CURRENT LITERATURE

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

General and Historical

Annas, G. J. and Elias, S. Thalidomide and the Titanic: reconstructing the technology tragedies of the twentieth century. Am. J. Public Health **89** (1999) 98–101.

The Titanic has become a metaphor for the disastrous consequences of an unqualified belief in the safety and invincibility of new technology. Similarly, the thalidomide tragedy stands for all of the "monsters" that can be inadvertently or negligently created by modern medicine. Thalidomide, once banned, has returned to the center of controversy with the Food and Drug Administration's (FDA's) announcement that thalidomide will be placed on the market for the treatment of erythema nodosum leprosum, a severe dermatological complication of Hansen's disease. Although this indication is very restricted, thalidomide will be available for off-label uses once it is on the market.

New laws regarding abortion and a new technology, ultrasound, make reasonable the approval of thalidomide for patients who suffer from the serious conditions it can alleviate. In addition, the FDA and the manufacturer have proposed the most stringent postmarketing monitoring ever used for a prescription drug, including counseling, contraception, and ultrasonography in the event of pregnancy.

The Titanic/thalidomide lesson for the FDA and public health is that rules and guidelines alone are not sufficient to guarantee safety. Continuous vigilance will be required to ensure that all reasonable postmarketing monitoring steps are actually taken to avoid predictable and preventable teratogenic disasters.—Authors' Abstract

Opromolla, D. V. A., ed. [IX Congress of the Brazilian Association of Hansenology/IV Congress of the College of Hansenology of the Endemic Countries, Foz do Iguaçu, Paraná, Brazil, 4–8 June 1997.] (in Portuguese)

This publication comprises 28 papers presented at a joint conference between the College of Hansenology of the Endemic Countries and the Brazilian Association of Hansenology held in Foz do Iguaçu, Paraná, Brazil, 4–8 June 1997. Some papers are in English and some are in Portuguese, with or without English abstracts. The following subjects are covered: monitoring of the post-elimination period; quality of patient care in the prevention of disabilities; leprosy elimination in Brazil, the Americas, the Southern Cone and Amazonia; therapeutics; epidemiology of reactions; pathogenesis of visceral leprosy; clinical aspects of the reversal reaction; epidemiology of disabilities; value of biopsy in the diagnosis of leprosy; nerve damage, surgery and rehabilitation; composition and antigenicity of *Mycobacterium leprae*; genetics of leprosy; heat shock protein in mycobacterial diseases; effects of the microenvironment on the local regulation of granulomas cased by *M. leprae*; IgG antibodies and their relation to Th1/Th2 responses; multidrug therapy in Europe; cytokine profiles in the serum and in the supernatant of cultures of peripheral blood mononuclear cells from leprosy patients; early detection of subclinical leprosy; immunology and hypersensitivity in the development of Hansen's disease; and social aspects.—Trop Dis. Bull. **95** (1998) 1317.

Chemotherapy

Borner, K., Hartwig, H., Leitzke, S., Hanh, H., Muller, R. H. and Ehlers, S. HPLC determination of clofazimine in tissues and serum of mice with intravenous administration of nanocrystalline or liposomal formulations. Int. J. Antimicrob. Agents **11** (1999) 75–79.

A simple HPLC method is described for the determination of clofazimine in mouse tissues and in serum. The main application of the method was the determination of the drug in mouse tissues after i.v. administration of nanocrystalline suspensions or liposomal encapsulated clofazimine. Tissues were extracted with a 10-fold (w/v) volume of an extraction solution consisting of methanol/glacial acetic acid 9:1 (v/v). Serum proteins were precipitated with a 2-fold volume of acetonitrile. Isocratic chromatography was performed using an anion exchange column (Nucleosil 100-5 SA, Macherey & Nagel) for separation. The mobile phase was a mixture of acetonitrile and 0.1 mol/l aqueous phosphoric acid (75:25, v/v) adjusted to pH 2.9 with sodium hydroxide solution. Absorption of the eluate was monitored at 495 nm. The assay was precise, simple to perform and fast. Recovery from tissues was ≥98%, from nanoparticles ≥98%, and from liposomes ≥96%. No interference was observed in extracts from mouse liver, spleen, lungs, and human serum.—Authors' Abstract

Horgen, L., Legrand, E. and Rastogi, N. Postantibiotic effects of rifampin, amikacin, clarithromycin and ethambutol used alone or in various two-, three- and four-drug combinations against *Mycobacterium avium.* FEMS Immunol. Med. Microbiol. **23** (1999) 37–44.

The postantibiotic effects (PAEs) of rifampin, amikacin, clarithromycin, and ethambutol were determined radiometrically against five AIDS-associated isolates of *Mycobacterium avium,* and were found to be 20.8 ± 3.4, 18.4 ± 2.5, 11.8 ± 1.7, and 2 ± 9 hr, respectively. Various two-, three- or four-drug combinations were also screened; the PAEs for a two-drug combination were generally longer than individual drugs (mean PAE of 13.8 ± 1.5 to 29.2 ± 7.4 hr instead of 2.4 ± 0.9 to 18.4 ± 2.5 hr for single drugs). The addition of a third drug further increased the mean PAE to a range of 21.0 ± 2.6 to 32.4 ± 6.1 hr. Both rifampin + clarithromycin and rifampin + amikacin were the most potent two-drug combinations resulting in longer PAEs than individual drugs; whereas rifampin + amikacin + clarithromycin was the most potent three-drug combination. Parallel viable count determinations showed a good correlation between the PAE results obtained by the radiometric method or by bacterial viability assessment. These results are useful in planning future clinical investigations to clarify the possible implication of PAE in drug schedule and dosage, a line of information that is urgently needed to guide the drug administration in *M. avium*-infected AIDS patients who are presently over-burdened with the administration of too many drugs for HIV-treatment and opportunistic infections.—Authors' Abstract

Huang, F., *et al.* [On duration of treatment in leprosy.] China Lepr. J. **14** (1998) 162–163. (in Chinese)

Analysis of their treatment durations among 557 persons cured of leprosy since 1955 in Qingdao, China, showed that the treatment durations had no relation to sex or the disease duration, but was longer in MB and in youngers than in PB and in elders. After the 1980s the treatment duration had shortened significantly and generally.—Authors' English Abstract

Jian, D., *et al.* [Efficacy of MDT on PB leprosy.] China Lepr. J. **14** (1998) 144–146. (in Chinese)

From 1986 to 1997, 775 PB leprosy patients were treated by WHO's MDT in 20 counties of Liangshan Prefecture and Panzhihua City, Sichuan Province, China. Among them, 537 patients took 6 months'

PB regimen and were followed up for at least 2 years, and 238 had 24 months' MB regimen and were followed up for at least 5 years. Among 758 patients who completed the MDT, the complete resolution of skin lesions was 69.52% in the former and 75.98% in the latter. There was a significant correlation between the number of skin lesions and the effectiveness of MDT ($p < 0.01$). The negative rate of skin smear at stopping MDT in those with positive smear before taking the MDT-PB regimen was 95% and in those with the MDT-MB regimen, 97.7%. In 19 patients with type 1 reaction during MDT and monitoring, including 18 BT, and 1 I, 13 reactions occurred during MDT. One tuberculoid patient with PB regimen relapsed 107 months after stopping MDT (0.28/1000 patient-years).—Authors' English Abstract

John, S. S. Fixed drug eruption due to rifampin. Lepr. Rev. **69** (1998) 397–399.

A case of fixed drug eruption due to rifampin in a leprosy patient is described. Fixed drug eruption due to rifampin with the classical residual hyper-pigmentation has not been described before.—Author's Summary

McMillan, D. C., Jensen, C. B. and Jollow, D. J. Role of lipid peroxidation in dapsone-induced hemolytic anemia. J. Pharmacol. Exp. Ther. **287** (1998) 868–876.

Dapsone hydroxylamine (DDS-NOH) is a direct-acting hemolytic agent responsible for dapsone-induced hemolytic anemia in the rat. The hemolytic activity of DDS-NOH is associated with the formation of disulfide-linked hemoglobin adducts on membrane skeletal proteins. We have postulated that this membrane protein "damage" is a consequence of DDS-NOH-induced oxidative stress within the red cell and that it serves as the trigger for premature removal of injured but intact red cells from the circulation by splenic macrophages. Oxidative stress has also been associated with the induction of lipid peroxidation, and it is possible that direct damage to the lipoidal membrane may play a role in the premature sequestration of the damaged cells in the spleen. To investigate this possibility, rat and human red cells were incubated with hemolytic concentrations of DDS-NOH and examined for evidence of lipid peroxidation using two independent assays: thiobarbituric acid-reactive substances formation and cis-paranaric acid degradation. Phenylhydrazine, which is known to induce lipid peroxidation in red cells, was used a s a positive control. the extent of thiobarbituric acid-reactive substances formation and cis-paranaric acid degradation in DDS-NOH-treated rat and human red cells was not significantly different from that in control cells. In contrast, thiobarbituric acid-reactive substances formation and cis-paranaric acid degradation were significantly increased in red cells treated with hemolytic concentrations of the positive control, phenylhydrazine. These data suggest that lipid peroxidation is not involved in the mechanism underlying dapsone-induced hemolytic anemia.—Authors' Abstract

Shoen, C. M., Choromanska, O., Reynolds, R. C., Piper, J. R., Johnson, C. A. and Cynamon, M. H. *In vitro* activities of several diaminomethylpyridopyrimidines against *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **42** (1998) 3315–3316.

Three recently synthesized dihydrofolate reductase (DHFR) inhibitors designated SoRI 8890, 8895, and 8897 were evaluated for their *in vitro* activities against 25 isolates of *Mycobacterium avium* complex. The MICs at which 50% and 90% of isolates were inhibited were 1 and 2, 4 and 8, and 4 and 8 μg/ml for SoRI 8890, 8895, and 8897, respectively. Although the addition of dapsone at 0.5 μg/ml did not significantly enhance the *in vitro* activities of these compounds, their activities alone were comparable to, if not better than, results seen with other DHFR inhibitors, such as pyrimethamine or WR99210.—Authors' Abstract

Yang, B., Koga, H., Ohno, H., Ogawa, K., Fukuda, M., Hirakata, Y., Maesaki, S., Tomono, K., Tashiro, T. and Kohno, S.

Relationship between antimycobacterial activities of rifampicin, rifabutin and KRM-1648 and rpoB mutations of *Mycobacterium tuberculosis*. J. Antimicrob. Chemother. **42** (1998) 621–628.

We compared the *in-vitro* antimycobacterial activities of rifabutin and KRM-1648, two rifamycin derivatives, with that of rifampin against 163 strains of *Mycobacterium tuberculosis*. We also evaluated the correlation between the level of resistance to rifampin, rifabutin and KRM-1648 and genetic alterations in the rpoB gene. All 82 strains susceptible to rifampin or resistant to rifampin with MICs ≤16 mg/l were susceptible to rifabutin and KRM-1648 with MICs ≤1 mg/l. Seventy-six of 81 strains resistant to rifampin with MICs ≥32 mg/l were resistant to both rifabutin and KRM-1648, but with lower MICs than those of rifampin. KRM-1648 showed more potent antimycobacterial activity than rifabutin against organisms with low MICs (≤1 mg/l), while rifabutin was more active than KRM-1648 against organisms with high MICs (≥2 mg/l). A total of 96 genetic alterations around the 69 bp core region of the rpoB gene were detected in 92 strains. Alterations at codons 515, 521 and 533 in the rpoB gene did not influence the susceptibility to rifampin, rifabutin and KRM-1648. Point mutations at codons 516 and 529, deletion at codon 518 and insertion at codon 514 influenced the susceptibility to rifampin but not that to rifabutin or KRM-1648. With the exception of one strain, all alterations at codon 513 and 531 correlated with resistance to the three test drugs. The resistant phenotype of strains with an alteration at codon 526 depended on the type of amino acid substitution. Our results suggest that analysis of genetic alterations in the rpoB gene might be useful not only for predicting rifampin susceptibility, but also for deciding when to use rifabutin for treating tuberculosis. Further studies may be required to determine the usefulness of KRM-1648.—Authors' Abstract

Zhang, X., *et al.* [Three relapsed cases among those cured of leprosy with MDT.] China Lepr. J. **14** (1998) 151–152. (in Chinese)

In Guangdong, since 1987 WHO-MDT has been generally used and several thousand patients were cured according to the Chinese criterion, of which three relapsed cases have been found from 1990 to the end of 1997 through regular follow up; all had PB leprosy and were treated for 1 to 2 years with the PB regimen of MDT. The relapses all occurred 1 or 2 years after completion of their courses. After the relapses, their leprosy types are BT, neuritic and BL being proved clinically, bacteriologically and pathologically. Among them retreatment using MDT showed still great efficiency, suggesting that they are not resistant to MDT.—Authors' English Abstract

Zhu, R. [Analysis of 100 relapsed cases of leprosy.] China Lepr. J. **14** (1998) 161–162. (in Chinese)

In Shaoxing County, Zhejiang Province, during the period 1975 to 1996, 1423 cases of leprosy were cured, of which 100 relapsed cases were found (7%), (including T 30, B 5 and L 65) who all took only DDS 50–100 mg a day for a long time. The relapses occurred after 1 to 17 years, averaging 7.1 years, after stopping the treatment and had no association with duration and regularity of antileprosy treatment received originally.—Author's English Abstract

Clinical Sciences

Campos, W. R., Rodrigues, C. A., Orefice, F. and Monteiro, L. G. Identification of *M. leprae* in conjunctiva of leprosy patients using the superior tarsal conjunctiva scrape technique. Indian J. Lepr. **70** (1998) 397–403.

The technique of superior tarsal conjunctiva scrape was used for identifying *M. leprae* in the conjunctiva in 56 leprosy patients (all of them multibacillary, some untreated and others treated with multidrug therapy). The technique of tarsal conjunctiva scrape

was shown to be more suitable than conjunctival biopsy for identifying leprosy bacilli. This technique is also easier to perform and has shown a statistical relation between the bacilloscopical index of skin (BI_{sk}) and bacilloscopical index of tarsal conjunctiva (BI_{conj}) values. Thus, if the bacilli can be identified at tarsal conjunctiva we can assume greater systemic bacillary load in the patients.—Authors' Abstract

Ishikawa, S., Ishikawa, A., Yoh, K., Tanaka, H. A. and Fujiwara, M. Osteoporosis in male and female leprosy patients. Calcif. Tissue Int. **64** (1999) 144–147.

We measured the bone mineral density (BMD) of 353 leprosy patients (197 males 50–89 years old, average age 70.2; 156 females 53–90 years old, average age 72.9) and the serum levels of free testosterone (FT) in 81 males. The BMD of the lumbar vertebrae (L2–L4), diaphysis of the radius (⅓ radius), and the neck of the femur (neck) was measured using DXA (QDR 4500). The BMD of –2.5 S.D. YAM (young adult mean) in Japanese men and women was used as the cut-off value for osteoporosis in the respective genders: BMD of L2–L4, 0.751 g/cm^2 (male), 0.747 g/cm^2 (female); ⅓ radius, 0.655 g/cm^2 (male), 0.550 g/cm^2 (female); neck, 0.581 g/cm^2 (female). The percentages of males with osteoporosis were: 31.3% in the 50th, 32.9% in the 60th, 44.9% in the 70th, and 40.7% in the 80th decade at L2–L4. Similarly, the percentages were 33.3%, 58.3%, 74.3%, and 75.0%, respectively, at ⅓ radius. Among females, the percentages were 22.2%, 41.3%, 44.9%, and 68.8%, respectively, at L2–L4; 0%, 42.9%, 89.5% and 78.6%, respectively, at ⅓ radius; 11.1%, 38.6%, 67.7%, and 84.6%, respectively, at neck. FT in men ranged from almost 0 to normal at each decade and BMD levels were significantly correlated with FT in all three regions of the skeleton ($p < 0.0001$). More than 30% of osteoporosis was found at each decade and FT may be one of the main factors affecting BMD in male leprosy patients.—Authors' Abstract

John, D. and Daniel, E. Infectious keratitis in leprosy. Br. J. Ophthalmol. **83** (1999) 173–176.

Aims: To describe leprosy characteristics, ocular features, and type of organisms that produce infective corneal ulcers in leprosy patients.

Method: The records of all leprosy patients admitted for treatment of corneal ulcers between 1992 and 1997 were reviewed.

Results: Sixty-three leprosy patients, 53 males and 10 females, are described: 16 were tuberculoid and 47 lepromatous. Twenty-five patients had completed multidrug therapy. Ten patients had face patches, 8 had type 1 reaction, and 10 had type 2 reaction. Forty-three (68%) patients had hand deformities.

In 54% of patients pain was absent as a presenting symptom. Nineteen patients gave a history of trauma. In 15 patients ulcers had also occurred on the other eye, five of them having occurred during the study period and the rest before 1992. Of the 68 eyes with corneal ulcers, 28 had madarosis, 34 had lagophthalmos, 9 had ectropion, 3 had trichiasis, 6 had blocked nasolacrimal ducts, and 39 decreased corneal sensation. In 14 eyes, a previous lagophthalmos surgery had been done. Sixteen patients were blind at presentation. Thirty-two percent of ulcers were located centrally. After treatment only 18% of the eyes showed visual improvement. Five types of fungus were cultured, two of them rare ocular pathogens.

Conclusions: Corneal ulcers occur more in males and in the lepromatous group of patients. Decreased corneal sensation, lagophthalmos and hand deformity are closely associated. Indigenous treatment and late presentations were notable in many patients. Visual outcome is not good. There is increased risk of developing an ulcer in the other eye. Fungal corneal ulcers are not uncommon.—Authors' Abstract

Mitra, S., Gombar, K. K. and Gombar, S. Anaesthetic considerations in a patient with lepromatous leprosy. Can. J. Anaesth. **45** (1998) 1103–1105.

Purpose: To consider the anesthetic problems in a patient with lepromatous leprosy undergoing general anesthesia.

Clinical features: A 52-year-old man with lepromatous leprosy for 5 years was booked for elective radical nephrectomy. He received 100 mg dapsone per day p.o. The patient was asymptomatic for cardiovascular disease but his electrocardiogram showed complete left bundle branch block, inferior wall ischemia with echocardiogram findings of 58% ejection fraction and left ventricular diastolic dysfunction. Other preoperative investigations (hemogram, serum urea and creatinine, liver function tests and chest X-ray) were normal. After premedication with diazepam, meperidine and promethazine, the patient received glycopyrrolate and anesthesia was induced with thiopentone. Atracurium was given to facilitate tracheal intubation. Anesthesia was maintained with intermittent positive pressure ventilation using N_2O in oxygen with halothane. Anesthesia and surgery were uneventful except that the patient had a fixed heart rate that remained unchanged in response to administration of anticholinergic, laryngoscopy, intubation and extubation.

Conclusion: Patients with lepromatous leprosy may have cardiovascular dysautonomia even when they are asymptomatic for cardiovascular disease.—Authors' Abstract

Sirmour, S. K., Verma, P. K., Singh, J. N. and Okhandiar, P. LDH isozymes with anomalous bands in semen of leprosy patients. Indian J. Lepr. **70** (1998) 405–409.

Activity of LDH isozymes was evaluated electrophoretically on 7% acrylamide gel in the semen of 37 leprosy patients (15 with borderline, 12 with borderline tuberculoid and 10 with lepromatous leprosy) and 10 fertile men 30–45 years of age. Significantly lower activities were recorded of LDH_1 in all categories of leprosy patients. Similarly, lowering of LDH_2 activity was noticed in borderline and lepromatous cases only, lowering of LDH_4 activity in lepromatous cases only, and LDH_5 activity was lowered in borderline leprosy patients. Lowest activity of LDH_3 and absence of LDH_x were found in lepromatous leprosy. However, in borderline tuberculoid patients, LDH_3 and LDH_x were significantly higher. This exceptional increase in activity was found to be due to the presence of additional (anomalous) isozymes bands of LDH_3, LDH_x and LDH_4 in 25% of borderline tuberculoid patients. Additional bands of LDH_3 have also been located in 40% of the borderline leprosy patients.—Authors' Abstract

van de Weg, N., Post, E. B., Lucassen, R., de Jong, J. T. V. M. and van den Broek, J. Explanatory models and help-seeking behaviour of leprosy patients in Adamawa State, Nigeria. Lepr. Rev. **69** (1998) 382–389.

In northern Nigeria 60 leprosy patients, 49 outpatients and 11 inpatients, were interviewed about their help-seeking behavior and explanatory models before their first contact with the leprosy services. Most patients showed a delay of more than 1 year. After leprosy was provisionally diagnosed by lay persons, 27% of patients found their way to the leprosy services within 3 months. Chemists (popular sector) and the professional sector frequently missed the diagnosis. If early case finding is to be improved, it is important to involve them in case-finding activities and to train them in adequate diagnostic skills.

No significant correlations were found between total delay and sex, age, religion or leprosy classification, except with visible deformity at the time of the interview and illiteracy.

Consultation of folk healers was the major reason for delay. Most patients consulted folk healers who, although they claimed to have a positive attitude toward modern medicine in the case of leprosy, never referred patients to the leprosy services.

While many patients held a variety of causes responsible for leprosy, most patients explained the disease in traditional terms (58%), while only a minority used modern concepts (20%). This emphasizes the need for continuous attention for health eduction of diagnosed patients and their families. No significant difference was found between male and female patients concerning their concept of leprosy.

Denial of the leprosy diagnosis was rare.—Authors' Summary

Zhou, A., *et al.* [Actualities of leprosy control in Ninghai City, Zhejiang.] China Lepr. J. **14** (1998) 156–158. (in Chinese)

In Ninghai City, Zhejiang, China, with a population of 642,000, up to 1997 there were 767 registered cases of leprosy. Since 1975 it has started to control leprosy, and from 1987 WHO-MDT has been adopted. Out of 535 persons cured with DDS or DDS plus RMP, 10 relapsed (1.86%) and there was no relapse in the users of MDT. Among them 224 had visible disability (29.2%) and 309 died, of which 162 had been cured. The detection rate decreased from 5.14/100,000 (before 1975) to 0.043/100,000 (1993) and after 1994 no new case was seen. Among 309 dead, cause of death was: cancer 48 (6.2%), cor pulmonale 37 (4.82%), angiocardiopathy 32 (4.17%), apoplexy 19 (2.84%), pneumonia or TB 17 (2.22%), accidents 16 (2.09%) and suicide 11 (1.43%). The average lifespan of the persons cured of leprosy was 73.95 years, nearing that of normal residents in 1997 when 405 of them were still living.—Authors' English Abstract

Immuno-Pathology

Agrewala, J. N. and Wilkinson, R. J. Differential regulation of Th1 and Th2 cells by p91–110 and p21–40 peptides of the 16-kDa alpha-crystallin antigen of *Mycobacterium tuberculosis.* Clin. Exp. Immunol. **114** (1999) 392–397.

Permissively recognized peptides which can activate lymphocytes from subjects with a variety of class II HLA types are interesting diagnostic and vaccine candidates. In this study we generated T helper clones reactive to the permissively recognized p21–40 and p91–110 peptides of the 16-kD heat shock protein of *Mycobacterium tuberculosis.* All of the clones specific for p91–110 secreted interferon-gamma (IFN-γ) and were of the TH1 phenotype. By contrast, the p21–40 peptide favored the generation of IL-4-producing clones. Antibody blockade established that the peptide-specific Th clones could be DR-, DP- or DQ-restricted. Thus, two permissively recognized sequences (p21–40 and p91–110) from the same mycobacterial antigen can drive the differentiation of functionally distinct T helper subsets. Attempts to immunize against tuberculosis should bear in mind epitope specificity if a favorable Th subtype response is to be generated.—Authors' Abstract

Facer, P., Mathur, R., Pandya, S. S., Landiwala, U., Singhal, B. S. and Anand, P. Correlation of quantitative skin tests of nerve and target organ dysfunction with skin immunohistology in leprosy. Brain **121** (1998) 2239–2247.

Loss of nociception and hypohidrosis in skin are hallmarks of leprosy, attributed to early invasion by *Mycobacterium leprae* of Schwann cells related to unmyelinated nerve fibers. We have studied skin lesions and contralateral clinically unaffected skin in 28 patients across the leprosy spectrum with a range of selective quantitative sensory and autonomic tests prior to biopsy of both sites. Unaffected sites showed normal skin innervation when antibodies to the pan-neuronal marker PGP (protein gene product) 9.5 were used, with the exception of intra-epidermal fibers which were not detected in the majority of cases. Elevation of thermal thresholds and reduced sensory axon-reflex flare responses in affected skin

correlated with decreased nerve fibers in the subepidermis, e.g., axon-reflex flux units (means ± S.E.M.) for no detectable innervation, decreased innervation, and clinically unaffected skin were 23 ± 3.1, 41.2 ± 7.3; and 84.5 ± 4.0, respectively. Reduced nicotine-induced, axon-reflex sweating was correlated with decreased innervation of sweat glands. Where methacholine-induced direct activation of sweat glands was affected, there was inflammatory infiltrate and loss of sweat gland structure. This study demonstrates a correlation between selective nerve dysfunction on clinical tests and morphological changes in skin, irrespective of the type of leprosy, and is the first to show that loss of sweating in leprosy may result either from decreased innervation and/or involvement of the sweat glands. The findings have implications for the selection and monitoring of patients with leprosy in clinical trials which aim to restore cutaneous function.—Authors' Abstract

Fiallo, P., Travaglino, C., Nunzi, E. and Cardo, P. P. β_2-Glycoprotein I-dependence of anticardiolipin antibodies in multibacillary leprosy patients. Lepr. Rev. **69** (1998) 376–381.

This study was undertaken to investigate the influence of β_2-glycoprotein I (GPI) on anticardiolipin antibody (aCL) titration in leprosy. The study group consisted of 140 sera from patients with multibacillary leprosy (46 borderline, 94 lepromatous). The group included newly diagnosed, previously untreated patients, patients under treatment and patients released from treatment. GPI addition enhanced significantly the aCL titers in sera from lepromatous leprosy but not in those from borderline leprosy. Moreover, when patients were classified according to their bacteriological status, aCL titers were found to be significantly higher in skin-smear-positive patients compared to bacteriologically negative patients. Thus, the present study demonstrates that aCL in multibacillary leprosy patients is mainly of the GPI-dependent type and emphasizes the importance of GPI addition for aCL titration in leprosy.—Authors' Summary

Juffermans, N. P., Verbon, A., van Deventer, S. J. H., Buurman, W. A., van Deutekom, H., Speelman, P. and van der Poll, T. Serum concentrations of lipopolysaccharide activity-modulating proteins during tuberculosis. J. Infect. Dis. **178** (1998) 1839–1842.

Lipopolysaccharide (LPS) is the principal stimulator of host defense against gram-negative bacteria. LPS-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), and soluble CD14 (sCD14) bind LPS and regulate its toxicity. Lipoarabinomannan, a cell wall component of *Mycobacterium tuberculosis,* resembles LPS with respect to induction of inflammatory responses through recognition by LBP and sCD14. LBP, BPI, and sCD14 were measured in serum of 124 patients with tuberculosis in various stages of disease, in persons who had been in close contact with patients with contagious pulmonary tuberculosis, and in healthy controls. Levels of these LPS toxicity-regulating proteins were elevated in patients with active tuberculosis compared with those in contacts and controls and declined during treatment. The levels of LBP and sCD14 were higher in patients with fever and anorexia. LPS-regulating proteins may play a role in host defense during tuberculosis, presumably through interaction with lipoarabinomannan.—Authors' Abstract

Kornfeld, H., Mancino, G. and Colizzi, V. The role of macrophage cell death in tuberculosis. Cell Death Differ. **6** (1999) 71–78.

Studies of host response to infection have traditionally focused on the direct antimicrobial activity of effector molecules (antibodies, complement, defensins, reactive oxygen and nitrogen intermediates) and immunocytes (macrophages, lymphocytes, and neutrophils among others). The discovery of the systems for programmed cell death of eukaryotic cells has revealed a unique role for this process in the complex interplay between microorganisms and their cellular targets or responding immunocytes. In particular, cells of the monocyte/macro-

phage lineage have been demonstrated to undergo apoptosis following intracellular infection with certain pathogens that are otherwise capable of surviving within the hostile environment of the phagosome or which can escape the phagosome. *Mycobacterium tuberculosis* is a prototypical "intracellular parasite" of macrophages, and the direct induction of macrophage apoptosis by this organism has recently been reported from several laboratories. This paper reviews the current understanding of the mechanism and regulation of macrophage apoptosis in response to *M. tuberculosis* and examines the role this process plays in protective immunity and microbial virulence.—Authors' Abstract

Lagranderie, M., Winter, N., Balazuc, A. M., Gicquel, B. and Gheorghiu, M. A cocktail of *Mycobacterium bovis* BCG recombinants expressing the SIV Nef, Env, and Gag antigens induces antibody and cytotoxic responses in mice vaccinated by different mucosal routes. AIDS Res. Hum. Retrovir. **14** (1998) 1625–1633.

Recombinant live *Mycobacterium bovis* BCG strains (rBCG) expressing different human immunodeficiency virus (HIV) or simian immunodeficiency virus (SIV) antigens could be good candidates for the development of vaccines against AIDS. To develop effective HIV/SIV vaccines, humoral and cellular immune responses directed against multiple antigens may be essential for the control of the infection. In this study we immunized BALB/c mice via different mucosal routes (oral, aerogenic, nasal, and rectal) with a mixture of three rBCG strains expressing, respectively, the entire SIVmac251 Nef protein, and large fragments of the Env and Gag proteins. All routes of immunization studied induced immunoglobulin A (IgA) antibodies against mycobacterial PPD, SIV Env, and SIV Gag antigens in feces and bronchial lavages as well as specific immunoglobulin G (IgG) in serum. Strong, specific cytotoxic responses of splenocytes against Nef, Env, and Gag were observed whatever the mucosal route of immunization. Therefore, mucosal vaccination with a cocktail of rBCG strains induces local, specific IgA, systemic IgG, and systemic CTLs against the three SIV antigens expressed. Rectal and oral routes seemed the most appropriate route of vaccination to be used to protect against SIV infection.—Authors' Abstract

Manandhar, R., le Master, J. W. and Roche, P. W. The development of new skin tests: a tool for the eradication of leprosy. J. Nepal Med. Assoc. **37** (1998) 535–540.

This review paper discusses tests for exposure to leprosy, skin tests and tuberculosis, and the lepromin and leprosin-soluble antigen skin tests in leprosy. New strategies are also considered including further fractionation of leprosin and peptide-based skin tests.—Trop. Dis. Bull. **95** (1998) 1317.

Roy, S., Frodsham, A., Saha, B., Hazra, S. K., Mascie Taylor, C. G. N. and Hill, A. V. S. Association of vitamin D receptor genotype with leprosy type. J. Infect. Dis. **179** (1999) 187–191.

Host genetic factors including major histocompatibility complex (MHC) polymorphisms influence both susceptibility to leprosy *per se* and also to leprosy type. Non-MHC genes may play an important role, but such genes remain undefined. The influence of two non-MHC candidate genes was assessed in a case-control study of Bengali leprosy patients from Calcutta. Recent studies have implicated variation in the vitamin D receptor (VDR) gene in susceptibility to several diseases, including osteoporosis and pulmonary tuberculosis. In this population, homozygotes for the alternate alleles of the VDR polymorphism are associated, respectively, with lepromatous and tuberculoid leprosy. The NRAMP1 (natural resistance associated macrophage protein 1) gene may influence human mycobacterial disease susceptibility based on studies with the murine homolog Nramp1. However, no significant association was found between NRAMP1 and leprosy susceptibility. This study suggests that the VDR polymorphism may influence susceptibility to some diseases by affecting the type and the strength

of the host immune responses.—Authors' Abstract

Sieling, P. A., Jullien, D., Dahlem, M., Tedder, T. F., Rea, T. H., Modlin, R. L. and Porcelli, S. A. CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. J. Immunol. **162** (1999) 1851–1858.

A potential role for the CD1 family of lipid antigen (Ag)-presenting molecules in antimicrobial immunity *in vivo* was investigated in human leprosy skin lesions. Strong induction of three CD1 proteins (CD1a, -b, and -c) was observed in dermal granulomas in biopsy samples of involved skin from patients with the tuberculoid form of leprosy or with reversal reactions, which represent clinical patterns of disease associated with active cellular immunity to *Mycobacterium leprae.* In contrast, lesions from patients with the lepromatous form of the disease who lack effective cell-mediated immunity to the pathogen did not show induction of CD1 proteins. Thus, expression of CD1 correlated directly with effective immunity to *M. leprae,* as assessed by the clinical course of infection. CD1a, -b, and -c could be induced to similar levels on monocytes from the blood of either tuberculoid or lepromatous leprosy patients. this suggested that the absence of expression in lepromatous lesions was most likely due to local factors at the site of infection as opposed to a primary defect of the CD1 system itself. The majority of cells expressing CD1 in leprosy lesions were identified as a population of CD83+ dendritic cells. Initial *in vitro* studies of the Ag-presenting function of CD1+ CD83+ monocyte-derived dendritic cells showed that such cells were highly efficient APCs for CD1-restricted T cells. These results indicate that the CD1 system can be up-regulated in human infectious diseases *in vivo,* and may play a role in augmenting host defense against microbial pathogens.—Authors' Abstract

Sreenivasan, P., Misra, R. S., Wilfred, D. and Nath, I. Lepromatous leprosy patients show T helper 1-like cytokine profile with differential expression of interleukin-10 during type 1 and 2 reactions. Immunology **95** (1998) 529–536.

Leprosy patients suffer from clinical episodes associated with tissue damage which are designated as type 1 (reversal reaction) when localized to the lesions and type 2 (erythema nodosum leprosum, ENL) when accompanied by systemic involvement. We had reported earlier that stable, nonreaction lepromatous leprosy subjects show T helper 2 (Th2)- and Th0- but not Th1-like responses in the peripheral blood. To further understand the development of Th-like responses during disease, 32 lepromatous patients undergoing reactions were studied using cytokine-specific, reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) in peripheral blood and some skin biopsies. Of interest was the evidence of a Th1-like response with presence of interferon-gamma (IFN-γ) and absence of interleukin-4 (IL-4) mRNA. In the peripheral blood mononuclear cells (PBMC) of 85% and 64% of type 1 and 2 reaction patients, respectively, and in all reaction sites; whereas a Th0-like response was seen in some, a Th2-like response was absent. IL-12p40 mRNA was seen in 21/25 ENL and all type 1 reaction subjects irrespective of the Th phenotype. IL-12p40 and IFN-γ were detectable in unstimulated PBMC, suggesting an *in vivo* priming during reactions. IL-10 was mainly associated with adherent cells and showed a differential expression in the two reactions. It was present in the PBMC of ENL but not in reversal reaction patients. Moreover, it was not detectable in the skin lesions of either type of reactions. A Th1-like cytokine profile was associated with immunopathology and persisted up to 6–7 months after the onset of reactions.—Authors' Abstract

Suneetha, S., Arunthathi, S., Chandi, S., Kurian, N. and Chacko, C. J. G. Histological studies in primary neuritic leprosy: changes in the apparently normal skin. Lepr. Rev. **69** (1998) 351–357.

The visually normal skin of 196 patients diagnosed clinically to have primary neuritic leprosy was studied histologically to

determine whether there were any specific changes due to the disease in this site. Histological changes due to leprosy were seen in 32.1% of the patients, and included indeterminate leprosy in 19.4%, borderline tuberculoid leprosy in 6.6% and borderline lepromatous leprosy in 6.1%. The remaining biopsies showed mild nonspecific dermal inflammation, mild nerve changes or no significant lesion. The nerve inflammation and/or granulomas were mostly in the deep dermal nerves or neurovascular complexes. This study shows that there is a cutaneous component to primary neuritic leprosy and the disease is not totally confined to nerves. The absence of visible hypopigmented patches in these patients is probably related to the deep location of the dermal inflammation.—Authors' Summary

Suneetha, S., Arunthathi, S., Job, A., Date, A., Kurian, N. and Chacko, C. J. G. Histological studies in primary neuritic leprosy: changes in the nasal mucosa. Lepr. Rev. **69** (1998) 358–366.

The nasal mucosae of 39 cases of primary neuritic leprosy (PNL) registered at Karigiri were studied histologically to determine nasal mucosal involvement in PNL and its relevance to the pathogenesis of the disease. Specific changes of leprosy were seen in 20 (51%) biopsies, ranging from macrophage granulomas with acid-fast bacilli to epithelioid granulomas and nerve inflammation. The remaining biopsies revealed chronic inflammatory changes of the mucosa or mild nonspecific nerve changes. These findings show that there are widespread effects of the disease even in PNL patients in whom the disease is believed to be confined to the peripheral nerves. The findings also show that early leprosy involvement can be found in the nasal mucosa even before lesions become apparent in the skin or other parts of the body. The nasal mucosa could be one of the sites for the primary lesion in leprosy. Clinical and histological examination of the nasal mucosa may be useful and important in the early diagnosis of leprosy and especially in contacts.—Authors' Summary

Microbiology

Harboe, M. and Wiker, H. G. Secreted proteins of *Mycobacterium leprae.* Scan. J. Immunol. **48** (1998) 577–584.

In mycobacteria, secreted proteins represent a distinct group, probably of particular importance for development of immune responses following infection. Quantification of individual proteins in culture fluid and corresponding disrupted bacilli permits determination of a localization index for identification of secreted proteins. This procedure cannot be applied to *Mycobacterium leprae* because secreted proteins are lost during isolation of bacilli from tissues. The DNA sequences of secreted proteins of *M. tuberculosis* were compared with sequences of *M. leprae.* Genes for homologs of the 85a, 85b, 85c, mpt32 (apa), mpt51, erp, mtc28, Rv2376c, Rv3354 and Rv0526 genes were identified. All of these contained signal sequences typical for secretion in *M. leprae.* In several instances the local distance between marker genes and occurrence on the same or the complementary DNA strand was similar in these two species. The genetic organization of genes for secreted proteins is thus very similar in *M. leprae* and *M. tuberculosis,* the homology being higher for the mature polypeptide chains than for the corresponding signal peptides.—Authors' Abstract

Hu, J. M. and Coates, A. R. M. Transcription of two sigma 70 homologue genes, sigA and sigB, in stationary-phase *Mycobacterium tuberculosis.* J. Bacteriol. **181** (1999) 469–476.

The sigA and sigB genes of *Mycobacterium tuberculosis* encode two sigma 70-like sigma factors of RNA polymerase. While transcription of the sigA gene is growth-rate independent, sigB transcription is increased during entry into stationary phase. The sigA gene transcription is unresponsive to environmental stress but that of sigB is very responsive, more so in stationary-phase growth than in log-phase cultures. These data suggest that sigA is a primary sigma factor which, like sigma^{80}, controls the transcription of the housekeeping type of promoters. In contrast, sigB, although showing some overlap in function with sigA, is more like the alternative sigma factor, sigma^{S}, which controls the transcription of the gearbox type of promoters. Primer extension analysis identified the RNA start sites for both genes as 129 nucleotides upstream to the GTG start codon of sigA and 27 nucleotides from the ATG start codon of sigB. The –10 promoter of sigA but not that of sigB was similar to the sigma^{70} promoter. The half-life of the sigA transcript was very long, and this is likely to play an important part in its regulation. In contrast, the half-life of the sigB transcript was short, about 2 min. These results demonstrate that the sigB gene may control the regulons of stationary phase and general stress resistance, while sigA may be involved in the housekeeping regulons.—Authors' Abstract

Lee, B. H., Murugasu Oei, B. and Dick, T. Upregulation of a histone-like protein in dormant *Mycobacterium smegmatis.* Mol. Gen. Genet. **260** (1998) 475–479.

The aerobic saprophyte *Mycobacterium smegmatis,* like its pathogenic counterpart *M. tuberculosis,* has the ability to adapt to anaerobiosis by shifting down to a dormant state. Here, we report the identification and molecular genetic characterization of the first dormancy-induced protein in *M. smegmatis.* Comparative SDS-polyacrylamide gel electrophoresis of protein extracts of aerobically growing and dormant anaerobic *M. smegmatis* cultures revealed the upregulation of a 27-kDa protein in the dormant state. Peptide sequencing showed that the induced protein is a homolog of the histone-like protein Hlp, predicted by the *M. tuberculosis* genome project. The corresponding hlp gene was cloned from *M. smegmatis* and sequenced. Disruption of the hlp gene eliminated the histone-like protein but did not affect the viability of the dormant culture.—Authors' Abstract

Michele, T. M., Ko, C. and Bishai, W. R. Exposure to antibiotics induces expression of the *Mycobacterium tuberculosis sigF* gene: implications for chemotherapy against mycobacterial persistors. Antimicrob. Agents Chemother. **43** (1999) 218–225.

The *sigF* gene encodes an alternate sigma factor found in *Mycobacterium tuberculosis* and related pathogenic mycobacteria. Determination of conditions of *sigF* expression is an important step in understanding the conditional gene regulation which may govern such processes as virulence and dormancy in mycobacteria. We constructed an in-frame translational *lacZ-kan* fusion within the *sigF* gene to determine the conditions of *sigF* expression. This reporter construct was expressed from a multicopy plasmid in a strain of BCG harboring an integrated luciferase reporter gene under the control of the mycobacteriophage L5 gp71 promoter. Antibiotic exposure, in particular, ethambutol, rifampin, streptomycin, and cycloserine treatment, increased the level of SigF reporter specific expression in a dose-dependent fashion. The level of SigF reporter-specific expression increased over 100-fold in late-stationary-phase growth compared to that in exponential growth. During the exponential phase, SigF-specific expression could be induced by a number of other stresses. Anaerobic metabolism induced SigF by greater than 150-fold, particularly in the presence of metronidazole. Cold shock increased the level of SigF-specific expression, while heat shock decreased it. Oxidative stress was also an important inducer of SigF-specific expression; a greater induction was seen with cumene hydroperoxide than with hydrogen peroxide. Comparisons of bacterial viability as determined by the luciferase assay or by plating serial dilutions revealed that luciferase gp71-dependent activity was an un-

reliable predictor of the numbers of CFU during stationary-phase growth and anaerobic metabolism. The induction of *sigF* following antibiotic exposure suggests that this bacterial transcription factor may control genes which are important for mycobacterial persistence in the host during chemotherapy.—Authors' Abstract

Rambukkana, A., Yamada, H., Zanazzi, G., Mathus, T., Salzer, J. L., Yurchenco, P. D., Campbell, K. P. and Vischetti, V. A. Role of alpha-dystroglycan as a Schwann cell receptor for *Mycobacterium leprae.* Science **282** (1998) 2076–2079.

alpha-Dystroglycan (alpha-DG) is a component of the dystroglycan complex, which is involved in early development and morphogenesis and in the pathogenesis of muscular dystrophies. Here, alpha-DG was shown to serve as a Schwann cell receptor for *Mycobacterium leprae,* the causative organism of leprosy. *M. leprae* specifically bound to alpha-DG only in the presence of the G domain of the alpha 2 chain of laminin-2. Native alpha-DG competitively inhibited the laminin-2-mediated *M. leprae* binding to primary Schwann cells. Thus, *M. leprae* may use linkage between the extracellular matrix and cytoskeleton through laminin-2 and alpha-DG for its interaction with Schwann cells.—Authors' Abstract

Smeulders, M. J., Keer, J., Speight, R. A. and Williams, H. D. Adaptation of *Mycobacterium smegmatis* to stationary phase. J. Bacteriol. **181** (199) 270–283.

Mycobacterium tuberculosis can persist for many years within host lung tissue without causing clinical disease. Little is known about the state in which the bacilli survive, although it is frequently referred to as dormancy. Some evidence suggests that cells survive in the nutrient-deprived stationary phase. Therefore, we are studying stationary-phase survival of *Mycobacterium smegmatis* as a model for mycobacterial persistence. *M. smegmatis* cultures could survive 650 days of either carbon, nitrogen, or phosphorus starvation. In carbon-limited medium, cells entered the stationary phase before the carbon source (glycerol) had been completely depleted and glycerol uptake from the medium continued during the early stages of stationary phase. These results suggest that the cells are able to sense when the glycerol is approaching limiting concentrations and initiate a shutdown into stationary phase, which involves the uptake of the remaining glycerol from the medium. During the early stationary phase, cells underwent reductive cell division and became more resistant to osmotic and acid stress and pool mRNA stabilized. Stationary-phase cells were also more resistant to oxidative stress, but this resistance was induced during the late exponential phase in a cell-density-dependent manner. Upon recovery in fresh medium, stationary-phase cultures showed an immediate increase in protein synthesis irrespective of culture age. Colony morphology variants accumulated in stationary-phase cultures. A flat colony variant was seen in 75% of all long-term stationary-phase cultures and frequently took over the whole population. Cryo scanning electron microscopy showed that the colony organization was different in flat colony strains, flat colonies appearing less well organized than wild-type colonies. Competition experiments with an exponential-phase-adapted wild-type strain showed that the flat strain had a competitive advantage in the stationary phase, as well as providing evidence that growth and cell division occur in stationary-phase cultures of *M. smegmatis.* These results argue against stationary-phase *M. smegmatis* cultures entering a quiescent state akin to dormancy but support the idea that they are a dynamic population of cells.—Authors' Abstract

Yin, Y., *et al.* [Preparation and expression of DNA fragments in various epitopes of α-antigen genes in *M. leprae.*] China Lepr. J. **14** (1998) 139–142. (in Chinese)

The DNA fragments with a different epitope from an α-antigen gene of *M. leprae* have been produced by using the methods of restriction endonuclease digestion and multiprimer PCR. Meanwhile the DNA

fragments obtained from restriction endonuclease digestion were preliminarily expressed.—Authors' English Abstract

Zhao, W. C., Schorey, J. S., Groger, R., Allen, P. M., Brown, E. J. and Ratliff, T. L. Characterization of the fibronectin binding motif for a unique mycobacterial fibronectin attachment protein, FAP. J. Biol. Chem. **274** (1999) 4521–4526.

Studies were performed to define the fibronectin binding motif of the previously identified *Mycobacterium avium* fibronectin attachment protein (FAP-A). Using synthetic peptides of a previously identified fibronectin binding region (amino acids 269–292), the minimal binding sequence was determined to be 12 amino acids, 269–280 (FAP-A-(269–280)). Synthetic peptides were prepared in which each amino acid in the 269–280 sequence was substituted with Ala. Assessment of the effect of Ala substitution on fibronectin binding showed that the presence of Ala at amino acids 273–276 (RWFV) completely abrogated fibronectin binding activity. Furthermore, the ability to inhibit the attachment of viable *M. bovis* BCG to fibronectin was abrogated by Ala substitution at the RWFV sites. To validate the function of RWFV, further studies were performed with recombinant FAP-A in which single Ala mutations were generated for the RWFV sites and as controls at amino acids 269 and 280. Mutant FAP-A containing single Ala substitutions at the RWFV sites (amino acids 273, 274, 275, or 276) showed significant abrogation of fibronectin binding function. Recombinant FAP-A with Ala substitutions at either 269 or 280 showed wild-type activity. When the four essential amino acids (RWFV) were either substituted *en bloc* with Ala or were all deleted, complete loss of fibronectin-binding function was observed. Control recombinant proteins with *en bloc* Ala substitutions or deletions at four positions outside the fibronectin-binding region (amino acids 255–257) retained functional activity. These data show that the RWFV sequence is necessary for fibronectin-binding function of FAP-A. Furthermore, the data suggest that mycobacterial FAP proteins, all of which share the RWFV binding motif, constitute a family of highly homologous proteins that bind fibronectin in a unique manner.—Authors' Abstract

Experimental Infections

Moura, A. C. N., Werneck Barroso, E., Rosas, E. C., Henriques, M. G. M. O. and Cordeiro, R. S. B. Opposite cellular accumulation and nitric oxide production *in vivo* after pleural immunization with *M. leprae* or *M. bovis* BCG. Int. J. Mol. Med. **3** (1999) 69–74.

Mycobacteria as intracellular pathogens have evolved mechanisms to survive within macrophages. Our previous data showed that *M. leprae* (ML), unlike *M. bovis* BCG, did not induce an inflammatory response in the mice subcutaneous tissue. Further, ML inhibited BCG-induced foot pad edema and seemed to transform macrophages into epithelioid cells. Since these mycobacteria share common antigens, here we sought to compare the acute and chronic cellular responses evoked by ML and BCG in pleurisy of mycobacteria-susceptible mice (BALB/c). The total leukocytes, the cell type that migrated to the pleural cavity and macrophage activation assayed by nitric oxide release were determined. Live or dead BCG Moreau recruited the same extent of cells, essentially monocytes and neutrophils, dose-dependently, in both acute and chronic pleurisy. BCG-induced eosinophilia was observed only in the acute response (after 24 hr of injection). A significant nitric oxide release by pleural macrophages was triggered by BCG Moreau without previous activation.

Nevertheless, ML failed to recruit leukocytes to the pleural space or to lead to nitric oxide production despite the number of bacilli used and the time studied (1, 7 or 14 days after injection). Although these mycobacteria have common antigens that cross-react, these data show a distinct ability of ML or BCG to recruit cells to the pleural space and to activate pleural macrophages for nitric oxide production *in vivo*.—Authors' Abstract

Xiong, J., *et al.* [BALB/c mouse model in screening of antileprosy drugs.] China Lepr. J. **14** (1998) 142–143. (in Chinese)

Leprosy bacilli taken from two newly detected patients with lepromatous leprosy have been inoculated into the rear foot pads of BALB/c pure bred mice. The contents of inoculated bacilli were 5×10^3 per foot pad. The mice of experimental groups have been given food containing 0.0001%, 0.001%, or 0.01% DDS, or 0.05% B663, or agar-water containing 20 mg RMP per ml, respectively. The latter was given a week. Six months after the inoculation, the number of bacilli in the foot pads of control mice reached 2.5×10^5, but in the foot pads of experimental mice no bacillus was seen except in one of five mice taking RMP. The authors think that BALB/c mice are cheap, easily raised, quickly bred, and so suitable for studying leprosy.—Authors' English Abstract

Epidemiology and Prevention

Archibald, H., Fitzpatrick, P. F. and Ree, G. H. Locally acquired Hansen's disease in North Queensland. Med. J. Aust. **170** (1999) 72–73.

Hansen's disease (leprosy) is rare in Australia and usually imported from endemic areas. We report a 23-year-old white male with multibacillary leprosy who had lived all his life in North Queensland and initially appeared to have no risk factors. However, historical records revealed his grandfather to have been infected; because of stigma, this was unknown to the patient. Since Hansen's disease has an incubation period of years, isolated cases may still occur as a result of previous endemicity in Queensland.—Authors' Abstract

Gupte, M. D., Vallishayee, R. S., Arantharaman, D. S., Nagaraju, B., Sreevatsa, Balasubramanyam, S., de Britto, R. L. J., Elango, N., Uthayakumaran, N., Mahalingam, V. N., Lourdusamy, G., Ramalingam, A., Kannan, S. and Arokiawamy, J. Comparative leprosy vaccine trial in South India. Indian J. Lepr. **70** (1998) 369–388.

This report provides results from a controlled, double blind, randomized, prophylactic leprosy vaccine trial conducted in South India. Four vaccines, i.e., BCG, BCG+ killed *M. leprae, Mycobacterium w* and ICRC, were studied in this trial in comparison with a normal saline placebo. From about 300,000 people, 216,000 were found eligible for vaccination and among them, 171,400 volunteered to participate in the study. Intake for the study was completed in 2½ years from January 1991. There was no instance of serious toxicity or side effects subsequent to vaccination for which premature decoding was required. All of the vaccine candidates were safe for human use. Decoding was done after the completion of the second resurvey in December 1998. Results for vaccine efficacy are based on examination of more than 70% of the original "vaccinated" cohort population in both the first and the second resurveys. It was possible to assess the overall protective efficacy of the candidate vaccines against leprosy as such. Observed incidence rates were not sufficiently high to ascertain the protective efficacy of the candidate vaccines against progressive and serious forms of leprosy. BCG+ killed *M. leprae* provided 64% pro-

tection (CI 50.4–73.9), ICRC provided 65.5% protection (CI 48.0–77.0), *Mycobacterium w* gave 25.7% protection (CI 1.9–43.8) and BCG gave 34.1% protection (CI 13.5–49.8). Protection observed with the ICRC vaccine and the combination vaccine (BCG+ killed *M. leprae*) meets the requirement of public health utility, and these vaccines deserve further consideration for their ultimate applicability in leprosy prevention.—Authors' Abstract

Huang, Y. [The way of case finding and early diagnosis in leprosy.] China Lepr. J. **14** (1998) 166–167. (in Chinese)

From 1957 to 1995, 856 cases of leprosy were found in Huadu City, Guangzhou, China, of which 366 were detected by various surveys and 490 were diagnosed in hospitals. Among survey-detected cases the mean disease duration was 4.24 years and the disability rate was 44.54%; among hospital-diagnosed cases, 2.89 years and 28.16%, respectively. Of the patients diagnosed in hospitals, 66.36% were from the town and 54.32% were from the countryside. The mean disease duration was 3.22 years among the town patients and 4.51 years for those from the countryside. Between town and countryside the rate of population was 0.57 to 1 and that of the numbers of the patients was 0.32 to 1. Since the patients with leprosy are fewer now, to find them, the author thinks that wide health education on leprosy is essential.—Author's English Abstract

Sreevatsa, Hari, M. and Gupte, M. D. Quality control tests for vaccines in leprosy vaccine trial, Avadi. Indian J. Lepr. **70** (1998) 389–395.

All of the vaccines supplied for the large-scale comparative leprosy vaccine trial of ICRC bacilli, *Mycobacterium w,* BCG plus killed *M. leprae* (candidate vaccines), BCG and normal saline (control arms) at CJIL Field Unit, Chennai, India, were tested for quality control by the suppliers following the procedures laid down in the WHO protocol for killed *M. leprae.* Quality control for BCG was carried out at a BCG vaccine laboratory as per protocol. Toxicity and sterility tests were done on all the vaccine batches/lots received. As part of the quality control, bacterial counts and protein estimations were also done. Studies showed that the bacterial content and protein concentration were comparable with the original preparations. Vaccines were free from micro-organisms, toxic materials and safe for human use. Thus, the quality of all vaccine preparations was satisfactory.—Authors' Abstract

Wu, P. [To be approaching basic eradication of leprosy in Guangxi Autonomous Region.] China Lepr. J. **14** (1998) 149–151. (in Chinese)

In Guangxi Region with a population of 15,300,000 and 89 counties, since 1956 started leprosy control, 26,575 leprosy patients have been found and treated with DDS monotherapy before 1986 and with WHO's MDT after 1987. By the end of 1995, the prevalence had declined from 0.28‰ to 0.005‰ and the incidence from 4.55/100,000 to 0.11/100,000. The relapse rate was 2.45. What were adopted are comprehensive measures and Grade 3 leprosy control network all along for that.—Author's English Abstract

Zhang, X. [Epidemiological meanings of the type ratio in leprosy.] China Lepr. J. **14** (1998) 165–166. (in Chinese)

On the basis of the data obtained from 16 counties (cities) in Guangdong, a curve of leprosy type ratio (MB/PB) since the 1950s assumed a U-shape, being its first peak in the beginning with a gradual decline, the lowest point in 1966 to 1971 and then a gradual going up to the second peak in the mid-1990s. The author thinks that the first peak implied that MB cases were easy to be found when there were more hidden patients and the second peak suggested that MB cases really accounted for the majority of leprosy patients because only persons without the resistance to leprosy should suffer from the disease when leprosy transmission has been controlled.—Author's English Abstract

Rehabilitation

Bainson, K. A. and van den Borne, B. Dimensions and process of stigmatization in leprosy. Lepr. Rev. **69** (1998) 341–350.

Leprosy is a disease which has struck fear into human beings for thousands of years. This is partly because it causes considerable deformities and disabilities. In 1991, the 44th World Health Assembly adopted a resolution to eliminate the disease as a public health problem by the year 2000. However, one of the major obstacles to achieving this objective is the stigma associated with the disease. Stigma against leprosy patients affects all aspects of leprosy control. This paper describes a model of the stigmatization process in leprosy. The process of stigmatization can be divided into two stages. The first stage describes how certain cognitive dimensions of leprosy lead to a variety of affective responses toward the disease. The second stage involve how these affective responses contribute to social devaluation of the leprosy patient and, consequently, the adoption of negative behavior toward them.—Authors' Summary

Chen, W., *et al.* [Simple artificial limb and its use in persons affected with leprosy.] China Lepr. J. **14** (1998) 160–161. (in Chinese)

To resolve difficulties for persons affected with leprosy in acquiring and mending artificial limbs, a shop for that was built in 1992 in Jiangyan City, Jiangsu, China. Up to now, 70 artificial limbs have been made for 68 persons with a mean age of 52 years and, after a follow up of 3 years, the evaluation from physicians and users was excellent in 75.3% and better in 25.7%. The users regarded wearing it as very comfortable, but for this the technician had to repair them every month.—Authors' English Abstract

Jiang, T., Watson, J. M., Zhang, G. C. and Wei, X. Y. A field trial of detection and treatment of nerve function impairment in leprosy—report from national POD pilot project. Lepr. Rev. **69** (1998) 367–375.

As part of a collaborative project between the Ministry of Health of China (MOH) and The Leprosy Mission International (TLMI) on leprosy rehabilitation and the prevention of disability (POD), a total of 1407 patients was monitored for possible nerve function impairment (NFI) through standardized clinical nerve function assessment between May 1995 and February 1998. Of these, 191 patients were found to have NFI and were put on a fixed regimen of prednisolone. In this study, 36.7% of NFI occurred before diagnosis of leprosy, 35.6% developed during MDT and 25.7% after their release from MDT. Overall, 7.5% (105 out of 1407) of all patients, or 55.9% of patients with NFI, suffered from silent neuropathy. Of the affected nerves, 62.6% had silent neuropathy. Sensory impairment responded to prednisolone satisfactorily, giving a recovery rate of 73.8%, 76.5% and 81.0% in the ulnar, median and posterior tibial nerves, respectively. Sensibility in patients even with a NFI duration of longer than 6 months made significant improvement ($p < 0.05$). Motor function improvement was less satisfactory, especially in ulnar and c. popliteal nerve. The possible reasons are analyzed. Our findings with regard to sensibility changes confirm that once it becomes clinically detectable, NFI is no longer at the "early" stage. More sensitive tests are necessary to detect real "early" sensory impairment in the field. Our study also indicates that with well-trained field staff and proper equipment for nerve function assessment, early detection and treatment of NFI can be practical and effective.—Authors' Summary

Shen, J. [Economical benefit of controlling leprosy.] China Lepr. J. **14** (1998) 169–170. (in Chinese)

Economic benefits of leprosy control in Yangcheng City, Jiangsu, China, during the period of 1956 to 1995 were analyzed. The results showed that the benefit-cost ratio of leprosy control was 9.09:1 and the net ben-

efit was 692,014,700 RMB Yuan with net benefit-cost ratio of 8.09:1, suggesting that leprosy control is a hygiene investment item with good economic results.—Author's English Abstract

Tang, X. [On couching for leprosy patients.] China Lepr. J. **14** (1998) 152–153. (in Chinese)

Cataracts in 26 eyes of 23 persons cured of leprosy and living in leprosaria have been extracted with the couching method, that is, to press the opaque lens down into the lower temporal part of the vitreous by a special needle, and the vision increased from less than 0.05 to more than 0.2. The author thinks that the operation is simple, safe and effective, and can be used in the field.—Author's English Abstract

Wu, W. [Reconstruction of thumb opposing function for leprosy patients with monkey hand.] China Lepr. J. **14** (1998) 146–148. (in Chinese)

Several methods of reconstructing the opposing muscle function for correcting ape hand in leprosy were described, including the use of tendon transplantation of the superficial flexor muscle of the fingers, changing the route of the short extensor muscle of the thumb and transplanting the long palmar muscle tendon to the tendon of the short extensor muscle of thumb. The author has corrected 20 hands for 17 persons and obtained excellent effects in 16 hands and good effects in four, i.e., their function and appearance have been obviously improved, of which six using the changing of the route of the short extensor muscle of thumb all obtained the best result.—Author's English Abstract

Wu, W. [Transfer of posterial tibial tendon for footdrop in leprosy.] China Lepr. J. **14** (1998) 163–164. (in Chinese)

A common surgical method—TMT—for the correction of dropfoot due to peroneal nerve paralysis in leprosy and its indication are described. The results of the operation for 36 cases the author had done showed that it was effective in all of them, being evaluated as excellent in 31 and good in five.—Author's English Abstract

Zhang, S. [Protective shoes and rehabilitation for disabled feet in leprosy.] China Lepr. J. **14** (1998) 167–168. (in Chinese)

The effects of protective shoes, using those for sportsmen made in Shanghai and Qingdao, on 38 superficial plantar ulcers and 12 complicated ones were described. Within 3 years, 31 superficial ulcers and 7 complicated ones have healed. In addition, of 56 rhagades in the feet 34 healed and 22 improved, and the wearers of the shoes felt comfort very much.—Author's English Abstract

Zhen, B. [Effect of self-care on 56 cases of leprosy.] China Lepr. J. **14** (1998) 159–160. (in Chinese)

Under the collaboration project for leprosy rehabilitation between the MOPH and TLMI in Lanxi City, Zhejiang, 65 persons afflicted with leprosy had done self-care for 3 years (64 peasants and 1 worker) 55 were men and 10 were women with a mean age of 54.23 years, and 23 were MB and 42 were PB with grade II disability in 64. After 3 years, red eyes, rhagades and ulcers of the hands and feet decreased by 62.1%, 100%, 100%, 77.8% and 53.33%, respectively. The author thinks that for success regular supervision and guidance of medical workers is the key.—Author's English Abstract

Zheng, Z., *et al.* [Effect of self-care in 856 persons affected with leprosy.] China Lepr. J. **14** (1998) 154–156. (in Chinese)

In stage I of Leprosy Rehabilitation Collaboration between MOPH and TLMI, out of 856 people with eye, hand and foot disabilities 70% of plantar ulcers were cured, 18 wounds on 15 patients' hands were healed and 80% of them have self-care habits, showing that self-care can be accepted and that disability can be prevented only if the health care and supervision are carried out well and intensively.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Abate, G. and Miorner, H. Susceptibility of multidrug-resistant strains of *Mycobacterium tuberculosis* to amoxicillin in combination with clavulanic acid and ethambutol. J. Antimicrob. Chemother. **42** (1998) 735–740.

Thirty clinical isolates of *Mycobacterium tuberculosis,* 20 of which were multidrug-resistant (MDR), were tested for susceptibility to different combinations of amoxicillin, clavulanic acid and subinhibitory concentrations of ethambutol. Beta-Lactamase production was assessed semiquantitatively with the nitrocefin method and susceptibility testing was performed with the BACTEC method. All isolates were beta-lactamase positive and were resistant to 16 mg/l amoxicillin. The MIC of amoxicillin in combination with clavulanic acid was ≥2 mg/l for 27/30 (90%) isolates. Addition of subinhibitory concentrations of ethambutol significantly reduced the MIC of amoxicillin for all tested isolates. Twenty-nine (97%) isolates had an MIC of amoxycillin of ≤0.5 mg/l when subinhibitory concentrations of ethambutol were added; this is well below the concentrations achievable in serum and tissue.—Authors' Abstract

Aceti, A., Zanetti, S., Mura, M. S., Sechi, L. A., Turrini, F., Saba, F., Babudieri, S., Mannu, F. and Fadda, G. Identification of HIV patients with active pulmonary tuberculosis using urine based polymerase chain reaction assay. Thorax **54** (1999) 145–146.

Background: Despite the increased dissemination of tuberculosis among HIV infected patients, the diagnosis is difficult to establish. Traditional microbiological methods lack satisfactory sensitivity. We have developed a highly sensitive and specific nested polymerase chain reaction (PCR) capable of detecting *Mycobacterium tuberculosis* DNA in urine specimens and have used this test to examine urine specimens from HIV patients with active pulmonary tuberculosis.

Methods: Urine specimens from 13 HIV infected patients with microbiologically proven active pulmonary tuberculosis, 10 AIDS patients with nontuberculous mycobacterial infection (documented by blood culture), 53 AIDS patients with no evidence of mycobacterial disease, and 80 healthy subjects (25 with positive skin test to purified protein derivative) were tested for *M. tuberculosis* using PCR, acid-fast staining (AFS), and culture.

Results: Of the urine specimens from patients with active tuberculosis, all tested positive by PCR, two by culture, and none by AFS. No reactivity was observed in urine specimens form patients with nontuberculous mycobacterial infection. Of the 53 AIDS patients without mycobacterial infection, one had a positive urine PCR. Normal subjects were all negative.

Conclusions: Urine based nested PCR for *M. tuberculosis* may be a useful test for identifying HIV patients with pulmonary tuberculosis.—Authors' Abstract

Behr, M. A., Warren, S. A., Salamon, H., Hopewell, P. C., de Leon, A. P., Daley, C. L. and Small, P. M. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. Lancet **353** (1999) 444–449.

Background: The microscopic examination of sputum for acid-fast bacilli (AFB) is a simple and rapid test that is used to provide a presumptive diagnosis of infectious tuberculosis. While patients with tuberculosis with sputum smears negative for AFB are less infectious than those with positive smears, both theoretical and empirical evidence suggest that they can still transmit *Mycobacterium tuberculosis.* We aimed to estimate the risk of transmission from smear-negative individuals.

Methods: As part of an ongoing study of the molecular epidemiology of tuberculosis in San Francisco, California, U.S.A., patients with tuberculosis with mycobacterial isolates with the same DNA fingerprint were assigned to clusters that were assumed

to have involved recent transmission. Secondary cases with tuberculosis, whose mycobacterial isolates had the same DNA, were linked to their presumed source case to estimate transmission from smear-negative patients. Sensitivity analyses were done to assess potential bias due to misclassification of source cases, unidentified source cases, and HIV-1 co-infection.

Findings: 1574 patients with culture-positive tuberculosis were reported and DNA fingerprints were available for 1359 (86%) of these patients. Of the 71 clusters of patients infected with strains that had matching fingerprints, 28 (39% [95% CI 28–52]) had a smear-negative putative source. There were 183 secondary cases in these 71 clusters, of whom a minimum of 32 were attributed to infection by smear-negative patients (17% [12–24]). The relative transmission rate of smear-negative compared with smear-positive patients was calculated as 0.22 (95% CI 0.16–0.32). Sensitivity analyses and stratification for HIV-1 status had no impact on these estimates.

Interpretation: In San Francisco, the AFB smear identifies the most infectious patients, but patients with smear-negative culture-positive tuberculosis appear responsible for about 17% of tuberculosis transmission.—Authors' Abstract

Bekker, L. G., Maartens, G., Steyn, L. and Kaplan, G. Selective increase in plasma tumor necrosis factor-α and concomitant clinical deterioration after initiating therapy in patients with severe tuberculosis. J. Infect. Dis. **178** (1998) 580–584.

The initiation of antituberculosis treatment in patients with severe tuberculosis may be accompanied by clinical deterioration and even death before any improvement occurs. To investigate this phenomenon, newly diagnosed, human immunodeficiency virus-negative adults with severe tuberculosis (in Cape Town, South Africa) were followed for the first 42 days of standard short-course therapy. Clinical status, serum lactate, plasma cytokine, and plasma cytokine receptor levels were monitored on days 0, 3, and 7 and then weekly for up to 42 days. Following 7 days of antituberculosis therapy, a significant transient decrease in mean Karnofsky score ($p < 0.001$), a concomitant increase in serum lactate ($p = 0.06$), a decrease in patient weight ($p = 0.02$), and an increase in plasma tumor necrosis factor-alpha (TNF-α) concentrations ($p = 0.04$) were observed. Plasma levels of soluble interleukin-2 receptor, interferon-γ, interleukin-6, and TNF-α receptor decreased over the 42-day study period. These observations suggest that increases in plasma TNF-α levels may be associated with clinical deterioration observed early in the treatment of severe tuberculosis.—Authors' Abstract

Bentley Hibbert, S. I., Quan, X., Newman, T., Huygen, K. and Godfrey, H. P. Pathophysiology of antigen 85 in patients with active tuberculosis: antigen 85 circulates as complexes with fibronectin and immunoglobulin G. Infect. Immun. **67** (1999) 581–588.

Antigen 85 (Ag85) complex proteins are major secretory products of *Mycobacterium tuberculosis* and induce strong cellular and humoral immune responses in infected experimental animals and human beings. We have previously shown that nanogram doses of these 30- to 32-kDa fibronectin-binding proteins inhibit local expression of delayed hypersensitivity by a T-cell fibronectin-dependent mechanism. Circulating levels of Ag85 might be expected to be elevated in patients with active tuberculosis and possibly to play a role in systemic anergy in these patients. To test this hypothesis, Ag85 was measured in serum and urine by a monoclonal antibody-based, dot immunobinding assay in 56 patients and controls with known skin test reactivity. Medium serum Ag85 levels were 50- to 150-fold higher in patients with active tuberculosis than in patients with active *M. avium-intracellulare* disease or other nontuberculous pulmonary disease or in healthy controls ($p < 0.001$). The median and range of serum Ag85 in patients with active tuberculosis was not significantly different between skin test-positive and -negative subjects. Patients with active *M.*

avium disease could be distinguished from those with disease due to *M. tuberculosis* by monoclonal anti-Ag85 antibodies of appropriate specificities. No increases in urinary Ag85 were detected in any patient, regardless of the Ag85 level in serum. Chromatographic analysis and immunoprecipitation studies of serum revealed that Ag85 existed in the serum of these patients complexed to either fibronectin or immunoglobulin G (IgG). Uncomplexed circulating Ag85 was demonstrable in serum from fewer than 20% of patients with active tuberculosis. In patients with active tuberculosis, Ag85 is therefore likely to circulate primarily as complexes with plasma fibronectin and IgG rather than in an unbound form. The existence of Ag85 complexes with plasma proteins would account for its lack of urinary clearance.—Authors' Abstract

Chen, L., Boomershine, C., Wang, T. Y., Lafuse, W. P. and Zwilling, B. S. Synergistic interaction of catecholamine hormones and *Mycobacterium avium* results in the induction of interleukin-10 mRNA expression by murine peritoneal macrophages. J. Neuroimmunol. **93** (1999) 149–155.

The results of this investigation provide evidence that catecholamine hormones interact with macrophages that are infected with *Mycobacterium avium,* resulting in the induction of IL-IO mRNA and protein. The effect of catecholamine hormones was prevented by treating the cells with the beta-adrenergic receptor antagonist propranolol but not by alpha-adrenergic antagonist phentolamine. The effect of catecholamine stimulation was mimicked by the addition of beta-2 adrenergic agonists and by the addition of cAMP to the infected macrophage cultures. These observations suggest that the sympathetic nervous system activation together with microbial infection results in a synergistic interaction that could result in the control of inflammatory processes.—Authors' Abstract

Dye, C., Garnett, G. P., Sleeman, A. and Williams, B. G. Prospects for worldwide tuberculosis control under the WHO DOTS strategy. Lancet **352** (1998) 1886–1891.

Background: The WHO advocates the use of directly observed treatment with a short-course drug regimen as part of the DOTS strategy, but the potential effect of this strategy worldwide has not been investigated.

Methods: We developed an age-structured mathematical model to explore the characteristics of tuberculosis control under DOTS, and to forecast the effect of improved case finding and cure on tuberculosis epidemics for each of the six WHO regions.

Findings: In countries where the incidence of tuberculosis is stable and HIV-1 absent, a control program that reaches the WHO targets of 70% case detection and 85% cure would reduce the incidence rate by 11% (range 8–12) per year and the death rate by 12% (9–13) per year. If tuberculosis has been in decline for some years, the same case detection and cure rates would have a smaller effect on incidence. DOTS saves a greater proportion of deaths than cases, and this difference is bigger in the presence of HIV-1. HIV-1 epidemics cause an increase in tuberculosis incidence, but do not substantially reduce the preventable proportion of cases and deaths. Without greater effort to control tuberculosis, the annual incidence of the disease is expected to increase by 41% (21–61) between 1998 and 2020 (from 7.4 million to 10.6 million cases per year). Achievement of WHO targets by 2010 would prevent 23% (15–30) or 48 million cases by 2020.

Interpretation: The potential effect of chemotherapy (delivered as DOTS) on tuberculosis is greater in many developing countries now than it was in developed countries 50 years ago. To exploit this potential, case detection and cure rates urgently need to be improved in the main endemic areas.—Authors' Abstract

Emoto, M., Emoto, Y., Buchwalow, I. B. and Kaufman, S. H. E. Induction of IFN-gamma-producing CD4+ natural killer T cells by *Mycobacterium bovis*

bacillus Calmette Guerin. Eur. J. Immunol. **29** (1999) 650–659.

The CD4+ natural killer (NK) T cells in the liver are potent IL-4 producers and hence may promote Th2 cell development. Following *Mycobacterium bovis* bacillus Calmette Guerin (BCG) infection. IL-4-producing CD4+ NK T cells become undetectable in liver mononuclear cells of normal density (interface between 40% and 70% Percoll) by flow cytometry. The present study shows that *M. bovis* BCG infection changes the density of liver CD4+ NK T cells and shifts cytokine production from IL-4 to interferon-gamma (IFN-γ). The number of CD4+ NK1+ TCR alpha/beta (intermediate) cells increased in the low-density fraction (<40% Percoll density gradient) in parallel to the reduction of this cell population in the fraction of normal density. The number of IL-4-producing cells, however, was small and high frequencies of IFN-γ-secreting cells were identified in the low-density fraction after TCR/CD3 ligation. Accordingly, selected low-density CD4+ NK T cells encompassed high numbers of IFN-γ producers and minute numbers of IL-4-secreting cells. Induction of low-density CD4+ NK T cells by *M. bovis* BCG was abrogated by endogenous IL-12 neutralization which also caused increased bacterial growth in the liver. We assume that *M. bovis* BCG infection changes cytokine secretion by the CD4+ NK T cell population from IL-4 to IFN-γ through IL-12 induction. Thus, CD4+ NK T cells may contribute to host resistance against intracellular bacteria prior to conventional IFN-γ-producing Th1 cells.—Authors' Abstract

Garbe, T. R., Hibler, N. S. and Deretic, V. Response to reactive nitrogen intermediates in *Mycobacterium tuberculosis*: induction of the 16-kilodalton alpha-crystallin homolog by exposure to nitric oxide donors. Infect. Immun. **67** (1999) 460–465.

In contrast to the apparent paucity of *Mycobacterium tuberculosis* response to reactive oxygen intermediates, this organism has evolved a specific response to nitric oxide (NO) challenge). Exposure of *M. tuberculosis* to NO donors induces the synthesis of a set of polypeptides that have been collectively termed Nos. In this work, the most prominent Nos polypeptide, Nox16, was identified by immunoblotting and by N-terminal sequencing as the alpha-crystallin-related, 16-kDa small heat shock protein, sHsp16. A panel of chemically diverse donors of nitric oxide, with the exception of nitroprusside, induced sHsp16 (Nox16). Nitroprusside, a coordination complex of Fe2+ with a nitrosonium (NO+) ion, induced a 19-kDa polypeptide (Nox19) homologous to the nonheme bacterial ferritins. We conclude that the NO response in *M. tuberculosis* is dominated by increased synthesis of the alpha-crystallin homolog sHsp16, previously implicated in stationary-phase processes and found in this study to be a major *M. tuberculosis* protein induced upon exposure to reactive nitrogen intermediates.—Authors' Abstract

George, K. M., Chatterjee, D., Gunawardana, G., Welty, D., Hayman, J., Lee, R. and Small, P. L. C. Mycolactone: a polyketide toxin from *Mycobacterium ulcerans* required for virulence. Science **283** (1999) 854–857.

Mycobacterium ulcerans is the causative agent of Buruli ulcer, a severe human skin disease that occurs primarily in Africa and Australia. Infection with *M. ulcerans* results in persistent severe necrosis without an acute inflammatory response. The presence of histopathological changes distant from the site of infection suggested that pathogenesis might be toxin mediated. A polyketide-derived macrolide designated mycolactone was isolated that causes cytopathicity and cell cycle arrest in cultured L929 murine fibroblasts. Intradermal inoculation of purified toxin into guinea pigs produced a lesion similar to that of Buruli ulcer in humans. This toxin may represent one of a family of virulence factors associated with pathology in mycobacterial diseases such as leprosy and tuberculosis.—Authors' Abstract

Guimaraes Peres, A., Portaels, F., de Rijk, P., Fissette, K., Pattyn, S. R., van

Vooren, J. P. and Fonteyne, P. A. Comparison of two PCRs for detection of *Mycobacterium ulcerans.* J. Clin. Microbiol. **37** (1999) 206–208.

Two nested PCRs for the detection of *Mycobacterium ulcerans* were compared by using a collection of 65 clinical specimens. The first method amplifies the gene coding for 16S rRNA, and the second method amplifies a repetitive DNA sequence. The sensitivities of bacterioscopy, culture, 16S rRNA gene PCR, and repetitive-sequence PCR were 29%, 34%, 80%, and 85%, respectively. Compared to the 16S rRNA gene PCR, the repetitive-sequence PCR was faster, easier to perform, and less expensive.—Authors' Abstract

Guinan, P., Shaw, M., Mirochnik, Y., Slobodskoy, L., Ray, V. and Rubenstein, M. Paclitaxel is more effective than thalidomide in inhibiting LNCaP tumor growth in a prostate cancer model. Methods Find. Exp. Clin. Pharmacol. **20** (1998) 739–742.

LNCaP tumors were treated by the administration of paclitaxel, thalidomide or by orchiectomy in order to determine their relationship with markers pertaining to the process of tumor growth, apoptosis or angiogenesis. Forty mice bearing LNCaP tumors were divided into 4 groups of 10 and treated by paclitaxel (20 mg/kg × 5 days), thalidomide (200 mg/kg × 5 days/week × 5 weeks), or orchiectomy. After 6 weeks, serum samples were removed for PSA determination and the animals sacrificed for evaluation of: a) tumor volume, b) tissue bcl-2, cyclin D, PSA and factor VIII immunohistochemically graded (0–5 scale) for marker expression, and c) serum PSA. Comparisons were made to untreated LNCaP tumors. Statistically significant differences were determined using the nonparametric Mann-Whitney test. Paclitaxel produced significant differences in volume ($p < 0.001$), expression of bcl-2 ($p < 0.043$), cyclin D ($p < 0.023$), tissue PSA ($p < 0.001$) and serum PSA ($p < 0.019$) levels. Thalidomide altered expression of bcl-2 ($p < 0.011$) and tissue PSA ($p < 0.002$). Orchiectomy altered volume ($p < 0.002$) and bcl-2 expression ($p < 0.001$). All three therapies have been suggested for prostate cancer and each produced alterations in accepted markers for treatment response (either reduced volume or serum PSA). Paclitaxel significantly influenced the most markers. Of interest was that all treatments, especially thalidomide, a known anti-angiogenesis agent, reduced factor VIII, although not significantly. Evidently each treatment evokes different pathways of activity.—Authors' Abstract

Hellyer, T. J., Des Jardin, L. E., Hehman, G. L., Cave, M. D. and Eisenach, K. D. Quantitative analysis of mRNA as a marker for viability of *Mycobacterium tuberculosis.* J. Clin. Microbiol. **37** (1999) 290–295.

Numerous assays which use conserved DNA or rRNA sequences as targets for amplification have been described for the diagnosis of tuberculosis. However, these techniques have not been applied successfully to the monitoring of therapeutic efficacy owing to the persistence of amplifiable nucleic acid beyond the point at which smears and cultures become negative. Semiquantitative analysis of rRNA has been used to reduce the time required for antimicrobial susceptibility testing of *Mycobacterium tuberculosis,* although growth for up to 5 days in the presence of some drugs is still required to discriminate resistant strains. The purpose of the present study was to determine whether quantitative analysis of *M. tuberculosis* mRNA could be used to assess bacterial viability and to illustrate the application of this technique to rapid determination of drug susceptibility. Levels of mRNA encoding the 85B protein (alpha-antigen), IS6110 DNA, and 16S rRNA were compared in parallel cultures of *M. tuberculosis* that were treated with either no drug, 0.2 μg of isoniazid per ml, or 1 μg of rifampin per ml. Exposure of sensitive strains to isoniazid or rifampin for 24 hr reduced the levels of 85B mRNA to <4% and <0.01%, respectively, of those present in control cultures without drug. In contrast, the levels of IS6110 DNA and 16S rRNA did not diminish over the same period. Strains which were resistant to either isoniazid or ri-

fampin demonstrated no reduction in 85B mRNA in the presence of the drug to which they were nonresponsive. Quantitative analysis of 85B mRNA offers a potentially useful tool for the rapid determination of *M. tuberculosis* drug susceptibility and for the monitoring of therapeutic efficacy.—Authors' Abstract

Hellyer, T. J., Des Jardin, L. E., Teixeira, L., Perkins, M. D., Cave, M. D. and Eisenach, K. D. Detection of viable *Mycobacterium tuberculosis* by reverse transcriptase-strand displacement amplification of mRNA. J. Clin. Microbiol. **37** (1999) 518–523.

Numerous assays have been described for the detection of DNA and rRNA sequences that are specific for the *Mycobacterium tuberculosis* complex. Although beneficial to initial diagnosis, such assays have proven unsuitable for monitoring therapeutic efficacy owing to the persistence of these nucleic acid targets long after conversion of smears and cultures to negative. However, prokaryotic mRNA has a typical half-life of only a few minutes, and we have previously shown that the presence of mRNA is a good indicator of bacterial viability. The purpose of the present study was to develop a novel reverse transcriptase-strand displacement amplification system for the detection of *M. tuberculosis* alpha-antigen (85B protein) mRANA and to demonstrate the use of this assay in assessing chemotherapeutic efficacy in patients with pulmonary tuberculosis. The assay was applied to sequential, noninduced sputum specimens collected from four patients: 10 of 11 samples (91%) collected prior to the start of therapy were positive for alpha-antigen mRNA, compared with 1 of 8 (13%), 2 of 8 (25%), 2 of 8 (25%), and 0 of 8 collected on days 2, 4, 7, and 11 of treatment, respectively. In contrast, 39 of 44 samples (89%) collected on or before day 14 were positive for alpha-antigen DNA. The loss of detectable mRNA corresponded to a rapid drop over the first 4 days of treatment in the number of viable organisms present in each sputum sample, equivalent to a mean fall of 0.43 $\log^{10}$ CFU/ml/day. Analysis of mRNA is a potentially useful method for monitoring therapeutic efficacy and for rapid *in vitro* determination of drug susceptibility.—Authors' Abstract

Kremer, L., Dupre, L., Riveau, G., Capron, A. and Locht, C. Systemic and mucosal immune responses after intranasal administration of recombinant *Mycobacterium bovis* bacillus Calmette-Guerin expressing glutathione S-transferase from *Schistosome haematobium.* Infect. Immun. **66** (1998) 5669–5676.

A major goal of current vaccine development is the induction of strong immune responses against protective antigens delivered by mucosal routes. One of the most promising approaches in that respect relies on the use of live recombinant vaccine carriers. In this study, *Mycobacterium bovis* BCG was engineered to produce an intracellular glutathione S-transferase from *Schistosoma haematobium* (Sh28GST). The gene encoding Sh28GST was placed under the control of the mycobacterial hsp60 promoter on a replicative shuttle plasmid containing a mercury resistance operon as the only selectable marker. The recombinant Sh28GST produced in BCG bound glutathione and expressed enzymatic activity, indicating that its active site was properly folded. Both intraperitoneal and intranasal immunizations of BALB/c mice with the recombinant BCG resulted in strong anti-Sh28GST antibody responses, which were enhanced by a boost. Mice immunized intranasally produced a mixed response with the production of Sh28GST-specific immunoglobulin G1 (IgG1), IgG2a, IgG2b, and IgA in the serum. In addition, high levels of anti-Sh28GST IgA were also found in the bronchoalveolar lavage fluids, demonstrating that intranasal delivery of the recombinant BCG was able to induce long-lasting secretory and systemic immune responses to antigens expressed intracellularly. Surprisingly, intranasal immunization with the BCG producing the Sh28GST induced a much stronger specific humoral response than intranasal immunization with BCG producing the glutathione S-transferase from *Schistosoma mansoni,* although the two antigens have over 90% identity. This difference was

not observed after intraperitoneal administration.—Authors' Abstract

Long, R., Light, B. and Talbot, J. A. Mycobacteriocidal action of exogenous nitric oxide. Antimicrob. Agents Chemother. **43** (1999) 403–405.

We tested the hypothesis that exposure of extracellular *Mycobacterium tuberculosis* to low concentrations (<100 ppm) of nitric oxide (NO) for short periods (24 hr or less) will result in microbial killing. We observed that NO had both dose- and time-dependent cidal effects that were very significant by two-way analysis of variance (*F* ratios of 13.4 [$p < 0.001$] and 98.1 [$p < 0.0001$], respectively). Conceivably, extracellular bacilli in patients with pulmonary tuberculosis might be vulnerable to exogenous NO.—Authors' Abstract

Manganelli, R., Dubnau, E., Tyagi, S., Kramer, F. R. and Smith, I. Differential expression of 10 sigma factor genes in *Mycobacterium tuberculosis.* Mol. Microbiol. **31** (1999) 715–724.

The ability of *Mycobacterium tuberculosis* to adapt to different environments in the infected host is essential for its pathogenicity. Consequently, this organism must be able to modulate gene expression to respond to the changing conditions it encounters during infection. In this paper we begin a comprehensive study of *M. tuberculosis* gene regulation, characterizing the transcript levels of 10 of its 13 putative sigma factor genes. We developed a real-time RT-PCR assay using a family of novel fluorescent probes called molecular beacons to quantitatively measure the different mRNAs. Three sigma factor genes were identified that have increased mRNA levels after heat shock, two of which also responded to detergent stress. In addition, we also identified a sigma factor gene whose mRNA increased after mild cold shock and a second that responded to conditions of low aeration.—Authors' Abstract

Moore, A. V., Kirk, S. M., Callister, S. M., Mazurek, G. H. and Schell, R. F. Safe determination of susceptibility of *Mycobacterium tuberculosis* to antimycobacterial agents by flow cytometry. J. Clin. Microbiol. **37** (1999) 479–483.

We showed previously that susceptibility testing for *Mycobacterium tuberculosis* labeled with fluorescein diacetate could be accomplished rapidly by using flow cytometry. However, safety was a major concern because mycobacteria were not killed prior to flow cytometric analysis. In this study, we developed a biologically safe flow cytometric susceptibility test that depends on detection and enumeration of actively growing *M. tuberculosis* organisms in drug-free and antimycobacterial agent-containing medium. The susceptibilities of 17 clinical isolates of *M. tuberculosis* to ethambutol, isoniazid, and rifampin were tested by the agar proportion and flow cytometric methods. Subsequently, all flow cytometric susceptibility test samples were inactivated by exposure to paraformaldehyde before analysis with a flow cytometer. Agreement between the results form the two methods was 98%. In addition, the flow cytometric results were available 72 hr after the initiation of testing. The flow cytometric susceptibility assay is safe, simple to perform, and more rapid than conventional test methods, such as the BACTEC system and the proportion method.—Authors' Abstract

Nakamura, R. M., Velmonte, M. A., Kawajiri, K., Ang, C. F., Frias, R. A., Mendoza, M. T., Montoya, J. C., Honda, I., Haga, S. and Toida, I. MPB64 mycobacterial antigen: a new skin-test reagent through patch method for rapid diagnosis of active tuberculosis. Int. J. Tuberc. Lung Dis. **2** (1998) 541–546.

Tuberculosis patients from four clinics in the vicinity of Manila, The Philippines, were examined [date not given] to develop a new, simple and rapid diagnostic method for active tuberculosis. Subjects were tested for skin reaction to a special antigen, MPB64, by the patch test method instead of intradermal injection of purified protein derivative (PPD). Fifty-three active tuberculosis patients and 43 healthy PPD-positive controls were tested to determine whether

or not the reaction to MPB64 was positive only in active tuberculosis patients; 52 of the 53 active tuberculosis patients showed a positive reaction to MPB64, while none of the 43 PPD-positive controls did. The specificity of MPB64 to active tuberculosis was 100%, and the sensitivity was 98.1%. The efficacy of the test was 98.9%. It is concluded that the patch test with MPB64 is a promising method for the diagnosis of active tuberculosis.—Authors' Abstract

Penna, G. O., Pinheiro, A. M. C. and Hajjar, L. A. [Thalidomide: mechanism of action, side effects and therapeutic use.] An. Bras. Dermatol. Rio de Janeiro **73** (1998) 501–504. (in Portuguese)

Thalidomide, an oral drug marketed in the 1950s as a sedative, has been used to treat a variety of diseases. Although initially withdrawn from the world market when its use was associated with teratogenicity, thalidomide has been gradually reintroduced in the treatment of autoimmune and inflammatory disorders. Its action on the immunologic system is pleomorphic, including potent inhibition of tumor necrosis factor-α, decrease in the ratio of CD4 to CD8 and suppression of phagocytosis. Diseases for which thalidomide has been found effective include: erythema nodosum leprosum, lupus erythematosus, graft-versus-host disease, aphtous stomatitis, Behçet's syndrome, actinic prurigo, prurigo nodularis and others. Side effects such as peripheral neuropathy and teratogenicity remain its limiting factor. Acquiring more knowledge about its mechanisms of action, pharmacokinetics and side effects will allow thalidomide to be used appropriately in many conditions.—Authors' English Summary

Ray, M., Kumar, L. and Prasad, R. Plasma zinc status in Indian childhood tuberculosis: impact of antituberculosis therapy. Int. J. Tuberc. Lung Dis. **1** (1998) 719–725.

The plasma zinc status in children from Chandigarh, India, with tuberculosis was measured and correlated with nutritional status, activity and severity of disease in relation to antituberculosis therapy [date not given]. The plasma zinc status of 50 children with different forms of tuberculosis was compared with 10 healthy and 10 malnourished children without tuberculosis at 0, 1, 2, 3 and 6 months of antituberculosis therapy. The mean plasma zinc concentration in children with pulmonary tuberculosis (N = 20) was 68.65 ± 2.50 μg/100 ml, central nervous system (CNS) tuberculosis (N = 10) was 64.20 ± 3.82 μg/100 ml, tuberculous lymphadenitis (N = 10) was 63.2 ± 3.77 μg/100 ml and disseminated tuberculosis (N = 10) was 59.0 ± 2.75 μg/100 ml at 0 months. The mean plasma zinc concentration of healthy children was 129.10 ± 3.01 μg/100 ml and in malnourished nontuberculous children it was 108.40 ± 3.16 μg/100 ml. Thus, children with tuberculosis had significantly lower plasma zinc concentrations than those without tuberculosis, irrespective of their nutritional status ($p < 0.001$). There was a significant increase in zinc concentration at the end of 6 months of antituberculosis therapy ($p < 0.001$). It was concluded that plasma zinc status may prove to be a good objective marker for monitoring the severity of the disease and the response to therapy.—Authors' Abstract

Reist, M., Carrupt, P. A., Francotte, E. and Testa, B. Chiral inversion and hydrolysis of thalidomide: mechanisms and catalysis by bases and serum albumin, and chiral stability of teratogenic metabolites. Chem. Res. Toxicol. **11** (1998) 1521–1528.

The chiral inversion and hydrolysis of thalidomide and the catalysis by bases and human serum albumin were investigated by using a steroeselective HPLC assay. Chiral inversion was catalyzed by albumin, hydroxyl ions, phosphate, and amino acids. Basic amino acids (Arg and Lys) had a superior potency in catalyzing chiral invesion compared to acid and neutral ones. The chiral inversion of thalidomide is thus subject to specific and general base catalysis, and it is suggested that the ability of HSA to catalyze the reaction is due to the basic groups of the amino acids Arg and Lys and not to a single catalytic site on the macromolecule. The hydrolysis of thalidomide was also base-catalyzed. However, albumin had no

effect on hydrolysis, and there was no difference between the catalytic potencies of acidic, neutral, and basic amino acids. This may be explained by different reaction mechanisms of the chiral inversion and hydrolysis of thalidomide. Chiral inversion is deduced to occur by eletrophilic substitution involving specific and general base catalysis; whereas hydrolysis is thought to occur by nucleophilic substitution involving specific and general base as well as nucleophilic catalysis. Since nucleophilic attack is sensitive to steric properties of the catalyst, steric hindrance might be the reason albumin is not able to catalyze hydrolysis. H-1 NMR experiments revealed that the three teratogenic metabolites of thalidomide, in sharp contrast to the drug itself, had complete chiral stability. This leads to the speculation that, were some enantioselectivity to exist in the teratogenicity of thalidomide, it could result from fast hydrolysis to chirally stable teratogenic metabolites.—Authors' Abstract

Sano, C., Shimizu, T., Sato, K., Kawauchi, H., Kawahara, S. and Tomioka, H. Therapeutic effects of benzoxazinorifamycin KRM-1648 administered alone or in combination with a half-sized secretory leukocyte protease inhibitor or the nonsteroidal anti-inflammatory drug diclofenac sodium against *Mycobacterium avium* complex infection in mice. Antimicrob. Agents Chemother. **43** (1999) 360–364.

The effects of half-sized secretory leukocyte protease inhibitor or diclofenac sodium administered alone or in combination with the benzoxazinorifamycin KRM-1648 on the therapeutic efficacy of KRM-1648 against *Mycobacterium avium* complex (MAC) in mice were studied. Neither of the two anti-inflammatory drugs affected the efficacy of KRM-1648, while they exerted significant modulating effects on tumor necrosis factor-alpha production by MAC-infected macrophages.—Authors' Abstract

Stenger, S. and Modlin, R. L. T cell mediated immunity to *Mycobacterium tuberculosis.* Curr. Opin. Microbiol. **2** (1999) 89–93.

Recent advances in the characterization of the protective immune response to mycobacteria have highlighted the central role of phenotypically and functionally distinct subsets of T cells. These T-cell subsets not only contribute to host defense by the secretion of macrophage-activating cytokines, but also by lysing the host cell. Besides releasing intracellular pathogens, which can then be taken up and killed by newly recruited macrophages, it has now been demonstrated that lysis of infected targets by one subset of cytolytic T cells can directly kill *Mycobacterium tuberculosis.*—Authors' Abstract

Sun, Z. and Zhang, Y. Reduced pyrazinamidase activity and the natural resistance to *Mycobacterium kansasii* to the antituberculosis drug pyrazinamide. Antimicrob. Agents Chemother. **43** (1999) 537–542.

Pyrazinamide (PZA), an analog of nicotinamide, is a prodrug that requires conversion to the bactericidal compound pyrazinoic acid (POA) by the bacterial pyrazinamidase (PZase) activity of nicotinamidase to show activity against *Mycobacterium tuberculosis.* Mutations leading to a loss of PZase activity cause PZA resistance in *M. tuberculosis. M. kansasii* is naturally resistant to PZA and has reduced PZase activity along with an apparently detectable nicotinamidase activity. The role of the reduction in PZase activity in the natural PZA resistance of *M. kansasii* is unknown. The MICs of PZA and POA for *M. kansasii* were determined to be 500 and 125 μg/ml, respectively. Using [^{14}C]PZA and [^{14}C]nicotinamide, we found that *M. kansasii* had about 5-fold less PZase activity and about 25-fold less nicotinamidase activity than *M. tuberculosis.* The *M. kansasii pncA* gene was cloned on a 1.8-kb *Bam*HI DNA fragment, using *M. avium pncA* probe. Sequence analysis showed that the *M. kansasii pncA* gene encoded a protein with homology to its counterparts from *M. tuberculosis* (69.9%), *M. avium* (65.6%), and *Escherichia coli* (28.5%). Transformation of naturally PZA-resistant *M. bovis* BCG with *M. kansasii pncA* conferred partial PZA susceptibility. Transformation of *M. kansasii*

with *M. avium pncA* caused functional expression of PZase and high-level susceptibility to PZA, indicating that the natural PZA resistance in *M. kansasii* results from a reduced PZase activity. Like *M. tuberculosis, M. kansasii* accumulated POA in the cells at an acidic pH; however, due to its highly active POA efflux pump, the naturally PZA-resistant species *M. smegmatis* did not. These findings suggest the existence of a weak POA efflux mechanism in *M. kansasii.*—Authors' Abstract

Teitelbaum, R., Glatman Freedman, A., Chen, B., Robbins, J. B., Unanue, E., Casadevall, A. and Bloom, B. R. A mAb recognizing a surface antigen of *Mycobacterium tuberculosis* enhances host survival. Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 15688–15693.

Murine monoclonal antibodies (mAbs) reactive with the surface of *Mycobacterium tuberculosis* were assayed for their ability to affect the course of infection in mice challenged with virulent organisms. An IgG3 mAb (9d8) specific for arabinomannan and reactive with purified antigen from a clinical isolate of *M. tuberculosis* conferred partial protection on mice after respiratory challenge (30%–60% survival >75 days; $p \leq 0.05$). Control mice pretreated with an irrelevant mAb of the same isotype succumbed to tuberculosis within 30 days. Mice with gene disruptions in interferon gamma and major histocompatibility complex Class II also were partially protected from challenge. The protective mAb was neither bactericidal nor inhibitory of infection or bacterial replication. Nevertheless, it profoundly altered the nature of the granulomas in the infected lungs. Mice treated with mAb 9d8 and challenged with *M. tuberculosis* localized the pathogen within granuloma centers, suggesting that the mAb conferred protection by enhancing a cellular immune response.—Authors' Abstract

Teran Escandon, D., Teran Ortiz, L., Camarena Olvera, A., Gonzalez Avila, G., Vaca Marin, M. A., Granados, J. and Selman, M. Human leukocyte antigen-associated susceptibility to pulmonary tuberculosis–molecular analysis of class II alleles by DNA amplification and oligonucleotide hybridization in Mexican patients. Chest **115** (1999) 428–433.

Background: Pulmonary tuberculosis (PTB) develops by a complex combination of environmental factors with genetic susceptibility. In this context, an association between human leukocyte antigens (HLAs) and tuberculosis has been examined in several populations, but results have been controversial.

Design and Measurements: A prospective evaluation of class II HLA genotypes was completed by the polymerase chain reaction (PCR) sequence-specific primer technique and PCR sequence-specific oligonucleotide hybridization in a Mexican population.

Setting: This study was conducted at the Clinical Service of Tuberculosis and the Department of Immunology, National Institute of Respiratory Diseases, Mexico City, Mexico.

Patients: Four groups were examined: 95 healthy subjects; 50 nonimmunosuppressed PTB patients; 15 HIV-infected patients (stage IVc in the Centers for Disease Control and Prevention [CDC] classification system for AIDS) with PTB; and 37 HIV-infected patients in the asymptomatic stage (CDC stage II).

Results: The frequencies of alleles DQA1*0101 (odds ratio [OR] 6.18; 95% confidence interval [CI] 2.38 to 16.08), DQB1*0501 (OR 6.16; 95% CI 2.44 to 17.71), and DRB1*1501 (OR 7.92; 95% CI 2.71 to 23.14) were significantly increased in nonimmunosuppressed patients with PTB when compared with healthy subjects. By contrast, frequencies of allele DQB1*0402 and antigens DR4 and DR8 were significantly decreased in patients with PTB. Additionally, a significantly higher frequency of the DRB1*1101 allele was found in HIV-positive subjects (OR, 6.67; 95% CI 2.13 to 20.83).

Conclusion: The genetic influence associated with the HLA system appears to have an important role in the development of PTB, although this susceptibility may not be relevant in patients with sever immunodeficiency diseases such as AIDS.—Authors' Abstract

Ulrichs, T., Munk, M. E., Mollenkopf, H., Behr Perst, S., Colangeli, R., Gennaro, M. L. and Kaufmann, S. H. E. Differential T cell responses to *Mycobacterium tuberculosis* ESAT6 in tuberculosis patients and healthy donors. Eur. J. Immunol. **28** (1998) 3949–3958.

Vaccination against and diagnosis of tuberculosis are still insufficient. Proteins secreted by *Mycobacterium tuberculosis* induce strong immune responses in tuberculosis and constitute prime candidates for development of novel vaccines against tuberculosis as well as for immunodiagnostic assays. We investigated the role of the secreted proteins MPT63, MPT64 and ESAT6 from *M. tuberculosis* in healthy individuals and tuberculosis patients. None of the secreted proteins stimulated peripheral blood mononuclear cells from healthy donors. In contrast, CD4+ T cells from many tuberculosis patients were stimulated in an MHC class II-restricted fashion by ESAT6, but not by MPT63 or MPT64. T-cell reactivities of tuberculosis patients were focused on the N-terminal region of ESAT6. The ESAT6 T-cell epitopes were presented by different HLA-DR phenotypes. Cell cultures responding to either ESAT6 or synthetic peptides thereof showed mRNA transcripts for macrophage inflammatory protein (MIP)-1 alpha, monocyte chemotactic protein (MCP)-1 or IL-8 and production of IFN-gamma and MIP-1 alpha. Our results suggest that the secreted *M. tuberculosis* proteins MPT63, MPT64 or ESAT6 do not stimulate unprimed T cells, and that ESAT6 may be a potential candidate antigen for detection of clinical disease.—Authors' Abstract

Urquhart, B. L., Cordwell, S. J. and Humphery Smith, I. Comparison of predicted and observed properties of proteins encoded in the genome of *Mycobacterium tuberculosis* H37Rv. Biochem. Biophys. Res. Comm. **253** (1998) 70–79.

Proteome studies complement current molecular approaches through analysis of the actively translated portion of the genome (the "functional proteome"). Two-dimensional gel electrophoresis (2-DGE) utilizing immobilized pH gradients of pH 2.3–5.0 and pH 6.0–11.0, developed with predetermined regions of overlap compatible with commercially available pH 4.0–7.0 gradients, permitted the display of a significant portion of the proteome of *Mycobacterium tuberculosis* H37Rv. A significant portion of the *M. tuberculosis* proteome, in the molecular mass (M-r) window 5 kDa to 200 kDa and with isoelectric point (pI) between pH 2.3 and 11.0, was visualized for the first time. A total of 493 protein spots were effectively resolved, including 126 spots that could not be seen using standard pH 4.0–7.0 gradients. These results were used to compare the physical properties of the observed proteins to the theoretical predictions of the recently completed *M. tuberculosis* H37Rv genome. Most proteins were found in the pI and mass window of pH 4.0–7.0 and 10–100 kDa. Analysis of the predicted proteome revealed a bimodal pI distribution, with substantial numbers of proteins in the pI regions 4.0–7.0 and 9.0–12.0 as has been seen for the majority of completed genomes. Such data may reveal current limitations in experimental extraction and separation of extremely basic, high M, and hydrophobic proteins via 2-DGE. Conversely, 13 acidic proteins were observed with pI less than the lowest value predicted by the genome. In addition, a subset of small proteins (<10 kDa) were observed within the pI region of pH 5.0–8.0 that were not predicted by the complete genomic sequence, reflecting the current inability to distinguish small genes from within DNA sequence. This work represents the foundation for comparing the protein expression patterns of different pathogenic and nonpathogenic *M. tuberculosis* strains. The characterization of *M. tuberculosis* protein expression, further facilitated by the recent completion of the genome sequence, could aid in developing more effective diagnostic or therapeutic reagents.—Authors' Abstract

Verheul, H. M. W., Panigrahy, D., Yuan, J. and DAmato, R. J. Combination oral antiangiogenic therapy with thalidomide

and sulindac inhibits tumour growth in rabbits. Br. J. Cancer **79** (1999) 114–118.

Neovascularization facilitates tumor growth and metastasis formation. In our laboratory, we attempt to identify clinically available oral efficacious drugs for antiangiogenic activity. Here, we report which nonsteroidal antiinflammatory drugs (NSAIDs) can inhibit corneal neovascularization induced by basic fibroblast growth factor (bFGF) or vascular endothelial growth factor (VEGF). This antiangiogenic activity may contribute to the known effects of NSAIDs on gastric ulcers, polyps and tumors. We found that sulindac was one of the most potent antiangiogenic NSAIDs, inhibiting bFGF-induced neovascularization by 50% and VEGF-induced neovascularization by 55%. Previously, we reported that thalidomide inhibited growth factor-induced corneal neovascularization. When we combined sulindac with thalidomide, we found a significantly increased inhibition of bFGF- or VEGF-induced corneal neovascularization (by 63% or 74%, respectively) compared with either agent alone ($p < 0.01$). Because of this strong antiangiogenic effect, we tested the oral combination of thalidomide and sulindac for its ability to inhibit the growth of V2 carcinoma in rabbits. Oral treatment of thalidomide or sulindac alone inhibited tumor growth by 55% and 35%, respectively. When given together, the growth of the V2 carcinoma was inhibited by 75%. Our results indicated that oral antiangiogenic combination therapy with thalidomide and sulindac may be a useful nontoxic treatment for cancer.—Authors' Abstract

Vijaya, S. The genetics of *Mycobacterium tuberculosis.* J. Genet. **77** (1998) 123–128.

Gene manipulation in *Mycobacterium tuberculosis* has been slow in coming of age owing to the inherent difficulties associated with working on this aerosol-transmitted pathogen, in addition to the paucity of molecular tools such as plasmids and transposons. One of the early approaches to overcome these difficulties was the development of phasmids, which combined the properties of phages and plasmids and allowed introduction of recombinant genes into mycobacteria. The lone plasmid pAL5000 of mycobacteria has been exploited to its fullest potential in the construction of a plethora of vectors. Above all, the single most important achievement has been the development of elegant and innovative approaches to overcome the problem of illegitimate recombination which threatened the success of allelic-exchange mutagenesis in the slow-growing pathogenic mycobacterial species. In this review I discuss the current status of conditionally replicating plasmid and transposon vectors and their application in generating targeted mutations in mycobacteria.—Author's Abstract

von Reyn, C. F., Marsh, B. J., Waddell, R., Lein, A. D., Tvaroha, S., Morin, P. and Modlin, J. F. Cellular immune responses to mycobacteria in healthy and human immunodeficiency virus-positive subjects in the United States after a five-dose schedule of *Mycobacterium vaccae* vaccine. Clin. Infect. Dis. **27** (1998) 1517–1520.

The safety and immunogenicity of heat-killed *Mycobacterium vaccae* vaccine were investigated in a pilot study assessing the feasibility of immunization to prevent mycobacterial disease in patients with human immunodeficiency virus (HIV) infection. Fifteen (seven healthy and eight HIV-positive subjects) received five doses of *M. vaccae* vaccine. Lymphocyte proliferation assays (LPAs) were performed using *M. avium* sensitin (MAS) and *M. vaccae* sonicate (MVS). Vaccine was well tolerated in all 15 subjects with minimal induration at the vaccine site. LPAs for 4 of 7 healthy vaccinees were positive for MAS after immunization. Median responses to MAS and MVS that were determined by LPAs were consistently higher for the eight HIV-positive vaccinees than for the seven healthy controls. A five-dose series of *M. vaccae* vaccine is safe for both healthy and HIV-positive subjects and deserves further evaluation as a vaccine to prevent HIV-associated mycobacterial disease.—Authors' Abstract

Weldingh, K. and Andersen, P. Immunological evaluation of novel *Mycobacterium tuberculosis* culture filtrate proteins. FEMS Immunol. Med. Microbiol. **23** (1999) 159–164.

Culture filtrate from *Mycobacterium tuberculosis* contains molecules which can promote protective immunity to tuberculosis in animal models. Six novel proteins in the region of 17–29 kDa were purified and investigated for their immunological relevance in *M. tuberculosis*-infected mice, guinea pigs and tuberculosis patients. The proteins CFP17, CFP21, CFP25 and CFP29 were all identified as strong interferon-gamma inducers in *M. tuberculosis*-infected mice and in tuberculosis patients. The CFP21 protein is encoded in the genomic legion RD-2 which is deleted from a number of BCG strains, and the diagnostic potential of this antigen was evaluated.—Authors' Abstract

Wilkinson, R. J., Zhu, X., Wilkinson, K. A., Lalvani, A., Ivanyi, J., Pasvol, G. and Vordermeier, H. M. 38,000 MW antigen-specific major histocompatibility complex class I restricted interferon-gamma-secreting CD8+ T cells in healthy contacts of tuberculosis. Immunology **95** (1998) 585–590.

CD+ T lymphocytes are required to protect mice against *Mycobacterium tuberculosis,* although in early infection the mechanism appears not to be via perforin or granzyme-mediated lysis of the infected target, and may be via interferon-gamma (IFN-γ) production. We therefore investigated whether CD8+ T cells specific for the immunoprotective 38,000 MW antigen of *M. tuberculosis* could be detected in infected humans. Using a recombinant vaccinia virus expressing the 38,000 MW antigen of *M. tuberculosis* (rV38) and a control vaccinia virus (rVras), we demonstrated that both viruses stimulated IFN-γ production from freshly isolated peripheral blood mononuclear cells (PBMC) in a 36-hr enzyme-linked immunospot assay. Cell depletion and antibody blockade established that the bulk of the 38,000 MW antigen-specific IFN-γ response was mediated by CD8+, major histocompatibility complex class I-restricted T cells; whereas the anti-vaccinia virus response was predominantly mediated by CD4+ T cells. In further evaluations PBMC from all seven healthy tuberculosis-exposed contacts had a 38,000 MW antigen-specific IFN-γ response; whereas seven patients with untreated sputum-positive pulmonary tuberculosis had very low levels of 38,000 antigen-specific IFN-γ-producing cells. These preliminary observations demonstrate the utility of recombinant vaccinia viruses in restimulating freshly isolated CD4+ and CD8+ T cells. The bias toward a higher frequency of IFN-γ-producing CD8+ T cells in contacts rather than patients may indicate a protective role for CD8+ cells in human tuberculosis.—Authors' Abstract

Zhoa, B. Y., Pine, R., Domagala, J. and Drlica, K. Fluoroquinolone action against clinical isolates of *Mycobacterium tuberculosis*: effects of a C-8 methoxyl group on survival in liquid media and in human macrophages. Antimicrob. Agents Chemother. **43** (1999) 661–666.

When the lethal action of a C-8 methoxyl fluoroquinolone against clinical isolates of *Mycobacterium tuberculosis* in liquid medium was measured, the compound was found to be three to four times more effective (as determined by measuring the 90% lethal dose) than a C-8-H control fluoroquinolone or ciprofloxacin against cells having a wild-type *gyrA* (gyrase) gene. Against ciprofloxacin-resistant strains, the C-8 methoxyl group enhanced lethality when alanine was replaced by valine at position 90 of the GyrA protein or when aspartic acid 94 was replaced by glycine, histidine, or tyrosine. During infection of a human macrophage model by wild-type *M. bovis* BCG, the C-8 methoxyl group lowered survival 20- to 100-fold compared with the same concentration of a C-8-H fluoroquinolone. the C-8 methoxyl fluoroquinolone was also more effective than ciprofloxacin against a *gyrA* Asn94 mutant of *M. bovis* BCG. In an *M. tuberculosis*-macrophage system the C-8 methoxyl

group improved fluoroquinolone action against both quinolone-susceptible and quinolone-resistant clinical isolates. Thus, a C-8 methoxyl group enhances the bactericidal activity of quinolones with N1-cyclopropyl substitutions; these data encourage further refinement of fluoroquinolones as antituberculosis agents.—Authors' Abstract