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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

Diffey, B., Vaz, M., Soares, M. J., Jacob, A. J. W. and Piers, L. S. The effect of leprosy-induced deformity on the nutritional status of index cases and their household members in rural South India: a socioeconomic perspective. Eur. J. Clin. Nutr. **54** (2000) 643–649.

Objectives: To determine whether the socioeconomic and nutritional status of cured leprosy patients with residual deformity, and their household members, was lower than that of cured leprosy patients without deformity.

Design: Cross-sectional study.

Subjects: One-hundred-fifty-five index cases with deformity; 100 without deformity. Also 616 household members comprising 48% of the total members enumerated.

Measurements: Nutritional status was evaluated using anthropometry. Dìsease characteristics, socioeconomic parameters and household information were recorded using a questionnaire.

Results: Index cases with deformity had lower community acceptance ($p < 0.001$) and employment ($p < 0.001$) than those cases without deformity. Households of index cases with deformity had a lower income ($p < 0.01$) and a lower expenditure on food ($p < 0.05$). The presence of deformity [odds ratio (OR): 2.1–3.2, $p < 0.01$], unemployment (OR: 2.3–4.3, $p < 0.01$) and female gender (OR: 2.4, $p < 0.01$) significantly increased the risk of index cases being undernourished, as judged by body mass index (BMI) alone or BMI and mid-upper arm circumference. A low BMI (<18.5) in the index case significantly increased the odds of other adults (OR 2.2), adolescents (OR 2.9–3.8) and children (OR 2.2) in the household being undernourished.

Conclusions: Cured leprosy index cases with physical deformity are more undernourished than index cases without deformity. This is associated with a reduced expenditure on food, possibly brought on by increased unemployment, and a loss of income. Undernutrition in the index case increases the risk of undernutrition in other members of the family.—Authors' Abstract

Haas, C. J., Zink, A., Palfi, G., Szeimies, U. and Nerlich, A. G. Detection of leprosy in ancient human skeletal remains by molecular identification of *Mycobacterium leprae*. Am. J. Clin. Pathol. **114** (2000) 428–436.

We isolated ancient DNA from skeletal remains obtained from a south German ossuary (approximately 1400–1800 A.D.) and from a 10th century Hungarian cemetery partially indicating macromorphologic evidence of leprosy. In samples taken of 2 skulls from Germany and of 1 hard palate from Hungary: *Mycobacterium leprae*-specific fragments of RLEP1 and RLEP3 were amplified using polymerase chain reaction (PCR), thereby confirming their specificity by sequencing. In another case, PCR with primers targeting IS6110 of *M. tuberculosis* gave positive results only for a mandibular specimen. No signal for any mycobacterial DNA was observed in samples from 2 Hungarian foot bones. In ancient material, osseous involvement of *M. leprae* may be detected and distinguished from other mycobacterial infections by specific PCR. In the small bones of leprous hands and feet, not enough *M. leprae* DNA seems to be present for detection. This supports the view that rhinomaxillary leprous alterations result from direct bacterial involvement, while osseous mutilation of hands and feet result from a nervous involvement and/or secondary infections due to small lacerations of the overlying soft tissues.—Authors' Abstract

Chemotherapy

Mirochnick, M., Clarke, D. F., McNamara, E. R. and Cabral, H. Bioequivalence of a propylene glycol-based liquid dapsone preparation and dapsone tablets. Am. J. Health-System Pharm. **57** (2000) 1775–1777.

The bioequivalence of a proprietary liquid dapsone preparation and commercially available dapsone tablets was studied. Twelve adult volunteers received dapsone doses with 8 oz of water 1 to 2 hr after their usual breakfast.

Each subject received an initial 100 mg dose of a propylene glycol-based liquid preparation of dapsone and, 2 weeks later, a 100 mg dapsone tablet (both from Jacobus Pharmaceutical Company, Princeton, New Jersey, U.S.A.). Blood samples were collected before and at intervals up to 96 hr after the administration of each dose. Serum dapsone concentrations were determined by high performance liquid chromatography, and pharmacokinetic values were calculated by model-independent analysis. The area under the concentration-versus-time curve and the maximum serum concentration for the two formulations met the criteria for bioequivalence. Time to maximum serum concentration tended to be lower for the liquid, but not significantly. The liquid and tablet formulations of dapsone studied were found to be bioequivalent and may be used interchangeably.—Authors' Abstract

Pattyn, S. and Grillone, S. A 6 week quadruple drug regimen for the treatment of multibacillary leprosy. Lepr. Rev. **71** (2000) 43–46.

Results of a regimen of 6 weeks' duration involving the supervised administration of 4 drugs in 136 patients with multibacillary leprosy, who were studied between November 1989 and December 1993 in Belgium, are presented. Patients received daily, 6 days a week, rifampin (600 mg), ofloxacin (400 mg), clofazimine (100 mg) and once a week minocycline (100 mg). At intake there were 18 (13.2%) patients with a bacterial index (BI) = 2, 21 (15.4%) with a BI = 3, 73 (53.6%) with a BI = 4 and 24 (17.6%) with a BI = 5. All patients showed a rapid favorable clinical evolution. No major complications such as hepatitis and complaints of photophobia were observed. Follow up later than 6 years concerned considerably <50% of patients. The mean follow up at year 6 was 4.7 years. During the first 6 years of follow up, 2 relapses were diagnosed at 56 and 60 months, respectively, after the start of treatment, giving rise to a cumulative relapse rate of 2%. Six more relapses were diagnosed at 72, 96, 96, 103, 106 and 111 months, respectively, after the start of therapy. At the start of therapy, the 2 patients relapsing at year 5 had a BI of 5. Among the 6 patients relapsing later, 2 had a BI of 4, the other had a BI of 5.—Trop. Dis. Bull. **97** (2000) 991

Pereira, J., Hidalgo, P., Ocqueteau, M., Blacutt, M., Marchesse, M., Nien, Y., Letelier, L. and Mezzano, D. Glycoprotein Ib/IX complex is the target in rifampicin-induced immune thrombocytopenia. Br. J. Haematol. **110** (2000) 907–910.

Thrombocytopenia is a major adverse effect of several drug treatments. Rifampin has been recognized as a cause of immune thrombocytopenia during intermittent high-dose therapy. We characterized the antibody of a patient who presented with purpura and thrombocytopenia during treatment of tuberculosis with rifampin. Drug-dependent binding of the antibody to platelets was demonstrated by flow cytometry. In a glycoprotein-specific immunoassay, the binding epitope of the IgG antibody was found in the glycoprotein Ib/IX complex, using four different monoclonal antibodies (mAbs) against various epitopes on the GPIb/IX complex, as well as mAbs against GPIIb/IIIa, GPIa/IIa and GPIV. By immunoprecipitation of biotin-labelled platelets, reactivity of the antibody with GPIb/IX was found only in the presence of the drug. These findings clearly demonstrate that rifampin induces the formation of drug-dependent antibodies capable of caus-

ing thrombocytopenia. The binding site of the rifampin-dependent antibody, located in the GPIb/IX complex, seems to be a favored target for antibodies induced by different drugs.—Authors' Abstract

Clinical Sciences

Cabrini, J. M., Macia, A., Sudarovich, M., Bressanelli, A. and Bottasso, O. A. [Bacteriologic evolution of lepromatous leprosy in relation to the presence of episodes of erythema nodosum leprosum.] Rev. Leprol. Fontilles **22** (2000) 451–462.

Erythema nodosum leprosum (ENL) is a common complication of lepromatous leprosy (LL) that appears to be triggered by immune complex deposition in the vascular wall along with the emergence of cell-mediated immune phenomena, some of them specific to *M. leprae.* To find out whether occurrence of ENL reactions could facilitate a better disease outcome, an evaluation was made on the time by which LL patients attained bacteriological negativity (TBN), depending on the appearance of ENL episodes during treatment. The study consisted of a retrospective analysis of clinical records from 106 patients, mostly sulfone recipients, 27 cases undergoing no ENL episodes and 79 patients who had developed reactions of distinct severity and frequency. Groups were similar as to age, sex distribution, treatment schedule and degree of involved skin, showing differences in clinical varieties, namely, more macular cases in LL without ENL. These patients had a significantly shorter TBN (3.2 ± 0.4 years) than LL with ENL (6.1 ± 0.3, mean ± S.E.M.). A further division of the latter group according to the intensity and frequency of ENL episodes revealed no differences in TBN, which appeared similar to the one recorded in the entire group. Occurrence of ENL reactions does not seem to accelerate the chemotherapy-mediated bacillary clearing.—Authors' English Summary

Elbeialy, A., Strassburger Lorna, K., Atsumi, T., Bertolaccini, M. L., Amengual, O., Hanafi, M., Khamashta, M. A. and Hughes, G. R. V. Antiphospholipid antibodies in leprotic patients: a correlation with disease manifestations. Clin. Exp. Rheumatol. **18** (2000) 492.

Objectives: Previous studies showed that antiphospholipid antibodies (aPL) are frequent in the sera of leprosy patients and are most probably directed against body tissue cardiolipins. Some groups have demonstrated differences between the binding specificity of "autoimmune-aPL" and "non-autoimmune-aPL." It is widely accepted that a plasma protein beta 2-glycoprotein I (beta 2-GPI) is required for the binding of autoimmune anticardiolipin antibodies (aCL) to cardiolipin. However, some reports suggested heterogeneity of leprosy aCL with respect to their beta 2-glycoprotein I (beta 2GPI) dependency, although no thromboembolic complications have been reported. This study was designed to assess the specificity of aPL by investigating the prevalence of aCL, anti-phosphatidylserine (aPS), anti-phosphatidylinositol (aPI), anti-beta 2GPI and antiprothrombin (aPT) antibodies, and evaluate their clinical significance in a group of patients with lepromatous leprosy.

Patients and methods: 35 lepromatous leprosy patients were selected randomly from an Egyptian leprosarium as a study group; 35 normal household contact controls were selected matching the study group for both sex and age. ACL, aPS, aPI, aPT, anti-beta 2GPI and beta 2-dependent aCL antibodies were investigated by ELISA in all patients and controls.

Results: aCL antibodies were more frequent in leprosy patients than in controls [13/35 (37%) vs 3/35 (9%), respectively, p = 0.02], and significantly correlated with Raynaud's phenomenon, skin nodules, chronic skin ulcers and urticarial skin rash. No association was found with hypopigmentation, hyperpigmentation and saddle nose. None of the patients presented aPS or

aPI. Only 1 subject from the control group presented aPI along with aCL. APT were present in 2/35 (5.7%) and anti-beta 2GPI in 1/35 (2.9%) leprotic patients. None of the individuals from the control group presented aPT or anti-beta 2GPI.

Conclusions: An association was found between the presence of aCL and certain dermatological manifestations of leprosy, such as Raynaud's phenomenon, skin nodules, chronic skin ulcers and urticarial skin rash. As in other infectious diseases, there was a lack of beta 2GPI-dependency and an absence of thrombotic complications.—Authors' Abstract

Lewallen, S., Tungpakorn, N. C., Kim, S. H. and Courtright, P. Progression of eye disease in "cured" leprosy patients: implications for understanding the pathophysiology of ocular disease and for addressing eye care needs. Br. J. Ophthalmol. **84** (2000) 817–821.

Background: Ocular damage in leprosy is due either to nerve damage or infiltration by mycobacteria. There is currently little information about the magnitude and nature of incident ocular pathology in cured leprosy patients. This information would increase our understanding of the pathophysiology of ocular involvement in leprosy and help in developing programs to address the eye care needs of leprosy patients who have been released from treatment. The cumulative incidence of leprosy-related ocular pathology and cataract was measured during an 11-year follow-up period in cured leprosy patients released from treatment in Korea.

Methods: In 1988 standardized eye examinations were performed on 501 patients in eight resettlement villages in central South Korea. In May 1999 standardized eye examinations were repeated in this population.

Results: Among the patients in whom there was no sight-threatening leprosy-related ocular disease (lagophthalmos, posterior synechia, or keratitis) in 1988, 14.7% developed one or more of these conditions. Overall, among those with no vision-reducing cataract in 1988, 26.4% had developed a vision-reducing lens opacity in at least one eye. Among patients examined in both 1988 and 1999, 14.3% developed visual impairment and 5.7% developed blindness.

Conclusion: This study demonstrates that leprosy-related ocular pathology progresses in some patients even after they are cured mycobiologically. The progressive leprosy-related lesions are the result of chronic nerve damage; ocular lesions due to infiltration by *Mycobacterium leprae* did not develop. Based on the factors found to be associated with development of the most visually significant findings (posterior synechia, keratitis, and cataract) certain patients should be targeted at discharge for active follow up eye care. We suggest that patients with lagophthalmos (even in gentle closure), trichiasis, small pupils, and posterior synechiae should be screened regularly for the development of lagophthalmos in forced closure, keratitis, and cataract.—Authors' Abstract

Nunez Marti, J. M. [Quality of dental care in patients with leprosy.] Rev. Leprol. Fontilles **22** (2000) 511–518. (in Spanish)

WHO insists that an effective health program should be based on the complete comprehension of the role of all the different factors involved in the development and maintainment of a particular way of life; health and other factors that can have influence in changing certain ways of living into other more healthier forms. A similar approach is applied to the needs of valuable information in relation to the environment and health. The importance of prevention and health is emphasized in this paper and a health quality control program in dentistry for the patients of Hansen's disease of the Sanatorium of Fontilles has been elaborated. In this article the procedure is explained together with a detailed analysis of the problem, whose final aim is to persuade the patient to adopt daily methods for maintaining a proper and healthy life style generally deteriorated in these patients due to disease.—Author's English Summary

Sow, S. O., Tiendrebeogo, A., Hamed Oould, B., Lienhart, C. and Ponnighaus, J. M. [Physical disabilities among new cases of leprosy diagnosed in the Bamako district (Mali) in 1994.]

Acta Leprol. **11** (1999) 161–170. (in French)

Our study concerns 244 new cases of leprosy diagnosed in the Bamako district [Mali] in 1994; 154/244 patients could be contacted and were examined in the Leprosy Department of the Marchoux Institute in Bamako. Results showed that the presence of leprosy-induced physical disabilities was associated with male gender (59%), advanced age (68%) and multibacillary disease (68%). Disabilities were also more frequent among patients having a rural or manual occupation at the time of screening or afterward. There was a significant increase ($p < 0.001$) in the prevalence of disabilities when comparing patients at the time of diagnosis (29%) and thereafter (48%). This means that in 40% of disability cases, lesions developed during or after the treatment. Disabilities were predominantly observed in hands (33%) and feet (29%) with more frequent lesions in lateral popliteal, superior ulnar and posterior tibial nerves. Our results seem to demonstrate the inadequacy of preventive measures and management. This stresses the need for adequate prevention and therapy of leprosy-induced disabilities in order to obtain proper eradication of leprosy-induced health problems.—Authors' English Summary

Tiendrebeogo, A., Coulibaly, I., Sarr, A. M. and Sow, S. O. [Nature and sensitivity of bacteria in leprosy plantar ulcers at Marchoux Institute, Bamako, Mali.] Acta Leprol. **11** (1999) 153–159. (in French)

To determine potential usefulness of antimicrobial agents and to guide their prescription in the treatment of leprosy plantar ulcers, we conduced an *in vitro* study about germs' nature and sensitivity to antibiotics. We took samples of plantar ulcer secretion from 107 patients at Marchoux Institute; 92.5% of those ulcers were infected. These samples revealed 145 strains of microorganisms among those, *Staphylococcus aureus* (70 strains) and genus *Pseudomonas* (41 strains) were the most frequent. These bacteria were resistant to several antibiotics currently used at Marchoux Institute (tetracyclin, penicillin, cotrimoxazol and erythromycin). Antibiotics, efficient at 80% on tested strains, were expensive for patients. They cannot be recommended for the treatment of local infections. These results outline that the main treatment in plantar ulcers is based upon antiseptic solutions and keeping feet at rest. Antibiotherapy in case of extension of local infection would be based on the results of a previous study of sensitivity.—Authors' English Summary

Immuno-Pathology

Adams, L. B., Job, C. K. and Krahenbuhl, J. L. Role of inducible nitric oxide synthase in resistance to *Mycobacterium leprae* in mice. Infect. Immun. **68** (2000) 5462–5465.

The manifestation of leprosy in humans is largely determined by host immunity to *Mycobacterium leprae* and is a model for immunoregulation in a human disease. However, animal models available for exploration of the leprosy spectrum are inadequate. This study explored *M. leprae* infection in mice deficient in inducible nitric oxide synthase, and this report describes elements resembling borderline tuberculoid leprosy in humans.—Authors' Abstract

Dalton, D. K., Haynes, L., Chu, C. Q., Swain, S. L. and Wittmer, S. Interferon gamma eliminates responding CD4 T cells during mycobacterial infection by inducing apoptosis of activated CD4 T cells. J. Exp. Med. **192** (2000) 117–122.

In *Mycobacterium bovis* Bacille Calmette-Guerin (BCG)-infected wild-type mice, there was a large expansion of an activated ($CD44^{hi}$) splenic CD4 T-cell population followed by a rapid contraction of this population to normal numbers. Contraction of the activated CD4 T-cell population in wild-type mice was associated with increased apoptosis of activated CD4 T cells. In BCG-infected interferon-gamma (IFN-γ)

knockout (KO) mice, the activated CD4 T-cell population did not undergo apoptosis. These mice accumulated large numbers of CD4+ CD44^{hi} T cells that were responsive to mycobacterial antigens. Addition of IFN-γ to cultured splenocytes from BCG-infected IFN-γ KO mice induced apoptosis of activated CD4 T cells. IFN-γ-mediated apoptosis was abolished by depleting adherent cells or Mac-1+ spleen cells or by inhibiting nitric oxide synthase. Thus, IFN-γ is essential to a regulatory mechanism that eliminates activated CD4 T cells and maintains CD4 T-cell homeostasis during an immune response.—Authors' Abstract

Demangel, C. and Britton, W. J. Interaction of dendritic cells with mycobacteria: where the action starts. Immunol. Cell Biol. **78** (2000) 318–324.

Dendritic cells (DC) are the major antigen-presenting cells in the induction of cellular responses to intracellular pathogens, such as mycobacteria. Recent studies have shown that they also play a critical role in the regulation of immune responses. The interaction of DC with microbial antigens may be the controlling factor in the development of a Th1-oriented protective immunity. Analysis of the innate response of DC to mycobacteria and the involvement of the DC receptors in antigen recognition have highlighted the pivotal role of these cells in T-cell activation. Mycobacteria-infected DC have an enhanced capacity to release pro-inflammatory cytokines and chemokines, and are potent inducers of interferon-gamma-producing cells *in vivo*. Therefore, DC manipulation for maximal antigen presentation and Th1 cytokine production may form the basis of a new generation of vaccines, with improved efficacy against mycobacterial infections.—Authors' Abstract

Garcia, I., Guler, R., Vesin, D., Olleros, M. L., Vassalli, P., Chvatchko, Y., Jacobs, M. and Ryffel, B. Lethal *Mycobacterium bovis* Bacillus Calmette Guerin infection in nitric oxide synthase 2-deficient mice: cell-mediated immunity requires nitric oxide synthase 2. Lab. Invest. **80** (2000) 1385–1397.

The role of nitric oxide (NO) in *Mycobacterium bovis* Bacillus Calmette Guerin (BCG) infection was investigated using nitric oxide synthase 2 (nos2)-deficient mice because NO plays a pivotal protective role in *M. tuberculosis* infection. We demonstrate that nos2-deficient mice were unable to eliminate BCG and succumbed within 8 to 12 weeks to BCG infection (10^6 CFU) with cachexia and pneumonia; whereas all infected wild-type mice survived. The greatest mycobacterial loads were observed in lung and spleen. Nos2-deficient mice developed large granulomas consisting of macrophages and activated T cells and caseous necrotic lesions in spleen. The macrophages in granulomas from nos2-deficient mice had reduced acid phosphatase activities, suggesting that NO is required for macrophage activation. The absence of NOS2 affected the cytokine production of the Th1-type of immune response, except IL-18. Serum amounts of IL-12p40 were increased and IFN-gamma was decreased compared with wild-type mice.

The lack of NOS2 resulted in an overproduction of TNF, observed throughout the infection period. Additionally, TNFR1 and TNFR2 shedding was altered compared with wild-type mice. Upregulation of TNF may be compensatory for the lack of NOS2. The late neutralization of TNF by soluble TNF receptors resulted in heightened disease severity and accelerated death in nos2-deficient mice but had no effect in wild-type mice. In conclusion, the inability of nos2-deficient mice to kill *M. bovis* BCG resulted in an accumulation of mycobacteria with a dramatic activation of the immune system and overproduction of pro-inflammatory cytokines which resulted in death.—Authors' Abstract

Mempel, M., Flageul, B., Suarez, F., Ronet, C., Dubertret, L., Kourilsky, P., Gachelin, G. and Musette, P. Comparison of the T cell patterns of leprous and cutaneous sarcoid granulomas—presence of V alpha 24-invariant natural killer T cells in T-cell-reactive leprosy together with a highly biased T cell receptor V alpha repertoire. Am. J. Pathol. **157** (2000) 509–523.

The T-cell-reactive (e.g., tuberculoid and reversal) forms of leprosy represent a well-defined granulomatous reaction pattern against an invading pathogen. The immune response in cutaneous sarcoidosis is a granulomatous condition that pathologically is very similar to T-cell reactive leprosy. However, it lacks a defined causative agent. In view of the role of NKT cells in murine granulomas induced by mycobacterial cell walls, we have searched for the presence of NKT cells in the cutaneous lesions of both leprosy and sarcoidosis. These cells were present in T-cell-reactive leprosy but were undetectable in cutaneous sarcoidosis. We have also studied the TCR V alpha repertoire in the two diseases. In addition to V alpha 24+ NKT cells, all patients with T-cell-reactive leprosy showed a very restricted T-cell-reactive V alpha repertoire with a strong bias toward the use of the V alpha 6 and V alpha 14 segments. V alpha 6 and V alpha 14+ T cells were polyclonal in terms of CDR3 length and J alpha usage. In contrasts, most sarcoidosis patients showed a diverse usage of V alpha chains associated with clonal or oligoclonal expansions reminiscent of antigen-driven activation of conventional T cells. Thus, the origin and perpetuation of the two kinds of granulomatous lesions appear to depend on altogether distinct T-cell recruiting mechanisms.—Authors' Abstract

Nau, G. J., Chupp, G. L., Emile, J. F., Jouanguy, E., Berman, J. S., Casanova, J. L. and Young, R. A. Osteopontin expression correlates with clinical outcome in patients with mycobacterial infection. Am. J. Pathol. **157** (2000) 37–42.

Osteopontin (OPN) is a protein that is expressed in chronic inflammatory diseases including tuberculosis, and its deficiency predisposes to more severe mycobacterial infections in mice. However, no reports have identified altered OPN expression in, or correlated these alterations to, infections in humans. The data presented herein identify alterations in the tissue expression of OPN protein and describe an inverse correlation between these levels and disease progression after inoculation of *Mycobacterium bovis* bacillus Calmette-Guerin vaccine in humans. Patients with regional adenitis and good clinical outcomes had abundant OPN in infected lymph nodes. This pattern of OPN accumulation was also observed in patients infected by *M. avium-intracellulare*. In contrast, patients with disseminated infection and histologically ill-defined granulomas had no significant osteopontin accumulation in infected lymph nodes; these patients had either deficiencies in the interferon-gamma receptor 1 or idiopathic immune defects. The level of OPN protein expression was inversely correlated with disseminated infection and, of particular interest, with death of the patient. We conclude that osteopontin expression correlates with an effective immune and inflammatory response when humans are challenged by a mycobacterial infection and that osteopontin contributes to human resistance against mycobacteria.—Authors' Abstract

Panuto Castelo, A., Almeida, I. C., Rosa, J. C., Greene, L. J. and Roque Barreira, M. C. The Rubino test for leprosy is a beta^2-glycoprotein 1-dependent antiphospholipid reaction. Immunology **101** (2000) 147–153.

We describe the isolation and identification of three components required for the Rubino reaction (RR), which is the rapid sedimentation of formalinized sheep red blood cells (SRBC) initiated by serum from leprosy patients with defective *Mycobacterium leprae*-specific cell immunity. The Rubino reaction factor (RRF) required for this phenomenon, previously identified as an immunoglobulin M (IgM), was purified from leprosy patient serum by adsorption to formalinized SRBC. Purified RRF IgM when added to formalinized SRBC did not produce a positive RR. However, when the contact was carried out in the presence of normal human serum (NHS), cells rapidly sedimented. The purified cofactor from NHS contained two components of 70,000 and 50,000 molecular weight (MW), as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The latter was recognized by the RRF IgM on immunoblot and its N-termi-

nal sequence indicated that it was beta^2-glycoprotein 1 (beta^2-GP1), an anionic phospholipid-binding protein. Methanol-treated formalinized SRBC did not support the RR. Thin-layer chromatography of an extract of membranes indicated that the SRBC ligand was a cell-surface phospholipid. Cardiolipin inhibited the RR. These data demonstrate that the RR involves a trimolecular interaction in which IgM, beta^2-GP1 and an SRBC phospholipid participate. By analogy with the antiphospholipid antibodies (anti-PL) that occur in autoimmune processes, serum samples from 29 systemic lupus erythematosus patients with high levels of anticardiolipin antibodies were submitted to the RR. A positive RR was obtained for 45% (13 of 29 patients). These results modify the paradigm of the absolute specificity of the RR for leprosy and demonstrate that RRF IgM is a beta^2-Gp1-dependent anti-PL.—Authors' Abstract

Rhoades, E. R. and Ullrich, H. J. How to establish a lasting relationship with your host: lessons learned from *Mycobacterium* spp. Immunol. Cell Biol. **78** (2000) 301–310.

Mycobacterium spp. enjoy an intracellular lifestyle that is fatal to most microorganisms. Bacilli persist and multiply within mononuclear phagocytes in the face of defenses ranging from toxic oxygen and nitrogen radicals, acidic proteases and bactericidal peptides. Uptake of *Mycobacterium* by phagocytes results in the *de novo* formation of a phagosome, which is manipulated by the pathogen to accommodate its needs for intracellular survival and replication. The present review describes the intracellular compartment occupied by *Mycobacterium* spp. and presents current ideas on how mycobacteria may establish this niche, placing special emphasis on the involvement of mycobacterial cell wall lipids.—Authors' Abstract

Saunders, B. M. and Cooper, A. M. Restraining mycobacteria: role of granulomas in mycobacterial infections. Immunol. Cell Biol. **78** (2000) 334–341.

The generation of prolonged immunity to *Mycobacterium tuberculosis* requires not only an antigen-specific interferon-gamma-producing T-cell response, including both CD4 and CD8 T cells, but also the generation of protective granulomatous lesions, whereby the close apposition of activated T cells and macrophages acts to contain bacterial growth. The importance of the granulomatous lesion in controlling this immune response and in limiting both tissue damage and bacterial dissemination has been considered a secondary event but, as the present review illustrates, is no less important in surviving mycobacterial infection than an antigen-specific T-cell response. The formation of a protective granuloma involves the orchestrated production of a host of chemokines and cytokines, the upregulation of their receptors along with upregulation of addressins, selectins and integrins to coordinate the recruitment, migration and retention of cells to and within the granuloma. In the present review, the principal components of the protective response are outlined, and the role of granuloma formation and maintenance in mediating prolonged containment of mycobacteria within the lung is addressed.—Authors' Abstract

Sharma, S., Verma, I. and Khuller, G. K. Antibacterial activity of human neutrophil peptide-1 against *Mycobacterium tuberculosis* H37Rv: *in vitro* and *ex vivo* study. Eur. Resp. J. **16** (2000) 112–117.

The aim of the study was to investigate the activity of human neutrophil peptide (HNP)-1 to kill *Mycobacterium tuberculosis* H37Rv *in vitro* and *ex vivo* in the murine macrophage cell line J744A.1 on the basis of colony forming units.

Macromolecular biosynthesis was studied by monitoring the incorporation of radioactive precursors into different macromolecules. The binding and localization studies were carried out with radioiodinated HNP-1; whereas the cytotoxicity of HNP-1 to macrophages was determined by trypan blue exclusion assay.

A concentration dependent inhibition in the growth of *M. tuberculosis* H37Rv was observed in the presence of HNP-1. The

minimum inhibitory concentration and median inhibitory concentration of HNP-1 were found to be 2.5 $\mu \cdot ml^{-1}$ and 0.8 $\mu \cdot ml^{-1}$. Treatment of both *in vitro* grown and phagocytosed mycobacterial cells with HNP-1 resulted in generalized inhibition in the macromolecular biosynthesis with maximum inhibition in deoxyribonucleic acid and lipid biosynthesis. HNP-1 exhibited equilibrium binding with respect to time and two-thirds of bound radioactivity was shown to be present inside the macrophages. Approximately 50% and 98% killing of intracellular mycobacteria was observed after 3 days of treatment with 5 $\mu g \cdot ml^{-1}$ and 40 $\mu g \cdot ml^{-1}$ of HNP-1, respectively. HNP-1 exhibited low cytotoxicity toward the macrophage cell line at the bactericidal concentration to mycobacteria. From the results of this study, it is concluded that human neutrophil peptide-1 possesses potent bactericidal activity against virulent mycobacteria *in vitro* as well as mycobacteria replicating within macrophages.—Authors' Abstract

Spierings, E., DeBoer, T., Zulianello, L. and Ottenhoff, T. H M. Novel mechanisms in the immunopathogenesis of leprosy nerve damage: the role of Schwann cells, T cells and *Mycobacterium leprae.* Immunol. Cell Biol. **78** (2000) 349–355.

The major complication of reversal (or type 1) reactions in leprosy is peripheral nerve damage. The pathogenesis of nerve damage remains largely unresolved. *In situ* analyses suggest an important role for type 1 T cells. *Mycobacterium leprae* is known to have a remarkable tropism for Schwann cells that surround peripheral axons. Reversal reactions in leprosy are often accompanied by severe and irreversible nerve destruction and are associated with increased cellular immune reactivity against *M. leprae.* Thus, a likely immunopathogenic mechanism of Schwann cell and nerve damage in leprosy is that infected Schwann cells process and present antigens of *M. leprae* to antigen-specific, inflammatory type 1 T cells and that these T cells subsequently damage and lyse infected Schwann cells. Previous studies using rodent CD8+ T cells and Schwann cells have revealed evidence for the existence of such a mechanism. Recently, a similar role has been suggested for human CD4+ T cells. These cells may be more important in causing leprosy nerve damage *in vivo,* given the predilection of *M. leprae* for Schwann cells and the dominant role of CD4+ serine esterase(+) Th1 cells in leprosy lesions. Antagonism of molecular interactions between *M. leprae,* Schwann cells and inflammatory T cells may therefore provide a rational strategy to prevent Schwann cell and nerve damage in leprosy.—Authors' Abstract

Vankayalapati, R., Wizel, B., Weis, S. E., Samten, B., Girard, W. M. and Barnes, P. F. Production of interleukin-18 in human tuberculosis. J. Infect. Dis. **182** (2000) 234–239.

To investigate the role of interleukin (IL)-18 in human tuberculosis, IL-18 production was evaluated in blood and at the site of disease in patients with tuberculosis. *Mycobacterium tuberculosis*-stimulated peripheral blood mononuclear cells (PBMC) from tuberculosis patients secreted less IL-18 and interferon-gamma (IFN-γ) than did PBMC from healthy persons reactive to tuberculin. *M. tuberculosis*-induced IFN-γ production was inhibited by anti-IL-18 and enhanced by recombinant IL-18. Alveolar macrophages secreted IL-18 in response to *M. tuberculosis* and IL-18 and IFN-γ concentrations were higher in pleural fluid of patients with tuberculosis than in pleural fluid of patients with nontuberculous diseases. These findings demonstrate that IL-18 production by PBMC correlates with IFN-γ production and effective immunity to tuberculosis, suggesting that IL-18 contributes to a protective type 1 cytokine response in persons with mycobacterial infection.—Authors' Abstract

Yamauchi, P. S., Bleharski, J. R., Uyemura, K., Kim, J., Sieling, P. A., Miller, A., Brightbill, H., Schlienger, K., Rea, T. H. and Modlin, R. L. A role for CD40-CD40 ligand interactions in the generation of type 1 cytokine responses in human leprosy. J. Immunol. **165** (2000) 1506–1512.

The interaction of CD40 ligand (CD40L) expressed by activated T cells with CD40 on macrophages has been shown to be a potent stimulus for the production of IL-12, an obligate signal for generation of Th1 cytokine responses. The expression and interaction of CD40 and CD40L were investigated in human infectious disease using leprosy as a model. CD40 and CD40L mRNA and surface protein expression were predominant in skin lesions of resistant tuberculoid patients compared with the highly susceptible lepromatous group. IL-12 release from PBMC of tuberculoid patients stimulated with *Mycobacterium leprae* was partially inhibited by mAbs to CD40 or CD40L, correlating with Ag-induced upregulation of CD40L on T cells. Cognate recognition of *M. leprae* Ag by a T-cell clone derived from a tuberculoid lesion in the context of monocyte APC resulted in CD40L-CD40-dependent production of IL-12. In contrast, *M. leprae*-induced IL-12 production by PBMC from lepromatous patients was not dependent on CD40L-CD40 ligation, nor was CD40L upregulated by *M. leprae*. Furthermore, IL-10, a cytokine predominant in lepromatous lesions, blocked the IFN-gamma upregulation of CD40 on monocytes. These data suggest that T-cell activation *in situ* by *M. leprae* in tuberculoid leprosy leads to local upregulation of CD40L, which stimulates CD40-dependent induction of IL-12 in monocytes. The CD40-CD40L interaction, which is not evident in lepromatous leprosy, probably participates in the cell-mediated immune response to microbial pathogens.—Authors' Abstract

Microbiology

Arenas Licea, J., van Gool, A. J., Keeley, A. J., Davies, A., West, S. C. and Tsaneva, I. R. Functional interactions of *Mycobacterium leprae* RuvA with *Escherichia coli* RuvB and RuvC on Holliday junctions. J. Mol. Biol. **301** (2000) 839–850.

The *Mycobacterium leprae* RuvA homolog (MlRuvA) was overexpressed in *Escherichia coli* and purified to homogeneity. The DNA-binding specificity and the functional interactions of MlRuvA with *E. coli* RuvB and RuvC (EcRuvB and EcRuvC) were examined using synthetic Holliday junctions. MlRuvA bound specifically to Holliday junctions and produced similar band-shift patterns as EcRuvA. Moreover, MlRuvA formed functional DNA helicase and branch-migration enzymes with EcRuvB, although the heterologous enzyme had a lower efficiency. These results demonstrate that the RuvA homolog of *M. leprae* is a functional branch-migration subunit. Whereas MlRuvA promoted branch-migration in combination with EcRuvB, it was unable to stimulate branch-migration-dependent resolution in a RuvABC complex. The inability to stimulate RuvC was not due to its failure to form heterologous RuvABC complexes on junctions, since such complexes were detected by co-immunoprecipitation. Most likely, the stability of the heterologous RuvABC complex and, possibly, the interactions between RuvA and RuvC were impaired since gel-shift experiments failed to show mixed MlRuvA-EcRuv-junction complexes. These results demonstrate that branch-migration *per se* and the assembly of a RuvABC complex on the Holliday junction are insufficient for RuvAB-dependent resolution of the junction by RuvC, suggesting that specific and intimate interactions between all three proteins are required for the function of a RuvABC "resolvasome."—Authors' Abstract

Av Gay, Y. and Sobouti, R. Cholesterol is accumulated by mycobacteria but its degradation is limited to nonpathogenic fast-growing mycobacteria. Can. J. Microbiol. **46** (2000) 826–831.

In this report we show that fast-growing nonpathogenic mycobacteria degrade cholesterol from liquid media, and are able to

grow on cholesterol as a sole carbon source. In contrast, slow-growing mycobacteria, including pathogenic *Mycobacterium tuberculosis* and bacillus Calmette-Guerin (BCG), do not degrade and use cholesterol as a carbon source. Nevertheless, pathogenic mycobacteria are able to uptake, modify, and accumulate cholesterol from liquid growth media, and form a zone of clearance around a colony when plated on solid media containing cholesterol. These data suggest that cholesterol may have a role in mycobacterial infection other than its use as carbon source.—Authors' Abstract

Blackwood, K. S., He, C., Gunton, J., Turenne, C. Y., Wolfe, J. and Kabani, A. M. Evaluation of recA sequences for identification of *Mycobacterium* species. J. Clin. Microbiol. **38** (2000) 2846–2852.

16S rRNA sequence data have been used to provide a molecular basis for an accurate system for identification of members of the genus *Mycobacterium.* Previous studies have shown that *Mycobacterium* species demonstrate high levels (>94%) of 16S rRNA sequence similarity and that this method cannot differentiate between all species, i.e., *M. gastri* and *M. kansasii.* In the present study, we have used the recA gene as an alternative sequencing target in order to complement 16S rRNA sequence-based genetic identification. The recA genes of 30 *Mycobacterium* species were amplified by PCR, sequenced, and compared with the published recA sequences of *M. tuberculosis, M. smegmatis,* and *M. leprae* available from GenBank. By recA sequencing the species showed a lower degree of interspecies similarity than they did by 16S rRNA gene sequence analysis, ranging from 96.2% between *M. gastri* and *M. kansasii* to 75.7% between *M. aurum* and *M. leprae.* Exceptions to this were members of the *M. tuberculosis* complex, which were identical. Two strains of each of 27 species were tested, and the intraspecies similarity ranged from 98.7% to 100%. In addition, we identified new *Mycobacterium* species that contain a protein intron in their recA genes, similar to *M. tuberculosis* and *M. leprae.* We propose that recA gene sequencing offers a complementary method to 16S rRNA gene sequencing for the accurate identification of the *Mycobacterium* species.—Authors' Abstract

Brosch, R., Gordon, S. V., Buchrieser, C., Pym, A. S., Garnier, T. and Cole, S. T. Comparative genomics uncovers large tandem chromosomal duplications in *Mycobacterium bovis* BCG Pasteur. Yeast **17** (2000) 111–123.

On direct comparison of minimal sets of ordered clones from bacterial artificial chromosome (BAC) libraries representing the complete genomes of *Mycobacterium tuberculosis* H37Rv and the vaccine strain, *M. bovis* BCG Pasteur, two major rearrangements were identified in the genome of *M. bovis* BCG Pasteur. These were shown to correspond to two tandem duplications, DU1 and DU2, of 29,668 bp and 36,161 bp, respectively. While DU1 resulted from a single duplication event, DU2 apparently arose from duplication of a 100 kb genomic segment that subsequently incurred an internal deletion of 64 kb. Several lines of evidence suggest that DU2 may continue to expand, since two copies were detected in a subpopulation of BCG Pasteur cells. BCG strains harboring DU1 and DU2 are diploid for at least 58 genes and contain two copies of oviC, the chromosomal origin of replication. These findings indicate that these genomic regions of the BCG genome are still dynamic. Although the role of DU1 and DU2 in the attenuation and/or altered immunogenicity of BCG is yet unknown, knowledge of their existence will facilitate quality control of BCG vaccine lots and may help in monitoring the efficacy of the world's most widely used vaccine.—Authors' Abstract

Brown, G. D., Dave, J. A., van Pittius, N. C. G., Stevens, L., Ehlers, M. R. W. and Beyers, A. D. The mycosins of *Mycobacterium tuberculosis* H37Rv: a family of subtilisin-like serine proteases. Gene **254** (2000) 147–155.

There is little information regarding the role of proteolysis in *Mycobacterium tuberculosis,* and no studies on the potential in-

volvement of proteases in the pathogenesis of tuberculosis. We identified five *M. tuberculosis* genes (mycP1–5) that encode a family of serine proteases (mycosins-1 to 5), ranging from 36% to 47% identity. Each protein contains a catalytic triad (Asp, His, Ser) within highly conserved sequences, typical of proteases of the subtilisin family. These genes are also present in *M. bovis* BCG and other virulent mycobacteria, but only one homolog (mycP3) was detected in *M. smegmatis*. The mycosins have N-terminal signal sequences and C-terminal transmembrane anchors, and the localization of the mycosins to the membrane/cell wall was verified by Western blot analysis of heterologously expressed proteins in cellular fractions of *M. smegmatis*. In *M. tuberculosis*, all the mycosins were expressed constitutively during growth in broth. Mycosins-2 and -3 were also expressed constitutively in *M. bovis* BCG, but no expression of mycosin-1 was detected. Mycosin-2 was modified by cleavage in all three mycobacterial species. The multiplicity and constitutive expression of these proteins suggests that they have an important role in the biology of *M. tuberculosis*.—Authors' Abstract

Choi, K. H., Kremer, L., Besra, G. S. and Rock, C. O. Identification and substrate specificity of beta-ketoacyl (Acyl carrier protein) synthase III (MtFabH) from *Mycobacterium tuberculosis*. J. Biol. Chem. **275** (2000) 28201–28207.

The long-chain alpha-alkyl-beta-hydroxy fatty acids, termed mycolic acids, which are characteristic components of the mycobacterial cell wall are produced by successive rounds of elongation catalyzed by a multifunctional (type I) fatty acid synthase complex followed by a dissociated (type II) fatty acid synthase. In bacterial type II systems, the first initiation step in elongation is the condensation of acetyl-CoA with malonyl-acyl carrier protein (ACP) catalyzed by beta-ketoacyl-ACP III (FabH). An open reading frame in the *Mycobacterium tuberculosis* genome (Rv0533c), now termed mtfabH, was 37.3% identical to *Escherichia coli* ecFabH and contained the Cys-His-Asn catalytic triad signature. However, the purified recombinant mtFabH clearly preferred long-chain acyl-CoA substrates rather than acyl-ACP primers and did not utilize acetyl-CoA as a primer in comparison to ecFabH. In addition, purified mtFabH was sensitive to thiolactomycin and resistant to cerulenin in an *in vitro* assay. However, mtFabH overexpression in *M. bovis* BCG did not confer thiolactomycin resistance, suggesting that mtFabH may not be the primary target of thiolactomycin inhibition *in vivo* and led to several changes in the lipid composition of the bacilli. The data presented is consistent with a role for mtFabH as the pivotal link between the type I and type II fatty acid elongation systems in *M. tuberculosis*. This study opens up new avenues for the development of selective and novel anti-mycobacterial agents targeted against mtFabH.—Authors' Abstract

Collins, D. M. New tuberculosis vaccines based on attenuated strains of the *Mycobacterium tuberculosis* complex. Immunol. Cell Biol. **78** (2000) 342–348.

The world urgently needs a better tuberculosis vaccine. Bacille Calmette-Guerin (BCG), an attenuated strain of *Mycobacterium bovis*, has been very widely used as a vaccine for many years but has had no major effect on reducing the incidence of tuberculosis. A number of alternative living and nonliving vaccines are being investigated. Live vaccine candidates include genetically modified forms of BCG, genetically attenuated strains of the *M. tuberculosis* complex and genetically engineered vaccinia virus and *Salmonella* strains. Nonliving vaccine candidates include killed mycobacterial species, protein subunits and DNA vaccines. One requirement for acceptance of any new vaccine will be a favorable comparison of the protection it induces relative to BCG in a range of animal models, some of which may need further development. Molecular genetic techniques are now available that enable production of live attenuated strains of the *M. tuberculosis* complex with vaccine potential. In the first of two broadly different approaches that are being used, large numbers of mutants are produced by transposon mutagenesis or illegitimate recombination and are screened

for properties that correlate with attenuation. In the second approach, putative genes that may be required for virulence are identified and subsequently inactivated by allelic exchange. In both approaches, mutants that are attenuated need to be identified and subsequently tested for their vaccine efficacy in animal models. Many mutants of the *M. tuberculosis* complex have now been produced and the vaccine properties of a substantial number will be assessed in the next 3 years.—Author's Abstract

de Miranda, A. B., Alvarez Valin, F., Jabbari, K., Degrave, W. M. and Bernardi, G. Gene expression, amino acid conservation, and hydrophobicity are the main factor shaping codon preferences in *Mycobacterium tuberculosis* and *Mycobacterium leprae*. J. Mol. Evol. **50** (2000) 45–55.

Mycobacterium tuberculosis and *M. leprae* are the etiological agents of tuberculosis and leprosy, respectively. After performing extensive comparisons between genes from these two GC-rich bacterial species, we were able to construct a set of 275 homologous genes. Since these two bacterial species also have a very low growth rate, translational selection could not be so determinant in their codon preferences as it is in other fast-growing bacteria. Indeed, principal-components analysis of codon usage from this set of homologous genes revealed that the codon choices in *M. tuberculosis* and *M. leprae* are correlated not only with compositional constraints and translational selection, but also with the degree of amino acid conservation and the hydrophobicity of the encoded proteins. Finally, significant correlations were found between GC^3 and synonymous distances as well as between synonymous and nonsynonymous distances.—Authors' Abstract

Dmitriev, B. A., Ehlers, S., Rietschel, E. T. and Brennan, P. J. Molecular mechanics of the mycobacterial cell wall: from horizontal layers to vertical scaffolds. Int. J. Med. Microbiol. **290** (2000) 251–258.

Current models depicting the structural organization of the mycobacterial cell wall assume peptidoglycan and galactan strands to run in parallel to the cytoplasmic membrane forming several horizontal layers beneath perpendicularly oriented mycolic acids. Following a thorough re-evaluation of the currently available chemical, biochemical and electron microscopical data, we propose a fundamentally distinct principle of the physical organization and biosynthesis of the mycobacterial cell wall skeleton. According to this new concept, the solid and elastic matrix that makes the mycobacterial cell wall a formidably impermeable barrier is the direct consequence of cross-linked glycan strands which all run in a direction perpendicular to the cytoplasmic membrane.—Authors' Abstract

Dockrell, H. M., Brahmbhatt, S., Robertson, B. D., Britton, S., Fruth, U., Gebre, N., Hunegnaw, M., Hussain, R., Manandhar, R., Murillo, L., Pessolani, M. C. V., Roche, P., Salgado, J. L., Sampaio, E., Shahid, F., Thole, J. E. R. and Young, D. B. A postgenomic approach to identification of *Mycobacterium leprae*-specific peptides as T-cell reagents. Infect. Immun. **68** (2000) 5846–5855.

To identify *Mycobacterium leprae*-specific human T-cell epitopes, which could be used to distinguish exposure to *M. leprae* from exposure to *M. tuberculosis* or to environmental mycobacteria or from immune responses following *M. bovis* BCG vaccination, 15-mer synthetic peptides were synthesized based on data from the *M. leprae* genome, each peptide containing three or more predicted HLA-DR binding motifs. Eighty-one peptides from 33 genes were tested for their ability to induce T-cell responses, using peripheral blood mononuclear cells (PBMC) from tuberculoid leprosy patients (N = 59) and healthy leprosy contacts (N = 53) from Brazil, Ethiopia, Nepal, and Pakistan and 20 United Kingdom blood bank donors. Gamma interferon (IFN-γ) secretion proved more sensitive for detection of PBMC responses to peptides than did lymphocyte proliferation. Many of the peptides giving the strongest responses in leprosy donors compared to subjects from the United Kingdom, where leprosy

is not endemic, have identical, or almost identical, sequences in *M. leprae* and *M. tuberculosis* and would not be suitable as diagnostic tools. Most of the peptides recognized by United Kingdom donors showed promiscuous recognition by subjects expressing differing HLA-DR types. The majority of the novel T-cell epitopes identified came from proteins not previously recognized as immune targets, many of which are cytosolic enzymes. Fifteen of the tested peptides had ≥5 of 15 amino acid mismatches between the equivalent *M. leprae* and *M. tuberculosis* sequences; of these, eight gave specificities of ≥90% (percentage of United Kingdom donors who were nonresponders for IFN-γ secretion), with sensitivities (percentage of responders) ranging from 19% to 47% for tuberculoid leprosy patients and 21% to 64% for healthy leprosy contacts. A pool of such peptides, formulated as a skin test reagent, could be used to monitor exposure to leprosy, or as an aid to early diagnosis.—Authors' Abstract

Hutter, B. and Dick, T. Analysis of the dormancy-inducible narK2 promoter in *Mycobacterium bovis* BCG. FEMS Microbiol. Lett. **188** (2000) 141–146.

Upon depletion of oxygen, the obligate aerobe 5 mycobacteria switch from growth to a state of nonreplicating persistence or dormancy. Here, we report the first functional analysis of a dormancy-dependent mycobacterial promoter in *Mycobacterium bovis* BCG. Promoter probing using a 'lacZ reporter detected a dormancy-inducible promoter activity upstream of the coding sequence for the putative nitrite extrusion protein NarK2. Primer extension analysis mapped a transcriptional start point 47 bp upstream of the narK2 start codon. Deletion analysis revealed that the sequence –222 to –133 bp upstream from the transcriptional start point was required for basal and dormancy-inducible reporter expression. The sequence +1 to +47 downstream of the transcriptional start point had a strong inhibitory effect on the level of dormancy-induced beta-galactosidase activity. The identification of apparent activating and inhibiting regions suggests that the narK2 promoter is at least under dual control.—Authors' Abstract

Jackson, M., Crick, D. C. and Brennan, P. J. Phosphatidylinositol is an essential phospholipid of mycobacteria. J. Biol. Chem. **275** (2000) 30092–30099.

Phosphatidylinositol (PI) and metabolically derived products such as the phosphatidylinositol mannosides and linear and mature branched lipomannan and lipoarabinomannan are prominent phospholipids/lipoglycans of *Mycobacterium* sp. believed to play important roles in the structure and physiology of the bacterium as well as during host infection. To determine if PI is an essential phospholipid of mycobacteria, we identified the pgsA gene of *M. tuberculosis* encoding the phosphatidylinositol synthase enzyme and constructed a pgsA conditional mutant of *M. smegmatis*. The ability of this mutant to synthesize phosphatidylinositol synthase and subsequently PI was dependent on the presence of a functional copy of pgsA gene carried on a thermosensitive plasmid. The mutant grew like the control strain under permissive conditions (30°C), but ceased growing when placed at 42°C, a temperature at which the rescue plasmid is lost. Loss of cell viability at 42°C was observed when PI and phosphatidylinositol dimannoside contents dropped to similar to 30% and 50% of the wild-type levels, respectively. This work provides the first evidence of the essentiality of PI to the survival of mycobacteria. PI synthase is thus an essential enzyme of *Mycobacterium* that shows promise as a drug target for anti-tuberculosis therapy.—Authors' Abstract

Mahapatra, S., Bhakta, S., Ahamed, J. and Basu, J. Characterization of derivatives of the high-molecular-mass penicillin-binding protein (PBP) 1 of *Mycobacterium leprae*. Biochem. J. **350** (2000) 75–80.

Mycobacterium leprae has two high-molecular-mass multi-modular penicillin-binding proteins (PBPs) of class A, termed PBP1 and PBP1* [Lepage, Dubois, Ghosh, Joris, Mahapatra, Kundu, Basu, Chakrabarti, Cole, Nguyen-Disteche and Ghuy-

sen (1997) J. Bacteriol. 179, 4627–4630]. PBP1-Xaa-beta-lactamase fusions generated periplasmic p-lactamase activity when Xaa (the amino acid of PBP1 at the fusion junction) was residue 314, 363, 407, 450 or 480. Truncation of the N-terminal part of the protein up to residue Leu-147 generated a penicillin-binding polypeptide which could still associate with the plasma membrane; whereas [Delta M1-R314]PBP1 (PBP1 lacking residues Met-1 to Arg-314) failed to associate with the membrane, suggesting that the region between residues Leu-147 and Arg-314 harbors an additional plasma membrane association site for PBP1. Truncation of the C-terminus up to 42 residues downstream of the KTG (Lys-Thr-Gly) motif also generated a polypeptide that retained penicillin-binding activity. [Delta MI-R314]PBP1 could be extracted from inclusion bodies and refolded under appropriate conditions to give a form capable of binding penicillin with the same efficiency as full-length PBP1. This is, to the best of our knowledge, the first report of a soluble derivative of a penicillin-resistant high-molecular-mass PBP of class A that is capable of binding penicillin. A chimaeric PBP in which the penicillin-binding (PB) module of PBP1 was fused at its N-terminal end with the non-penicillin-binding (n-PB) module of PBP1* retained penicillin-binding activity similar to that of PBP1, corroborating the finding that the n-PB module of PBP1 is dispensable for its penicillin-binding activity.—Authors' Abstract

Mariani, F., Cappelli, G., Riccardi, G. and Colizzi, V. *Mycobacterium tuberculosis* H37Rv comparative gene-expression analysis in synthetic medium and human macrophage. Gene **253** (2000) 281–291.

Mycobacteria are intracellular pathogens that survive and grow in host macrophages. Following phagocytosis, sustained intracellular bacterial growth depends on its ability to avoid destruction by macrophage-mediated host defenses such as lysosomal enzymes, reactive oxygen and the reactive nitrogen intermediates.

This suggests that the interaction between host cell and microbe is delicately balanced, and can be tipped in favor of either organism. The identification of *Mycobacterium tuberculosis* H37Rv (MTB) genes expressed within host cells would contribute greatly to the development of new strategies to fight tuberculosis. In the present study, we compared MTB gene expression in the course of intra- (human macrophages) and extracellular growth (Sauton's medium) to ascertain whether differences might occur between gene-expression patterns in the two habitats of replication. Using reverse-transcriptase polymerase chain reaction (RT-PCR) on a group of 14 MTB-Complex-specific genes, we found that MT10Sa (a small stable RNA), 35 kDa (unknown), ahpC (alkyl hydroperoxide reductase, AhpC), sigF (alternative RNA Polymerase sigma factor), and katG (catalase-peroxidase, HPI) genes are expressed in both the environments, while Ag85B, Ag85C (members of the antigen 85 Complex): rpoV (RNA polymerase sigma factor) and ESAT6 (early secretory antigen, 6 kDa) are expressed only in the *in vitro* culture; on the other hand, Ag85A (antigen 85 Complex); rpoB (RNA polymerase beta sub-unit), pab (protein antigen b), invA and invB genes (encoding proteins that show homologies with p60 of *Listeria monocytogenes*) are expressed only inside the macrophage. Positive RT-PCR products on cDNAs for these genomic regions were not obtained from approximately 1000-fold more bacteria grown in laboratory broth. Identification of *M. tuberculosis* genes expressed in response to phagocytosis by human macrophages increases our basic understanding of the host-pathogen interaction, and helps to identify bacterial factors necessary for *in vivo* survival and growth.—Authors' Abstract

Mukhopadhyay, B. and Purwantini, E. Pyruvate carboxylase from *Mycobacterium smegmatis*: stabilization, rapid purification, molecular and biochemical characterization and regulation of the cellular level. Biochim. Biophys. Acta-Gen. Subjects **1475** (2000) 191–206.

This is the first report on the purification and characterization of an anaplerotic enzyme from a *Mycobacterium.* The anaplerotic reactions play important roles in the biochemical differentiation of mycobac-

teria into nonreplicating stages. We have purified and characterized a pyruvate carboxylase (PYC) from *Mycobacterium smegmatis* and cloned and sequenced its gene. We have developed a very rapid and efficient purification protocol that provided PYC with very high specific activities (up to 150 U/mg) that remained essentially unchanged over a month. The enzyme was found to be a homomultimer of 121 kDa subunits, mildly thermophilic, absolutely dependent on acyl-CoAs for activity and inhibited by ADP, by excess Mg2+, Co2+, and Mn2+, by aspartate, but not by glutamate and alpha-ketoglutarate. Supplementation of minimal growth medium with aspartate did not lower the cellular PYC level, rather doubled it; with glutamate the level remained unchanged. These observations would not fit the idea that the *M. smegmatis* enzyme fulfills a straightforward anaplerotic function: in a closely related organism, *Corynebacterium glutamicum,* PYC is the major anaplerotic enzyme. Growth on glucose provided a twofold higher cellular PYC level than that observed with glycerol. The PYCs of *M. smegmatis* and *M. tuberculosis* were highly homologous to each other. In *M. smegmatis, M. tuberculosis* and *M. leprae,* PYC was flanked by a putative methylase and a putative integral membrane protein gene in an identical operon-like arrangement. Thus, *M. smegmatis* could serve as a model for studying PYC-related physiological aspects of mycobacteria. Also, the ease of purification and the extraordinary stability could make the *M. smegmatis* enzyme a model for studying the structure-function relationships of PYCs in general. It should be noted that no crystal structure is available for this enzyme of paramount importance in all three domains of life, archaea, bacteria, and eukarya.—Authors' Abstract

Primm. T. P., Andersen, S. J., Mizrahi, V., Avarbock, D., Rubin, H. and Barry, C. E. The stringent response of *Mycobacterium tuberculosis* is required for long-term survival. J. Bacteriol. **182** (2000) 4889–4898.

The stringent response utilizes hyperphosphorylated guanine [(p)ppGpp] as a signaling molecule to control bacterial gene expression involved in long-term survival under starvation conditions. In gram-negative bacteria, (p)ppGpp is produced by the activity of the related RelA and SpoT proteins. *Mycobacterium tuberculosis* contains a single homolog of these proteins [rel(Mtb)] and responds to nutrient starvation by producing (p)ppGpp. A rel(Mtb) knockout strain was constructed in a virulent strain of *M. tuberculosis,* H37Rv, by allelic replacement. The rel(Mtb) mutant displayed a significantly slower aerobic growth rate than the wild type in synthetic liquid media, whether rich or minimal. The growth rate of the wild type was equivalent to that of the mutant when citrate or phospholipid was employed as the sole carbon source. These two organisms also showed identical growth rates within a human macrophage-like cell line. These results suggest that the *in vivo* carbon source does not represent a stressful condition for the bacilli, since it appears to be utilized in a similar rel(Mtb)-independent manner. *In vitro* growth in liquid media represents a condition that benefits from rel(Mtb)-mediated adaptation. Long-term survival of the rel(Mtb) mutant during *in vitro* starvation or nutrient run out in normal media was significantly impaired compared to that in the wild type. In addition, the mutant was significantly less able to survive extended anaerobic incubation than the wild-type virulent organism. Thus, the rel(Mtb) protein is required for long-term survival of pathogenic mycobacteria under starvation conditions.—Authors' Abstract

Singh, H. B., Katoch, K., Natrajan, M., Sharma, R. K., Gupta, U. D., Sharma, V. D., Singh, D., Chauhan, D. S., Srivastava, K. and Katoch, V. M. Effect of treatment on PCR positivity in multibacillary leprosy patients treated with conventional and newer drugs ofloxacin and minocycline. Acta Leprol. **11** (1999) 179–182.

In order to develop objective criteria to monitor trends of therapeutic responses, positivity of PCR signals and ATP assay methods has been compared in multibacillary (MB) leprosy patients. Biopsies from lesions of 95 BL/LL patients before and af-

ter 1 year of treatment with a new drug regimen composed of conventional and newer drugs ofloxacin and minocycline have been studied. These biopsies were processed for bacillary ATP assay and PCR positivity for a 36 kDa gene target by earlier published methods. In the untreated patients bacillary ATP levels were detectable in all specimens and ranged from 0.02 to more than 36 pg/million organisms. After 1 year of treatment ATP levels were not detectable in any of the 57 biopsy specimens available for analysis. However, PCR signals were detectable in 3 out of 57 biopsies. In 2 specimens signals were very weakly detectable only by hybridization. It may be concluded that the DNA-based PCR assay may be useful in monitoring the trends of therapeutic responses in MB patients under treatment.—Authors' Summary

Experimental Infections

Consigny, S., Bentoucha, A., Bonnafous, P., Grosset, J. and Ji, B. H. Bactericidal activities of HMR 3647, moxifloxacin, and rifapentine against *Mycobacterium leprae* in mice. Antimicrob. Agents Chemother. **44** (2000) 2919–2921.

Bactericidal activities of HMR 3647 (HMR), moxifloxacin (MXFX), and rifapentine (RPT) against *Mycobacterium leprae,* measured by the proportional bactericidal technique in the mouse foot pad system, were compared with those of the established antileprosy drugs clarithromycin (CLARI), ofloxacin (OFLO), and rifampin (RMP). Administered in five daily doses of 100 mg/kg of body weight, HMR appeared slightly more bactericidal than CLARI. In a single dose, MXFX at 150 mg/kg was more active than the same dose of OFLO and displayed exactly the same level of activity as RMP at 10 mg/kg; the combination MXFX-minocycline (MINO) (MM) was more bactericidal than the combination OFLO-MINO (OM); RPT at 10 mg/kg was more bactericidal than the same dose of RMP and even more active than the combination RMP-OFLO-MINO (ROM); the combination RPT-MXFX-MINO (PMM) killed 99.9% of viable *M. leprae* and was slightly more bactericidal than RPT alone, indicating that the combination PMM showed an additive effect against *M. leprae.*—Authors' Abstract

Gormus, B. J., Murphey Corb, M., Baskin, G. B., Uherka, K., Martin, L. N., Marx, P. A., Xu, K. and Ratterree, M. S. Interactions between *Mycobacterium leprae* and simian immunodeficiency virus (SIV) in rhesus monkeys. J. Med. Primatol. **29** (2000) 259–267.

Groups of rhesus monkeys were inoculated with: 1) simian immunodeficiency virus (SIV) (B670) alone; 2) *Mycobacterium leprae* alone; 3) SIV plus *M. leprae* on the same day; and 4) *M. leprae* 2 weeks after SIV. Animals were monitored at intervals for virus loads, antibody responses to *M. leprae* glycolipid antigens and to SIV Gp120, T-cell CD4+ anti-CD4+ CD29+ subset percentages, leprosy and acquired immunodeficiency syndrome (AIDS) clinical symptoms. Five out of 6 animals developed leprosy in each co-inoculated group compared to 1 out of 6 in the *M. leprae*-only-inoculated group, indicating that *M. leprae*/SIV co-infection increases the susceptibility to leprosy, regardless of the timing of the two infections. Animals in the co-infected group that received *M. leprae* 2 weeks after SIV had a significantly slower rate of AIDS progression and long-term survival was significantly greater (3 out of 6) compared to the group inoculated with SIV alone (0 out of 7). All *M. leprae*-only-inoculated animals (6 out of 6) survived. Post-SIV-inoculation, a rapid decrease in the percentages of CD4+ and CD4+ CD29+ T-cells was observed in the SIV-only-inoculated group that was significantly blocked by co-inoculation with *M. leprae* 2 weeks after SIV, but not by SIV on the same day. The virus load set point was increased by approximately two logs in the group inoculated with *M. leprae* and SIV on the same day compared to SIV 2 weeks prior to *M.*

leprae or the SIV-only-inoculated group. The results indicate that *M. leprae*, inoculated 2 weeks after SIV, decreased the pathogenicity of SIV compared to inoculation of *M. leprae* and SIV on the same day or SIV alone. The decreased pathogenicity correlated with a diminished loss of CD4+ and CD4+ CD29+ T-cell subsets in the group inoculated with *M. leprae* 2 weeks after SIV compared to the group inoculated with SIV alone. IgG antibody responses to *M. leprae*-specific cell wall phenolic glycolipid-I antigen were inhibited by 2-week-prior or same-day SIV co-inoculation compared to *M. leprae*-only inoculated animals. The IgG anti-lipoarabinomannan antibody response was enhanced in the group inoculated with *M. leprae* and SIV on the same day compared to the groups inoculated with *M. leprae* alone or SIV 2 weeks prior to *M. leprae*. Antibody responses to SIV Gp120 antigen were unimpaired in both co-inoculated groups compared to SIV-only-inoculated groups. The antibody results show that the immune responses to SIV and *M. leprae* are interrelated in SIV/*M. leprae* co-infected animals.—Authors' Abstract

Epidemiology and Prevention

Bruce, S., Schroeder, T. L., Ellner, K., Rubin, H., Williams, T. and Wolf, J. E. Armadillo exposure and Hansen's disease: an epidemiologic survey in southern Texas. J. Am. Acad. Dermatol. **43** (2000) 223–228.

Background: Naturally occurring leprosy has been demonstrated in wild nine-banded armadillos (*Dasypus novemcinctus*). This suggests a possible mode of transmission of human leprosy in regions where armadillo contact is prevalent.

Objective: Our purpose was to study the possible relationship between armadillo exposure and Hansen's disease.

Method: One-hundred-one patients (67 men, 34 women) with established Hansen's disease seen in the Hansen's Disease Clinic in Houston, Texas, were questioned about their exposure to armadillos. These patients were divided into two groups: Asian (N = 32) and non-Asian (N = 69).

Results: Seventy-one percent of the non-Asian patients surveyed reported either direct or indirect armadillo exposure. None of the Asian patients reported armadillo exposure ($p < 0.001$). Of the non-Asian patients, 75.4% had lepromatous disease versus 50.0% of the Asian patients ($p < 0.001$). The average age at diagnosis for the non-Asian group with Hansen's disease in this study was 51 versus 38 years for the Asian group ($p < 0.001$).

Conclusion: Although it is yet to be determined whether direct transmission from the armadillo to human occurs, it is likely based on the high incidence of armadillo exposure in non-Asian patients with Hansen's disease in our study population that this animal acts as a reservoir for human disease. However, the Asian patients reporting no known armadillo exposure likely obtained the disease from person-to-person contact in their respective countries of origin where Hansen's disease has a much higher prevalence.—Authors' Abstract

Crouzat, M. [Leprosy in New Caledonia: evolution from 1983 to 1998.] Acta Leprol. **11** (1999) 139–144. (in French)

The authors report the results of a retrospective study on the evolution of leprosy in New Caledonia, French island in Oceania, between 1983, time of the onset of polychemotherapy (PCT), and 1998. Since 1996, the prevalence is and remains less than 1/10,000. The annual rate detection fell from 15.6 in 1983 to 2.48/100,000 in 1998. Less than 10 new cases are detected annually since 1994. During this period, the number of new patients less than 15 years old decreased, and the percentage of multibacillary (MB) patients increased, assessing *a priori* the improvement of the endemicity. One case of relapse was detected in an MB patient treated for 2 years among the 231 new patients.—Author's English Summary

de Carsalade, G. Y., Achirafi, A. and Flageul, B. [Hansen's disease in Mayotte island, French territorial collectivity in the Comoro Islands: a retrospective study from 1990–1998.] Acta Leprol. **11** (1999) 133–137. (in French)

Mayotte, a French island of the Comoro Islands in the Indian Ocean, is located in a leprosy-endemic area including the other islands of the archipelago and Madagascar island. As the last Hansen's disease epidemiological study in the island was reported in 1982, we achieved a new valuation by a retrospective study on the 1990–1998 period. Our investigation showed that the disease was still endemic with a prevalence of 32/100,000 population in 1998 and a high annual new case detection rate (14 to 31/100,000 population). The profile of the newly detected cases was the same as that reported at the world level (predominance of males, less than 45 years old adults and paucibacillary forms) with two exceptions: the high percentage of children below 15 years of age (28.2%) and of family cases (25.3%). Moreover, 12.6% of the new cases exhibited disabilities of grade 2 at the time of the diagnosis. These features emphasize the need for an enhanced leprosy control in this island which has a well-developed medical assistance.—Authors' English Summary

Gomez Echevarria, J. R. and Hernandez Ramos, J. M. [Tuberculoid leprosy.] Rev. Leprol. Fontilles **22** (2000) 497–510. (in Spanish)

Since 1991, the Sanatorium of Fontilles has been involved in public health programs with a special dedication to dermatological pathology and leprosy in the state of Mato Grosso (Brazil). Work has been carried out in the northeast area of this Brazilian state (Santa Terezinha, Sao Félix do Araguaia, Porto Alegre do Norte and Alto do Boa Vista), a very endemic area for leprosy in Brazil.

From January 1991 to the present 822 individuals were diagnosed and treated for this disease (585 men and 297 women, 66 patients were children under 14 years); 624 patients were treated according to WHO-Nacional de Saude recommendations. At this moment 158 patients remain active and on treatment.

The prevalence of the disease in the area is over 45/10,000 population. In this paper the personal experience of the two doctors who carried out the study is explained. The number of individuals diagnosed as tuberculoid leprosy was 305 (190 men, 115 females, 12 children less than 14 years old); 232 were treated and discharged as cured, with 36 patients still on treatment at this moment.—Authors' English Summary

Leprosy elimination campaigns (LEC). Lepr. Rev. **70** (1999) 404–407.

The objectives and elements of leprosy elimination campaigns (LECs) are discussed. Some activities carried out under LEC are considered, including: orientation workshops for local health workers and community volunteers; community awareness creation and participation; case finding; treating every detected case with multidrug therapy and making efforts to ensure that each one is cured.—Trop. Dis. Bull. **97** (2000) 743–744

Revankar, C. R., Samy, M. S. A., Bulchand, H. O. and Ganapati, R. Leprosy elimination campaign in a metropolitan leprosy project, Bombay, India. Lepr. Rev. **70** (1999) 448–451.

An account of the leprosy elimination campaign undertaken by the Bombay Leprosy Project as a part of the statewide campaign in Maharashtra state [India] between 30 January and 5 February 1998 to identify hidden cases and locate endemic pockets is given. The impact of the campaign on leprosy case detection is discussed.—Trop. Dis. Bull. **97** (2000) 745

Umbarhande, D., Pharande, A. M., Thaker, U. H. and Naik, S. S. Voluntarily reporting leprosy cases in rural area. (Letter) Indian J. Lepr. **71** (1999) 483–484.

The reasons for the increase in voluntary reporting of leprosy at leprosy centers was

investigated by interviewing 115 multibacillary cases (53 smear-positive and 62 smear-negative) who were registered in the last 5 years and had reported voluntarily in the rural area in Panvel taluka or Raigad district of Maharashtra, India [date not given]. Only 26 (23%) leprosy patients said they knew about the leprosy worker in their area. Multiple patches (56 persons or 49%) and nodules (28 persons or 24%) were their major concern; 40 (35%) of them were referred to leprosy centers by other cured leprosy patients, 24 (21%) were sent by medical practitioners, another 24 by relatives, 12 (10%) by friends and 10 (9%) had reported voluntarily as a result of health education. The lag period between noticing the patches and reporting for diagnosis and treatment was zero (immediate reporting) in 42 (37%) patients and 6 months in 28 patients (24%). Treatment completion was reported in 108 patients (94%).—Trop. Dis. Bull. **97** (2000) 990

Rehabilitation

Boucher, P., Millan, J., Parent, M. and Moulia-Pela, J. P. [Compared and randomized trial of medical and medico-surgical treatment in leprosy neuritis.] Acta Leprol. **11** (1999) 171–177. (in French)

The aim of the study was to compare the results of the medical treatment alone and of the medico-surgical treatment on leprous neuritis. The patients were followed up during 2 years with regular neurological evaluations. The statistical study was performed using the Tukey test.

Ninety-three nerves (ulnar, median, common peroneal and posterior tibial) with a deficit of less than 6 months duration have been studied in 31 leprosy patients. All the patients were treated by steroids but in some of them a nerve surgical decompression was performed. An improvement of the sensitive and motor deficit was observed in both groups. No significant statistical differences appeared between the two groups according to the nerve involved, the duration of the deficit, the form of leprosy and the type of antibacillary treatment. However, the medico-surgical treatment had a significantly better result on pain and on major but incomplete nervous involvement.

This study included a limited number of nerves; thus, it would be useful to perform other randomized assays to better define the indications of surgical decompression in the management of leprous neuritis.—Authors' English Summary

Grauwin, M. Y., Cartel, J. L. and Lepers, J. P. [How to treat osteitis and septic arthritis of the extremities in patients with long-standing leprosy using ordinary granulated sugar.] Acta Leprol. **11** (1999) 147–152. (in French)

A common problem of osteitis and septic arthritis is the recurrent bone infection after surgical debridement, a problem frequently encountered in patients with sequellar leprosy. In these cases the authors propose the use of an ancient method of post-surgical wound care based on the treatment with ordinary granulated sugar. The hyperosmolar climate created this way in the wounds inhibits the bacterial growth, enhances bacterial death and therefore permits the growth of granulation tissue in order to recover the debrided nude bones. At ILAD (Leprosy Institute of Dakar), 36 osteitis and septic arthritis patients were treated and healed during the last 2 years from March 1995 to March 1997 using this technique. All the wounds healed in the mean time of 44 days. Only two of them needed a second debridement and healed afterward. Up to now the method using ordinary sugar was applied in the treatment of infected wounds, eschars and post-surgical infections. Our experience shows that it also can be indicated to treat bone infections. This method is easy to apply also under often difficult field conditions and is very cheap.—Authors' English Summary

Hietaharju, A., Croft, R., Alam, R., Birch, P., Mong, A. and Haanpaa, M. Chronic neuropathic pain in treated leprosy. Lancet **356** (2000) 1080–1081.

The existence of chronic neuropathic pain in treated leprosy has received scant attention. We describe the clinical findings of 16 patients with multibacillary leprosy who had chronic stimulus-independent pain despite finishing their treatment. With confirmation, our results could be of importance in the establishment of "care after cure" programs for patients with leprosy.—Authors' Abstract

Other Mycobacterial Diseases and Related Entities

Abolhassani, M., Lagranderie, M., Chavarot, P., Balazuc, A. M. and Marchal, G. *Mycobacterium bovis* BCG induces similar immune responses and protection by rectal and parenteral immunization routes. Infect. Immun. **68** (2000) 5657–5662.

We compared cellular immune responses to rectal, subcutaneous, and intradermal administration of *Mycobacterium bovis* BCG for 5 to 20 weeks in mice, guinea pigs, and macaques. Strong lymphoproliferative responses were induced in spleen cells after *in vitro* stimulation with purified protein derivative in guinea pigs and macaques, whatever the route of immunization. Comparable high numbers of gamma interferon- and tumor necrosis factor alpha-producing cells were found in the spleen after rectal, subcutaneous, and intradermal immunization of mice and macaques. Similar levels of precursors of cytotoxic T lymphocytes specific for mycobacterial antigens were observed in mice for all immunization routes. In macaques, cytotoxic activity, determined only at the end of the experiment (20 weeks), was similar after rectal and intradermal immunization. Six months after immunization, rectal and subcutaneous routes induced in mice similar levels of protective immunity against challenge with a virulent *M. tuberculosis* strain (H37Rv). Rectal immunization gave immune responses and protective capacity similar to those for parenteral immunization and seemed to be a promising new route of vaccination against tuberculosis; in our study, immunization via the rectal route never induced side effects associated with parenteral routes (axillary adenitis) and could also effectively reduce the risks of viral transmission associated with unsafe injections in the developing world.—Authors' Abstract

Al Zahrani, K., Al Jahdali, H. and Menzies, D. Does size matter? Utility of size of tuberculin reactions for the diagnosis of mycobacterial disease. Am. J. Respir. Crit. Care Med. **162** (2000) 1419–1422.

It is a common belief that larger tuberculin reactions are more serious and more likely to indicate patients with active tuberculosis (TB) or at high risk of disease in the future. Among 182 close contacts and 502 patients suspected of possible active TB, 529 underwent tuberculin skin testing (TST) and 605 had a chest radiograph. Final diagnoses, based on all available clinical, microbiological, histological, and radiographic information, were active TB, 68; inactive TB, 274; nontuberculous mycobacterial disease, 14; conditions associated with anergy, 36; no detectable abnormality (except a positive TST) or condition unrelated to TB, 213; and negative TST, no further evaluation, 79. Among these patients, a TST of 5 mm or larger was significantly more likely to indicate active or inactive TB ($p < 0.001$). However, among patients with a TST of 5 mm or greater, the size and frequency distribution of tuberculin reactions were not different between subjects with different diagnoses, nor between subjects with different types or extent of radiographic findings. As well, TST reactions were no different in 121 subjects with or 176 subjects without a history of BCG vaccination. In close contacts of patients suspected of active TB, reactions <5

mm indicated a lower likelihood of active or inactive disease, but above that threshold, the size of tuberculin reaction did not matter.—Authors' Abstract

Baca, A. M., Sirawaraporn, R., Turley, S., Sirawaraporn, W. and Hol, W. G. J. Crystal structure of *Mycobacterium tuberculosis* 6-hydroxymethyl-7,8-dihydropteroate synthase in complex with pterin monophosphate: new insight into the enzymatic mechanism and sulfa-drug action. J. Mol. Biol. **302** (2000) 1193–1212.

The enzyme 6-hydroxymethyl-7,8-dihydropteroate synthase (DHPS) catalyzes the condensation of para-aminobenzoic acid (pABA) with 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate to form 6-hydroxymethyl-7,8-dihydropteroate and pyrophosphate. DHPS is essential for the *de novo* synthesis of folate in prokaryotes, lower eukaryotes, and in plants, but is absent in mammals. Inhibition of this enzyme's activity by sulfonamide and sulfone drugs depletes the folate pool, resulting in growth inhibition and cell death. Here, we report the 1.7 Angstrom resolution crystal structure of the binary complex of 6-hydroxymethylpterin monophosphate (PtP) with DHPS from *M. tuberculosis* (Mtb), a pathogen responsible for the death of millions of human beings each year. Comparison to other DHPS structures reveals that the Mtb DHPS structure is in a unique conformation in which loop 1 closes over the active site. The Mtb DHPS structure hints at a mechanism in which both loops 1 and 2 play important roles in catalysis by shielding the active site from bulk solvent and allowing pyrophosphoryl transfer to occur. A binding mode for pABA, sulfonamides and sulfones is suggested based on: (i) the new conformation of the closed loop 1; (ii) the distribution of dapsone and sulfonamide resistance mutations; (iii) the observed direction of the bond between the 6-methyl carbon atom and the bridging oxygen atom to the alpha-phosphate group in the Mtb DHPS : PtP binary complex; and (iv) the conformation of loop 2 in the *Escherichia coli* DHPS structure. Finally, the Mtb DHPS structure reveals a highly conserved pterin binding pocket that may be exploited for the design of novel antimycobacterial agents.—Authors' Abstract

Bermudez, L. E., Nash, K., Petrofsky, M., Young, L. S. and Inderlied, C. B. Clarithromycin-resistant *Mycobacterium avium* is still susceptible to treatment with clarithromycin and is virulent in mice. Antimicrob. Agents Chemother. **44** (2000) 2619–2622.

Resistance to clarithromycin in breakthrough *Mycobacterium avium* complex (MAC) isolates typically occurs 3 to 4 months after the initiation of monotherapy in bacteremic AIDS patients. It has been suggested that continuation of clarithromycin therapy still results in clinical and microbiological improvement. To study this paradox, C57BL/6 beige mice were infected with a clarithromycin-resistant (MIC ≥128 μg/ml) strain of MAC 101 (CLA-R MAC 101) and treated with 200 mg of clarithromycin per kg of body weight/day alone or in combination with ethambutol (100 mg/kg/day) for 2 weeks. Mice infected with a clarithromycin-susceptible strain of MAC 101 had bacterial loads reduced by 90% in the liver and 91% in the spleen ($p < 0.05$ compared with the control). Clarithromycin treatment of CLA-R MAC 101 resulted in a 65% reduction of bacterial loads in the liver ($p = 0.009$) and a 71% reduction in the spleen ($p = 0.009$) compared with the results for the untreated control. CLA-R MAC 101 and MAC 101 (isogenic strains) had comparable growth rates in murine tissue, ruling out a loss of virulence of CLA-R MAC 101. Strains of MAC currently defined as macrolide resistant may still respond to treatment with an agent such as clarithromycin within infected tissues.—Authors' Abstract

Boshoff, H. I. M. and Mizrahi, V. Expression of *Mycobacterium smegmatis* pyrazinamidase in *Mycobacterium tuberculosis* confers hypersensitivity to pyrazinamide and related amides. J. Bacteriol. **182** (2000) 5479–5485.

A pyrazinamidase (PZase)-deficient pncA mutant of *Mycobacterium tuberculosis,*

constructed by allelic exchange, was used to investigate the effects of heterologous amidase gene expression on the susceptibility of this organism to pyrazinamide (PZA) and related amides. The mutant was highly resistant to PZA (MIC >2000 μg/ml) in accordance with the well-established role of pncA in the PZA susceptibility of *M. tuberculosis* (A. Scorpio and Y. Zhang, Nat. Med. 2: 662–667, 1996). Integration of the pzaA gene encoding the major PZase/nicotinamidase from *M. smegmatis* (H. I. Boshoff and V. Mizrahi. J. Bacteriol. 180:5809–5814, 1998) or the *M. tuberculosis* pncA gene into the pncA mutant complemented its PZase/nicotinamidase defect. In both pzaA- and pncA-complemented mutant strains, the PZase activity was detected exclusively in the cytoplasm, suggesting an intracellular localization for PzaA and PncA. The pzaA-complemented strain was hypersensitive to PZA (MIC ≤10 μg/ml) and nicotinamide (MIC ≥20 μg/ml) and was also sensitive to benzamide (MIC 20 μg/ml), unlike the wild-type and pncA-complemented mutant strains which were highly resistant to this amide (MIC >500 μg/ml). This finding was consistent with the observation that benzamide is hydrolyzed by PzaA but not by PncA. Overexpression of PzaA also conferred sensitivity to PZA, nicotinamide, and benzamide on *M. smegmatis* (MIC 150 μg/ml in all cases) and rendered *Escherichia coli* hypersensitive for growth at low pH.—Authors' Abstract

Bosne David, S., Barros, V., Verde, S. C., Portugal, C. and David, H. L. Intrinsic resistance of *Mycobacterium tuberculosis* to clarithromycin is effectively reversed by subinhibitory concentrations of cell wall inhibitors. J. Antimicrob. Chemother. **46** (2000) 391–395.

Subinhibitory concentrations of bacitracin, vancomycin and other inhibitors of cell wall synthesis reversed to varying extents the intrinsic resistance of *Mycobacterium tuberculosis* to clarithromycin. Ethambutol reversed clarithromycin resistance in all of the *M. tuberculosis* strains studied regardless of their susceptibility to this drug.—Authors' Abstract

Briscoe, H., Roach, D. R., Meadows, N., Rathjen, D. and Britton, W. J. A novel tumor necrosis factor (TNF) mimetic peptide prevents recrudescence of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) infection in CD4+ T cell-depleted mice. J. Leuk. Biol. **68** (2000) 538–544.

Tumor necrosis factor (TNF) is required to control mycobacterial infections, but its therapeutic value is limited by its *in vivo* instability and toxicity. The efficacy of a non-toxic TNF-mimetic peptide (TNF70–80) was tested in mice infected with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). *In vitro* TNF70–80 and recombinant human TNF (hTNF) acted with interferon gamma (IFN-γ) to reduce bacterial replication and to induce synthesis of bactericidal nitric oxide (NO) in BCG-infected, bone marrow-derived murine macrophages. The dose-dependent inhibitory effect on bacterial replication was blocked by neutralizing anti-IFN-γ and anti-hTNF monoclonal antibodies. Further, n-mono-methyl-L-arginine (n-MMA) and a soluble TNF-receptor I (TNFRI-IgG) blocked bacterial growth and NO synthesis. Therefore, the peptide acted with IFN-γ via induction of NO synthase and signaled through TNFRI receptors. Concomitant *in vivo* treatment with TNF70–80 or hTNF prevented reactivation of chronic BCG infection in mice depleted of CD4+ T cells by injecting anti-CD4 antibodies. Granuloma number and bacterial load were comparable in treated, T cell-depleted mice and in chronically infected, intact animals. Thus, TNF70–80 and hTNF can modulate recrudescent BCG infection in CD4+ T cell-deficient mice.—Authors' Abstract

Chambers, M. A., Vordermeier, H. M., Whelan, A., Commander, N., Tascon, R., Lowrie, D. and Hewinson, R. Vaccination of mice and cattle with plasmid DNA encoding the *Mycobacterium bovis* antigen MPB83. Clin. Infect. Dis. **30** Suppl. 3 (2000) S283–S287.

A scientific review of bovine tuberculosis in Great Britain has concluded that the development of a cattle vaccine holds the best prospect for long-term disease control.

Recent reports of successful DNA vaccination against *Mycobacterium tuberculosis* in small animal models have raised the possibility of using a similar strategy to produce vaccines against *Mycobacterium bovis* infection in cattle. To test this possibility, BALB/c mice were immunized with DNA encoding the *M. bovis* antigen MPB83. The mice responded to vaccination with a mixed IgG1/IgG2a response to the antigen and were protected from intravenous challenge with virulent *M. bovis* to a similar extent as those vaccinated with bacille Calmette-Guerin. The immunogenicity of the DNA vaccine in cattle was tested after having established that DNA encoding MPB83 was immunogenic and elicited protective immunity in mice. In these studies, vaccinated animals had strong proliferative responses to MPB83.—Authors' Abstract

Clemens, D. L., Lee, B. Y. and Horwitz, M. A. *Mycobacterium tuberculosis* and *Legionella pneumophila* phagosomes exhibit arrested maturation despite acquisition of Rab7. Infect. Immun. **68** (2000) 5254–5266.

Rab7 is a small GTPase that regulates vesicular traffic from early to late endosomal stages of the endocytic pathway. Phagosomes containing inert particles have also been shown to transiently acquire Rab7 as they mature. Disruption in the pathway prior to the acquisition of Rab7 has been suggested as playing a role in the altered maturation of *Mycobacterium bovis* BCG phagosomes. As a first step to determine whether disruption in the delivery or function of Rab7 could play a role in the altered maturation of *Legionella pneumophila* and *M. tuberculosis* phagosomes, we have examined the distribution of wild-type Rab7 and the GTPase-deficient, constitutively active mutant form of Rab7 in HeLa cells infected with *L. pneumophila* or *M. tuberculosis*. We have found that the majority of *L. pneumophila* and *M. tuberculosis* phagosomes acquire relatively abundant staining for Rab7 and for the constitutively active mutant Rab7 in HeLa cells that overexpress these proteins. Nevertheless, despite acquisition of wild-type or constitutively active Rab7, both the *L. pneumophila* and the *M. tuberculosis* phagosomes continue to exhibit altered maturation as manifested by a failure to acquire lysosome-associated membrane glycoprotein 1. These results demonstrate that *L. pneumophila* and *M. tuberculosis* phagosomes have receptors for Rab7 and that the altered maturation of these phagosomes is not due to a failure to acquire Rab7.—Authors' Abstract

Coler, R. N., Skeiky, Y. A. W., Ovendale, P. J., Vedvick, T. S., Gervassi, L., Guderian, J., Jen, S., Reed, S. G. and Campos Neto, A. Cloning of a *Mycobacterium tuberculosis* gene encoding a purified protein derivative protein that elicits strong tuberculosis-specific delayed-type hypersensitivity. J. Infect. Dis. **182** (2000) 224–233.

The purified protein derivative (PPD) skin test has been used for the diagnosis of tuberculosis for more than 75 years. However, the test lacks specificity because all mycobacteria share antigens present in PPD. Therefore, sensitization with nontuberculous pathogenic or with environmental nonpathogenic mycobacteria can lead to positive skin tests. This communication describes a novel PPD protein present only in tuberculous complex mycobacteria. A recombinant protein was obtained and named DPPD on the basis of the first 4 amino acids of its N-terminus sequence. DPPD elicited delayed-type hypersensitivity (DTH) in 100% of *Mycobacterium tuberculosis*-infected guinea pigs but in no animals sensitized with several organisms representative of all members of the *Mycobacterium* genus. Preliminary results indicate that DPPD induces strong and specific DTH in humans. This work points to the definition of a single recombinant *M. tuberculosis* protein that may be an alternative to the PPD test.—Authors' Abstract

Das, S. and Chattopadhyay, U. K. Role of silicon in modulating the internal morphology and growth of *Mycobacterium tuberculosis*. Indian J. Tuberc. **47** (2000) 87–91.

Silicon is known to enhance the growth and pathogenicity of *M. tuberculosis.* In this study the pattern of growth of different strains of *M. tuberculosis* was studied on a carbon-free silicon-based culture medium and compared with that on conventional media. Thirty-two strains of *M. tuberculosis* from mostly clinical isolates were serially propagated on a carbon-free silicate medium and on different conventional media using standard inocula. There was initial good growth on the silicon-based medium in comparison with the conventional media. However, the growth was considerably reduced after early serial transfers but improved again in late serial cultures. The initial good growth appears to be due to increased activity of silicon-induced fatty acid synthase, and the late improvement due to slow adaptation of *M. tuberculosis* to the carbon-free metabolism by the formation of silicic acid esterified cell wall and silicon induced genetic alteration. All these changes were probably responsible for the formation of fibrous, rope-like structures and dense granules seen under electron microscope. Since the silicon content of the lung tissue is comparatively higher than many other tissues of the human body, it could play a role in the pathogenicity of tuberculosis in the lungs.—Trop. Dis. Bull. **97** (2000) 1000

Davies, A. P., Billington, O. J., McHugh, T. D., Mitchison, D. A. and Gillespie, S. H. Comparison of phenotypic and genotypic methods for pyrazinamide susceptibility testing with *Mycobacterium tuberculosis.* J. Clin. Microbiol. **38** (2000) 3686–3688.

Mycobacterium tuberculosis converts pyrazinamide to its active form by using the enzyme pyrazinamidase. This enzyme is coded for on the pncA gene, and mutations in the pncA gene result in absence of active enzyme, conferring resistance to the drug pyrazinamide. We investigated 27 strains of *M. tuberculosis* suspected of being multidrug resistant. Each isolate was tested for susceptibility to pyrazinamide by the BACTEC 460TB method, and 19 were pyrazinamide resistant. The presence of active pyrazinamidase enzyme was sought by using the Wayne assay, which was positive in all of the sensitive isolates and four of the resistant isolates. The pncA gene was amplified by PCR, and mutations were sought by single-strand conformation polymorphism (SSCP) analysis. We identified four isolates which were phenotypically resistant to pyrazinamide, but which had active pyrazinamide enzyme on the Wayne assay and normal pncA gene SSCP. MICs measured by BACTEC 460TB and susceptibility testing at a lower pH of 5.5 confirmed genuine resistance. The pncA gene was sequenced in these four isolates and found not to have any mutations. This implies that an alternative mechanism of resistance exists in these strains. We conclude that genotypic assessment of pyrazinamide resistance is unreliable, because it depends on the identification of a single resistance mechanism. Phenotypic methods such as the BACTEC 460TB technique remain the best methods for pyrazinamide susceptibility testing.—Authors' Abstract

Dega, H., Robert, J., Bonnafous, P., Jarlier, V. and Grosset, J. Activities of several antimicrobials against *Mycobacterium ulcerans* infection in mice. Antimicrob. Agents Chemother. **44** (2000) 2367–2372.

Mycobacterium ulcerans inoculated into the foot pads of mice at 6×10^3 CFU was shown to have a generation time of 6.5 days when estimated from weekly changes in microscopic counts of acid-fast bacilli (AFB) and 7.5 days when calculated from actual CFU enumerated on Lowenstein-Jensen egg medium incubated at 32°C. Foot pads became swollen at week 10 (W10) after infection, and all infected control mice were dead at W15 after infection. Daily (5 days/week) treatment with 100 mg of clarithromycin (CLR)/kg of body weight beginning the day after infection prevented swelling of foot pads at W10. When initiation of treatment was delayed until obvious foot pad swelling was observed, there was a reduction in both the increase in AFB counts and deterioration of swollen foot pads and also a prolonged survival of the mice to W18. Mice infected in the hind foot pads with 5×10^5 CFU of *M. ulcerans*

were divided into an untreated control group and six treatment groups that received one of the following therapies for 8 weeks: 100 mg of CLR/kg, 25 mg of minocycline (MIN)/kg, 50 mg of sparfloxacin (SPX)/kg, 10 mg of rifampin (RIF)/kg, 10 mg of rifabutin (RBT)/kg, or 100 mg of amikacin (AMK)/kg. After completion of therapy, treated animals were observed for an additional 17 weeks. All control mice and mice treated with CLR, MIN, or SPX exhibited swollen foot pads during the observation period. In contrast, of those animals treated with RIF, RBT, or AMK, none had foot pad swelling and all inoculated cultures done after the W17 observation remained negative. These results suggest that RIF, RBT, and AMK may be effective in the treatment of human infection with *M. ulcerans*.—Authors' Abstract

DSouza, C. D., Cooper, A. M., Frank, A. A., Ehlers, S., Turner, J., Bendelac, A. and Orme, I. M. A novel nonclassic beta 2-microglobulin-restricted mechanism influencing early lymphocyte accumulation and subsequent resistance to tuberculosis in the lung. Am. J. Respir. Cell Mol. Biol. **23** (2000) 188–193.

In this study, we compared the course of a low-dose aerosol *Mycobacterium tuberculosis* infection in mice bearing gene disruptions for the beta 2-microglobulin molecule, the CD8 molecule, and the CD1 molecule. Over the first 50 days of infection, the CD8- and CD1-disrupted mice were no more susceptible to infection than were the control mice. In contrast, the bacterial load in beta 2-microglobulin gene-disrupted mice increased rapidly and attained much higher levels than that observed in the other gene-disrupted mice and in control mice. A second major difference between the beta 2-microglobulin gene-disrupted mice and the other animals was the development of lung granulomas; both the CD8- and CD1-disrupted mice developed essentially normal granulomas except for an apparent increased lymphocyte influx in the CD8-disrupted mice. The beta 2-microglobulin gene-disrupted mice, on the other hand, developed granulomas virtually devoid of lymphocytes, with these cells instead localized within prominent perivascular cuffing adjacent to the lesions. These data support the hypothesis that a beta 2-microglobulin-dependent, non-CD8- and non-CD1-dependent mechanism controls the early and efficient influx of protective lymphocytes into infected lesions, and that the absence of this mechanism decreases the capacity of the animal to initially deal with pulmonary tuberculosis.—Authors' Abstract

Eckstein, T. M., Inamine, J. M., Lambert, M. K. and Belisle, J. T. A genetic mechanism for deletion of the ser2 gene cluster and formation of rough morphological variants of *Mycobacterium avium*. J. Bacteriol. **182** (2000) 6177–6182.

A major phenotypic trait of the *Mycobacterium avium* complex is the ability to produce rough and smooth colony variants. The chemical basis of this morphological variation is the loss of an antigenic surface structure, termed glycopeptidolipid (GPL), by rough variants. Using *M. avium* serovar 2 strain 2151 as a model system, this laboratory previously reported that rough variants arise via the deletion of large genomic regions encoding GPL biosynthesis. One such deletion encompasses the gene cluster (ser2) responsible for production of the serovar 2 GPL haptenic oligosaccharide.

In this study, nucleotide sequencing revealed that both ends of the ser2 gene cluster are flanked by a novel insertion sequence (IS1601) oriented as direct repeats. Detailed analyses of the site of deletion in the genome of *M. avium* 2151 Rg-1 demonstrated that a single copy of IS1601 remained and that the ser2 gene cluster was deleted by homologous recombination. This same deletion pattern was observed for 10 out of 15 rough colony variants tested. Additionally, these studies revealed that IS1601 contains portions of three independent insertion sequences. This report is the first to define the precise genetic basis of colony variation in *Mycobacterium* spp., and provides further evidence that homologous recombination between insertion sequence elements can be a primary determinant of genome plasticity in these bacteria.—Authors' Abstract

Faber, W. R., Arias Bouda, L. M. P., Zeegelaar, J. E., Kolk, A. H. J., Fonteyne, P. A., Toonstra, J. and Portaels, F. First reported case of *Mycobacterium ulcerans* infection in a patient from China. Trans. R. Soc. Trop. Med. Hyg. **94** (2000) 277–279.

Buruli ulcers have not been previously described in China, and only once at higher latitudes on the northern hemisphere. A patient who travelled in the Shandong Province in the People's Republic of China developed an ulcer which was proven to be a Buruli ulcer. The clinical picture and histopathological findings from biopsy specimens are characteristic for a Buruli ulcer, and also the growth in culture (Coletsos medium) at a restricted temperature of 30°C. A multiplex polymerase chain reaction (PCR) based on the amplification of the gene encoding for 16S ribosomal RNA and a nested PCR based on the *Mycobacterium ulcerans* specific repeated sequence 2404 were performed. These PCR investigations identified the bacteria as *M. ulcerans* subspecies shinshuense. The patient was initially treated with clarithromycin and rifampin, which was changed to ciprofloxacin and rifabutin when rifampin resistance of the first isolate was established. There were no signs of reactivation of the disease 6 months after the end of treatment. *M. ulcerans* infection occurs above 30° latitude on the northern hemisphere in China, and is caused by *M. ulcerans* subspecies shinshuense. This case appears to be cured by chemotherapy alone in contrast to the general experience that surgical treatment is indicated. The granulomatous reaction with only fragments of acid-fast bacteria in the biopsy at the end of treatment may indicate the development of an adequate cell-mediated immune response leading to resistance to the infection.—Authors' Abstract

Falero Diaz, G., Challacombe, S., Banerjee, D., Douce, G., Boyd, A. and Ivanyi, J. Intranasal vaccination of mice against infection with *Mycobacterium tuberculosis*. Vaccine **18** (2000) 3223–3229.

The intranasal (i.n.) route of immunization has recently been of active interest in endeavours to improve the efficacy of vaccination against a number of respiratory infections. Here, we examined the outcome of tuberculous infection in BALB/c mice. I.n. application of the BCG-Pasteur strain was found to be highly protective against challenge infection with the pathogenic H37Rv strain given after a 4-week interval, reflected by the 100-fold reduction of CFUs in both lungs and spleens. Vaccination with the recombinant PstS-1 antigen and cholera toxin significantly protected against the challenge given 10 days later, but only marginally after 12 weeks. Histological examination showed that i.n. vaccination abrogated the confluent infiltration of lungs with inflammatory cells which surrounds the granulomas in H37Rv challenged control mice. In conclusion, the strong protection demonstrated by BCG suggests that the i.n. route of vaccine delivery deserves further attention toward improving vaccination against tuberculosis.—Authors' Abstract

Fayyazi, A., Eichmeyer, B., Soruri, A., Schweyer, S., Herms, J., Schwarz, P. and Radzun, H. J. Apoptosis of macrophages and T cells in tuberculosis associated gaseous necrosis. J. Pathol. **191** (2000) 417–425.

Immunity against mycobacteria is almost exclusively confined to epithelioid cell granulomas, where a long-lasting but labile balance exists between host and bacilli. The relationship between immunity and mycobacteria results in regression, growth, or caseation of granulomas. To prove whether caseation is associated with apoptosis, biopsy specimens of patients with tuberculosis were analyzed by electron microscopy and by *in situ* end-labelling combined with immunofluorescence. Apoptotic cells were not detected in regressive granulomas. Whereas productive granulomas without histologically recognizable caseous necrosis revealed only single apoptotic cells, large numbers of apoptotic CD68+ macrophages and apoptotic CD3+, CD45RO+ T cells were observed within caseous foci. As prime candidates undergoing and/or eliciting apoptosis, vital cells surrounding caseous foci were characterized. Immunohistochemistry showed that the majority

of vital CD68+ macrophages surrounding caseous foci are negative for the anti-apoptotic protein bcl2 but positive for the pro-apoptotic protein bax. *In situ* hybridization combined with immunofluorescence demonstrated that the majority of the adjacent lymphocytes are activated CD3+, CD45RO+ cells expressing the pro-inflammatory cytokine interferon gamma and the death ligand FasL. These results suggest that caseation is strongly associated with apoptosis of macrophages and T lymphocytes; that the onset of apoptosis in macrophages may be promoted by the lack of bcl2 and the abundance of bax; and that activation-induced cell death (AICD) may be responsible for the apoptosis of T cells.—Authors' Abstract

Federman, G. L. and Federman, D. G. Recalcitrant pyoderma gangrenosum treated with thalidomide. Mayo Clin. Proc. **75** (2000) 842–844.

Pyoderma gangrenosum is a painful, noninfectious, ulcerating skin disorder often associated with systemic disease. Thalidomide has been used to treat many inflammatory dermatologic conditions and has been reintroduced in the United States to treat immune-modulated diseases such as pyoderma gangrenosum. The patient described, a 47-year-old man, had histologically confirmed pyoderma gangrenosum that did not respond to treatment with several courses of methylprednisolone. The ulcer healed with 10 weeks of oral thalidomide administration.—Authors' Abstract

Fratazzi, C., Manjunath, N., Arbeit, R. D., Carini, C., Gerken, T. A., Ardman, B., Remold-O'Donnell, E. and Remold, H. G. A macrophage invasion mechanism for mycobacteria implicating the extracellular domain of CD43. J. Exp. Med. **192** (2000) 183–192.

We studied the role of CD43 (leukosialin/sialophorin), the negatively charged sialoglycoprotein of leukocytes, in the binding of mycobacteria to host cells. CD CD43-transfected HeLa cells bound *Mycobacterium avium* but not *Salmonella typhimurium* or *Shigella flexneri*. Quantitative bacteriology showed that macrophages (Mϕ) from wild-type mice [CD43(+/+)] bound *M. avium, M. bovis* (bacillus Calmette-Guerin), and *M. tuberculosis* (strain H37Rv); whereas Mϕ from CD43 knockout mice [CD43(–/–)] did not. Fluorescence microscopy demonstrate that the associated *M. avium* had been ingested by the CD43(+/+) Mϕ. The inability of CD43(–/–) Mϕ to bind *M. avium* could be restored by the addition of galactoglycoprotein (Galgp), the extracellular mucin portion of CD43. The effect of Galgp is not due to opsonization of the bacteria, but required its interaction with the Mϕ; other mucins had no effect. CD43 expression by the Mϕ was also required for optimal induction by *M. avium* of tumor necrosis factor-alpha (TNF-α) production, which likewise could be reconstituted by Galgp. In contrast, interleukin (IL)-10 production by *M. avium*-infected Mϕ was CD43 independent, demonstrating discordant regulation of TNF-α and IL-10. These findings describe a novel role of CD43 in promoting stable interaction of mycobacteria with receptors on the Mϕ enabling the cells to respond specifically with TNF-α production.—Authors' Abstract

Gomez, J. E. and Bishai, W. R. whmD is an essential mycobacterial gene required for proper septation and cell division. Proc. Natl. Acad. Sci. U.S.A. **97** (2000) 8554–8559.

A study of potential mycobacterial regulatory genes led to the isolation of the *Mycobacterium smegmatis* whmD gene, which encodes a homolog of WhiB, a *Streptomyces coelicolor* protein required for sporulation. Unlike its *Streptomyces* homolog, WhmD is essential in *M. smegmatis*. The whmD gene could be disrupted only in the presence of a plasmid supplying whmD in trans. A plasmid that allowed chemically regulated expression of the WhmD protein was used to generate a conditional whmD mutant. On withdrawal of the inducer, the conditional whmD mutant exhibited irreversible, filamentous, branched growth with diminished septum formation and aberrant septal placement; whereas WhmD overex-

pression resulted in growth retardation and hyperseptation. Nucleic acid synthesis and levels of the essential cell division protein FtsZ were unaltered by WhmD deficiency. Together, these phenotypes indicate a role for WhmD in mycobacterial septum formation and cell division.—Authors' Abstract

Govindarajan, R., Heaton, K. M., Broadwater, R., Zeitlin, A., Lang, N. P. and Hauer Jensen, M. Effect of thalidomide on gastrointestinal toxic effects of irinotecan. Lancet **356** (2000) 566–567.

Irinotecan is the only accepted second-line treatment for colorectal cancer in the U.S.A. Doses are, however, frequently limited by associated late-onset diarrhea. Thalidomide has antiangiogenic and immunomodulatory properties and is being investigated as an antineoplastic. We did a pilot study of combination therapy with thalidomide and irinotecan for metastatic colorectal cancer. In an interim analysis of nine patients, thalidomide had almost eliminated the dose-limiting gastrointestinal toxic effects of irinotecan, especially diarrhea and nausea (each $p < 0.0001$), and 8 of 9 patients were able to complete the chemotherapy course.—Authors' Abstract

Harrington, J. J., III, Ho, J. L., Lapa e Silva, J. R., Conde, M. B., Kritski, A. L., Fonseca, L. S. and Saad, M. H. F. *Mycobacterium tuberculosis* lipid antigens: use of multi-antigen based enzyme immunoassay for free and complex dissociated antibodies. Int. J. Tuberc. Lung Dis. **4** (2000) 161–167.

The sensitivity and specificity of 4 lipid antigens of *M. tuberculosis* (BDA-TDA, DAT, SL-I and PIMs, adsorbed in the same microplate well) to detect the reactive IgG by enzyme-immunoassay (EIA) from plain serum (MA-EIA) and dissociated immune complexes (ICMA-EIA) were tested. Serum samples were obtained from 155 tuberculous (TB) cases without human immunodeficiency virus (HIV) at the Chest Service of the University Hospital in Rio de Janeiro, Brazil [date not given]: 78 patients with positive bacilloscopy and culture, 33 patients with positive culture and 44 patients diagnosed by clinical and radiological criteria; and from 211 HIV-negative control subjects: 32 with other pulmonary diseases, 100 healthy people and 79 close contacts. MA-EIA had an overall sensitivity and specificity of 61% (94 of 155) and 95% (200 of 211), respectively. It was further examined whether the dissociation of immune complexes increases the number of positive reactions in those initially found to be seronegative (SN). The subset of 112 (76 controls and 36 TB) MA-EIA SN samples tested using ICMA-EIA yielded an overall sensitivity and specificity of 83% and 100%. The ICMA-EIA results improved the overall sensitivity from 61% to 80% without changing specificity. It is concluded that the MA-EIA followed by ICMA-EIA, for SN samples, might serve as a fast, cheap, and easy method for the diagnosis of TB in less than 48 hours.—Trop. Dis. Bull. **97** (2000) 751

Heinz, C. and Niederweis, M. Selective extraction and purification of a mycobacterial outer membrane protein. Analyt. Biochem. **285** (2000) 113–120.

MspA forms water-filled channels in the mycolic acid layer of *Mycobacterium smegmatis* thereby allowing the diffusion of hydrophilic solutes through this permeability barrier into the periplasm. MspA is the first member of a new family of porins and is extremely stable against chemical and thermal denaturation. We developed a purification procedure based on selective extraction of MspA with detergents from whole cells of *M. smegmatis* at high temperatures. Anion-exchange and size-exclusion chromatography yielded about 230 μg apparently pure and highly active MspA per liter of culture. This was a 20-fold increased yield compared to previous purification protocols. Similar amounts of pure MspA were obtained with the detergents isotridecylpolyethyleneglycolether, lauryldimethylamine oxide, and octylpolyethylene oxide, indicating that this purification procedure is not restricted to a specific detergent. This study will promote the structural and functional analysis of MspA and might be valuable for the isolation of porins from other mycolic acid-containing bacteria. —Authors' Abstract

Hoft, D. F., Brown, R. M. and Belshe, R. B. Mucosal bacille Calmette-Guerin vaccination of humans inhibits delayed-type hypersensitivity to purified protein derivative but induces mycobacteria-specific interferon-gamma responses. Clin. Infect. Dis. **20** Suppl. 3 (2000) S217–S222.

We conducted a placebo-controlled double-dose-escalation trial of oral bacille Calmette-Guerin (BCG) vaccination in 48 healthy volunteers. Seven of 32 BCG recipients became purified protein derivative (PPD)-positive after dose 1 and only 1 remained positive after dose 2, which suggests that oral BCG has inhibitory effects on delayed-type hypersensitivity (DTH) responses. Ten of the original placebo recipients and 11 oral BCG recipients were recruited to return for an intradermal BCG booster vaccination. Five of 10 original placebo recipients developed PPD responses ≥10 mm, but none of the 11 oral BCG recipients developed PPD induration after they received an intradermal BCG booster ($p < 0.05$; Fisher's exact test). These results document persistent inhibitory effects of oral BCG vaccination on mycobacteria-specific DTH responses. Despite inhibition of DTH, oral BCG induced significant increases in mycobacteria-specific interferon-gamma (IFN-γ) responses in peripheral blood mononuclear cells. More detailed studies of cytokine and homing molecule expression indicated that differential mucosal versus cutaneous trafficking may explain the dissociation between IFN-γ and DTH responses.—Authors' Abstract

Horgen, L., Barrow, E. L. W., Barrow, W. W. and Rastogi, N. Exposure of human peripheral blood mononuclear cells to total lipids and serovar-specific glycopeptidolipids from *Mycobacterium avium* serovars 4 and 8 results in inhibition of TH-1-type responses. Microb. Pathogen. **29** (2000) 9–16.

Previous studies have suggested that large quantities of bacterial lipids may accumulate and persist within host cells during chronic stages of *Mycobacterium avium* infections. This study intended to assess the ability of purified *M. avium* lipids to affect TH-1-type responses in human peripheral blood mononuclear cells (PBMC) from healthy donors. PBMC were exposed to total lipids and serovar-specific glycopeptidolipids (GPL) extracted from *M. avium* serovars 4 and 8, which have been reported to predominate as opportunistic infection among AIDS patients. After 24 hr exposure to lipids followed by PHA/PMA treatment, IL-2 and IFN-γ were assayed in the supernatants. Reverse transcriptase polymerase chain reaction (RT-PCR) was used for a semiquantitative estimation of mRNA for IL-2 and IFN-γ in cell pellets at various time points. Exposure of PBMC to *M. avium* total lipids significantly suppressed PHA/PMA-induced secretion of IL-2 and IFN-γ as determined by ELISA. The GPL antigens from serovar 4 were more efficient at inhibiting TH-1 responses than GPL from serovar 8. CD4+ T-lymphocyte enrichment of PBMC demonstrated that suppression by *M. avium* lipids was intact without the presence of other cell populations such as monocytes and B cells. Preliminary RT-PCR experiments showed that the secretion of TH-1 cytokines was partially affected at the transcriptional level. The results obtained showed that *M. avium* lipids are indeed able to modify the induction of TH-1-type cytokines by human PBMC, and suggest that accumulation of *M. avium* lipids in the chronic stages of infection may play an important role in the pathogenesis of HIV infection.—Authors' Abstract

Huttunen, K., Ruotsalainen, M., Iivanainen, E., Torkko, P., Katila, M. L. and Hirvonen, M. R. Inflammatory responses in RAW264.7 macrophages caused by mycobacteria isolated from moldy houses. Environ. Toxicol. Pharmacol. **8** (2000) 237–244.

Mycobacterial strains (nonpathogenic *Mycobacterium terrae,* potentially pathogenic *M. avium* complex and *M. scrofulaceum*), isolated from a moldy building, were studied with respect to their ability to stimulate macrophages (RAW264.7) to produce inflammatory mediators and to cause cytotxocity. Reactive oxygen species (ROS) were measured by chemiluminescence, cytokines (TNF-alpha, IL-6, IL-1, IL-10) im-

munochemically, nitric oxide (NO) by Griess-method, expression of inducible NO-synthase (iNOS) with Western blot analysis and cytotoxicity with MTT-test. All the strains induced dose- and time-dependent production of NO, IL-6 and TNF-alpha in macrophages; whereas IL-1 or IL-10 production was not detected. The production of ROS and cytotoxicity was increased with the highest doses. Interestingly, different strains had significant differences in their ability to induce these responses, *M. terrae* being the most potent and *M. avium* complex the weakest one. These results indicate that both non- and potentially pathogenic strains of mycobacteria present in moldy buildings are capable of activating inflammatory mechanisms in macrophages.—Authors' Abstract

Huys, G., Rigouts, L., Chemlal, K., Portaels, F. and Swings, J. Evaluation of amplified fragment length polymorphism analysis for inter- and intraspecific differentiation of *Mycobacterium bovis, M. tuberculosis* and *M. ulcerans.* J. Clin. Microbiol. **38** (2000) 3675–3680.

The usefulness of amplified fragment length polymorphism (AFLP) analysis was evaluated for the discrimination of *Mycobacterium bovis* (17 strains), *M. tuberculosis* (15 strains), and *M. ulcerans* (12 strains) at the inter- and intraspecific level. The AFLP technique is a whole-genome coverage genotypic fingerprinting method based on the selective PCR amplification of modified restriction fragments obtained through a double enzymatic digest and subsequent ligation of double-stranded restriction site-specific adapter oligonucleotides. Selective amplification of ApaI/TaqI templates with primer combination A02-T02 (both having an additional C at their 3′ end) generated autoradiographic AFLP fingerprints that were grouped by numerical analysis in two main AFLP clusters allowing clear separation of *M. ulcerans* (cluster I) from the *M. tuberculosis* complex members *M. bovis* and *M. tuberculosis* (cluster D). Calculation of similarities using the band-based Dice correlation coefficient instead of the Pearson product-moment correlation coefficient revealed a further subgrouping in cluster II. The two resulting subclusters corresponded with the phenotypic identity of *M. bovis* and *M. tuberculosis,* respectively, and could also be visually identified by two AFLP marker bands. Because of the relatively low degree of genotypic variation among the AFLP band patterns of the latter two taxa, no correlation could be found with previously reported molecular typing data or with geographical origin. The use of primer combination A02-T01 (the latter having an A as selective base) did not increase the resolving power within the *M. tuberculosis* complex but resulted in a visual subgrouping of the *M. ulcerans* strains that was not observed with primer combination A02-T02. Based on the presence or absence of a single AFLP marker band, the *M. ulcerans* isolates could be unambiguously classified in two continental types corresponding with the African and Australian origin of the strains, respectively. In conclusion, the radioactive AFLP method proved to be a reproducible and reliable taxonomic tool for the differentiation of the three mycobacterial species under study and also demonstrated its potential use for typing of *M. ulcerans* strains when employing multiple primer combinations.—Authors' Abstract

Igbal, N., Zayed, M. and Boden, G. Thalidomide impairs insulin action on glucose uptake and glycogen synthesis in patients with type 2 diabetes. Diabetes Care **23** (2000) 1172–1176.

Objective: To investigate the effect of thalidomide on glucose turnover (glucose production and uptake), on intracellular pathways of glucose utilization [glycogen synthesis (GS), glycolysis (GLS), carbohydrate oxidation, and nonoxidative GLS], and on free fatty acid (FFA) turnover (lipolysis, FFA oxidation, and FFA re-esterification).

Research design and methods: A total of 6 patients with type 2 diabetes were studied with 4-h isoglycemic-hyperinsulinemic clamps (similar to 8 mmol/l and 500–600 pmol/l, respectively) before treatment (prestudy), after 3 weeks of thalidomide (100 mg orally at bedtime), and after 3 weeks of placebo.

Results: Thalidomide reduced insulin-stimulated glucose uptake by 31% (from 27.7 to 19.2 μmol kg^{-1} min^{-1}, $p < 0.05$) compared with the prestudy and by 21% (from 24.2 to 19.2 μmol kg^{-1} min^{-1}, $p < 0.05$) compared with placebo. Thalidomide also reduced insulin stimulated GS by 48% (from 14.1 to 8.2 μmol kg^{-1} min^{-1}, $p < 0.05$) compared with the prestudy and by 40% (from 13.6 to 8.2 μmol kg^{-1} min^{-1}, $p < 0.5$) compared with placebo. Thalidomide had no effect on rates of GLS, carbohydrate oxidation, nonoxidative GLS, lipolysis, FFA oxidation, and re-esterification.

Conclusions: We conclude that thalidomide increased insulin resistance in obese patients with type 2 diabetes by inhibiting insulin-stimulated GS and that patients taking thalidomide should be monitored for possible deterioration in their glucose tolerance.—Authors' Abstract

Jacobs, M., Brown, N., Allie, N., Gulert, R. and Ryffel, B. Increased resistance to mycobacterial infection in the absence of interleukin-10. Immunology **100** (2000) 494–501.

Interleukin-10 (IL-10) downregulates T-helper type 1 cell and macrophage functions. As IL-10 is induced along with tumor necrosis factor (TNF) and IL-12 in mycobacterial infection, we asked whether endogenous IL-10 plays a role in the antimycobacterial response. We demonstrate here that IL-10-deficient mice eliminate *Mycobacterium bovis* Calmette-Guerin bacillus faster than wild-type mice. Granulomas are significantly larger, containing more CD-11b- and CD11c-positive antigen-presenting cells and T cells, and the expression of major histocompatibility complex class II and intracellular adhesion molecule-1 is increased. Macrophages in granulomas of IL-10-deficient mice express high levels of TNF, acid phosphatase and inducible nitric oxide synthase (iNOS). Finally, an increased cutaneous delayed-type hypersensitivity reaction to mycobacterial proteins is further evidence of an augmented cell-mediated immune response. In conclusion, the cell-mediated immunity is enhanced in the absence of IL-10, resulting in a robust granuloma response, which accelerates the clearance of mycobacteria. Therefore, endogenous IL-10 attenuates mycobacterial immunity.—Authors' Abstract

Jagannath, C., Hoffmann, H., Sepulveda, E., Actor, J. K., Wetsel, R. A. and Hunter, R. L. Hypersusceptibility of A/J mice to tuberculosis is in part due to a deficiency of the fifth complement component (C5). Scand. J. Immunol. **52** (2000) 369–379.

Mycobacterium tuberculosis (MTB) causes tuberculosis in man, which occurs as an acute, chronic or dormant disease reactivating over several years. The mechanisms of persistence and reactivation are not well understood and there is a need for animal models. Moderate-dose, aerosol infection killed A/J mice earlier than partially resistant C57BL/6 mice, whereas a low-dose, aerosol-induced chronic infection exacerbated earlier in A/J mice. A/J mice lethally infected with MTB but drug cured of disease underwent reactivation of tuberculosis at least 100 days before similarly infected C57BL/6 mice. Because A/J mice were C5 deficient, congenic B10 mice sufficient and deficient for C5 were infected intravenously with MTB to define the role of C5. C5-deficient mice again showed enhanced growth of MTB in the lungs. MTB-infected macrophages from C5-deficient mice showed enhanced growth of MTB coinciding with a reduced secretion of both cytokines (TNF-alpha, IL-1 beta, IL-6, IL-12) and chemokines (KC, MIP-2 and MIP-1 alpha) in A/J and TNF-alpha and chemokines in C5-deficient mice. Because C5-deficient macrophages could be activated from extraneous C5 and TNF-alpha, we suggest that both play a role in the macrophage-mediated killing as well as containment mechanisms in tuberculosis.—Authors' Abstract

Kaplan, G., Thomas, S., Fierer, D. S., Mulligan, K., Haslett, P. A. J., Fessel, W. J., Smith, L. G., Kook, K. A., Stirling, D. and Schambelan, M. Thalidomide for the treatment of AIDS-associated wasting. AIDS Res. Hum. Retroviruses **16** (2000) 1345–1355.

A double-blind, placebo-controlled trial of efficacy and safety of thalidomide in AIDS-associated wasting was carried out. Ninety-nine of 103 male patients had at least one on-study measurement [intent-to-treat (ITT) cohort]. Patients were randomized to thalidomide at 100 mg/day (T-100) or 200 mg/day (T-200), or placebo for 8 weeks. By ITT analysis, the mean change in body weight of the placebo, T-100, and T-200 treatment groups was 0.3 kg (0.4%), 2.0 kg (3.0%), and 0.9 kg (1.4%), respectively ($p = 0.021$ for T-100 versus placebo; $p = 0.53$ for T-200 versus placebo). Of the 64 patients who completed the 8 weeks of study treatment, significant weight gain was observed in both the T-100 group [2.2 kg, (33%); $p = 0.008$ versus placebo] and the T-200 group [1.5 kg (2.5%); $p = 0.019$ versus placebo]. Approximately half the weight gain was fat-free mass (bioimpedance analysis). Patients in the T-100 or T-200 groups had no significant change in CD4+ cell counts, neutrophil counts, or TNF-alpha levels, compared with placebo. HIV viral load measured as $\log_{10}$ copies/ml decreased by a median of 0.07 in the placebo group, and increased by a median of 0.29 (T-100 group) and 0.23 (T-200 group) ($p = 0.024$ and $p = 0.018$ versus placebo, respectively). Thalidomide therapy was associated with mild-to-moderate rashes and fevers, but not peripheral neuropathy. Although the anabolic benefits of high-dose thalidomide are limited by drug intolerance, 8 weeks of low-dose thalidomide results in significant weight gain in patients with AIDS-associated wasting.—Authors' Abstract

Kaufmann, S. H. E. and Hess, J. Immune response against *Mycobacterium tuberculosis*: implications for vaccine development. J. Biotechnol. **83** (2000) 13–17.

Tuberculosis remains a major health problem globally. Although this threat would best be controlled by a combination of chemotherapy and vaccination, satisfactory vaccines are not available yet. Rational design of a novel vaccine generation against tuberculosis has become possible on the basis of recent achievements in molecular genetics of the pathogen and immunology of the host. Currently, two different strategies are pursued. First, the subunit vaccine approach attempting to induce efficacious immunity by unique antigens in defined adjuvants. Second, the whole bacterial vaccine approach relying on multiple antigens and built-in adjuvanticity. Time will tell which type of vaccine is best suited for eradication of tuberculosis.—Authors' Abstract

Keer, J., Smeulders, M. J., Gray, K. M. and Williams, H. D. Mutants of *Mycobacterium smegmatis* impaired in stationary-phase survival. Microbiology U.K. **146** (2000) 2209–2217.

A bank of 600 insertional mutants of *Mycobacterium smegmatis* was screened for mutants defective in stationary-phase survival. Of 74 mutants picked by the initial screen, 21 had stationary-phase survival defects and 7 of these were studied in more detail. In general, mutants survived stationary phase significantly less well in rich medium than under carbon-starvation conditions. In all cases the loss of viability in stationary phase was not complete even after prolonged incubation. All mutants showed an initial decrease in viability, during the first 40 days in stationary phase, followed by an increase in viable counts that returned viability close to the levels of the wild-type. Southern hybridization experiments showed that recovery of viability was not a consequence of precise excision or movement of the transposon. Two of the survival mutants differed from the wild-type in their colony morphology, and recovery of their viability in stationary phase was coincident with the return of wild-type colony morphology. It is possible that second-site suppressor mutations accumulate that alleviate the effects of the original mutation. For five of the mutants the DNA flanking the site of transposition was amplified by ligation-mediated PCR and sequenced to identify the disrupted locus. In each case, homologous genes were identified in the *M. tuberculosis* genome, three of which have clearly predicted functions in *M. tuberculosis* as a penicillin-binding protein, in biotin biosynthesis and as a polyketide synthase. This is the first identification of genes implicated in the stationary-phase survival of mycobacteria.—Authors' Abstract

Kramnik, I., Dietrich, W. F., Demant, P. and Bloom, B. R. Genetic control of resistance to experimental infection with virulent *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U.S.A. **97** (2000) 8560–8565.

Over 2 billion people are estimated to be infected with virulent *Mycobacterium tuberculosis,* yet fewer than 10% progress to clinical tuberculosis within their lifetime. Twin studies and variations in the outcome of tuberculosis infection after exposure to similar environmental risks suggest genetic heterogeneity among individuals in their susceptibility to disease. In a mouse model of tuberculosis, we have established that resistance and susceptibility to virulent *M. tuberculosis* is a complex genetic trait. A new locus with a major effect on tuberculosis susceptibility, designated sst1 (susceptibility to tuberculosis 1), was mapped to a 9-centimorgan (cM) interval on mouse chromosome 1. It is located 10–19 cM distal to a previously identified gene, Nramp1, that controls the innate resistance of mice to the attenuated bacillus Calmette-Guerin vaccine strain. The phenotypic expression of the newly identified locus is distinct from that of Nramp1 in that sst1 controls progression of tuberculosis infection in a lung-specific manner. Mice segregating at the sst1 locus exhibit marked differences in the growth rates of virulent tubercle bacilli in the lungs. Lung lesions in congenic sst1-susceptible mice are characterized by extensive necrosis and unrestricted extracellular multiplication of virulent mycobacteria; whereas sst1-resistant mice develop interstitial granulomas and effectively control multiplication of the bacilli. The resistant allele of sst1, although powerful in controlling infection, is not sufficient to confer full protection against virulent *M. tuberculosis,* indicating that other genes located outside of the sst1 locus are likely also to be important for controlling tuberculosis infection.—Authors' Abstract

Lever, M. S., Williams, A. and Bennett, A. M. Survival of mycobacterial species in aerosols generated from artificial saliva. Lett. Appl. Microbiol. **31** (2000) 238–241.

Tuberculosis is transmitted primarily by the aerosol route and the aim of this study was to measure the ability of pathogenic mycobacteria to survive in aerosols generated from artificial saliva. Aerosols of *Mycobacterium avium, M. intracellulare* and *M. tuberculosis* were generated and maintained in air under controlled conditions using a Henderson apparatus and a rotating drum. There were no differences in aerosol survival between the three species, and all had a poor survival rate over a period of 1 hr. These data confirm epidemiological studies that close and prolonged contact with a TB patient is required for transmission of infection.—Authors' Abstract

Lewinsohn, D. M., Briden, A. L., Reed, S. G., Grabstein, K. H. and Alderson, M. R. *Mycobacterium tuberculosis*-reactive CD8+ T lymphocytes: the relative contribution of classical versus nonclassical HLA restriction. J. Immunol. **165** (2000) 925–930.

Previous studies in mice and human models have suggested an important role for CD8+ T cells in host defense to *Mycobacterium tuberculosis* (Mtb). In humans, CD8+ Mtb-reactive T cells have been described that are HLA-A2-, B52-, as well as CD1-restricted. Recently, we have described Mtb-specific CD8+ T cells that are neither HLA-A-, B-, or C- nor group 1 CD1-restricted. At present, little is known about the relative contribution of each of these restriction specificities to the overall CD8+ response to Mtb. An IFN-gamma enzyme-linked immunospot assay was used to determine the frequency of Mtb-reactive CD8+ T cells directly from PBMC. The effector cell frequency among five healthy, purified protein derivative-positive subjects was 1/7, 600 ± 4300 compared with 1/16,000 ± 7000 in six purified protein derivative-negative controls. To determine the frequencies of classically, CD1-, and nonclassically restricted cells, a limiting dilution analysis was performed. In one purified protein derivative-positive subject, 192 clones were generated using Mtb-infected dendritic cells (DC). Clones were assessed for reactivity against control autologous DC, Mtb-infected autologous DC, and HLA-mismatched CD1– targets (macrophages). Of

the 96 Mtb-reactive CD8+ T-cell clones, 4 (4%) were classically restricted and 92 (96%) were nonclassically restricted. CD1-restricted cells were not detected. Of the classically restricted cells, 2 were HLA-B44 restricted and 1 was HLA-B14 restricted. These results suggest that while classically restricted CD8+ lymphocytes can be detected, they comprise a relatively small component of the overall CD8+ T-cell response to Mtb. Further definition of the nonclassical response may aid development of an effective vaccine against tuberculosis.—Authors' Abstract

Lokensgard, J. R., Hu, S. X., van Fenema, E. M., Sheng, W. S. and Peterson, P. K. Effects of thalidomide on chemokine production by human microglia. J. Infect. Dis. **182** (2000) 983–987.

Thalidomide, a psychoactive drug that readily crosses the blood-brain barrier, has been shown to possess immunomodulatory attributes, including the inhibition of cytokine production by monocytes and microglia. In this study, we investigated the effect of thalidomide on chemokine production by human microglial cells. Microglial cells were stimulated with lipopolysaccharide, a key cell-wall component of gram-negative bacteria responsible for meningitis, and production of chemokines [regulated upon activation normally T cell expressed and secreted (RANTES), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 beta, and interleukin (IL)-8] was examined by ELISA. Thalidomide treatment was found to cause potent and selective inhibition of IL-8 production in a dose-responsive manner. This inhibition was associated with decreased intracellular IL-8 staining as well as reduced transcription of IL-8 mRNA. In addition, thalidomide treatment of lipopolysaccharide-stimulated microglia inhibited the activation of protein NF-kappa B, a transcription factor known to be important for IL-8 production. These results suggest thalidomide could have a therapeutic role in acute bacterial meningitis through inhibition of IL-8-mediated neutrophil chemotaxis.—Authors' Abstract

McKinney, J. D., zu Bentrup, K. H., Munoz Elias, E. J., Miczak, A., Chen, B., Chan, W. T., Swenson, D., Sacchettini, J. C., Jacobs, W. R. and Russell, D. G. Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature **406** (2000) 735–738.

Mycobacterium tuberculosis claims more human lives each year than any other bacterial pathogen. Infection is maintained in spite of acquired immunity and resists eradication by antimicrobials. Despite an urgent need for new therapies targeting persistent bacteria, our knowledge of bacterial metabolism throughout the course of infection remains rudimentary. Here we report that persistence of *M. tuberculosis* in mice is facilitated by isocitrate lyase (ICL), an enzyme essential for the metabolism of fatty acids. Disruption of the ICL gene attenuated bacterial persistence and virulence in immune-competent mice without affecting bacterial growth during the acute phase of infection. A link between the requirement for ICL and the immune status of the host was established by the restored virulence of Delta ICL bacteria in interferon-gamma knockout mice. This link was apparent at the level of the infected macrophage: activation of infected macrophages increased expression of ICL, and the Delta ICL mutant was markedly attenuated for survival in activated but not resting macrophages. These data suggest that the metabolism of *M. tuberculosis in vivo* is profoundly influenced by the host response to infection, an observation with important implications for the treatment of chronic tuberculosis.—Authors' Abstract

Middleton, A. M., Chadwick, M. V., Nicholson, A. G., Dewar, A., Groger, R. K., Brown E. J. and Wilson, R. The role of *Mycobacterium avium* complex fibronectin attachment protein in adherence to the human respiratory mucosa. Mol. Microbiol. **8** (2000) 381–391.

Mycobacterium avium complex (MAC) are opportunistic respiratory pathogens that infect non-immunocompromised patients

with established lung disease, although they can also cause primary infections. The ability to bind fibronectin is conserved among many mycobacterial species. We have investigated the adherence of a sputum isolate of MAC to the mucosa of organ cultures constructed with human tissue and the contribution of *M. avium* fibronectin attachment protein (FAR) to the process. MAC adhered to fibrous, but not globular mucus, and to extracellular matrix (ECM) in areas of epithelial damage, but not to intact extruded cells and collagen fibers. Bacteria occasionally adhered to healthy unciliated epithelium and to cells that had degenerated exposing their contents, but never to ciliated cells. The results obtained with different respiratory tissues were similar. Two ATCC strains of MAC gave similar results. There was a significant reduction ($p < 0.05$) in the number of bacteria adhering to ECM after preincubation of bacteria with fibronectin and after preincubation of the tissue with *M. avium* FAP in a concentration-dependent manner. The number of bacteria adhering to fibrous mucus was unchanged. Immunogold labelling demonstrated fibronectin in ECM as well as in other areas of epithelial damage, but only ECM bound FAP.

An *M. smegmatis* strain had the same pattern of adherence to the mucosa as MAC. When the FAP gene was deleted, the strain demonstrated reduced adherence to ECM, and adherence was restored when the strain was transfected with an *M. avium* FAP expression construct. We conclude that MAC adheres to ECM in areas of epithelial damage via FAP and to mucus with a fibrous appearance via another adhesin. Epithelial damage exposing ECM and poor mucus clearance will predispose to MAC airway infection.—Authors' Abstract

Mustafa, A. S., Oftung, F., Amoudy, H. A., Madi, N. M., Abal, A. T., Shaban, F., Krands, I. R. and Andersen, P. Multiple epitopes from the *Mycobacterium tuberculosis* ESAT-6 antigen are recognized by antigen-specific human T cell lines. Clin. Infect. Dis. **30** Suppl. 3 (2000) S201–S205.

A synthetic-peptide approach was used to map epitope regions of the *Mycobacterium tuberculosis* 6-kDa early secreted antigen target (ESAT-6) by testing human CD4+ T-cell lines for secretion of interferon-gamma (IFN-γ) in response to recombinant ESAT-6 (rESAT-6) and overlapping 20-mer peptides covering the antigen sequence. The results demonstrate that all of the ESAT-6 peptides screened were able to induce IFN-γ secretion from one or more of the T-cell lines tested. Some of the individual T-cell lines showed the capacity to respond to all peptides. Human leukocyte antigen (HLA-DR) typing of the donors showed that rESAT-6 was presented to T cells in association with multiple HLA-DR molecules. The results suggest that frequent recognition of the *M. tuberculosis* ESAT-6 antigen by T cells from patients with tuberculosis is due to the presence of multiple epitopes scattered throughout the ESAT-6 sequence.—Authors' Abstract

Niemann, S., Richter, E., Dalugge-Tamm, H., Schlesinger, H., Graupner, D., Konigstein, B., Gurath, G., Greinert, U. and Rusch-Gerdes, S. Two cases of *Mycobacterium microti*-derived tuberculosis in HIV-negative immunocompetent patients. Emerging Infect. Dis. **6** (2000) 539–542.

We describe two cases of *Mycobacterium microti* infection causing pulmonary tuberculosis (TB) in HIV-seronegative immunocompetent patients in Germany. The isolates were identified as *M. microti* of the llama and vole types, according to spoligotype patterns. Our data demonstrate that *M. microti* can cause severe pulmonary TB in immunocompetent patients.—Authors' Abstract

Niemann, S., Richter, E., Rusch-Gerdes, S., Schlaak, M. and Greinert, U. Double infection with a resistant and a multidrug-resistant strain of *Mycobacterium tuberculosis.* Emerging Infect. Dis. **6** (2000) 548–551.

An immunocompetent patient was dually infected with a resistant and a multidrug-resistant strain of *Mycobacterium tuberculo-*

sis (TB). The multidrug-resistant strain, which belongs to the W-strain/Beijing family, was first isolated after 3 months of therapy. Inappropriate treatment led to further drug resistance and unsuccessful therapy. Thus, additional infections with resistant *M. tuberculosis* strains should be considered when tuberculosis therapy fails.—Authors' Abstract

Parish, T. and Stoker, N. G. Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis* tlyA plcABC mutant by gene replacement. Microbiology U.K. **146** (2000) 1969–1975.

Progress in the field of mycobacterial research has been hindered by the inability to readily generate defined mutant strains of the slow-growing mycobacteria to investigate the function of specific genes. An efficient method is described that has been used to generate several mutants, including the first double unmarked deletion strain of *Mycobacterium tuberculosis.* Four mutants were constructed: a marked deletion of the plcABC cluster, which encodes three phospholipases C; separate unmarked deletions in plcABC and tlyA (encoding a haemolysin); and a double unmarked mutant tlyA Delta plcABC Delta. To accomplish this, two series of vectors were designed; the first of which, named pNIL, allows manipulation of the target gene sequence at a variety of convenient restriction sites. The second series, named pGOAL, contains marker cassettes flanked by Pad restriction enzyme sites. The final suicide plasmid vectors were then obtained by cloning a marker cassette from a pGOAL vector into the single PacI site of the pNIL vector with the modified gene of interest. Finally, a two-step strategy was employed whereby single cross-over events were first selected, then screening for the second cross-over was carried out to yield the mutant strains. This technique will now allow the construction of potential vaccine strains without the inclusion of antibiotic resistance markers, the ability to make multiple defined mutations and the possibility of making more subtle defined mutations, such as point mutations.—Authors' Abstract

Piddington, D. L., Kashkouli, A. and Buchmeier, N. A. Growth of *Mycobacterium tuberculosis* in a defined medium is very restricted by acid pH and Mg2+ levels. Infect. Immun. **68** (2000) 4518–4522.

Mycobacterium tuberculosis grows within the phagocytic vacuoles of macrophages, where it encounters a moderately acidic and possibly nutrient-restricted environment. Other mycobacterial species encounter acidic conditions in soil and aquatic environments. We have evaluated the influence of pH and divalent cation levels on the growth of *M. tuberculosis* and seven other mycobacterial species in a defined medium. The growth of *M. tuberculosis* was very restricted by acidic pH. Higher levels of Mg2+ were required for growth of *M. tuberculosis* in mildly acidic media (pH 6.0 to 6.5) compared to pH 7.0 medium. The divalent cations Ca2+, Zn2+, or Mn2+ could not replace Mg2+ during growth at pH 6.25, but Ca2+ could at least partially substitute for Mg2+ during growth at pH 7.0. Among 8 species of mycobacteria tested, there was a diversity of growth rates in media with acidic pH and low Mg2+ levels. *M. tuberculosis* was the most restricted in growth at pH 6.0, and all of this growth required elevated levels of Mg2+. *M. kansasii* and *M. smegmatis* also grew very poorly in acidic media with limiting Mg2+. *M fortuitum, M. marinum, M. scrofulaceum, M. avium,* and *M. chelonae* grew at pH 6.0 in an unrestricted manner. These results demonstrate that *M. tuberculosis* is unique among the mycobacteria in its extreme sensitivity to acid and indicate that *M. tuberculosis* must acquire sufficient Mg2+ in order to grow in a mildly acidic environment, such as within the phagosome of macrophages.—Authors' Abstract

Rajkumar, S. V., Fonseca, R., Dispenzieri, A., Lacy, M. Q., Lust, J. A., Witzig, T. E., Kyle, R. A., Gertz, M. A.

and Greipp, P. R. Thalidomide in the treatment of relapsed multiple myeloma. Mayo Clin. Proc. **75** (2000) 897–901.

Objective: To describe the efficacy of therapy with thalidomide, a drug that has antiangiogenic properties, in patients with relapsed multiple myeloma.

Patients and Methods: We studied 16 patients (median age 64 years) who received thalidomide for relapsed myeloma at the Mayo Clinic in Rochester, Minnesota, U.S.A., between November 1998 and August 1999. Treatment consisted of thalidomide given orally at a dose of 200 mg/d for 2 weeks, then increased by 200 mg/d every 2 weeks, up to a maximal dose of 800 mg/d.

Results: The stage of myeloma at treatment was Durie-Salmon IIIA in 9 patients (56%) and IIIB in 7 (44%). The median time from myeloma diagnosis to initiation of thalidomide therapy was 32 months. In 4 patients (25%) prior stem-cell transplantation had failed, and 14 (88%) had received 2 or more prior chemotherapeutic regimens before institution of thalidomide. All patients were evaluable for response. Four (25%) achieved a partial response to therapy, with a greater than 50% reduction in the serum or urine M protein level. Responses lasted 2, 4+, 8, and 10+ months. Major adverse effects included constipation, sedation, rash, and peripheral neuropathy.

Conclusion: Thalidomide is an active agent in the treatment of patients with advanced myeloma.—Authors' Abstract

Rojas, M., Garcia, L. F., Nigou, J., Puzo, G. and Olivier, M. Mannosylated lipoarabinomannan antagonizes *Mycobacterium tuberculosis*-induced macrophage apoptosis by altering Ca+ 2-dependent cell signaling. J. Infect. Dis. **182** (2000) 240–251.

Mycobacterium tuberculosis-induced macrophage apoptosis can be inhibited by mannosylated lipoarabinomannan (ManLAM), although it induces tumor necrosis factor-alpha (TNF-α) and NO production, which participate in apoptosis induction. ManLAM also modulates Ca+2-dependent intracellular events, and Ca+2 participates in apoptosis in different systems. Ca+2 was assessed for involvement in *M. tuberculosis*-induced macrophage apoptosis and for modulation by ManLAM. The role of Ca+2 was supported by the blockade of apoptosis by cAMP inhibitors and the Ca+2 chelator, BAPTA/AM. These agents also inhibited caspase-1 activation and cAMP-responsive element-binding protein translocation without affecting TNF-α production. Infection of macrophages with *M. tuberculosis* induced an influx of Ca+2 that was prevented by ManLAM. Similarly, *M. tuberculosis* infection-altered mitochondrial permeability transition was prevented by ManLAM and BAPTA/AM. Finally, ManLAM and BAPTA/AM reversed the effects of *M. tuberculosis* on p53 and Bcl-2 expression. ManLAM counteracts the alterations of calcium-dependent intracellular events that occur during *M. tuberculosis*-induced macrophage apoptosis.—Authors' Abstract

Saita, N., Fujiwara, N., Yano, I., Soejima, K. and Kobayashi, K. Trehalose 6,6′-dimycolate (cord factor) of *Mycobacterium tuberculosis* induces corneal angiogenesis in rats. Infect. Immun. **68** (2000) 5991–5997.

Neovascularization or angiogenesis is required for the progression of chronic inflammation. The mechanism of inflammatory neovascularization in tuberculosis remains unknown. Trehalose 6,6′-dimycolate (TDM) purified from *Mycobacterium tuberculosis* was injected into rat corneas. TDM challenge provoked a local granulomatous response in association with neovascularization. Neovascularization was seen within a few days after the challenge, with the extent of neovascularization being dose dependent, although granulomatous lesions developed 14 days after the challenge. Cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), IL-1 beta, and vascular endothelial growth factor (VEGF), were found in lesions at the early stage (within a few days after the challenge) and were detectable until day 21. Neovascularization was inhibited substantially by neutralizing antibodies to VEGF

and IL-8 but not IL-1 beta. Treatment with anti-TNF-α antibodies resulted in partial inhibition. TDM possesses pleiotropic activities, and the cytokine network plays an important role in the process of neovascularization.—Authors' Abstract

Sharma, V., Sharma, S., Bentrup, K. H. Z., McKinney, J. D., Russell, D. G., Jacobs, W. R. and Sacchettini, J. C. Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis*. Nature Struct. Biol. **7** (2000) 663–668.

Isocitrate lyase (ICL) plays a pivotal role in the persistence of *Mycobacterium tuberculosis* in mice by sustaining intracellular infection in inflammatory macrophages. The enzyme allows net carbon gain by diverting acetyl-CoA from beta-oxidation of fatty acids into the glyoxylate shunt pathway. Given its potential as a drug target against persistent infections, we solved its structure without ligand and in complex with two inhibitors. Covalent modification of an active site residue, Cys 191, by the inhibitor 3-bromopyruvate traps the enzyme in a catalytic conformation with the active site completely inaccessible to solvent. The structure of a C191S mutant of the enzyme with the inhibitor 3-nitropropionate provides further insight into the reaction mechanism.—Authors' Abstract

Shoen, C. M., DeStefano, M. S. and Cynamon, M. H. Durable cure for tuberculosis: rifalazil in combination with isoniazid in a murine model of *Mycobacterium tuberculosis* infection. Clin. Infect. Dis. **30** Suppl. 3 (2000) S288–S290.

Rifalazil (formerly known as KRM-1648) in combination with isoniazid has been found to be more active than rifampin/isoniazid. Administration of rifalazil/isoniazid for 12 weeks resulted in continued apparent sterilization of organs 6 months after cessation of therapy. In this study we evaluated the durability of rifalazil/isoniazid treatment. Female CD-1 mice were infected with *Mycobacterium tuberculosis* ATCC 35801 (strain Erdman). Rifalazil and isoniazid were given in combination for 6 and 12 weeks; no mycobacteria could be cultured from spleens and lungs at both the 6-week and 12-week time points. After completing treatment, groups of mice treated with rifalazil/isoniazid for 6 or 12 weeks were observed without any additional treatment. These observation groups were compared to groups of rifalazil/isoniazid-treated mice (6 and 12 weeks) given dexamethasone for 7 and 8 weeks, respectively. Modest regrowth was noted in the spleens and lungs of the group treated with rifalazil/isoniazid for 6 weeks. Regrowth in the 6-weeks group was enhanced slightly by treatment with dexamethasone. In contrast, no regrowth was noted in the 12-weeks rifalazil/isoniazid group, and treatment with dexamethasone did not result in any regrowth.—Authors' Abstract

Singh, K. K., Muralidhar, M., Kumar, A., Chattopadhyaya, T. K., Kusum, K., Singh, M. K., Sharma, S. K., Jain, N. K. and Tyagi, J. S. Comparison of in house polymerase chain reaction with conventional techniques for the detection of *Mycobacterium tuberculosis* DNA in granulomatous lymphadenopathy. J. Clin. Pathol. **53** (2000) 355–361.

The objective was to evaluate the usefulness of the devR-based polymerase chain reaction (PCR) in the detection of *Mycobacterium tuberculosis* in lymph node aspirates and tissues of lymphadenitis and to compare PCR with conventional diagnostic techniques. Coded specimens of fine needle aspirates and biopsies from 22 patients with tuberculous lymphadenitis, 14 patients with nontubercular lymphadenitis, and 9 patients with granulomatous lymphadenitis were processed and subjected to analysis by PCR, smear microscopy, *M. tuberculosis* culture, histology, and cytology. Tuberculous lymphadenitis was correctly diagnosed by PCR in 18 patients, by culture in 5 patients, by histology in 13 patients, and by cytology in 7 patients. PCR gave two false-positive results in 14 patients with nontubercular lymphadenitis. The sensitivity of the conventional techniques was significantly higher with biopsies (17 of 22 speci-

mens; 77%) than with fine needle aspirates (9 of 22 specimens; 41%). However, the sensitivity of PCR was not significantly higher with biopsies (68%) in comparison with fine needle aspirates (55%). The sensitivity of either biopsy PCR or fine needle aspirate PCR was not significantly different from that of either histology combined with culture or cytology combined with culture. The overall combined specificity of PCR was 86%. *M. tuberculosis* DNA was detected in 6 of 9 patients with granulomatous lymphadenitis. PCR is the most sensitive single technique available to date for the demonstration of *M. tuberculosis* in specimens derived from patients with a clinical suspicion of tuberculous lymphadenitis. The value of PCR lies in its use as an adjunct test in the diagnosis of tuberculous lymphadenitis, particularly in those patients where conventional methods fail. Because fine needle aspiration is not an invasive procedure, it is the procedure of choice, and PCR should be performed initially on these samples. Excisional biopsy histology and PCR should be recommended only for patients in whom fine needle aspirate PCR is negative or when there is discrepancy with the clinical impression.—Trop. Dis. Bull. **97** (2000) 1003

Stinear, T., Davies, J. K., Jenkin, G. A., Hayman, J. A., Oppedisano, F. and Johnson, P. D. R. Identification of *Mycobacterium ulcerans* in the environment from regions in southeast Australia in which it is endemic with sequence capture-PCR. Appl. Environ. Microbiol. **66** (2000) 3206–3213.

We recently described the use of PCR to identify the environmental source of *Mycobacterium ulcerans* during an outbreak of ulcerative disease that occurred in a localized region of southeast Australia. The PCR used was based on amplification of the *M. ulcerans*-specific insertion sequence, IS2404. In this study we developed a new test that is a substantial improvement over the original PCR method in terms of sensitivity, reliability, and ease of use. In the new method magnetic head sequence capture-PCR is used to detect two *M. ulcerans* sequences (IS2404 and IS2606) and total mycobacterial 16S ribosomal DNA. We used sequence capture-PCR to test water and plant material collected over a 12-month period during 1998 and 1999 from sites near the centers of two distinct foci of *M. ulcerans* infections. A golf course irrigation system in one area and a small shallow lake in another area repeatedly were PCR positive for *M. ulcerans*. Nearby sites and sites unrelated to the endemic areas were negative. Based on the PCR data, a most-probable-number method was used to estimate the concentration of *M. ulcerans* cells in positive samples from both regions. This procedure resulted in average concentrations of 0.5 cell per 100 ml of water and 40 cells per 100 g of detritus. Loss of the PCR signal coincided with a decrease in ulcerative disease in each area. These results provide further evidence that *M. ulcerans* may be transmitted from a point environmental source and demonstrate the utility of magnetic bead sequence capture-PCR for identification of nonculturable microbial pathogens in the environment.—Authors' Abstract

Suling, W. J., Seitz, L. E., Pathak, V., Westbrook, L., Barrow, E. W., Zywno Van Ginkel, S., Reynolds, R. C., Piper, J. R. and Barrow, W. W. Antimycobacterial activities of 2, 4-diamino-5-deazapteridine derivatives and effects on mycobacterial dihydrofolate reductase. Antimicrob. Agents Chemother. **44** (2000) 2784–2793.

Development of new antimycobacterial agents for *Mycobacterium avium* complex (MAC) infections is important particularly for persons coinfected with the human immunodeficiency virus. The objectives of this study were to evaluate the *in vitro* activity of 2,4-diamino-5-methyl-5-deazapteridines (DMDPs) against MAC and to assess their activities against MAC dihydrofolate reductase recombinant enzyme (rDHFR). Seventy-seven DMDP derivatives were evaluated initially for *in vitro* activity against 1 to 3 strains of MAC (NJ168, NJ211, and/or NJ3404). MICs were determined with 10-fold dilutions of drug and a colorimetric (Alamar blue) microdilution broth assay. MAC rDHFR 50% inhibitory concentrations versus those of human rD-

HFR were also determined. Substitutions at position 5 of the pteridine moiety included -CH3, -CH2CH3, and -CH20CH3 groups. Additionally, different substituted and unsubstituted aryl groups were linked at position 6 through a two-atom bridge of either -CH2NH, -CH2N(CH3), -CH2CH2, or -CH2S. All but 4 of the 77 derivatives were active against MAC NJ168 at concentrations of ≤13 μg/ml. Depending on the MAC strain used, 81% to 87% had MICs of ≤1.3 μg/ml. Twenty-one derivatives were >100-fold more active against MAC rDHFR than against human rDHFR. In general selectivity was dependent on the composition of the two-atom bridge at position 6 and the attached aryl group with substitutions at the 2′ and 5′ positions on the phenyl ring. Using this assessment, a rational synthetic approach was implemented that resulted in a DMDP derivative that had significant intracellular activity against a MAC-infected Mono Mac 6 monocytic cell line. These results demonstrate that it is possible to synthesize pteridine derivatives that have selective activity against MAC.—Authors' Abstract

Triccas, J. A. and Gicquel, B. Life on the inside: probing *Mycobacterium tuberculosis* gene expression during infection. Immunol. Cell Biol. **78** (2000) 311–317.

The identification of *Mycobacterium tuberculosis* genes specifically expressed during infection is a key step in understanding mycobacterial pathogenesis. Such genes most likely encode products required for survival within the host and for progressive infection. Recent advances in mycobacterial genetics have permitted the development of new techniques and the adaptation of existing methods to analyze mycobacterial *in vivo* gene expression and virulence. This has revealed a subset of *M. tuberculosis* genes that are differentially expressed during infection and has demonstrated that a number of components contribute to the virulence of the organism. This information is expected to provide new strategies to prevent tuberculosis infection, new targets for antimicrobial therapy and new insights into the infectious process.—Authors' Abstract

Verbon, A., Jufferans, N. P., Speelman, P., van Deventer, S. J. H., ten Berge, I. J. M., Guchelaar, H. J. and vander Poll, T. A single oral dose of thalidomide enhances the capacity of lymphocytes to secrete gamma interferon in healthy humans. Antimicrob. Agents Chemother. **44** (2000) 2286–2290.

Thalidomide is increasingly being used as adjuvant therapy for patients with mycobacterial and human immunodeficiency virus (HIV) infections. The T-helper (Th)1 cytokine-Th2 cytokine balance critically determines the outcomes of these diseases. To obtain insight into the effect of thalidomide on the capacity of lymphocytes to produce Th1 and Th2 cytokines, 6 healthy volunteers received an oral dose (400 mg) of thalidomide. Before and at 3, 6, and 24 hr after ingestion of thalidomide, peripheral blood mononuclear cells (PBMCs) were isolated and stimulated for 24 hr with the T-cell stimulant staphylococcal enterotoxin B (SEB) or anti-CD3/CD28. In all 6 volunteers ingestion of thalidomide was associated with enhanced SEB- and anti-CD3/CD28-induced production of the Th1 cytokine gamma interferon ($p < 0.05$) and a decrease in the level of anti-CD3/CD28-induced interleukin-5 (IL-5) production ($p < 0.05$). The levels of IL-2 (Th1) and IL-4 (Th2) released remained unchanged.

These changes were accompanied by an increase in the amount of IL-12p40 released by the PBMCs 6 hr after ingestion of thalidomide ($p < 0.05$). Thus, a single oral dose of thalidomide causes a Th1-type response in healthy humans. This finding offers a potential explanation for the positive effect of thalidomide in patients with mycobacterial and HIV infections.—Authors' Abstract

Werneck-Barroso, E., Bonecini de Almeida, M. da G., Vieria, M. A. M. S., Carvalho, C. E., Teixeira, A. K., Kritski, A. L. and Ho, J. L. Preferential recruitment of phagocytes into the lung of patients with advanced acquired immunodeficiency syndrome and tuberculosis. Respir. Med. **94** (2000) 64–70.

The immune host response against tuberculosis in early HIV-infection may differ from that in later stages of HIV disease, as is strongly suggested by different clinical and radiographic patterns. The cellular elements in the lungs of 15 HIV-infected patients with advanced immunosuppression and pulmonary tuberculosis. [tuberculosis (TB)/AIDS] were investigated in Rio de Janeiro, Brazil. The findings were compared with data from 4 other groups: (1) 15 HIV-seronegative patients with pulmonary TB; (2) 12 HIV-seropositive TB patients without previous AIDS-defining illnesses and with CD4+ >200 cells mm^{-3}; (3) 5 AIDS patients without pulmonary lesions; and (4) 5 healthy controls. Bronchoalveolar lavage (BAL) fluid and differential cell counts, as well as lymphocyte subsets, were determined. Despite a low CD4/CD8 ratio, the TB/AIDS group had a higher absolute number of CD8+ lymphocytes in the BAL fluid than the other groups. Alveolar macrophages and neutrophils were significantly increased in TB/AIDS patients compared to control groups. The number of eosinophils was increased in TB/HIV-patients but not in TB/AIDS patients. It is concluded that tuberculosis in late stage HIV-infected patients has a distinct inflammatory cell profile, suggesting an enhanced compensatory mechanism that amplifies the unspecific inflammatory reaction.—Trop. Dis. Bull. **97** (2000) 868

Zhang, L. X., Tu, D. H., He, G. X., Ma, Z. Q., Nagelkerke, N. J., Borgdorff, M. W., Enarson, D. A. and Broekmans, J. F. Risk of tuberculosis infection and tuberculous meningitis after discontinuation of Bacillus Calmette-Guerin in Beijing. Am. J. Respir. Crit. Care Med. **162** (2000) 1314–1317.

In Beijing, the notification rate of smear-positive tuberculosis (TB) has been below 20 per 100,000 since 1986, and continues to decline. To accurately measure the risk of TB infection in a population in which the results of tuberculin skin testing were not confounded by vaccination with Bacillus Calmette-Guerin (BCG). BCG vaccination at birth was discontinued from 1988 in Shunyi County. In 1995, the prevalence of TB infection among 12,836 primary school children aged 6 to 7 yr and without BCG scars was 1.4%, giving an estimated annual risk of infection of 0.19% (95% confidence interval: 0.16% to 0.22%). The prevalence of TB infection in children aged 5 to 9 yr in Beijing in 1950 was 46%. The number of cases of tuberculous meningitis did not increase after discontinuation of BCG. We conclude that discontinuation of BCG had no detectable harmful effects, and that control of TB in Beijing has markedly reduced the prevalence of TB infection since 1950.—Authors' Abstract

Zimhony, O., Cox, J. S., Welch, J. T., Vilcheze, C. and Jacobs, W. R. Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of *Mycobacterium tuberculosis.* Nature Med. **6** (2000) 1043–1047.

Tuberculosis treatment is shortened to 6 months by the indispensable addition of pyrazinamide (PZA) to the drug regimen that includes isoniazid and rifampin. PZA is a pro-drug of pyrazinoic acid (POA) whose target of action has never been identified. Although PZA is active only against *Mycobacterium tuberculosis,* the PZA analog 5-chloro-pyrazinamide (5-Cl-PZA) displays a broader range of anti-mycobacterial activity. We have found that the eukaryotic-like fas1 gene (encoding fatty acid synthetase I, FASI) from *M. avium, M. bovis* BCG or *M. tuberculosis* confers resistance to 5-CI-PZA when present on multicopy vectors in *M. smegmatis.* 5-CI-PZA and PZA markedly inhibited the activity of *M. tuberculosis* FASI, the biosynthesis of C-16 to C-24/C26 fatty acids from acetyl-CoA. Importantly, PZA inhibited FASI in *M. tuberculosis* in correlation with PZA susceptibility. These results indicate that FASI is a primary target of action for PZA in *M. tuberculosis.* Further characterization of FASI as a drug target for PZA may allow the development of new drugs to shorten the therapy against *M. tuberculosis* and may provide more options for treatment against *M. bovis, M. avium* and drug-resistant *M. tuberculosis.*—Authors' Abstract

Zomas, A., Anagnostopoulos, N. and Dimopoulos, M. A. Successful treatment of multiple myeloma relapsing after high-dose therapy and autologous transplantation with thalidomide as a single agent. Bone Marrow Transplant. **25** (2000) 1319–1320.

A 52-year-old dentist with kappa light-chain multiple myeloma relapsed 6 months after 180 mg/m^2 melphalan and an autograft. A partial remission had been attained after the autograft. Relapse occurred while he was on dexamethasone maintenance therapy. Chemotherapy was not an option due to low blood counts. Thalidomide was administered at relatively high doses (escalated up to 700 mg daily and continued for 4 months). There was a prompt decline in urine protein from 6067 mg/day to 2177 mg/day within a month. The response continued to improve with achievement of near-complete remission within 6 months and a decline in urine protein to 413 mg/day. Subsequently, grade 3 neutropenia and peripheral neuropathy required dose reduction to 200 mg/day. Disease activity parameters continued to improve on the lower dose of thalidomide. Nine months after starting thalidomide, the patient is in near-complete remission, enjoys an excellent quality of life, and has returned to work. We conclude that thalidomide can effectively control myeloma relapsing after high-dose chemotherapy, and may be especially useful in resistant cases or those unable to tolerate further chemotherapy.—Authors' Abstract