Mycobacterium chelonae* (subspecies *chelonae*). Polymerase chain reaction-restriction enzyme pattern analysis (PRA) was used ofr molecular identification of mycobacteria. *In vitro* sensitivity tests showed sensitivity to clarithromycin, amikacin, tobramycin, doxycycline, and erythromycin, and resistance to ciprofloxacin, ofloxacin, trimethoprim-sulfamethoxazole, imipenem, and cefoxitin. Oral clarithromycin (500 mg b.d.) was started and after 3 months of therapy, the lesions had cleared completely.—Authors' Abstract

Bannantine, J. P., Huntley, J. F., Miltner, E., Stabel, J. R., and Bermudez, L. E. The *Mycobacterium avium* subsp. paratuberculosis 35 kDa protein plays a role in invasion of bovine epithelial cells. Microbiology **149(Pt 8)** (2003) 2061–2069.

Mycobacterium avium subsp. paratuberculosis (*M. paratuberculosis*) enters intestinal epithelial cells of cattle and other ruminants via a mechanism that remains to be fully elucidated. This study showed that a gene encoding the *M. paratuberculosis* 35 kDa major membrane protein (MMP) is expressed at a higher level in low-oxygen and high-osmolarity conditions that are similar to the environment of the intestine. In addition, cattle with Johne's disease produced antibodies against MMP, suggesting that the protein is present during infection. The gene encoding MMP was cloned and expressed as a fusion protein with the maltose-binding protein (MBP-MMP) in *Escherichia coli*. Rabbit antisera were raised against a *M. paratuberculosis* whole-cell sonicate and MMP-specific antibodies were purified from these sera by affinity chromatography. MMP was localized to the surface of *M. paratuberculosis* by immunoelectron microscopy and by immunoblot analysis of fractionated protein lysates. Both anti-MMP antibodies and MBP-MMP protein inhibited *M. paratuberculosis* invasion of cultured Madin-Darby bovine kidney cells by 30%. In similar invasion experiments with *M. paratuberculosis* incubated in low oxygen tension, these antibodies and protein decreased invasion by 60%. Collectively, these data show that the 35 kDa MMP is a surface exposed protein that plays a role in invasion of epithelial cells. The authors suggest that the MMP is a virulence factor of *M. paratuberculosis* that may be important in the initiation of infection *in vivo*.—Authors' Abstract

Bull, T. J., McMinn, E. J., Sidi-Boumedine, K., Skull, A., Durkin, D., Neild, P., Rhodes, G., Pickup, R., and Hermon-Taylor, J. Detection and verification of *Mycobacterium avium* subsp. paratuberculosis in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. J. Clin. Microbiol. **41(7)** (2003) 2915–2923.

Mycobacterium avium subsp. paratuberculosis is a robust and phenotypically versatile pathogen which causes chronic inflammation of the intestine in many species, including primates. *M. avium* subsp. paratuberculosis infection is widespread in domestic livestock and is present in retail pasteurized cows' milk in the United Kingdom and, potentially, elsewhere. Water supplies are also at risk. The involvement of *M. avium* subsp. paratuberculosis in Crohn's disease (CD) in humans has been uncertain because of the substantial difficulties in detecting this pathogen. In its Ziehl-Neelsen staining-negative form, *M. avium* subsp. paratuberculosis is highly resistant to chemical and enzymatic lysis. The present study describes the development of optimized sample processing and DNA extraction procedures with fresh human intestinal mucosal biopsy specimens which ensure access to *M. avium* subsp. paratuberculosis DNA and maximize detection of these low-

abundance pathogens. Also described are two nested PCR methodologies targeted at IS900, designated IS900[L/AV] and IS900[TJ1-4], which are uniquely specific for IS900. Detection of *M. avium* subsp. paratuberculosis in mucosal biopsy specimens was also evaluated by using mycobacterial growth indicator tube (MGIT) cultures (Becton Dickinson). IS900[L/AV] PCR detected *M. avium* subsp. paratuberculosis in 34 of 37 (92%) patients with CD and in 9 of 34 (26%) controls without CD (noninflammatory bowel disease [nIBD] controls) ($p = 0.0002$; odds ratio = 3.47). *M. avium* subsp. paratuberculosis was detected by IS900[L/AV] PCR in MGIT cultures after 14 to 88 weeks of incubation in 14 of 33 (42%) CD patients and 3 of 33 (9%) nIBD controls ($p = 0.0019$; odds ratio = 4.66). Nine of 15 (60%) MGIT cultures of specimens from CD patients incubated for more than 38 weeks were positive *for M. avium* subsp. paratuberculosis. In each case the identity of IS900 from *M. avium* subsp. paratuberculosis was verified by amplicon sequencing. The rate of detection of *M. avium* subsp. paratuberculosis in individuals with CD is highly significant and implicates this chronic enteric pathogen in disease causation.—Authors' Abstract

Cynamon, M. H., Elliott, S. A., DeStefano, M. S., and Yeo, A. E. Activity of clarithromycin alone and in combination in a murine model of *Mycobacterium kansasii* infection. J. Antimicrob. Chemother. **52(2)** (2003) 306–307.

Activities of clarithromycin alone and in combination with rifampicin, gatifloxacin or linezolid were evaluated against *Mycobacterium kansasii* in a murine infection model. Clarithromycin was the most active single agent. Rifampicin and gatifloxacin had similar activities, but were less active than clarithromycin. Clarithromycin in combination with rifampicin was the most active combination therapy.—Authors' Abstract

Fujita, J., Ohtsuki, Y., Shigeto, E., Suemitsu, I., Yamadori, I., Bandoh, S., Shiode, M., Nishimura, K., Hirayama, T., Matsushima, T., Fukunaga, H., and Ishida, T. Pathological findings of bronchiectases caused by *Mycobacterium avium* intracellulare complex. Respir. Med. **97(8)** (2003) 933–938.

It has been argued whether bronchiectasis is truly caused by MAC infection or just a predisposed condition in which MAC colonizes. Our present study was designed to evaluate the pathological findings of bronchiectases caused by *Mycobacterium avium* intracellulare complex (MAC) lung infection and to demonstrate MAC in the lesion of bronchiectases. A retrospective study was performed in nine cases with positive cultures for MAC in whom lung resections were performed. A determination of whether or not MAC caused pulmonary disease was made using the 1997 criteria required by the American Thoracic Society. In addition, MAC were cultured from all nine lung specimens. Pathological findings of bronchiectases were evaluated in these nine patients. Destruction of bronchial cartilage and smooth muscles layer, obstruction of airway by granulomas, and ulceration of bronchial mucosa were frequently observed. Our present study demonstrates that destruction of fundamental bronchial structure due to extensive granuloma formation throughout the airways was likely the main cause of bronchiectases in MAC infection.—Authors' Abstract

Garofalo, R., Chadwick, E. G., and Yogev, R. *Mycobacterium gordonae* bacteremia in an asymptomatic adolescent with acquired immunodeficiency syndrome. Pediatr. Infect. Dis. J. **22(6)** (2003) 569–570.

Mycobacterium gordonae is historically viewed as an organism with low pathogenic potential, but it has increasingly become implicated in clinical disease in immunocompromised hosts. Illness related to *M. gordonae* infection ranges from localized infections to rare cases of disseminated disease. This report describes treatment of the first case of occult *M. gordonae* bacteremia in an adolescent with AIDS.—Authors' Abstract

Guarner, J., Bartlett, J., Whitney, E. A., Raghunathan, P. L., Stienstra, Y., Asamoa, K., Etuaful, S., Klutse, E., Quarshie, E., van der Werf, T. S., van der Graaf, W. T., King, C. H., and Ashford, D. A. Histopathologic features of *Mycobacterium ulcerans* infection. Emerg. Infect. Dis. **9(6)** (2003) 651–656.

Because of the emergence of Buruli ulcer disease, the World Health Organization launched a Global Buruli Ulcer Initiative in 1998. This indolent skin infection is caused by *Mycobacterium ulcerans*. During a study of risk factors for the disease in Ghana, adequate excisional skin-biopsy specimens were obtained from 124 clinically suspicious lesions. Buruli ulcer disease was diagnosed in 78 lesions since acid-fast bacilli (AFB) were found by histopathologic examination. Lesions with other diagnoses included filariasis (3 cases), zygomycosis (2 cases), ulcerative squamous cell carcinomas (2 cases), keratin cyst (1 case), and lymph node (1 case). Thirty-seven specimens that did not show AFB were considered suspected Buruli ulcer disease cases. Necrosis of subcutaneous tissues and dermal collagen were found more frequently in AFB-positive specimens compared with specimens from suspected case-patients ($p < 0.001$). Defining histologic criteria for a diagnosis of Buruli ulcer disease is of clinical and public health importance since it would allow earlier treatment, leading to less deforming sequelae.—Authors' Abstract

Haverkort, F. Australian Mycobacterium Reference Laboratory Network; Special Interest Group in Mycobacteria within the Australian Society for Microbiology. National atypical mycobacteria survey, 2000. Commun. Dis. Intell. **27(2)** (2003) 180–189.

Infections with atypical mycobacteria in Australia during 2000 occurred at a rate of 1.8 cases per 100,000 population. The main sites of disease were the respiratory tract, soft tissue, and the lymphatics. The *Mycobacterium avium* complex was the most common group of mycobacteria isolated from respiratory, lymphatic sites, and blood. The rapidly growing mycobacteria, predominantly the *M. fortuitum-M. abscessus-M. chelonae* group were the most common soft tissue infections. Atypical mycobacteria were isolated from significant numbers of sputum 'smear positive' patients, requiring further tests to exclude *M. tuberculosis*. Geographical differences were observed for some *Mycobacterium* species, notably the isolation of *M. haemophilum* from Western Australia, and *M. ulcerans* from Victoria and Queensland. Newer molecular techniques, while improving precision and accuracy of identification, raise additional questions about the ecology of the atypical mycobacteria and their role in disease.—Author's Abstract

Horwitz, M. E., Uzel, G., Linton, G. F., Miller, J. A., Brown, M. R., Malech, H. L., and Holland, S. M. Persistent *Mycobacterium avium* infection following nonmyeloablative allogeneic peripheral blood stem cell transplantation for interferon-gamma receptor-1 deficiency. Blood **102(7)** (2003) 2692–2694.

Interferon-gamma receptor-1 (IFNgammaR1) deficiency is a rare inherited immunodeficiency. We performed a nonmyeloablative allogeneic stem cell transplantation on a boy with complete IFNgammaR1 deficiency and refractory disseminated *Mycobacterium avium* infection. Despite the patient's profound immune defect, early donor stem cell engraftment was low. Full donor engraftment was accomplished only following multiple donor lymphocyte infusions. Detection of IFNgammaR1 expression on peripheral blood monocytes and neutrophils corresponded with establishment of stable, complete donor hematopoietic chimerism. However, expression of, and signaling through IFNgammaR1 disappeared shortly thereafter. Disseminated *Mycobacterium avium* infection persisted and the patient died. Coculture of *Mycobacterium avium* with normal myeloid cells resulted in an IFNgamma signaling defect similar to that observed *in vivo*. Active disseminated *Mycobacterium avium* infection may significantly compromise normal immune reconstitution following allogeneic stem cell transplantation. Patients with IFNgammaR1 deficiency should receive transplants

before developing refractory mycobacterial infections.—Authors' Abstract

Kullavanijaya, P., Rattana-Apiromyakij, N., Sukonthapirom-Napattalung, P., Sirimachand, S., and Duangdeeden, I. Disseminated *Mycobacterium chelonae* cutaneous infection: recalcitrant to combined antibiotic therapy. J. Dermatol. **30(6)** (2003) 485–491.

A 25-year-old Thai housewife had a history of tuberculosis of the lymph nodes for six years that had been successfully treated with a course of anti-TB drugs. She developed several red, circumscribed, infiltrative plaques composed of umbilicated papules and pustules on her face and upper part of the body with cervical lymphadenopathy six months later. A pus smear from the lesion grew acid fast bacilli (AFB). Histopathological examination showed a mixed cell granuloma suggestive of infection. A T cell study showed a low CD4 count, and multi skin tests indicated cutaneous anergy. Culture from a biopsy specimen taken from the skin lesion grew *M. chelonae;* the cultures from blood, urine, and bone marrow. The lesions were not responsive to an anti TB drug given for 2 months based upon the results of the AFB positive pus smear before the culture and sensitivity reports were obtained. Since then the patient was treated with antibiotics according to the results of the sensitivity tests. A combination of amikacin and clarithromycin was started and hyperthermic therapy was later added with a partial response. Based upon the sensitivity test, kanamycin was introduced but had to be stopped because of ototoxicity. Sparfloxacin was used with an effective result but was discontinued for economic reasons. Finally, clarithromycin in combination with clofazimine and cryotherapy were given for a year before the lesions healed completely. It took a three years duration for the total course of treatment for this patient. She is still in remission after two years of follow-up period. This extensive cutaneous *M. chelonae* infection needed a prolonged combination of antibiotics with the addition of cryotherapy for the non-responsive lesions.—Authors' Abstract

Liou, J. H., Huang, P. Y., Hung, C. C., and Hsiao, C. H. Mycobacterial spindle cell pseudotumor of skin. J. Formos. Med. Assoc. **102(5)** (2003) 342–345.

Spindle cell pseudotumors may occur due to mycobacterial infection in immunocompromised hosts, particularly those with acquired immunodeficiency syndrome (AIDS). Most of the reported mycobacterial spindle cell pseudotumors were found in the lymph nodes. We report a case of spindle cell pseudotumor in a 37-year-old man with AIDS who presented with a firm nodule over his right arm. Histologically, the tumor was composed of proliferative spindle cells admixed with histiocytes and inflammatory cells. Ziehl-Neelsen stain revealed many acid-fast bacilli in the spindle cells and histiocytes. The acid-fast bacilli were shown to be *Mycobacterium avium* intracellulare by culture and sequencing of the polymerase chain reaction product of mycobacterial 65-kDa heat shock protein gene. Immunohistochemically, the spindle cells were reactive to CD68, suggesting macrophage differentiation of these cells. It is important for pathologists to recognize this unusual manifestation of mycobacterial infection in immunocompromised patients and avoid mistaking the lesion for a mesenchymal neoplasm.—Authors' Abstract

Mauriello, J. A. Jr. and Atypical Mycobacterial Study Group. Atypical mycobacterial infection of the periocular region after periocular and facial surgery. Ophthal. Plast. Reconstr. Surg. **19(3)** (2003) 182–188.

PURPOSE: To delineate the clinicopathologic features of patients who have atypical mycobacterial infections of the periorbital region after periocular and facial surgery and to define the sequelae after treatment and their management. METHODS: A case series of patients from 7 practices of ophthalmic plastic and reconstructive surgeons was analyzed retrospectively. RESULTS: Thirteen patients had infection in the following clinical settings: 8 patients had infections after blepharoplasty, 2 patients had infections that involved the anophthalmic socket, 1 patient had orbital

cellulitis after orbital fracture repair with an alloplastic implant, and 2 patients had infections involving the lacrimal system, one after silicone tube insertion and the other after dacryocystorhinostomy with silicone tube intubation. Sequelae of infection included eyelid retraction and ectropion requiring surgical repair (two patients) and enophthalmos (one patient). Twelve of 13 patients required extensive antibiotic therapy. One infection resolved after local excision of eyelid lesions. Another patient had recurrent infection after 4 weeks of antibiotic treatment. CONCLUSIONS: Delayed infection with erythematous nodules, particularly when a foreign body is implanted weeks after periocular surgery, should arouse suspicion of an atypical mycobacterial infection. Delayed infection after blepharoplasty may mimic a chalazion, develop in a sutured incision, or occur without any inflammatory signs. Orbital abscess formation may occur in the setting of transconjunctival blepharoplasty. Cultures for acid-fast bacilli and excisional biopsy of nodules with performance of acid-fast stains may be necessary for diagnosis. The selection of systemic antibiotic therapy, usually clarithromycin, and the length of treatment should be guided by results of culture and sensitivity laboratory studies, biopsy results, and clinical response to treatment. Surgical removal of any implanted foreign bodies should be performed expeditiously. Consultation with an infectious disease specialist may be useful in selected cases. Sequelae of infection may include eyelid scarring and retraction and enophthalmos.—Authors' Abstract

Molecular & Genetic Studies

Agarwal, N. and Tyagi, A. K. Role of 5′-TGN-3′ motif in the interaction of mycobacterial RNA polymerase with a promoter of 'extended -10' class. FEMS Microbiol. Lett. **225(1)** (2003) 75–83.

In a systematic approach to understand the transcriptional machinery of mycobacteria, we had previously isolated and characterized mycobacterial promoter regions. In this study, we have investigated molecular interactions between mycobacterial RNA polymerase holoenzyme, reconstituted with different sigma subunits and the promoter element of the *Mycobacterium tuberculosis* gene pknH (Rv1266c), a representative of promoters belonging to the 'extended-10' class. *In vitro* transcription assays using the pknH promoter and reconstituted RNA polymerase holoenzyme demonstrated that transcription from the pknH promoter is specifically initiated by sigmaA, the principal sigma factor of mycobacteria. DNase I protection assay and deletion studies with the pknH promoter revealed that the minimal region required for optimal transcription carries the sequence from position –37 to position +6. Moreover, mutation in the TGN motif of the pknH promoter resulted in the loss of >75% of its activity. Binding of RNA polymerase with wild-type promoter as well as its TG- mutant revealed that the TGN motif is required for the transition from a close complex into an open complex. Further, it was observed that the presence of the TGN motif reduces the thermal energy required for the conversion of a close complex into an open complex, necessary for initiation of transcription.

Ahmed, N., Alam, M., Abdul Majeed, A., Asad Rahman, S., Cataldi, A., Cousins, D., and Hasnain, S. E. Genome sequence based, comparative analysis of the fluorescent amplified fragment length polymorphisms (FAFLP) of tubercle bacilli from seals provides molecular evidence for a new species within the *Mycobacterium tuberculosis* complex. Infect Genet Evol. **2(3)** (2003) 193–199.

Tuberculosis in seals is caused by a member of the *Mycobacterium tuberculosis* complex referred to as the 'seal bacillus.' Fluorescent amplified-fragment length polymorphism (FAFLP) analysis was applied to isolates from four Australian and

six Argentinean seals and compared with FAFLP pattern for standard strains belonging to the *M. tuberculosis* complex. The FAFLP profiles derived from EcoRI/MseI restricted fragments of blind coded DNA samples differentiated the seal bacillus from other members of the *M. tuberculosis* complex. According to the phylogenetic analysis performed using FAFLP data, seal bacilli appear to have diverged significantly from other members of *the M. tuberculosis* complex. We describe the suitability of a panel of 19 highly polymorphic markers for rapid identification and comparative genomic analyses of the seal bacillus strains. It is likely that these bacilli got separated from the *M. tuberculosis* lineage as a result of different insertion deletion events occurring on a genome wide scale. Our analysis reveals that the seal bacillus and *M. bovis* are genetically related and therefore, might have originated from a common ancestor. Our data additionally support the hypothesis that seal bacillus occupies a unique taxonomic position within the *M. tuberculosis* complex.—Authors' Abstract

Bamaga, M. S., Wright, D. J., and Zhang, H. An assessment of a multiplex PCR assay for differentiating clinically important mycobacteria based on pncA gene variation. Mol. Cell. Probes. **17(2–3)** (2003) 69–75.

The pncA genes in mycobacteria are responsible for the production of pyrazinamidase (PZase). In *Mycobacterium tuberculosis*, PZase hydrolyses pyrazinamide (PZA) to pyrazonic acid, a compound that possesses bactericidal activity against tubercle bacilli. Nucleotide sequences of pncA genes found within mycobacteria where aligned in an effort to ascertain the significance of any variability in sequence. Three sets of primers (one degenerate and five consensus sequences) were designed and employed in a multiplex PCR assay to amplify the pncA region in seven clinically common mycobacteria. The banding patterns generated from each species in conjunction with PZase activity tests demonstrated that the mycobacterial species examined could be clearly identified and differentiated from one another. Although not yet tested with clinical isolates, the combination of these two assays has provided a promising discriminatory tool for the identification of commonly encountered clinical mycobacteria species.—Authors' Abstract

Betts, J. C., McLaren, A., Lennon, M. G., Kelly, F. M., Lukey, P. T., Blakemore, S. J., and Duncan, K. Signature gene expression profiles discriminate between isoniazid-, thiolactomycin-, and triclosan-treated *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **47(9)** (2003) 2903–2913.

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Blokpoel, M. C., O'Toole, R., Smeulders, M. J., and Williams, H. D. Development and application of unstable GFP variants to kinetic studies of mycobacterial gene expression. J. Microbiol. Methods. **54(2)** (2003) 203–211.

Unstable variants of green fluorescent protein (GFP) tagged with C-terminal extensions, which are targets for a tail specific protease, have been described in *Escherichia coli* and *Pseudomonas putida* [Appl. Envir. Microbiol. 64 (1998) 2240]. We investigated whether similar modifications to flow cytometer optimized GFP (GFPmut2) could be used to generate unstable variants of GFP for gene expression studies in mycobacteria. We constructed GFP variants in a mycobacterial shuttle vector under the control of the regulatory region of the inducible *Mycobacterium smegmatis* acetamidase gene. GFP expression was induced by the addition of acetamide and the stability of the GFP variants in *M. smegmatis*, following the removal of the inducer to switch off their expression, was determined using spectrofluorometry and flow cytometry. We demonstrate that, compared to the GFPmut2 (half-lives >7 days), the modified GFP variants exhibit much lower half-lives (between 70 and 165 min) in *M. smegmatis*. To investigate their utility in the measurement of mycobacterial gene expression, we cloned the promoter region of a putative amino acid efflux pump gene, lysE (Rv1986), from *Myco-*

bacterium tuberculosis together with the divergently transcribed, putative lysR-type regulator gene (Rv1985c) upstream of one of the unstable GFP variants. We found that the expression kinetics of the lysRE-gfp fusion were identical throughout the *M. smegmatis* growth curve to those measured using a conventional lysRE-xylE reporter fusion, peaking upon entry into stationary phase. In addition, it was established that the tagged GFP variants were also unstable in *Mycobacterium bovis* BCG. Thus, we have demonstrated that unstable GFP variants are suitable reporter genes for monitoring transient gene expression in fast- and slow-growing mycobacteria.—Authors' Abstract

Boitel, B., Ortiz-Lombardia, M., Duran, R., Pompeo, F., Cole, S. T., Cervenansky, C., and Alzari, P. M. PknB kinase activity is regulated by phosphorylation in two Thr residues and dephosphorylation by PstP, the cognate phospho-Ser/Thr phosphatase, in *Mycobacterium tuberculosis*. Mol. Microbiol. **49(6)** (2003) 1493–1508.

Bacterial genomics revealed the widespread presence of eukaryotic-like protein kinases and phosphatases in prokaryotes, but little is known on their biochemical properties, regulation mechanisms and physiological roles. Here we focus on the catalytic domains of two trans-membrane enzymes, the Ser/Thr protein kinase PknB and the protein phosphatase PstP from *Mycobacterium tuberculosis*. PstP was found to specifically dephosphorylate model phospho-Ser/Thr substrates in a Mn2+-dependent manner. Autophosphorylated PknB was shown to be a substrate for Pstp and its kinase activity was affected by PstP-mediated dephosphorylation. Two threonine residues in the PknB activation loop, found to be mostly disordered in the crystal structure of this kinase, namely Thr171 and Thr173, were identified as the target for PknB autophosphorylation and PstP dephosphorylation. Replacement of these threonine residues by alanine significantly decreased the kinase activity, confirming their direct regulatory role. These results indicate that, as for eukaryotic homologues, phosphorylation of the activation loop provides a regulation mechanism of mycobacterial kinases and strongly suggest that PknB and PstP could work as a functional pair *in vivo* to control mycobacterial cell growth.—Authors' Abstract

Carvalho, W. S., Spindola de Miranda, S., Costa, K. M., Araujo, J. G., Augusto, C. J., Pesquero, J. B., Pesquero, J. L., and Gomes, M. A. Low-stringency single-specific-primer PCR as a tool for detection of mutations in the rpoB gene of rifampin-resistant *Mycobacterium tuberculosis*. J. Clin. Microbiol. **41(7)** (2003) 3384–3386.

By the low-stringency single-specific-primer PCR technique, a highly sensitive and rapid method for diagnosis of rifampin resistance in *Mycobacterium tuberculosis* was established. Seven rifampin-resistant and five rifampin-susceptible specimens were analyzed. Rifampin resistance was determined by MIC measurement. A complex electrophoretic pattern consisting of many bands was obtained for both susceptible and rifampin-resistant isolates. The same pattern was obtained for all of the susceptible specimens, but differences between resistant and susceptible isolates were found. DNA sequencing showed that a particular mutation produces a specific electrophoretic pattern.—Authors' Abstract

Duan, H. F., Zhou, X. H., Ma, Y., Li, C. Y., Chen, X. Y., Gao, W. W., Zheng, S. H. [A study on the association of 3′UTR polymorphisms of NRAMP1 gene with susceptibility to tuberculosis in Hans.] Zhonghua Jie He He Hu Xi Za Zhi. **26(5)** (2003) 286–289. [Article in Chinese].

OBJECTIVE: To determine whether 3′UTR polymorphisms of the NRAMP1 gene are associated with tuberculosis in Hans. METHODS: 3′UTR polymorphisms of NRAMP1 gene were typed by PCR-RFLP among 147 patients with active tuberculosis and 145 healthy individuals. The relationship between 3′UTR polymorphisms and susceptibility to tuberculosis was studied, and cases were grouped according to genotypes. RESULTS: In the tu-

berculosis patients, genotype TGTG/TGTG, TGTG/TGTG deleted, and TGTG deleted/TGTG deleted were observed in 95, 50 and 2 cases respectively, while the genotypes of the healthy controls were TGTG/TGTG in 115, TGTG/TGTG deleted in 29 and TGTG deleted/TGTG deleted in 1 case. The frequency of the genotype TGTG/TGTG was found more often among controls than that in patients (chi(2) = 7.79, p <0.01). The frequency of allele TGTG and the frequency of variant allele were 0.85 and 0.15 respectively. CONCLUSIONS: 3′UTR polymorphisms of NRAMP1 gene are associated with susceptibility to tuberculosis in Hans. The variant allele observed in Hans is more common than that in Caucasians. These observations might explain in part why Hans have greater susceptibility to tuberculosis than Caucasians.—Authors' Abstract

Dubnau, E. and Smith, I. *Mycobacterium tuberculosis* gene expression in macrophages. Microbes Infect. **5(7)** (2003) 629–637.

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Gaafar, A., Unzaga, M. J., Cisterna, R., Clavo, F. E., Urra, E., Ayarza, R., and Martin, G. Evaluation of a modified single-enzyme amplified-fragment length polymorphism technique for fingerprinting and differentiating of Mycobacterium kansasii type I isolates. J. Clin. Microbiol. **41(8)** (2003) 3846–3850.

The usefulness of single-enzyme amplified-fragment length polymorphism (AFLP) analysis for the subtyping of *Mycobacterium kansasii* type I isolates was evaluated. This simplified technique classified 253 type I strains into 12 distinct clusters. The discriminating power of this technique was high, and the technique easily distinguished between the epidemiologically unrelated control strains and our clinical isolates. Overall, the technique was relatively rapid and technically simple, yet it gave reproducible and discriminatory results. This technique provides a powerful typing tool which may be helpful in solving many questions concerning the reservoirs, pathogenicities, and modes of transmission of these isolates.—Authors' Abstract

Ganesh, N. and Muniyappa, K. Characterization of DNA strand transfer promoted by *Mycobacterium smegmatis* RecA reveals functional diversity *with Mycobacterium tuberculosis* RecA. Biochemistry. **42(23)** (2003) 7216–7225.

The RecA-like proteins constitute a group of DNA strand transfer proteins ubiquitous in eubacteria, eukarya, and archaea. However, the functional relationship among RecA proteins is poorly understood. For instance, *Mycobacterium tuberculosis* RecA is synthesized as a large precursor, which undergoes an unusual protein-splicing reaction to generate an active form. Whereas the precursor was inactive, the active form promoted DNA strand transfer less efficiently compared to EcRecA. Furthermore, gene disruption studies have indicated that the frequencies of allele exchange are relatively lower in *Mycobacterium tuberculosis* compared to *Mycobacterium smegmatis*. The mechanistic basis and the factors that contribute to differences in allele exchange remain to be understood. Here, we show that the extent of DNA strand transfer promoted by the *M. smegmatis* RecA *in vitro* differs significantly from that of *M. tuberculosis* RecA. Importantly, *M. smegmatis* RecA by itself was unable to promote strand transfer, but cognate or noncognate SSBs rendered it efficient even when added prior to RecA. In the presence of SSB, MsRecA or MtRecA catalyzed strand transfer between ssDNA and varying lengths of linear duplex DNA with distinctly different pH profiles. The factors that were able to suppress the formation of DNA networks greatly stimulated strand transfer reactions promoted by MsRecA or MtRecA. Although the rate and pH profiles of dATP hydrolysis catalyzed by MtRecA and MsRecA were similar, only MsRecA was able to couple dATP hydrolysis to DNA strand transfer. Together, these results provide insights into the functional diversity in DNA strand transfer promoted by RecA proteins of pathogenic and nonpathogenic species of mycobacteria.—Authors' Abstract

Garcia de Viedma, D. Rapid detection of resistance in *Mycobacterium tuberculosis*: a review discussing molecular approaches. Clin. Microbiol. Infect. **9(5)** (2003) 349–359.

The last few years have seen the development of several molecular designs to search for mutations encoding resistance to antituberculous drugs in *Mycobacterium tuberculosis*. Most of these are highly efficient for RIF-r detection and are well adapted to search for the most relevant INH-R mutations. In this review, these new molecular approaches are explained and are presented according to the molecular strategies on which they are based. In this sense, techniques based on DNA-sequencing, electrophoresis and hybridization are reviewed and the newer designs based on real-time PCR and microarrays are also included. Molecular methods are sure to transform standard approaches to the issue of resistance in the mycobacteriology laboratory. This will allow laboratories to speed up the performance of resistance assays and provide access to essential information for highly refined detection, follow-up and management of antibiotic resistance in *M. tuberculosis*.—Author's Abstract

Gilleron, M., Quesniaux, V. F., and Puzo, G. Acylation state of the phosphatidylinositol hexamannosides from *Mycobacterium bovis* bacillus Calmette Guerin and *Mycobacterium tuberculosis* H37Rv and its implication in Toll-like receptor response. J. Biol. Chem. **278(32)** (2003) 29880–29889.

The dimannoside (PIM2) and hexamannoside (PIM6) phosphatidyl-myo-inositol mannosides are the two most abundant classes of PIM found in *Mycobacterium bovis* bacillus Calmette Guerin, *Mycobacterium tuberculosis* H37Rv, and *Mycobacterium smegmatis* 607. Recently, these long known molecules received a renewed interest due to the fact that PIM2 constitute the anchor motif of an important constituent of the mycobacterial cell wall, the lipoarabinomannans (LAM), and that both LAM (phosphoinositol-capped LAM) and PIM are agonists of Toll-like receptor 2 (TLR2), a pattern recognition receptor involved in innate immunity. Due to the biological importance of these molecules, the chemical structure of PIM was revisited. The structure of PIM2 was recently published (Gilleron, M., Ronet, C., Mempel, M., Monsarrat, B., Gachelin, G., and Puzo, G. (2001) J. Biol. Chem. 276, 34896–34904). Here we report the purification and molecular characterization of PIM6 in their native form. For the first time, four acyl forms of this molecule have been purified, using hydrophobic interaction chromatography. Mono- to tetra-acylated molecules were identified in *M. bovis* bacillus Calmette Guerin, *M. tuberculosis* H37Rv, and *M. smegmatis* 607 using a sophisticated combination of analytical tools, including matrix-assisted laser desorption/ionization-time of flight-mass spectrometry and two-dimensional homo- and heteronuclear NMR spectroscopy. These experiments revealed that the major acyl forms are similar to the ones described for PIM2. Finally, we show that PIM6, like PIM2, activate primary macrophages to secrete TNF-alpha through TLR2, irrespective of their acylation pattern, and that they signal through the adaptor MyD88.—Authors' Abstract

Hawkey, P. M., Smith, E. G., Evans, J. T., Monk, P., Bryan, G., Mohamed, H. H., Bardhan, M., and Pugh, R. N. Mycobacterial interspersed repetitive unit typing of *Mycobacterium tuberculosis* compared to IS6110-based restriction fragment length polymorphism analysis for investigation of apparently clustered cases of tuberculosis. J. Clin. Microbiol. **41(8)** (2003) 3514–3520.

An evaluation of the utility of IS6110-based restriction fragment length polymorphism (RFLP) typing compared to a combination of variable number tandem repeat (VNTR) typing and mycobacterial interspersed repetitive unit (MIRU) typing was undertaken. A total of 53 patient isolates of *Mycobacterium tuberculosis* from four presumed episodes of cross-infection were examined. Genomic DNA was extracted from the isolates by a cetyl trimethylammonium bromide method. The number of copies of tandem repeats of the five loci ETR(A) to

ETR(E) and 12 MIRU loci was determined by PCR amplification and agarose gel electrophoresis of the amplicons. VNTR typing identified the major clusters of strains in the three investigations in which they occurred (each representing a different evolutionary clade: 32333, 42235, and 32433). The majority of unrelated isolates (by epidemiology and RFLP typing) were also identified by VNTR typing. The concordance between the RFLP and MIRU typing was complete, with the exception of two isolates with RFLP patterns that differed by one band each from the rest of the major epidemiologically linked groups of isolates in investigation A. All of these isolates had identical MIRU and VNTR types. A further pair of isolates differed in the number of tandem repeat copies at two MIRU alleles but had identical RFLP patterns. The speed of the combined VNTR and MIRU typing approach enabled results for some of the investigations to be supplied in "real time," influencing choices in contact tracing. The ease of comparison of results of MIRU and VNTR typing, which are recorded as single multidigit numbers, was also found to greatly facilitate investigation management and the communication of results to health care professionals.—Authors' Abstract

He, X. Y., Zhuang, Y. H., Zhang, X. G., and Li, G. L. Comparative proteome analysis of culture supernatant proteins of *Mycobacterium tuberculo*sis H37Rv and H37Ra. Microbes Infect. **5(10)** (2003) 851–856.

To examine the virulence factors of *Mycobacterium tuberculosis* H37Rv, the proteome was used to characterize the differences in protein expression between virulent *M. tuberculosis* H37Rv and attenuated *M. tuberculosis* H37Ra. Two-dimensional gel electrophoresis was performed to separate culture supernatant proteins extracted from *M. tuberculosis* H37Rv and *M. tuberculosis* H37Ra. The protein spots of interest were identified by mass spectrometry, and then the genes encoding the identified proteins were cloned and sequenced. Comparison of silver-stained gels showed that three well-resolved protein spots were present in *M. tuberculosis* H37Rv but absent from *M. tuberculosis* H37Ra. Protein spot no. 1 was identified as Rv2346c. Protein spot no. 2 was identified as Rv2347c, Rv1197, Rv1038c, and Rv3620c, which shared significant homology and had the same peptide fingerprinting using tryptic digestion. No *M. tuberculosis* protein matched protein spot no. 3. Rv2346c, Rv2347c, Rv1038c, and Rv3620c of *M. tuberculosis* H37Rv were located on the *M. tuberculosis* H37Ra chromosome, and multiple mutations were observed in the corresponding areas of *M. tuberculosis* H37Ra. Codon 59 (CAG, Gln) of Rv2347c and Rv3620c was replaced by termination codon (TAG) in *M. tuberculosis* H37Ra, which probably terminated the polypeptide elongation. These results demonstrate the importance of studying the gene products of *M. tuberculosis* and show that subtle differences in isogenic mutant strains might play an important role in identifying the attenuating mutations.—Authors' Abstract

Iwamoto, T., Sonobe, T., and Hayashi, K. Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis* complex, *M. avium*, and *M. intracellulare* in sputum samples. J. Clin. Microbiol. **41(6)** (2003) 2616–2622.

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method in which reagents react under isothermal conditions with high specificity, efficiency, and rapidity. We used LAMP for detection of *Mycobacterium tuberculosis* complex, *Mycobacterium avium*, and *Mycobacterium intracellulare* directly from sputum specimens as well as for detection of culture isolates grown in a liquid medium (MGIT; Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) or on a solid medium (Ogawa's medium). Species-specific primers were designed by targeting the gyrB gene, and their specificities were validated on 24 mycobacterial species and 7 nonmycobacterial species. The whole procedure is quite simple, starting with the mixing of all reagents in a single tube, followed by an isothermal reaction during which the reaction mixture is held at 63 degrees C. The resulting amplicons are visualized by adding SYBR Green I

to the reaction tube. The only equipment needed for the amplification reaction is a regular laboratory water bath or heat block that furnishes a constant temperature of 63 degrees C. The assay had a detection limit of 5 to 50 copies of purified DNA with a 60-min incubation time. The reaction time could be shortened to 35 min for the species identification of *M. tuberculosis* complex, *M. avium*, and *M. intracellulare* from a solid-medium culture. Residual DNA lysates prepared for the Amplicor assay (Roche Diagnostics GmbH) from 66 sputum specimens were tested in the LAMP assay. Although the sample size used for the latter assay was small, 2.75 micro l of the DNA lysates, it showed a performance comparable with that of the Amplicor assay, which required 50 micro l of the lysates. This LAMP-based assay is simple, rapid, and sensitive; a result is available in 35 min for a solid-medium culture and in 60 min for a liquid-medium culture or for a sputum specimen that contains a corresponding amount of DNA available for testing.—Authors' Abstract

Kakinuma, K., Fukushima, M., and Kawaguchi, R. Detection and identification of *Escherichia coli*, Shigella, and Salmonella by microarrays using the gyrB gene. Biotechnol. Bioeng. **83(6)** (2003) 721–728.

Commonly, 16S ribosome RNA (16S rRNA) sequence analysis has been used for identifying enteric bacteria. However, it may not always be applicable for distinguishing closely related bacteria. Therefore, we selected gyrB genes that encode the subunit B protein of DNA gyrase (a topoisomerase type II protein) as target genes. The molecular evolution rate of gyrB genes is higher than that of 16S rRNA, and gyrB genes are distributed universally among bacterial species. Microarray technology includes the methods of arraying cDNA or oligonucleotides on substrates such as glass slides while acquiring a lot of information simultaneously. Thus, it is possible to identify the enteric bacteria easily using microarray technology. We devised a simple method of rapidly identifying bacterial species through the combined use of gyrB genes and microarrays. Closely related bacteria were not identified at the species level using 16S rRNA sequence analysis, whereas they were identified at the species level based on the reaction patterns of oligonucleotides on our microarrays using gyrB genes.—Authors' Abstract

Kang, T. J., Kim, S. K., Lee, S. B., Chae, G. T., and Kim, J. P. Comparison of two different PCR amplification products (the 18-kDa protein gene vs. RLEP repetitive sequence) in the diagnosis of *Mycobacterium leprae*. Clin. Exp. Dermatol. **28(4)** (2003) 420–424.

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Kurabachew, M., Sandaa, R. A., Enger, O., and Bjorvatn, B. Sequence analysis in the 23S rDNA region of *Mycobacterium tuberculosis* and related species. J. Microbiol. Methods. **54(3)** (2003) 373–380.

We sequenced a 516 base pair segment in the 23S rRNA gene of 54 *Mycobacterium tuberculosis* isolates, 52 of which were clinical isolates from Ethiopia. Sequence polymorphism was observed with 19 of the 54 strains; the polymorphic sites occurred in less than 2% (9/516) of the total sequence positions. The sequence variations represented base pair substitutions (14/23), insertions (9/23) or both (1/23). Insertions occurred at one site only, whereas substitutions were observed in various regions of the gene. There was no relation between mutational sites and drug susceptibility. However, using information from the GenBank database, comparison between the 23S rDNA sequences of *M. tuberculosis* and the corresponding sequences of other mycobacteria and of related non-mycobacterial species revealed considerable variation, suggesting that this region may provide a target for rapid detection and identification of mycobacteria both at the genus or species level.—Authors' Abstract