

CURRENT LITERATURE

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

General and Historical

Rafi, A., Spigelman, M., Stanford, J., Lemma, E., Donoghue, H. and Zias, J. *Mycobacterium leprae* DNA from ancient bone detected by PCR. (Letter) *Lancet* **343** (1994) 1360–1361.

We have extracted and amplified ancient DNA of *Mycobacterium leprae* from a bone dating from 600 A.D. The specimens were prepared with techniques developed by Spigelman and Lemma, who found ancient DNA in tuberculosis (TB)-infected bones from Europe, Turkey, and pre-European-contact Borneo. Salo and colleagues have used identical primers, but on lung tissue from a 1000-year-old Peruvian mummy, and isolated *M. tuberculosis*, which (like *M. leprae*) leaves morphologically identifiable lesions, and has a thick protective wall protecting the DNA after death. A ^{14}C date for our site, based on wood found in the grave, is 600 A.D. \pm 50 years. The grave is in the grounds of the Monastery of St. John the Baptist, on the river Jordan, the site of a massacre of Christians by Persians in 614 A.D. It is the spot where it is believed that John baptized Jesus, which is also the site for the ceremony of the washing of the leper in Christian sources.—From the Letter

Yoder, L. J. Epidemiology and treatment of leprosy. *Curr. Opin. Infect. Dis.* **6** (1993) 349–354.

In 1991 the World Health Organization established a goal to eliminate leprosy (Hansen's disease) as a public health problem by the year 2000. Multidrug short-term chemotherapy in the past decade has reduced the number of registered cases significantly, but in many countries new case rates have changed very little. Current research efforts are directed toward introducing new bactericidal drugs, shorter treatment regimens, vaccine trials, and new diagnostic techniques such as polymerase chain reaction. However, the stigma experienced by leprosy patients is still widespread and remains an obstacle to the ultimate elimination of this disease. Whether the goal proposed by the World Health Organization is achievable remains to be seen, but with the efforts presently underway there is at least some basis for cautious optimism.—Author's Abstract

Chemotherapy

Chen, D. [Effect of MDT on 1139 cases of leprosy.] *China Lepr. J.* **10** (1994) 4–5. (in Chinese)

After 6 months of MDT, among 223 new PB leprosy cases 42 were cured and 142 improved, and out of 240 PB cases who had been treated with dapsone (DDS) or DDS plus rifampin 98 were cured and 131 improved. After 1 year, among both the new and the treated PB cases the cured and im-

proved ones were 116 and 102, respectively, and 7 out of 21 relapsed cases after DDS monotherapy were cured. After 2 years, 39 out of 181 new MB cases, 142 out of 319 DDS-treated MB cases and 35 out of 155 relapsed after DDS monotherapy were cured, and improved ones were 127, 148 and 94, respectively. Therefore, three suggestions are put forward: 1) The course of MDT for PB cases should be 1 year. 2) MB cases should be treated until skin smears

become negative if there is no significant improvement after completion of 2-year MDT. 3) All of the relapsed after DDS monotherapy should be treated with MB regimen of MDT.—Author's English Abstract

Damato, R. J., Loughnan, M. S., Flynn, E. and Folkman, J. Thalidomide is an inhibitor of angiogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **91** (1994) 4082–4085.

Thalidomide is a potent teratogen causing dysmelia (stunted limb growth) in humans. We have demonstrated that orally administered thalidomide is an inhibitor of angiogenesis induced by basic fibroblast growth factor in a rabbit cornea micropocket assay. Experiments including the analysis of thalidomide analogs revealed that the antiangiogenic activity correlated with the teratogenicity but not with the sedative or the mild immunosuppressive properties of thalidomide. Electron-microscopic examination of the corneal neovascularization of thalidomide-treated rabbits revealed specific ultrastructural changes similar to those seen in the deformed limb bud vasculature of thalidomide-treated embryos. These experiments shed light on the mechanism of thalidomide's teratogenicity and hold promise for the potential use of thalidomide as an orally administered drug for the treatment of many diverse diseases dependent on angiogenesis.—Authors' Abstract

Domagala, J. M. Structure-analysis and structure-side-effect relationships for the quinolone antibacterials. *J. Antimicrob. Chemother.* **33** (1994) 685–706.

The fluoroquinolones represent a major class of antibacterials with great therapeutic potential. Over the years, several structure-activity and side-effect relationships have been developed, covering thousands of analogs, in an effort to improve overall antimicrobial efficacy while reducing undesirable side effects. In this review, the various structural features of the quinolones which govern antibacterial efficacy and influence the side-effect profile are delineated and summarized at the molecular level. Those features which most remarkably enhance antimicrobial effectiveness are: a halogen (F

or Cl) at the 8-position which improves oral absorption and activity against anaerobes; an alkylated pyrrolidine or piperazine at C₇ which increases serum half-life and potency vs Gram-positive bacteria; and a cyclopropyl group at N₁ and an amino substituent at C₅, both of which improve overall potency. Some side effects of the quinolones are class effects, and cannot be modulated by molecular variation. These include gastrointestinal irritation and arthropathy. Several other potential side effects are directly influenced by structural modification. For example, CNS effects and drug interactions with theophylline and NSAIDs are strongly influenced by the C₇ substituent with simple pyrrolidines and piperazines the worst actors. Increasing steric bulk through alkylation ameliorates these effects. Phototoxicity is determined by the nature of the 8-position substituent with halogen causing the greatest photo reaction while hydrogen and methoxy show little light induced toxicity. Genetic toxicity is controlled in additive fashion by the choice of groups at the 1, 7 and 8 positions. From the analysis, those groups which mutually improve efficacy while reducing side effects are identified. In addition, preclinical models for determining potential side effects are discussed.—Author's Abstract

Franzblau, S. G., Chan, G. P., Garcia-Ignacio, B. G., Chavez, V. E., Livelo, J. B., Jimenez, C. L., Parrilla, M. L. R., Calvo, R. F., Williams, D. L. and Gillis, T. P. Clinical trial of fusidic acid for lepromatous leprosy. *Antimicrob. Agents Chemother.* **38** (1994) 1651–1654.

Fusidic acid was assessed for antileprosy activity in nine lepromatous leprosy patients. Patients received fusidic acid at either 500 mg/day for 12 weeks or 750 mg/day for 4 weeks followed by 500 mg/day for 8 weeks. All patients showed time-dependent clinical improvement and decreases in bacillary morphological index, radiorespirometric activity and PCR signal, and in serum phenolic glycolipid-I. Fusidic acid appears to be a weakly bactericidal antileprosy agent which may have a role in the multidrug treatment of leprosy pending an evaluation of lepra-reaction-suppressive activity.—Authors' Abstract

Irshaid, Y., Adedoyin, A., Lotze, M. and Branch, R. A. Monoacetyldapsone inhibition of dapsone N-hydroxylation by human and rat liver microsomes. *Drug Metabol. Dispos.* **22** (1994) 161–164.

Dapsone (DDS) is metabolized by N-hydroxylation and N-acetylation to DDS hydroxylamine (DDS-NOH) and monoacetyldapsone (MAD), respectively. The activities of these two alternative and independent reactions vary widely between individuals and show an inverse relationship during chronic DDS therapy. Toxicity observed during DDS therapy has been attributed to DDS NOH. The observation of reduced toxicity in rapid acetylators, who are also poor hydroxylators, therefore raised the possibility that MAD may be inhibiting DDS-NOH formation. This hypothesis was tested in human and rat liver microsomes. Human liver microsomes hydroxylated DDS with a lower affinity (K_m 2-fold greater) and lower maximal catalytic activity (V_{max} 12-fold lower) than that of the rat. The relative catalytic activity (V_{max}/K_m) was 22-fold higher in rat compared with human liver microsomes. Furthermore, MAD was a potent inhibitor of DDS N-hydroxylation by rat liver microsomes (52% inhibition at 0.01 mM MAD) compared with human liver microsomes (23% inhibition at 0.4 mM MAD). Human, but not rat, liver microsomes deacetylated MAD to DDS by an NADPH-independent mechanism. These results show that substantial differences exist in DDS N-hydroxylase between rats and humans, with respect to substrate affinity, enzyme activity, and susceptibility to inhibition, such that information obtained from the rat should not be extrapolated to humans. We conclude that MAD is a potent inhibitor of DDS-NOH formation in rat liver microsomes. The degree of inhibition in human microsomes, however, suggests that MAD is unlikely to be a significant modulator of enzyme activity *in vivo*. — Authors' Abstract

Janier, M., Guillevin, L. and Badillet, G. Pulmonary eosinophilia associated with dapsone. (Letter) *Lancet* **343** (1994) 860–861.

Pulmonary eosinophilia (Loëffler's syndrome) can be caused by drugs such as sul-

fonamides, penicillin, nitrofurantoin, hydralazine, or chlorpropamide and may be life-threatening. We report a case of pulmonary eosinophilia associated with dapsone.

This case documents pulmonary eosinophilia that was likely to be induced by dapsone. Dapsone is in widespread use in clinical situations, such as leprosy, malaria, AIDS, and in various dermatological conditions. The adverse reactions caused by dapsone include hemolysis, methemoglobinemia, white blood cell toxicity, and central nervous system toxicity but this drug has not been implicated in Loëffler's syndrome previously, to our knowledge. Four previous reports of pulmonary eosinophilia caused by maloprim (a combination of dapsone and pyrimethamine) favored the role of pyrimethamine. In our view, dapsone should be added to the list of drugs that induce this potentially life-threatening side effect. Blood eosinophils should be carefully monitored in all patients treated with dapsone. — From the Letter

Klug, S., Felies, A., Sturje, H., Nogueira, A. C., Neubert, R. and Frankus, E. Embryotoxic effects of thalidomide derivatives in the nonhuman primate *Callithrix jacchus*. 5. Lack of teratogenic effects of phthalimidophthalimide. *Arch. Toxicol.* **68** (1994) 203–205.

The teratogenic potency of the thalidomide (Thd) derivative phthalimidophthalimide (Phtpht) was assessed in the common marmoset (*Callithrix jacchus*), by oral administration of the relatively high daily dose of 50 mg Phtpht/kg body wt, during the susceptible period (days 48–61 of pregnancy). Since in this species daily doses of only 100 μ g/kg body wt of the Thd derivative EM12 already induce typical gross structural abnormalities in nearly 100% of the fetuses, investigations with a small number of these New World monkeys allow a rough estimation of the teratogenic potency of Thd-type substances. Macroscopic inspection and skeletal evaluation of ten fetuses gave no indication of dysmorphogenesis following treatment with Phtpht. We conclude that Phtpht has little, if any, Thd-type teratogenic potency in this nonhuman primate. — Authors' Abstract

Knoche, B. and Blaschke, G. Investigations on the *in vitro* racemization of thalidomide by high-performance liquid chromatography. *J. Chromatogr. A* **666** (1994) 235–240.

The racemization of thalidomide enantiomers was examined in various aqueous media. The determination of the enantiomeric ratio was carried out by high-performance liquid chromatography (HPLC) on a poly[(S)-N-(1-cyclohexylethyl)methacrylamide] stationary phase. A significant increase in the rate of thalidomide racemization was observed in human citric plasma when compared with incubations in buffer. Further investigations using physiological concentrations of human serum albumin (HSA) in phosphate buffer (0.067 M, pH 7.4) demonstrated the influence of albumin on racemization. This observation was confirmed by inhibition experiments with capric acid anions.—Authors' Abstract

Krishnan, T. R. and Abraham, I. Comparative bioavailability of clofazimine coevaporate in the pig. *Biopharm. Drug Dispos.* **15** (1994) 329–339.

The systemic availability of a solid dispersion (coevaporate) of clofazimine (CLF) in poly(vinyl methyl ether maleic anhydride) copolymer (PVM/MA) was tested in the pig. Single 100-mg oral doses of the coevaporate and the commercial product, Lamprene® were administered on separate occasions (separated by a 2-week washout period) to 4 pigs (2 males, 2 females) in a random crossover study. Multiple plasma samples, obtained from an indwelling jugular-vein cannula, following drug administration, were analyzed by an HPLC method for CLF. Pharmacokinetic analyses of the plasma CLF concentration-time data were performed. A paired t-test indicated significant differences ($p < 0.05$) between the coevaporate and Lamprene® in the C_{pmax}, t_(max), and AUC. The calculated relative systemic bioavailability (F_{rel}) of CLF from the coevaporate, relative to that from Lamprene®, was three. It is concluded that formulation of CLF, as a solid dispersion, may provide enhanced aqueous dissolution and systemic absorption and may also provide high therapeutic blood levels. These could lead to reduction in the current

therapeutic doses and, consequently, minimization of drug-related side effects.—Authors' Abstract

Leprosy Unit, Division of Control of Tropical Diseases, WHO. Risk of relapse in leprosy. WHO/CTD/LEP/94.1.

Until the introduction by WHO of the standard regimens using multidrug therapy (MDT) for the treatment of leprosy, there was a general unwillingness to release patients from treatment. This was mainly due to the high risk of relapse after dapsone monotherapy. After almost a decade of MDT implementation and after releasing more than 4 million patients, it was necessary for WHO to review the risk of relapse following WHO-recommended MDT. The results of this study, carried out on more than 20,000 MB and 50,000 PB patients, revealed that the risk of relapse is very low, 0.77% for MB and 1.07% for PB, 9 years after stopping MDT. In comparison to dapsone monotherapy, the risk is 10-times lower. Thus, over the last decade, MDT implementation has probably prevented close to half-a-million relapses.—Summary

May, D. G., Porter, J., Wilkinson, G. R. and Branch, R. A. Frequency distribution of dapsone N-hydroxylase, a putative probe for P4503A4 activity, in a white population. *Clin. Pharmacol. Ther.* **5** (1994) 492–500.

Phenotypic trait values in 166 healthy white subjects (age range 18 to 88 years) were determined for dapsone N-hydroxylation, dapsone N-acetylation, debrisoquin 4-hydroxylation, and S-mephenytoin 4'-hydroxylation after single oral dose administration of the probe drugs dapsone (100 mg), debrisoquin (10 mg), and mephenytoin (100 mg). No associations or evidence of cosegregation were found between the individual routes of metabolism. Dapsone N-hydroxylation exhibited a unimodal distribution, with marked (tenfold) intersubject variability, and aging was associated with reduced N-oxidation. However, the other measured routes of metabolism were age independent, but intersubject variability in all of the trait measurements increased with age. In subjects younger than 50 years, S-mephenytoin 4'-hydroxylation was mod-

estly (34%) less in men than in women. In contrast, dapsone N-acetylation, dapsone N-hydroxylation, and debrisoquin 4-hydroxylation were not influenced by gender. Previous smoking habit and alcohol consumption were not associated with a difference in any of the four routes of metabolism. Accordingly, the measured phenotypic traits of drug oxidation and N-acetylation appear to be quite robust in regard to some common demographic variabilities present in population studies, with the exception of dapsone N-hydroxylase, which is affected by aging.—Authors' Abstract

O'Reilly, J. R., Corrigan, O. I. and O'Driscoll, C. M. The effect of simple micellar systems on the solubility and intestinal absorption of clofazimine (B663) in the anaesthetised rat. *Int. J. Pharm.* **105** (1994) 137–146.

Clofazimine (B663) is a highly lipophilic drug used in the treatment of leprosy. The solubility and gastrointestinal membrane permeability (P_{app}) of B663 in buffer and in micellar solutions were examined. Membrane permeability was determined using a rat gut perfusion model and, in addition, these studies incorporated the hydrophilic marker PEG 4000. The micellar systems included the bile salts; sodium deoxycholate, sodium cholate and sodium taurocholate, and the synthetic surfactants; sodium dodecyl sulphate (anionic) and cremophor EL (non-ionic). The low P_{app} of B663 in buffer ($0.98 \times 10^{-5} \text{ cm s}^{-1}$) was ascribed to a combination of the low flow rate used, and the high degree of ionization of B663 at pH 7.2 which resulted in the production of an impermeable ion. Limited absorption of PEG 4000, in buffer, was also observed. All micellar systems investigated enhanced the solubility of B663. Maximum solubility (> 350-fold) was observed in the non-ionic surfactant. The P_{app} of B663 was unaffected by low concentrations of bile salts. However, at the higher concentration of sodium cholate (80 mM) an increase was observed. The P_{app} of PEG 4000 increased, with increasing bile salt concentration. Both synthetic surfactants enhanced the P_{app} of PEG 4000. In contrast, B663 was enhanced only by the non-ionic surfactant.

These results have shown that micellar systems can enhance the absorption of B663 from saturated solutions. Solubilization did not inhibit absorption, rather the increase in solubility was reflected by increases in absorption rates.—Authors' Abstract

Pattyn, S. R., Ghys, P., Janssens, L., Tshilumba, K., Kuykens, L., Karibushi, N. and Denis, P. A randomized clinical trial of two single-dose treatments for paucibacillary leprosy. *Lepr. Rev.* **65** (1994) 45–57.

We compared two single-dose regimens for the treatment of paucibacillary leprosy in a randomized clinical trial in Zaïre. The regimens were: C2 (rifampin 40 mg/kg and 1200 mg clofazimine once) and C4 (rifampin 40 mg/kg, clofazimine 100 mg, DDS 100 mg and ethionamide 500 mg once). An analysis of the results of patients enrolled between May 1987 and December 1988, with a maximum follow up of 4 years, is presented. A total of 622 patients were enrolled and 14 paucibacillary and 1 multibacillary relapses occurred. The overall paucibacillary relapse rate was 2.4 per 100 person years. This relapse rate was higher for older patients as well as for patients with three or more lesions. The probability of cure at 3 years is 0.816 for C2 and 0.823 for C4, the difference not being statistically significant. The probability of cure at 3 years with either regimen is higher for patients with 1 or 2 lesions (0.872) than for patients with 3 or more lesions (0.787), and it is higher for patients with a bacterial index of 0 (0.831) than for patients with a bacterial index of 1 (0.699). These results are compared to other studies. We also discuss the potential of single-dose treatment regimens for paucibacillary leprosy.—Authors' Summary

Randhawa, B., Harris, E. B., Prabhakaran, K. and Hastings, R. C. Bactericidal action of ampicillin/sulbactam against *Mycobacterium leprae*. *J. Antimicrob. Chemother.* **33** (1994) 1035–1037.

Mycobacteria, in general, are resistant to β -lactam antibiotics because of their ability to synthesize β -lactamase which degrades penicillins and cephalosporins. *Mycobac-*

terium tuberculosis produces a constitutive β -lactamase. We demonstrated de-repression of the enzyme in *M. leprae*. In a later study, a β -lactam/ β -lactamase-inhibitor combination (ampicillin/sulbactam) was shown to suppress growth of *M. leprae* in mouse foot pads, including strains of bacteria resistant to dapsone or rifampin. The study used the "continuous method" of drug administration; this does not differentiate between bacteriostatic and bactericidal drugs. Sulbactam, a potent inhibitor of the bacterial β -lactamase, by itself had no antibacterial activity. In the work reported here, we adopted the "kinetic method" of drug administration. The results demonstrate that ampicillin/sulbactam is an effective bactericidal agent against *M. leprae*.—From the Introduction

van Brakel, W. H. and Drever, W. Side-effects of Isoprodian compared with WHO-MDT in rural Nepal. (Letter) *Lepr. Rev.* **64** (1993) 276–280.

Many leprosy patients in Nepal live too far from a treatment unit to be able to attend for regular multidrug therapy. The correspondents treated 43 patients (who had either been previously treated with dapsone monotherapy or were previously untreated) with Isoprodian tablets (containing dapsone, prothionamide and isoniazid), alone for paucibacillary cases and together with clofazimine and supervised rifampin for multibacillary cases. After an average time of 3½ years, 14 (32.5%) of these patients were lost to follow up (as also were 20.7% of 63 patients on multidrug therapy) and 5 patients (11.6) had had to change regimens because of severe gastrointestinal disturbances. The relative risk of developing severe gastrointestinal disturbances when taking Isoprodian compared with WHO multidrug therapy was 7.33. The corre-

spondents conclude that Isoprodian should be prescribed only to patients who can be properly monitored. Patients who cannot frequently attend the clinic in Nepal will be treated with unsupervised multidrug therapy in blister calendar packs rather than with Isoprodian.—C. A. Brown (*Trop. Dis. Bull.*)

van Rensburg, C. E. J., De Bruyn, E. E. and Anderson, R. The anti-proliferative riminophenazine agents clofazimine and B669 promote lysophospholipid-mediated inhibition of Na⁺, K⁺-adenosine triphosphatase activity in cancer cell lines *in vitro*. *Int. J. Oncol.* **4** (1994) 1115–1119.

The effects of the anti-proliferative, phospholipase A (2) (PLA(2))-activating riminophenazine agents, clofazimine and B669, on the Na⁺, K⁺-adenosine triphosphatase activity of the FaDu human pharynx squamous carcinoma cell line have been investigated *in vitro*. At concentrations of 1.25–10 μ g/ml both agents caused dose-related enhancement of PLA(2), as measured by increased release of lysophosphatidylcholine (LPC), and inhibition of Na⁺, K⁺-ATPase in intact cells and isolated membrane preparations. The inhibitory effects of both riminophenazines on the Na⁺, K⁺-ATPase activity of FaDu cells were mimicked by reagent LPC and prevented by treatment of the cells with the lysophospholipid-neutralizing agents alphasatocopherol and lysophospholipase. Riminophenazine-mediated inhibition of Na⁺, K⁺-ATPase activity was also observed with the HeLa (human cervix epitheloid carcinoma) and T24 (human transitional cell bladder carcinoma) cell lines. The anti-proliferative activity of clofazimine and B669 is therefore probably achieved by lysophospholipid-mediated inactivation of Na⁺, K⁺-ATPase.—Authors' Abstract

Clinical Sciences

Dana, M. R., Hochman, M. A., Viana, M. A. G., Hill, C. H. and Sugar, J. Ocular manifestations of leprosy in a noninstitutionalized community in the United States. *Arch. Ophthalmol.* **112** (1994) 626–629.

Our goal was to delineate the epidemiologic and clinical patterns of ocular leprosy in an outpatient setting in the United States. Examinations were performed in 61 consecutive outpatients seen in a Midwestern leprosy clinic. Forty-three male and 18 fe-

male patients were examined. The patients' origins included Southeast Asia (24 patients [39%]), Latin America (23 patients [38%]), India (9 patients [15%]), Europe or North America (2 patients [3%]), Africa (2 patients [3%]), and the Middle East (1 patient [2%]).

Thirty-nine percent of patients were classified as having polar lepromatous leprosy; 18%, borderline lepromatous leprosy; 3%, borderline borderline leprosy; 36%, borderline tuberculoid leprosy; 2%, polar tuberculoid leprosy; and 2% indeterminate leprosy. Ninety-six percent of patients had a best-corrected visual acuity of 20/40 or better. Ocular findings included madarosis (28 patients [46%]), subconjunctival fibrosis (18 patients [30%]), punctate epithelial keratopathy (17 patients [28%]), posterior subcapsular cataract (10 patients [16%]), corneal hypesthesia (10 patients [16%]), lagophthalmos (7 patients [11%]), corneal pannus (6 patients [10%]), entropion (5 patients [8%]), prominent or beaded corneal nerves (4 patients [7%]), iridocyclitis (4 patients [7%]), focal avascular keratitis (3 patients [5%]), scleritis (3 patients [5%]), interstitial keratitis (2 patients [3%]), iris pearls (2 patients [3%]), and ocular clofazimine crystals (2 patients [3%]). Madarosis, corneal hypesthesia, and posterior subcapsular cataracts were significantly associated with disease duration ($p < 0.05$).

We report herein a relatively low frequency of visual impairment attributable to leprosy in our series compared with that seen among institutionalized leprosy patients. However, since 48% of subjects had one or more sight-threatening complications as a result of their disease, a program of regular ophthalmic follow up is strongly advocated for all patients with leprosy.—Authors' Abstract

Denis, F., Aussel, L., Ranger, S., Martin, P., Itouangaporo, A., Frommel, D., Tecle-Haimanot, R. T., Sangare, A., Mboup, S., Millan, J. and Al-Qubati, Y. Prevalence of antibodies to hepatitis C virus among patients with leprosy in several African countries and the Yemen. *J. Med. Virol.* **43** (1994) 1–4.

The prevalence of anti-HCV was determined in 1309 leprosy patients and a control group of 1469 subjects from 6 sub-Sa-

haran African countries and the Yemen. Sera found positive by an initial second generation ELISA were subjected to 3 additional confirmatory tests. The anti-HCV prevalence in leprosy patients (7.1%) was significantly higher than in the control group (2.6%). HCV seroprevalence increased with age in both the control and leprosy groups. No statistically significant difference could be found between anti-HCV prevalence and the several clinical forms of leprosy among patients. The results of this study indicate a high degree of exposure or chronic carriage of hepatitis C among leprosy patients.—Authors' Abstract

Fiallo, P., Nunzi, E., Bishighini, G. and Vaccari, G. Leprosy in an Italian tourist visiting the tropics. *Trans. R. Soc. Trop. Med. Hyg.* **87** (1993) 675.

The authors report a case of leprosy acquired by a 38-year-old Italian tourist during short vacations to the tropics. It is believed he acquired the paucibacillary disease while in India or Cuba. The case is considered unusual because the patient had apparently no history of "contact." The authors conclude that "leprosy must be considered as a communicable disease that can be transmitted even by casual contact in the right set of circumstances."—S. Verall (*Trop. Dis. Bull.*)

Goodless, D. R., Vician, A. L., Pardo, R. J. and Ruiz, P. Borderline tuberculoid Hansen's disease in AIDS. *J. Am. Acad. Dermatol.* **30** Suppl. Part 2 (1994) 866–869.

We performed an immunohistochemical analysis of a skin lesion from a patient with AIDS who had borderline tuberculoid Hansen's disease. We also evaluated other laboratory features and performed peripheral blood flow cytometric analysis. The *in situ* immunologic response to *Mycobacterium leprae* was minimally affected by concomitant infection and immunosuppression by HIV. The skin demonstrated the typical characteristics of borderline tuberculoid lesions. These results indicate that although a patient with HIV infection may have laboratory evidence typical of the immunosuppression seen in AIDS, the immunologic

response to *M. leprae* is essentially unchanged.—Authors' Abstract

Mishra, B., Mukherjee, A., Girdhar, A., Husain, S., Malaviya, G. N. and Girdhar, B. K. Evolution of early lesions in leprosy. *Lepr. Rev.* **64** (1993) 259–266.

[The authors] observed 29 patients presenting [in India] with vague peripheral neurological symptoms for 6 months or more. During this period, 16 developed clinical leprosy, 3 developed borderline tuberculoid leprosy, and the other 13 developed neuritic leprosy. Of these 13 cases, 11 subsequently developed skin lesions similar to those seen in indeterminate and in borderline tuberculoid leprosy. Based on the above observations, an attempt has been made to explain the evolution of early lesions of leprosy.—Authors' Summary

Nouira, R., Zili, J., Denguezli, M., Belajouza, C. and Jomaa, B. [Plantar ulcer occurring in a 15-year-old tuberculoid leprosy patient.] *Acta Leprol.* **9** (1994) 31–34. (in French)

Perforating ulcers of the foot occurring in leprosy are frequent, chronic, often giving mutilations. They usually affect the adult. Plantar ulcer occurred in a 15-year-old young patient affected by tuberculoid leprosy has incited us to report it.—Authors' English Summary

Premkumar, R., Sathis Kumar, K. and Dave, S. L. Understanding the attitude of multidisciplinary teams working in leprosy. *Lepr. Rev.* **65** (1994) 66–77.

This study investigated the attitude of health personnel who were working for the National Leprosy Eradication Programme (NLEP) in India to their leprosy patients. These personnel were studied individually and as homogeneous groups so that comparisons were possible within and among the groups, and between the groups in different regions who were conducting similar health programs with a difference in length of between 1 and 5 years.

The sample population was the NLEP employees of two state governments, consisting of eight health professional groups. A questionnaire was developed for each of

these groups to elicit information on five aspects of the relationships with their patients.

The main outcome of the study was that two thirds of the personnel tested possessed the "minimum desirable" interaction with their patients. The quality of their relationships differed only among work specialities, but was consistent within the same speciality in different regions; this pattern was unchanged after 5 years of a multidrug (MDT) program. A further analysis showed that although they possessed a caring attitude toward patients from low socioeconomic classes, a domineering attitude toward these same patients was also prevalent. Analysis according to speciality revealed that laboratory technicians had the highest "desirable attitude" (74.67%) and health educators had the lowest (57.5%), while the rest of the team members fell in between. The stigma shown toward leprosy was higher among doctors when compared to the rest of the team members.

Discussion is based on the performance, overall and in each of its five facets, of each of the professional groups with reference to their job descriptions and with similar studies undertaken earlier.—Authors' Summary

Wu, X., et al. [Impact of leprosy on the patients' marriage.] *China Lepr. J.* **10** (1994) 17–19. (in Chinese)

Influence of leprosy on the patient's marriage in 542 adult cases of leprosy in Sichuan Province has been studied with method of one-to-one pairing as control. The result showed that 107 out of 198 patients unmarried when their leprosy was detected have married, accounting for 54%, while the marriage rate in the healthy control group was 78.8% ($p < 0.01$). But leprosy did not make a significant impact on the patient's marriage in women and the Yi nationality. Compared with healthy persons, the age of marriage in the patients was postponed for 4.58 years. Divorce rate in married leprosy patients was 8.4%, while it was 0.9% in control group. Remarriage rate in the divorced patients was 14.5%, while it was 37.1% in healthy persons. The cause of unmarriage and divorce of leprosy patients is that their spouses are afraid of acquiring the disease

and being discriminated against.—Authors' English Abstract

Zhao, A., et al. [Early detection and treatment of silent neuritis in leprosy.] *China Lepr. J.* **10** (1994) 6–8. (in Chinese)

Two-hundred cases of leprosy being under MDT and surveillance had been observed for 3 to 30 months. Among 12 cases

silent neuritis was seen, involving ulnar (2), common peroneal (2), facial (2) and tibial nerves (8). After treated with prednisone recovery of sensation and motion functions are in 60% (6/10) and in 66.7% (4/6), respectively. There was no side effect in the period of taking prednisone.—Authors' English Abstract

Immuno-Pathology

Corsico, B., Croce, M. V., Mukherjee, R. and Segaleiras, A. Identification of myelin basic proteins in circulating immune complexes associated with lepromatous leprosy. *Clin. Immunol. Immunopathol.* **71** (1994) 38–43.

Circulating immune complexes (CIC) were first measured in lepromatous patients (LL) by the I-125-C1q binding assay and the polyethylene glycol (PEG) precipitation test. High levels were found by both methods (95% and 90% of positives, respectively). LL-CIC were investigated for the presence of neural antigens. CIC were precipitated in 3.5% PEG, filtered through protein A-Sepharose affinity chromatography, eluted with glycine-HCl, pH 2.8, and washed with PBS; fractions after CIC dissociation were studied by SDS-PAGE and Western blotting. The LL-CIC PEG precipitates and the glycine-HCl eluates were positive in 76% and 71%, respectively, against anti-myelin basic proteins (MBP) monoclonal antibody, showing a single band at 15–25 kDa similar to the one obtained incubating MBP with anti-MBP. No reaction was detected with CIC-PBS fractions; strips were incubated with other anti-neural antibodies such as anti-glial fibrillary acidic proteins, anti-S-100, and anti-neurofilaments, without any reactivity. Our results demonstrate that LL-CIC contain MBP as an antigen; its significance could be related to the pathogenesis of leprosy since the liberation of MBP after *Mycobacterium leprae* nerve damage may elicit anti-MBP auto-antibodies to myelin breakdown, which reacts with peripheral nerve MBP inducing CIC formation. This mechanism may be

important in demyelination and destruction of nerve in leprosy.—Authors' Abstract

Holland, S. M., Eisenstein, E. M., Kuhns, D. B., Turner, M. L., Fleisher, T. A., Strober, W. and Gallin, J. I. Treatment of refractory disseminated nontuberculous mycobacterial infection with interferon gamma—a preliminary report. *N. Engl. J. Med.* **330** (1994) 1348–1355.

Studies conducted *in vitro* and in animals suggest that cytokine signals to monocytes or macrophages by interferon-gamma are important in the containment and clearance of disseminated nontuberculous mycobacterial infections. We studied seven patients with refractory disseminated nontuberculous mycobacterial infections who were not infected with the human immunodeficiency virus. Three patients were from a family predisposed to the development of *Mycobacterium avium* complex infections; four patients had idiopathic CD4+ T-lymphocytopenia. Their infections were culture- or biopsy-proved, involved at least two organ systems, and had been treated with the maximal tolerated medical therapy. Cellular proliferation, cytokine production, and phagocyte function were assessed in peripheral-blood cells. Interferon-gamma was administered subcutaneously two or three times weekly in a dose of 25 to 50 µg per square meter of body-surface area in addition to antimycobacterial medications. Clinical effects were monitored by cultures, biopsies, radiographs, and in one patient a change in the need for paracentesis.

In response to phytohemagglutinin, the production of interferon-gamma by mono-

nuclear cells from the patients was lower than in normal subjects ($p < 0.001$); whereas stimulation with ionomycin and phorbol myristate acetate led to normal production of interferon-gamma in the patients. Within 8 weeks of the start of interferon-gamma therapy, all seven patients had marked clinical improvement, with abatement of fever, clearing of many lesions and quiescence of others, radiographic improvement, and a reduction in the need for paracentesis. Interferon-gamma in combination with conventional therapy may be effective for some cases of refractory disseminated nontuberculous mycobacterial infection.—Authors' Abstract

Ilangumaran, S., Robinson, P., Shanker-narayan, N. P., Ramu, G., Mahadevan, P. R. and Muthukkaruppan, V. R. T lymphocyte reactivity of leprosy patients and healthy contacts from a leprosy-endemic population to delipidified cell components of *Mycobacterium leprae*. *Lepr. Rev.* **65** (1994) 34–44.

In this study, we measured *in vitro* proliferative responses of peripheral blood mononuclear cells from both leprosy patients across the clinical spectrum and also healthy contacts from a leprosy-endemic population to delipidified cell components of *Mycobacterium leprae* (DCC) and Dharmendra lepromin. Dharmendra lepromin was poor in inducing *in vitro* T-cell proliferation in all the study groups, even though it elicited marked *in vivo* skin-test reaction in tuberculoid leprosy patients and healthy contacts. In contrast, Dharmendra preparation of BCG induced marked T-cell response in tuberculoid as well as bacterial-index-negative lepromatous patients. DCC induced a significantly higher lymphoproliferative response than Dharmendra lepromin in all study groups. A significant positive correlation was observed between the lymphoproliferative responses to DCC and BCG. The present study, based on a large number of leprosy patients and healthy contacts, clearly demonstrates that DCC, depleted of glycolipids and lipopolysaccharides, is a good antigenic preparation for evaluating T-cell reactivity to *M. leprae*.—Authors' Summary

Janssen, R., Wauben, M., Vanderzee, R. and Tommassen, J. Immunogenicity of a mycobacterial T-cell epitope expressed in outer membrane protein PhoE of *Escherichia coli*. *Vaccine* **12** (1994) 406–409.

The outer membrane protein PhoE of *Escherichia coli* can be used for the expression of foreign antigenic determinants. Previously, a T-cell epitope of the 65-kDa heat-shock protein (hsp65) of *Mycobacterium tuberculosis*, comprising amino acids 180 to 188, was expressed in PhoE. The hybrid protein induced proliferation of epitope-specific T-cell clones *in vitro*. In this report, the potential of the hybrid protein to induce an *in vivo* T-cell response against the 180–188 T-cell epitope was assessed. Popliteal lymph node cells, isolated from rats immunized with PhoE containing the hsp65 epitope, showed high proliferative responses to a synthetic peptide consisting of amino acids 180 to 188 of hsp65, indicating that the epitope is immunogenic in the PhoE-associated conformation.—Authors' Abstract

Khanolkar-Young, S., Young, D. B., Colston, M. J., Stanley, J. N. A. and Lockwood, D. N. J. Nerve and skin damage in leprosy is associated with increased intralesional heat shock protein. *Clin. Exp. Immunol.* **96** (1994) 208–213.

Leprosy is frequently complicated by the development of reversal reactions in which peripheral nerve and skin lesions become inflamed and irreversible nerve damage may ensue. Increased expression of proteins belonging to the 70-kDa heat-shock family (hsp70) occurs in cells of the central nervous system exposed to hyperthermia, physical damage or drug-induced trauma. In the present study we have used immunocytochemical staining to monitor hsp70 levels in peripheral nerves infected by *Mycobacterium leprae*. Hsp70 was detected in skin and nerve lesions from all leprosy patients, but was particularly prominent in lesions from patients undergoing reversal reactions. Hsp70 immunocytochemistry can thus be used as a marker of neural injury in the peripheral as well as in the central nervous system. The cellular dynamics of nerve damage in leprosy are currently poorly un-

derstood, and we postulate that the immunopathology of leprosy may be partly due to an autoimmune response to heat-shock proteins.—Authors' Abstract

Kramnik, I., Radzioch, D. and Skamene, E. T-Helper 1-like subset selection in *Mycobacterium bovis* bacillus Calmette-Guerin-infected resistant and susceptible mice. *Immunology* **81** (1994) 618–624.

The *Bcg* gene has been shown to control natural resistance of mice to intravenous infection with low doses of *Mycobacterium bovis* (bacillus Calmette-Guerin; BCG). In the present study, we evaluated the impact of the *Bcg* gene on the development of T-cell reactivity during the early stages of infection. Congenic strains of mice, bearing "r" and "s" alleles of the *Bcg* gene on B10.A and BALB/c backgrounds, were studied at different time-points for 2 weeks after infection. The *in vitro* proliferative response of spleen cells, induced by mycobacteria or concanavalin A, was depressed in the *Bcg*(s) mice compared to the *Bcg*(r) congenic mice 14 days after infection with 10^5 colony-forming units (CFU) of BCG. Polymerase chain reaction (PCR)-based methodology was used to compare the level of lymphokine gene expression in the spleens of infected congenic mice both *ex vivo* and after *in vitro* stimulation. In both cases, preferential expression of interferon-gamma (IFN- γ), lymphotoxin, interleukin-2 (IL-2) and IL-2 receptor genes was observed. The lymphokine gene expression profiles indicated that T lymphocytes activated in the course of the BCG infection preferentially expressed the T-helper 1-specific pattern, irrespective of the allele of the *Bcg* gene. We showed that this bias in T-cell differentiation could not be attributed to either down-regulation of IL-4 gene expression or modulation of the macrophage co-stimulatory activity by live *M. bovis* BCG. We conclude that the mechanism of phenotypic expression of the *Bcg* gene resides in the differential ability of macrophages to be activated by lymphokines produced by protective T cells, rather than in the lack of these lymphokines in susceptible animals.—Authors' Abstract

Mathew, J. M. and Muthukkaruppan, V. Class-specific immunoglobulins and antibodies to mycobacterial sonicates and autoantigens in leprosy patients and contacts. *Microb. Pathogen.* **15** (1993) 367–376.

A comprehensive analysis of the humoral immune response in leprosy patients and contacts was undertaken. Class-specific antibodies to four mycobacterial sonicates, three autoantigens and three haptens were estimated by ELISA. It was found that IgG levels varied more extensively than IgM or IgA and that total serum IgG was significantly higher in lepromatous bacterial-index-positive (LL + ve) and -negative LL – ve) leprosy patients than in tuberculoid (TT/BT) patients and controls. The high levels of antimycobacterial antibodies found in untreated LL + ve patients were significantly reduced in LL – ve patients after effective chemotherapy. Considerable amount of antimycobacterial IgG was also detected in TT/BT patients. Each serum when assayed against sonicates of *Mycobacterium leprae*, *M. tuberculosis*, ICRC bacilli and BCG gave a similar antibody profile, suggesting that these antibodies were directed predominantly against crossreactive antigens. Up to 75% of LL patients and 35% of TT/BT patients were found to be positive for antibodies to histone, collagen and fibronectin. However, antibodies to several haptens were not detected in any of the patients and controls studied. Taken together, these results suggested that the amount of IgG antibodies is directly correlated with the antigenic load in the system, and that there is no evidence for polyclonal activation. It may be speculated that the regulatory mechanism of antibody production is severely deranged in lepromatous leprosy patients.—Authors' Abstract

Nishimura, K., Hashimoto, Y. and Iwasaki, S. Enhancement of phorbol ester-induced production of tumor necrosis factor alpha by thalidomide. *Biochem. Biophys. Res. Comm.* **199** (1994) 455–460.

The effect of thalidomide [racemic (DL-) form and optically pure (D- and L-) forms] on tumor necrosis factor (TNF)-alpha production by human leukemia cell lines (HL-

60, K562 and U937) stimulated with 12-O-tetradecanoylphorbol-13-acetate (TPA) was investigated. Although thalidomide has been regarded as a specific inhibitor of TNF- α production, our study indicated that all forms of thalidomide enhanced (but did not inhibit) the TPA-induced TNF- α production by the human leukemia cell lines investigated. The effects of thalidomide on TNF- α production might be cell type-specific.—Authors' Abstract

Nishimura, M., Kwon, K. S., Shibuta, K., Yoshikawa, Y., Oh, C. K., Suzuki, T., Chung, T. A. and Hori, Y. An improved method for DNA diagnosis of leprosy using formaldehyde-fixed, paraffin-embedded skin biopsies. *Mod. Pathol.* 7 (1994) 253–256.

To improve the sensitivity of the previously reported polymerase chain reaction (PCR) for the detection of *Mycobacterium leprae* in the formaldehyde-fixed, paraffin-embedded tissues, we adapted the PCR designed to amplify an internal 372-bp fragment of a *M. leprae*-specific repetitive sequence to 39 skin biopsies taken from patients with leprosy of the lepromatous type and tuberculoid type. Crude DNA samples were prepared from tissue sections that were deparaffinized and subjected to proteinase-K digestion without any further treatment for DNA purification. Overcoming a false-negative reaction by an elongation of the period for enzymatic digestion and an appropriate dilution of the samples, an amplification of the target sequence was obtained as a single band with all 39 skin biopsies tested. The fragments specifically amplified by the PCR were subjected to direct sequencing and were confirmed to be identical with an internal 372 bp of *M. leprae*-specific repetitive sequence. Although in 9 of 24 nonleprosy control samples, a false-positive amplification was observed as from one to several bands, they were distinguishable from the specific one by the electrophoretic pattern. This PCR makes up for the classic histological methods used in the diagnosis of leprosy.—Authors' Abstract

O'Donnell, M. A., Aldovini, A., Duda, R. B., Yang, H., Szilvasi, A., Young, R. A.

and de Wolf, W. C. Recombinant *Mycobacterium bovis* BCG secreting functional interleukin-2 enhances gamma interferon production by splenocytes. *Infect. Immun.* 62 (1994) 2508–2514.

Mycobacterium bovis BCG was genetically engineered to express and secrete mouse interleukin-2 (IL-2) and rat IL-2. Genes encoding IL-2 were inserted into an *Escherichia coli*-BCG shuttle plasmid under the control of the BCG HSP60 promoter. To facilitate study of proteins produced in this system, the IL-2 gene product was expressed (i) alone, (ii) with the mycobacterial alpha-antigen secretion signal sequence at the amino terminus, (iii) with an influenza virus hemagglutinin epitope tag at the amino terminus, and (iv) with both the secretion signal sequence and the epitope tag. When expressed with the alpha-antigen signal sequence, biologically active IL-2 was secreted into the extracellular medium. Western blot (immunoblot) analysis of the intracellular IL-2 and extracellular IL-2 revealed that the secretion signal was appropriately cleaved from the recombinant lymphokine upon secretion. To assess the ability of recombinant BCG to stimulate cytokine production in a splenocyte population, mouse splenocytes were cultured together with wild-type or IL-2-producing BCG. IL-2-secreting BCG clones stimulated substantial increases in gamma-interferon production, which could be reproduced by the addition of exogenous IL-2 to BCG. Levels of IL-6, IL-10, tumor necrosis factor- α , and granulocyte-macrophage colony-stimulating factor were not significantly changed, while IL-4 and IL-5 remained undetectable (less than 50 pg/ml). The enhanced production of gamma-interferon in response to IL-2-secreting BCG was strain independent. Recombinant BCG expressing mammalian cytokines provides a novel means to deliver cytokines and may augment the immunostimulatory properties of BCG in immunization and cancer therapy.—Authors' Abstract

Pechhold, K., Wesch, D., Schondelmaier, S. and Kabelitz, D. Primary activation of V gamma 9-expressing gamma/delta T cells by *Mycobacterium tuberculosis*—require-

ment for Th1-type CD4 T cell help and inhibition by IL-10. *J. Immunol.* **152** (1994) 4984–4992.

Purified peripheral blood gamma/delta T cells proliferated vigorously in response to killed *Mycobacterium tuberculosis* (M. tb.) in the presence of PBMC but not in the presence of T-cell-depleted [E(-)] feeder cells. Addition of graded numbers of autologous CD4 T cells to E(-) feeder cells reconstituted in a dose-dependent fashion the response of V gamma 9-expressing gamma/delta T cells to M. tb. IL-2 was identified as the major CD4 T-cell-derived helper factor required for gamma/delta T-cell proliferation after stimulation with M. tb. In addition, neutralizing anti-IFN-gamma but not anti-IFN-alpha Ab inhibited the responsiveness of V gamma 9 T cells, suggesting that endogenously produced IFN-gamma was involved in the activation of gamma/delta T cells by M. tb. Although gamma/delta T cells could not proliferate on their own in the absence of CD4 T cells (or exogenous IL-2), the appearance of IL-2 receptors (CD25) was triggered in the absence of CD4 T cells. Furthermore, IL-10 strongly inhibited the activation of V gamma 9 T cells among unfractionated PBMC responder cells. Similarly, the responsiveness of purified gamma/delta T cells to M. tb. occurring in the presence of CD4 T cells was strongly inhibited by IL-10; whereas the activation occurring in the presence of exogenous IL-2 was not impaired. These results show that interactions with Th1-type CD4 T cells are required for efficient activation of peripheral blood gamma/delta T cells by M. tb. In addition, our results have practical implications for creating experimental conditions aimed at identifying V gamma 9-selective (myco)bacterial ligands.—Authors' Abstract

Ramirez, G. M. L., Rom, W. N., Ciotoli, C., Talbot, A., Martiniuk, F., Cronstein, B. and Reibman, J. *Mycobacterium tuberculosis* alters expression of adhesion molecules on monocytic cells. *Infect. Immun.* **62** (1994) 2515–2520.

The host response to *Mycobacterium tuberculosis* is characterized by interactions between mononuclear cells, with recruit-

ment and fusion of these cells culminating in granuloma formation. In addition, the host response to *M. tuberculosis* requires CD4(+) T-cell reactivity, mediated by antigen-independent as well as antigen-dependent mechanisms. Thus, we hypothesized that cell adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1; CD54) would participate in the response to infection with *M. tuberculosis*. Exposure of THP-1 cells derived from a monocyte/macrophage cell line to *M. tuberculosis* (1:1 bacterium/cell ratio) elicited a sustained increase ($660\% \pm 49\%$ above resting level) in the expression of ICAM-1 that continued for at least 72 hr. Neither the expression of vascular cell adhesion molecule 1 (VCAM-1; CD106) nor that of the integrins lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18) or CR3 (CD11b/CD18) was increased to a similar extent at corresponding time points. The increase in ICAM-1 protein expression was accompanied by an increase in steady-state mRNA [Northern (RNA) analysis]. Neutralizing monoclonal antibodies directed against tumor necrosis factor-alpha but not interleukin-1a or interleukin-1-beta substantially abrogated the response to *M. tuberculosis* consistent with a paracrine or autocrine response. Continuous upregulation of the expression of ICAM-1 on mononuclear phagocytes induced by *M. tuberculosis* may mediate the recruitment of monocytes and enhance the antigen presentation of *M. tuberculosis*, thus permitting the generation and maintenance of the host response.—Authors' Abstract

Silva, C. L. and Lowrie, D. B. A single mycobacterial protein (hsp 65) expressed by a transgenic antigen-presenting cell vaccinates mice against tuberculosis. *Immunology* **82** (1994) 244–248.

We used a retroviral shuttle vector [pZIPNeoSV(X)] to transfect a monocyte-like murine tumor cell line (J774.G8) with the *Mycobacterium leprae* gene encoding heat-shock protein (hsp) 65. The antigen was expressed and presented on the surface of the transfected cell in association with major histocompatibility complex (MHC) class I and class II for recognition by T cells from

specifically sensitized mice. We show here that when these transfected cells were used as a vaccine and introduced parentally into syngeneic (BALB/c) mice they conferred a remarkably high degree of protective immunity against subsequent challenge with either *M. bovis* bacillus Calmette-Guerin (BCG) or *M. tuberculosis* H37Rv.—Authors' Abstract

Warwick-Davies, J., Dhillon, J., O'Brien, L., Andrew, P. W. and Lowrie, D. B. Apparent killing of *Mycobacterium tuberculosis* by cytokine-activated human monocytes can be an artefact of a cytotoxic effect on the monocytes. Clin. Exp. Immunol. **96** (1994) 214–217.

A protocol using combined exposure to interferon-gamma (IFN- γ), calcitriol and tumour necrosis factor-alpha (TNF- α) has been reported to activate human monocytes *in vitro* to kill *Mycobacterium tuberculosis*. We have attempted to repeat the findings in two laboratories, with negative results; treated cells were no different from untreated cells in this respect. However, the treated cells were more sensitive to a toxic effect of the bacteria. We suggest that the reported dramatic mycobacterial killing may have been an illusory consequence of the toxicity leading to cell lysis and loss of the liberated bacteria from the assay.—Authors' Abstract

Zhang, M., Gately, M. K., Wang, E., Gong, J. H., Wolf, S. F., Lu, S. H., Modlin, R. L. and Barnes, P. F. Interleukin 12 at the site of disease in tuberculosis. J. Clin. Invest. **93** (1994) 1733–1739.

Interleukin 12 (IL-12), a heterodimeric cytokine composed of p40 and p35 chains, has potent immunologic effects *in vitro*. We used tuberculous pleuritis as a model to study the immunoregulatory potential of IL-12 *in vivo* at the site of human infectious disease. Messenger RNAs for p40 and p35 were detected in pleural fluid from 6 of 6 patients by reverse-transcription polymerase chain reaction. By using an ELISA that detected both free p40 and heterodimeric IL-12, we found that mean concentrations were 585 ± 89 pg/ml in pleural fluid of patients with tuberculous pleuritis, which were significantly higher than those in serum of the same patients (54 ± 36 pg/ml), or in malignant pleural effusions (123 ± 35 pg/ml). By using an ELISA specific for heterodimeric IL-12, we found that mean concentrations in pleural fluid of patients with tuberculous pleuritis were 165 ± 28 pg/ml and undetectable in serum of the same patients, or in malignant pleural effusions. Bioactive IL-12 was detectable in 5 of 5 supernatants of pleural fluid cells stimulated with *Mycobacterium tuberculosis*. Addition of anti-IL-12 antibodies suppressed proliferative responses of pleural fluid cells to *M. tuberculosis* by $36 \pm 7\%$. These data indicate that IL-12 may play a role in the human immune response to infectious agents *in vivo*. We hypothesize that IL-12 contributes to the antimycobacterial immune response by enhancing production of interferon-gamma, facilitating development of Th1 cells and augmenting cytotoxicity of antigen-specific T cells and natural killer cells.—Authors' Abstract

Microbiology

Alugupalli, S., Portaels, F., and Larson, L. Systematic study of the 3-hydroxy fatty acid composition of mycobacteria. J. Bacteriol. **176** (1994) 2962–2969.

Mycobacterial cell walls have been shown to contain type-specific antigenic glycolipids, such as phenolic glycolipids, glycopeptidolipids (GPL), and trehalose-contain-

ing lipooligosaccharides (LOS) (4, 5). Long-chain 3-hydroxy fatty acids (3-OH-FAs) have been identified in several glycolipids, for example, in GPL from *Mycobacterium peregrinum* and *M. smegmatis* (7, 12), in LOS from *M. szulgai* and *M. tuberculosis* Canetti (9, 10), and in acyltrehaloses from *M. tuberculosis* Canetti and H37Rv (3, 6). Furthermore, 3-OH-FAs have been detect-

ed in other bioactive mycobacterial structures, i.e., in ornithine-amide lipids and in lipopeptides in rough mutants of *M. avium* (2, 11). The present work was undertaken to systematically study the 3-OH-FA composition of several clinically important mycobacteria.—Authors' Abstract

Amorim, A. G., Cardoso-de Almeida, M. L., Carrington, M., Morga, D. P., Riezman, H., Zerbini, L. F., Piestun, V. S. and Castilhovalavicius, B. A. Expression of *Mycobacterium leprae* 18-kDa antigen in yeast in a GPI-anchored form. *Braz. J. Med. Biol. Res.* **27** (1994) 623–626.

The 18-kDa protein from *Mycobacterium leprae* is a major target for the immune response in leprosy. We have developed a system to express this antigen in yeast as a fusion protein with the C-terminal region of the yeast membrane protein GAS1, which would render the recombinant protein anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. Cells lacking the GAS1 gene and transformed with the hybrid 18-kDa-GAS1 construct express a polypeptide that reacts with an 18-kDa-specific monoclonal antibody. In addition, these cells react with an alpha-CRD antibody after GPI-PLC treatment. The nontransformed cells are negative. These data indicate that our system may be suitable for the expression of foreign proteins in yeast in a GPI-anchored form.—Authors' Abstract

Bergh, S. and Cole, S. T. MycDB: an integrated mycobacterial database. *Mol. Microbiol.* **12** (1994) 517–534.

As part of ongoing efforts to investigate the molecular biology of the human pathogens in the genus *Mycobacterium*, a customized database was developed specifically for these organisms and implemented in ACEDB database manager software. The data loaded include the IMMYC Antigen List, details of reagents available from the CDC/WHO Antibody Bank, more than 1 Mb of sequences of mycobacterial genes and proteins from public databases, the physical maps of *Mycobacterium leprae* and *M. tuberculosis* developed at the Institut Pasteur, as well as a subset of the references found

in MedLine. The ACEDB software allows both quick and intuitive access to the data and to connections between facts by a simple mouse-driven interface, as well as by more powerful query mechanisms.—Authors' Abstract

Dhandayuthapani, S., Banu, M. J. and Kashiwabara, Y. Cloning and sequence determination of the gene coding for the elongation factor Tu of *Mycobacterium leprae*. *J. Biochem.* **115** (1994) 664–669.

Elongation factor Tu (EF-Tu) plays an important role in protein biosynthesis and is susceptible to antibiotics in prokaryotes like *Escherichia coli*. In order to understand the primary structure of EF-Tu in the intracellular pathogenic bacterium *Mycobacterium leprae*, the gene (*tuf* gene) coding for this protein was cloned and sequenced. The gene contains a coding region of 1,188 bp with GUG as start codon. The deduced amino acid sequence has 396 amino acids with a molecular weight of 43.6 kDa. Putative GTP-binding sites are located at amino acid positions 19–24, 83–87, and 138–141. Comparison of *M. leprae* EF-Tu amino acid sequence with those of *M. tuberculosis*, *Micrococcus luteus*, *E. coli*, and *Salmonella typhimurium* reveals 74%–95% homology. Mitochondrial EF-Tu of *Saccharomyces cerevisiae* (62%) and chloroplast EF-Tu of *Arabidopsis thaliana* (65.6%) also show strong homology with that of *M. leprae*. In contrast, the EF-Tu of the archaebacterium *Halobacterium marismortui* exhibits relatively less homology (36.7%). Southern hybridization of *M. leprae* *tuf* gene with genomic DNA of slow growing and fast growing mycobacteria and related species like *Corynebacterium fascians* and *Nocardia asteroides* suggests that the gene is highly conserved in these organisms.—Authors' Abstract

Frothingham, R., Hills, H. G. and Wilson, K. H. Extensive DNA sequence conservation throughout the *Mycobacterium tuberculosis* complex. *J. Clin. Microbiol.* **32** (1994) 1639–1643.

The *Mycobacterium tuberculosis* complex includes the four species *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*. We

sequenced 13 *M. tuberculosis* complex strains in the 16S-to-23S rDNA internal transcribed spacer (ITS). The ITS has a high rate of nucleotide substitution. Previous reports found three nucleotide substitutions in the ITS between two *M. tuberculosis* complex strains. In contrast, we found the same ITS sequence in all 13 *M. tuberculosis* complex strains (including all four species and *M. bovis* BCG). This finding confirms the conservation of 16S rDNA sequence and the high DNA-DNA relatedness found in previous studies. By the usual criteria, the four species of the *M. tuberculosis* complex would be considered a single species. In a phylogenetic analysis based on the ITS sequence, the four species of the *M. tuberculosis* complex were distinct from nontuberculous mycobacteria. The ITS contains at least seven potential sites for oligonucleotide probes with specificity for the *M. tuberculosis* complex.—Authors' Abstract

Gan, S. C. Identification of immunologically reactive proteins specified by the recombinant phages of a λ gt11::*M. leprae* genomic library. *Immunol. Infect. Dis.* **3** (1993) 215–218.

A genomic library of *Mycobacterium leprae* isolated directly from leprosy patients' skin biopsies (in contrast to the available *M. leprae* genomic libraries which consist of DNA from *M. leprae* grown in armadillos experimentally) was constructed. Thirty-nine immunologically reactive clones were identified by pooled leprosy patients' serum. Three of the 39 immunologically reactive clones were also identified by pooled tuberculoid leprosy (TT) and borderline tuberculoid leprosy (BT) patients' serum. Further work is being carried out to identify antigenic epitopes that would stimulate protective immunity.—Author's Abstract

Hamid, M. E., Chun, J. S., Magee, J. G., Minnikin, D. E. and Goodfellow, M. Rapid characterization and identification of mycobacteria using fluorogenic enzyme tests. *Zentr. Bakteriol.* **280** (1994) 476–487.

Sixty representatives of selected *Mycobacterium* and *Nocardia* species were examined for their ability to cleave 79 fluo-

rogenic synthetic enzyme substrates based on the fluorophores 7-amino-4-methylcoumarin and 4-methylumbelliferone. The resultant data were analyzed using the simple matching coefficient and clustering achieved using the unweighted pair group method with arithmetic averages algorithm. Clusters corresponding to the validly described species *Mycobacterium bovis*, *M. chelonae*, *M. chitae*, *M. farcinogenes*, *M. fortuitum*, *M. peregrinum*, *M. senegalense*, *M. smegmatis*, *Nocardia asteroides*, and *N. farcinica* were circumscribed at or above the 83% similarity level. Fluorogenic probes prepared from 7-amino-4-methylcoumarin and 4-methylumbelliferone provide a rapid means of detecting taxonomically useful enzymes in small amounts of whole mycobacteria and nocardiae.—Authors' Abstract

Hartmann, S., Minnikin, D. E., Mallet, A. I., Ridell, M., Rigouts, L. and Portaels, F. Fast atom bombardment mass spectrometry of mycobacterial phenolic glycolipids. *Biol. Mass Spectrom.* **23** (1994) 362–368.

Fast atom bombardment mass spectra were successfully recorded for intact glycosylphenolphthiocerol dimycocerosates (phenolic glycolipids, PGLs) from *Mycobacterium kansasii*, *M. leprae*, *M. tuberculosis*, *M. marinum*, *M. bovis* and *M. haemophilum*. Characteristic fragment ions from the loss of the oligosaccharide moiety and one of the long-chain multimethyl-branched mycocerosic acids were observed in most cases. A tandem mass spectrometric experiment was carried out on the PGL from *M. tuberculosis*, revealing the type of mycocerosic acids esterified to individual homologs. Mass spectra of homologs separated by reversed-phase high-performance liquid chromatography gave information on the substitution pattern in certain cases. The potential of matrix-assisted laser desorption ionization spectroscopy was demonstrated by a successful analysis of the PGL from *M. tuberculosis*.—Authors' Abstract

Ji, Y. E., Colston, M. J. and Cox, R. A. Nucleotide sequence and secondary structures of precursor 16S rRNA of slow-growing mycobacteria. *Microbiology U.K.* **140** Part 1 (1994) 123–132.

Slow-growing mycobacteria have a single ribosomal RNA (rrn) operon, with the genes for 16S, 23S and 5S rRNA being present in that order. The transcription start site of the rrn operon of *Mycobacterium tuberculosis* was identified in *Escherichia coli*. PCR methodology was used to amplify parts of the rrn operon, namely, the leader region and the spacer-1 region separating the 16S rRNA and 23S rRNA genes of *M. avium*, *M. paratuberculosis*, *M. intracellulare*, "*M. lufu*," *M. simiae* and *M. marinum*. The amplified DNA was sequenced. The sequence data, together with those obtained previously for *M. leprae* and *M. tuberculosis*, were used to identify putative antitermination signals and RNase III processing sites within the leader region. Notable features include a highly conserved Box B element and a sequence of 31 nucleotides which is common to all eight slow-growers which were scrutinized. A secondary structure for mycobacterial precursor-16S rRNA was devised, based on sequence homologies and homologous nucleotide substitutions. The 18 nucleotides at the 5'-end of spacer-1 have the capacity of binding sequences close to the 5'- and 3'-ends of mature 16S rRNA, suggesting that secondary structure is important to the maturation process. All the slow-growers, including *M. leprae*, conform to the same scheme of secondary structure. The scheme proposed for *M. tuberculosis* is a variant of the main theme. The leader and spacer sequences may prove a useful supplement to 16S rRNA sequences in establishing phylogenetic relationships between very closely related species. "*M. lufu*" appears to be a close relative of *M. intracellulare*.—Authors' Abstract

Kamala, T., Paramasivan, C. N., Herbert, D., Venkatesan, P. and Prabhakar, R. Isolation and identification of environmental mycobacteria in the *Mycobacterium bovis* BCG trial area of South India. *Appl. Environ. Microbiol.* **60** (1994) 2180–2183.

The isolation profiles of environmental mycobacteria present in soil, water, and dust samples, and sputum samples of persons with symptoms of chest infection in the South Indian *Mycobacterium bovis* BCG (bacillus Calmette-Guerin) trial area were

compared. Isolates belonging to the *M. avium-intracellulare-scrofulaceum* complex were predominant in water, dust, and sputum samples and *M. fortuitum*-complex organisms were predominant in soil samples irrespective of the season of the year.—Authors' Abstract

Kolk, A. H. J., Noordhoek, G. T., De Leeuw, O., Kuijper, S. and van Embden, J. D. A. *Mycobacterium smegmatis* strain for detection of *Mycobacterium tuberculosis* by PCR used as internal control for inhibition of amplification and for quantification of bacteria. *J. Clin. Microbiol.* **32** (1994) 1354–1356.

For the detection of *Mycobacterium tuberculosis* by polymerase chain reaction (PCR), the IS6110 sequence was used. A modified target was constructed by insertion of 56 nucleotides in the IS6110 insertion element of *M. bovis* BCG. This modified insertion sequence was integrated into the genome of *M. smegmatis*, a mycobacterium species which does not contain the IS6110 element. When DNA from the modified *M. smegmatis* 1008 strain was amplified with IS6110-specific primers INS1 and INS2, a band of 301 bp was seen on agarose gel; whereas the PCR product of *M. tuberculosis* complex DNA was a 245-bp fragment with these primers. The addition of a small number of *M. smegmatis* 1008 cells to clinical samples before DNA purification enables the detection of problems which may be due to the loss of DNA in the isolation procedure or to the presence of inhibitors. The presence of inhibitors of the amplification reaction can be confirmed by the addition of *M. smegmatis* 1008 DNA after the DNA isolation procedure. Furthermore, competition between the different target DNAs of *M. smegmatis* 1008 DNA and *M. tuberculosis* complex DNA enables the estimation of the number of IS6110 elements in the clinical sample.—Authors' Abstract

Norman, E., de Smet, K. A. L., Stoker, N. G., Ratledge, C., Wheeler, P. R. and Dale, J. W. Lipid synthesis in mycobacteria: characterization of the biotin carboxyl carrier protein genes from *Mycobacterium leprae* and *M. tuberculosis*. *J. Bacteriol.* **176** (1994) 2525–2531.

The causative agents of leprosy and tuberculosis, *Mycobacterium leprae* and *M. tuberculosis*, have a lipid-rich cell envelope which contributes to virulence and antibiotic resistance. Acyl coenzyme A carboxylase, which catalyzes the first committed step of lipid biosynthesis, consists in mycobacteria of two subunits, one of which is biotinylated. Genes from *M. leprae* and *M. tuberculosis* encoding a biotinylated protein have been cloned and sequenced. Analysis of the derived protein sequences demonstrated the presence of biotin-binding sites and putative ATP-bicarbonate interaction sites, consistent with the proteins having a biotin carboxylase function as well as with their being biotin carrier proteins.—Authors' Abstract

Oettinger, T. and Andersen, A. B. Cloning and B-cell-epitope mapping of Mpt64 from *Mycobacterium tuberculosis* H37Rv. *Infect. Immun.* **62** (1994) 1058–1064.

The gene of the immunogenic protein MPT64 found in culture filtrates of *Mycobacterium tuberculosis* H37Rv was cloned and sequenced. A comparison showed mpt64 and the gene encoding MPB64 from *M. bovis* BCG Tokyo to be identical except for one silent mutation. The regions encoding the promoter and the signal peptide were also well conserved for the two sequences. Southern blot experiments on genomic mycobacterial DNA showed the presence of mpt64 in the *M. tuberculosis* substrains H37Rv, H37Ra, and Erdman and in the *M. bovis* BCG substrains Tokyo, Moreau, and Russian; whereas the *M. bovis* BCG substrains Glaxo, Pasteur, Canadian, Tice, and Danish 1131 and *M. leprae* lack the gene. Southern blot analyses revealed differences in the restriction enzyme patterns within the *M. tuberculosis* substrains as well as within the *M. bovis* BCG substrains, indicating either different chromosomal localization of mpt64 or that mutations have occurred at different locations on the chromosomes. N-terminal and C-terminal deletion mutants were constructed for the mapping of B-cell epitopes on MPT64 with five monoclonal antibodies, C24b1, C24b2, C24b3, L24b4, and L24b5. Western blot (immunoblot) analysis revealed that the murine

antibodies bind to one linear and three conformational epitopes.—Authors' Abstract

Öner, F., Tian, X.-X., Son, K., Klegerman, M. E. and Groves, M. J. Characterization of the antigen 85 complex fibronectin-binding proteins derived from aged culture filtrates of *Mycobacterium bovis* BCG, Tice® substrain. *Microbios* **78** (1994) 69–81.

The antigen 85 (Ag 85) complex are major T-cell and B-cell antigens and fibronectin-binding proteins secreted by *Mycobacterium tuberculosis*, *M. leprae* and attenuated *M. bovis* (BCG vaccine). The Ag 85 complex was found to comprise a high proportion of the extracellular protein in filtrates of surface-pellicle cultures of Tice®-substrain BCG vaccine, attaining a maximum of 25%. This proportion began to decrease prior to the end of the logarithmic growth phase, about 3 weeks after the start of the culture, mainly due to apparent degradation of the Ag 85 complex. Isolation of the main Ag 85 protein and determination of the first 36 residues of the NH₂-terminus showed identity with the 85A protein isolated by others from various mycobacteria. Both the Ag 85A and B components were secreted in nearly constant proportions over a 6-week period. No Ag 85C protein was detected.—Authors' Abstract

Pang, Y., Brown, B. A., Steingrube, V. A., Wallace, R. J., Jr., and Roberts, M. C. Tetracycline resistance determinants in *Mycobacterium* and *Streptomyces* species. *Antimicrob. Agents Chemother.* **38** (1994) 1408–1412.

Two of seven tetracycline-resistant (Tc^r) *Mycobacterium fortuitum* group isolates and six Tc^r clinical *Streptomyces* isolates carried gram-positive Tc^r determinants (Tet K and Tet L) and *Streptomyces* resistance determinants (Otr A, Otr B, and Otr C). This represents the first documentation of the acquisition by mycobacteria of determinants coding for antibiotic resistance and suggests the potential for the spread of antibiotic resistance determinants within mycobacterial species.—Authors' Abstract

Paul, T. R. and Beveridge, T. J. Preservation of surface lipids and determination of ultrastructure of *Mycobacterium kansasii* by freeze-substitution. *Infect. Immun.* **62** (1994) 1542–1550.

The cell wall architecture of a slowly growing mycobacterium, *Mycobacterium kansasii*, was examined by freeze-substitution following growth *in vitro*. Freeze-substituted bacteria were marked by the presence of an electron-translucent space [or electron-transparent zone (ETZ) described by previous workers] surrounding the majority of cells. At least two morphotypes of mycobacteria were revealed by freeze-substitution. In the first, a relatively thin (11 ± 2.3 to 35 ± 3.1 nm), uniform ETZ surrounded intact cells which contained cytoplasm filled with well-stained ribosomes and a DNA nucleoid distributed throughout the cell. The second morphotype consisted of a small proportion of organisms that were distorted in shape and were surrounded by a much thicker (59 ± 2.6 to 198 ± 2.5 nm) ETZ in areas of the cell which appeared to have retracted from the space it had originally occupied, leaving depressions in the ETZ. The lipid nature of the ETZ was demonstrated because cells were devoid of an ETZ when organisms were freeze-substituted in the absence of osmium tetroxide in the substitution medium or treated with neutral lipid solvents (acetone or ethanol) before freeze-substitution. Moreover, thin-layer chromatography of acetone or ethanol extracts obtained from solvent-treated cells identified a lipid component which corresponded to the *M. kansasii*-specific phenolic glycolipid. In contrast, negligible amounts of glycolipids were detected in extracts obtained from control HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer-treated cells, and these cells retained an ETZ. These results demonstrate that species-specific phenolic glycolipids are essential components in the architecture of the *M. kansasii* ETZ. Furthermore, we show that freeze-substitution is a reliable technique for the retention and precise preservation of lipid-containing polymers in the mycobacterial cell wall. — Authors' Abstract

Rivoire, B., Pessolani, M. C. V., Bozic, C. M., Hunter, S. W., Hefta, S. A., Mehra,

V. and Brennan, P. J. Chemical definition, cloning, and expression of the major protein of the leprosy bacillus. *Infect. Immun.* **62** (1994) 2417–2425.

The decline in prevalence of leprosy is not necessarily matched by a fall in incidence, emphasizing the need for new antigens to measure disease transmission and reservoirs of infection. *Mycobacterium leprae* obtained from armadillo tissues was disrupted and subjected to differential centrifugation to arrive at preparations of cell wall, cytoplasmic membrane, and cytosol. By committing 0.3 g of *M. leprae* to the task, it was possible to isolate from the cytosol and fully define the major cytosolic protein. Amino-terminus sequencing and chemical and enzymatic cleavage, followed by more sequencing and fast atom bombardment-mass spectrometry of fragments, allowed description of the entire amino acid sequence of a protein of 10,675-Da molecular mass. The sequence derived by chemical means is identical to that deduced previously from DNA analysis of the gene of a 10-kDa protein, a GroES analog. The work represents the first complete chemical definition of an *M. leprae* protein. PCR amplification of the 10-kDa protein gene, when cloned into *Escherichia coli* with a pTRP expression vector, allowed production of the recombinant protein. Chemical analysis of the expressed protein demonstrated that it exactly reflected the native protein. The recombinant major cytosolic protein appears to be a promising reagent for skin testing, still probably the most appropriate and pragmatic means of measuring incidence of leprosy. — Authors' Abstract

Silbaq, F. and Bercovier, H. Nucleotide sequence of *Mycobacterium leprae* elongation factor (EF-Tu) gene. *Nucleic Acids Res.* **21** (1993) 3327.

The elongation factor EF-Tu is essential in bacterial translation and has sequences which are highly conserved even in phylogenetically distant bacteria. This allowed us to show that gram-negative bacteria had two copies of the *tuf* gene whereas most gram-positive bacteria including Mycobacteria had one copy of this gene. The agent of leprosy, *Mycobacterium leprae*, has been iso-

lated from naturally infected man, armadillo and mangabey monkey. A genomic library of *M. leprae* isolated from a naturally infected mangabey monkey (*Cercocebus* spp.) was prepared in lambda Dash vector (Stratagene, U.S.A.). The library was screened with a 30-bp oligonucleotide probe (CAACAACTACCGTCCGCAGTTCT-CTTCCG) deduced from the sequences of a conserved region of the elongation factor gene in *Eubacteria* (GenBank M17788 and GenBank J01717). One recombinant phage with a 15-kb insert which hybridized with the probe was further digested with BamHI to produce a 9-kb fragment containing the *tuf* gene. This DNA fragment was subcloned into BLS plasmid (Stratagene) for sequence determination. The resulting DNA sequence (done on both strands) revealed an open reading frame (nucleotides 94–1299) containing a typical translation initiation site, including a putative Shine-Dalgarno consensus sequence (GGAGG) at –13 from the start codon GUG at position 108 and a stop codon (UAA) at position 1296. The *M. leprae* DNA sequence was compared to that of the elongation factor of *M. leprae* isolated from a man (EMBL Z14314), of *M. tuberculosis* (GenBank S40925, X63539), of *Micrococcus luteus* (GenBank M17788) and of *Escherichia coli* (GenBank J01717). The comparison showed identity of 99.5%, 88%, 78% and 72%, respectively. The data that we present here add further proof of the identity of *M. leprae* whether isolated from man or from a naturally infected monkey.—From the article

Spencer, S. A., Broughton, E. S., Hamid, S. and Young, D. B. Immunoblot studies in the differential diagnosis of porcine brucellosis—an immunodominant 62 kDa protein is related to the mycobacterial 65 kDa heat shock protein (HSP-65). *Vet. Microbiol.* **39** (1994) 47–60.

Smooth *Brucella* spp. share certain lipopolysaccharide antigens with other bacteria, resulting in serological crossreactions which can prevent the definitive diagnosis of brucellosis. To identify other antigens with serodiagnostic potential, immunoblot studies following sodium dodecyl sulphate-polyacrylamide gel electrophoresis were carried out. Sera from pigs experimentally infected

with *Brucella suis* and naturally infected feral pigs, sera from pigs from a farm with a known history of *Yersinia enterocolitica* 0:9 infection, Brucella Complement Fixation Test (CFT) reactor pigs (etiology unknown) and pigs from consistently Brucella CFT negative farms were examined. Although *B. suis*-infected pigs recognized a total of nine *B. melitensis* antigens, individual pigs rarely recognized more than three antigens in the range. A 62-kDa antigen was recognized by the majority (73%) of the Brucella-infected pigs, but only by 10% to 23% of pigs from the other groups. This antigen was shown to be the Brucella homolog of the ubiquitous 65-kDa heat-shock protein (HSP-65) family by immunoblot studies with 14 monoclonal antibodies to the *Mycobacterium leprae* HSP-65. Only four of these monoclones (Y1.2, ML-30, D7C and IIC8) identified the *B. melitensis* 62-kDa protein, suggesting that unshared, potentially Brucella-specific, regions exist.

Sera from *Y. enterocolitica* 0:9 infected pigs, CFT reactor pigs (etiology unknown), CFT-negative pigs and hyperimmune pig serum raised to *Y. enterocolitica* 0:9 also recognized *B. melitensis* antigens, most notably a 17-kDa protein. This antigen appears to be a common crossreactive protein.—Authors' Abstract

Timm, J., Perilli, M. G., Duez, C., Trias, J., Orefici, G., Fattorini, L., Amicosante, G., Oratore, A., Joris, B., Frere, J. M., Pugsley, A. P. and Gicquel, B. Transcription and expression analysis, using *lacZ* and *phoA* gene fusions, of *Mycobacterium fortuitum* β -lactamase genes cloned from a natural isolate and a high-level β -lactamase producer. *Mol. Microbiol.* **12** (1994) 491–504.

The gene encoding a class A β -lactamase was cloned from a natural isolate of *Mycobacterium fortuitum* (*blaF*) and from a high-level amoxicillin-resistant mutant that produces large amounts of β -lactamase (*blaF**). The nucleotide sequences of the two genes differ at 11 positions, including two in the region upstream from the coding sequence. Gene fusions to *Escherichia coli lacZ* and transcription and expression analysis of the cloned genes in *Mycobacterium smegmatis* indicated that high-level pro-

duction of the β -lactamase in the mutant is mainly or wholly due to a single base-pair difference in the promoter. These analyses also showed that transcription and translation start at the same position. A comparison of the amino acid sequence of BlaF, as predicted from the nucleotide sequence, with the determined *N*-terminal amino acid sequence indicated the presence of a typical signal peptide. The fusion of *blaF* (or *blaF**) to the *E. coli* gene *phoA* resulted in the production of BlaF-PhoA hybrid proteins that had alkaline phosphatase activity. These results demonstrate that *phoA* can be used as a reporter gene for studying protein export in mycobacteria.—Authors' Summary

Tomioka, H., Saito, H. and Sato, K. Evaluation of BACTEC 460 TB system for measurement of *in vitro* anti-*Mycobac-*

terium leprae activity of various antimicrobials. *Jpn. J. Lepr.* **61** (1992) 157–164.

This paper provides independent confirmation of observations by Franzblau that inhibition of the oxidation of labelled palmitate to release $^{14}\text{CO}_2$ can be used to assess drug susceptibility of *Mycobacterium leprae*, using BACTEC technology. Rapid, bacteriostatic drugs such as rifampin give the greatest inhibition. Results are quoted as “growth index” because this is the conventional way of expressing BACTEC results; there is no claim for, or indication of, axenic growth of leprosy bacilli. A new observation is that “growth index” determinations after a rather long period (18 or 27 days) are more sensitive in showing drug susceptibility than determinations after a shorter period (7 days). The methods described require at least 10^8 *M. leprae* per determination.—P. Wheeler (Trop. Dis. Bull.)

Experimental Infections

Wiid, I. J. F., Werely, C., Beyers, N., Donald, P. and van Helden, P. D. Oligonucleotide (GTG)₅ as a marker for *Mycobacterium tuberculosis* strain identification. *J. Clin. Microbiol.* **32** (1994) 1318–1321.

Culture of *Mycobacterium tuberculosis* provides no information on the identity of a strain or the distribution of such a strain in the community. Strain identification of *M. tuberculosis* can help to address important epidemiological questions, e.g., the origin of an infection in a patient's household or community, whether reactivation of infection is endogenous or exogenous in origin, and the spread and early detection of organisms with acquired antibiotic resistance. To research this problem, strain identification must be reliable and accurate. Although genetic identification techniques already exist, it is valuable to have genetic identification techniques based on a number of genetic markers to improve the accurate identification of *M. tuberculosis* strains. We show that oligonucleotide (GTG)₅ can be successfully applied to the

identification of *M. tuberculosis* strains. This technique may be particularly useful in cases in which *M. tuberculosis* strains have few or no insertion elements (e.g., IS6110) or in identifying other strains of mycobacteria when informative probes are lacking.—Authors' Abstract

Dhople, A. M. and Ibanez, M. A. *In vivo* susceptibility of *Mycobacterium leprae* to ofloxacin either singly or in combination with rifampicin and rifabutin—antileprosy activity of ofloxacin and ansamycins in mice. *Arzneimittelforschung* **44-1** (1994) 563–565.

The antimicrobial effects of ofloxacin against *Mycobacterium leprae*, either alone or in combination with rifampin and rifabutin, were studied using the mouse foot pad assay technique. When used singly, the minimum concentrations of the drugs needed to completely inhibit the growth of *M. leprae* in foot pads of mice were 50 mg/kg body weight for ofloxacin and 0.003% and 0.0001%, respectively, for rifampin and rifabutin. However, excellent synergistic ef-

fects were observed when mice were fed with 25 mg/kg body weight of ofloxacin along with 0.00003% rifabutin, but not rifampin. Thus, incorporation of ofloxacin and rifabutin in the multiple drug therapy of leprosy patients is suggested.—Authors' Abstract

Gosselin, D., Turcotte, R. and Lemieux, S.
Influence of the Ity/Lsh/Bcg gene on the development of suppressor cell precursors in the early phase of the infection of mice with *Mycobacterium lepraemurium*. Clin. Exp. Immunol. **96** (1994) 218–224.

In vitro inducible suppressor-cell precursors were detected in the spleen of BALB/c but not in DBA/2 mice infected intraperitoneally with 10^8 *Mycobacterium lepraemurium* bacilli, thus suggesting that their development is genetically controlled. Two pairs of mouse strains congenic at the Ity/Lsh/Bcg locus (BALB/c-C.D2 and B10.A-B10.A.Bcg(r)) were used to investigate whether this phenomenon is influenced by this gene known to control the relative susceptibility of mice to *M. lepraemurium* infection. This seems likely, as the detection of culture-induced suppressor activity was

delayed for 5–6 weeks in C.D2 and B10.A.Bcg(r) mice infected intravenously with 10^4 *M. lepraemurium* bacilli. However, despite the retardation in the detection of suppressor-cell precursors, the level of *in vitro* induced suppressor activity at onset in spleen cell suspensions of mice carrying the resistant allele was higher than in cell cultures derived from susceptible mice. Since the resistant allele has a different effect when found on BALB/c or DBA/2 background, other genetic factors are apparently involved in the development of suppressor-cell precursors. We finally observed that, in spleen cell cultures from intravenously infected Ity/Lsh/Bcg congenic mice on the BALB/c background, adherent and nonadherent cells were required in the inductive phase of suppressor-cell development; whereas *in vitro* induced suppressor activity was found exclusively in the adherent cell fraction. Given these properties, we thus conclude that suppressor-cell precursors detected in the spleen of these intravenously infected mice are similar to those previously observed in C3H mice infected intraperitoneally with a thousand times more bacilli.—Authors' Abstract

Epidemiology and Prevention

Gil Suarez, R. E., Baez Rodriguez, H., Gonzalez Segredo, A. B., Gonzalez-Abreu Castells, E. and Rodriguez Silveira, J.
[Study of a leprosy focus in an industrial center in Cuba.] Rev. Leprol. Fontilles **19** (1994) 343–354. (in Spanish)

The study population comprised 1200 workers of a factory where two cases of lepromatous leprosy had been detected. All these workers were clinically examined and blood samples were taken for the determination of anti-phenolic glycolipid I (PGL-I) antibodies. The cut-off point for the serological test was established at an OD value of 0.100. The procedure applied to seropositive individuals according to the OD readings included a second clinical examination, Mitsuda and skin-smear tests, and a follow-up serological test. It was foreseen

the institution of chemoprophylaxis according to the test results and of multidrug therapy (MDT) if the diagnosis of leprosy was confirmed in any case. The overall seropositivity rate was 18.3%; there was a highly significant decrease in the frequency of individuals showing elevated antibody levels as the lepromin reactivity increased; the risk of developing a low-level humoral response in those who were in contact with the patients for more than 47 months was 4.39-fold higher than in those with a lesser time. However, in those with a high response the risk increased steadily with time since the onset of the contact. But, in either group seropositivity was transient in some individuals decreasing in value or disappearing; the proportion seropositive was greater in the feminine sex but only by dint of the low-level response group; anti PGL-I antibodies rose to a peak at the age of 20–39 years and then fell.

At the clinical examination performed by dermatologists a slight infiltration of one earlobe was found in one individual of the high seropositivity group; the result of his Mitsuda test was 0 mm and the skin-smear test was positive with a BI of 1+.—Authors' English Summary

Gonzalez-Abreu, E., Rodriguez, M. E., Perez, J., Rodriguez, J. and Gonzalez, A. PGL-I antibody in HIV infected patients. (Letter) *Lepr. Rev.* **64** (1993) 275–276.

The correspondents determined by indirect ELISA the presence of antibodies to a *Mycobacterium leprae*-related antigen (ND-A-BSA) in 437 patients in an AIDS hospital in Havana, Cuba, with confirmed HIV infection, and in 313 age-matched blood donors without HIV infection and with no known contact with leprosy. The proportion of people with test results higher than the established cut-off was higher among the HIV patients (14.9%) than among the blood donors (1.3%). (A value of 3.0% is given for a leprosy-endemic area.) None of the seropositive people has developed leprosy [time period is not given]. The correspondents speculate that HIV-induced immunosuppression may increase the prevalence of multibacillary forms of leprosy; however, because of the long incubation time for leprosy the patients may die from HIV-associated causes before the leprosy becomes apparent.—C. A. Brown (*Trop. Dis. Bull.*)

Li, T. and Feng, X. [Analysis of factors for leprosy endemicity with stepwise regression model.] *China Lepr. J.* **10** (1994) 11–15. (in Chinese)

Multiple regression models of two dependent variables—incidence (Y_1) and prevalence (Y_2)—of leprosy have been set up, using data of Baise Prefecture of Guangxi in 1956 to 1992 and taking gross output value of industry and agriculture per capita (X_1), national income per capita (X_2), proportions of agricultural population (X_3) and health workers (X_4), annual mean air temperature (X_5) and annual rainfall (X_6) as independent variables. Analyses of simple correlations of the variables with independent variables used in models showed that factors of population, economy and climate

could exert some influence on the endemicity of leprosy and the impacts of factors X_4 , X_2 and X_3 on the incidence and of factors X_1 , X_2 and X_3 on the prevalence are more notable.—Authors' English Abstract

Lienhardt, C. and Fine, P. E. M. Type 1 reaction, neuritis and disability. What is the current epidemiological situation? *Lepr. Rev.* **65** (1994) 9–33.

Type 1 reaction is one of the major causes of nerve damage in leprosy patients leading to disabilities of varying severity. Although this complication of leprosy has been extensively described, we still know very little of its natural history and of the factors which may predispose to it. This paper examines the descriptive and analytic epidemiology of these reactions in leprosy. We find that they vary greatly in clinical expression, time of onset, duration and severity, which has important implications for the way they are handled in the context of leprosy-control programs. We review the various risk factors that have been suggested over the last 30 years and the evidence of their utility in identifying “high-risk” patients is assessed. We then review the specific aspects of neuritis and disability in leprosy and examine the contribution of type 1 reaction to leprosy-associated disabilities. The prospects for early detection and prevention of type 1 reaction are examined in the light of current knowledge, both at research and at the leprosy control level.—Authors' Summary

Lombardi, C., Ramirez, R. and Gil Suarez, R. E. [Temporal tendencies in the detection of leprosy in the state of São Paulo (Brazil) 1934–1983.] *Rev. Leprol. Fontilles* **19** (1994) 369–386. (in Spanish)

From a number of cases detected in the State of São Paulo between 1934 and 1983 the tendency of the endemia is evaluated and described. The tendency of the case detection has been studied by sex and age groups together with the different proportions of the distinct clinical cases and other countries with similar trends. In the period studied the global tendency of detection has decreased but only in the two decades following sulfone therapy can a real decline in the incidence be considered. An attempt is

made to evaluate this epidemiological phenomenon based on the characteristics of the organization and strategy of the control program in the different stages considered.—Authors' English Summary

Smith, T. C. and Richardus, J. H. Leprosy in northern Thailand: 1951–1990. *Southeast Asian J. Trop. Med. Pub. Health* **24** (1993) 3–10.

Data of new, previously untreated leprosy patients from six northern provinces of Thailand, diagnosed at McKean Rehabilitation Center, Chiang Mai, and associated clinics between 1951 and 1990, were analyzed. The following trends were found: (1) decreasing numbers of new, previously untreated patients; (2) increasing average age of patients at onset and presentation of disease; (3) decreasing duration between onset and presentation; (4) increasing percentage of patients presenting within the first year of symptoms; (5) increasing percentage of paucibacillary cases; (6) decreasing percentage of patients presenting with deformity. These trends are a reflection of those seen for the whole of Thailand, and indicate that leprosy control is being effective. Patients are presenting at an earlier stage than before, with consequent reduction in disability and infectivity. Better usage of chemotherapy since 1976 has helped to reduce the transmission of bacilli from person to person, combined with effective health education activities which have dispelled some wrong ideas about leprosy and encouraged patients to seek help early in the course of the disease. Additional factors related to public health and living standards have also contributed.—AS/A.C. McDougall (*Trop. Dis. Bull.*)

Wu, X. and Ning, Y. [Incidence of leprosy in the contacts after implementation of MDT.] *China Lepr. J.* **10** (1994) 30–31. (in Chinese)

During 1987 to 1992, 225 new cases of leprosy were detected in examinations of leprosy contacts in Liangshan Prefecture and

Panzhihua City, Sichuan, of whom 119 and 12 cases are household contacts of MB and PB patients, respectively, and 94 are neighbors of the patients. In the three groups, the numbers of newly detected patients were decreasing by 29.9%, 41.1%, and 40.2% year after year on the average. Among the new patients the proportion of MB cases have been increasing and the proportion of children have been decreasing year by year. In children, MB cases were fewer than PB cases. The authors believe that MDT is effective in controlling transmission of leprosy on the basis of the data presented.—Authors' English Abstract

Yang, Z., et al. [Calculation of leprosy endemic trend in Hetian Prefecture with mathematical model.] *China Lepr. J.* **10** (1994) 15–17. (in Chinese)

Linear regression and exponential function equation were used to fit leprosy prevalence in Hetian Prefecture, Xinjiang, and to calculate the trend of leprosy endemicity there. In the equation, $Y = 3.031 - 0.033X$ and $\ln Y = 31.0439 - 0.3769X$. The results show that the goodness of fit and calculation effect of the exponential function equation are better than those of linear regression. According to the calculation, leprosy would be basically eradicated by 1995 there.—Authors' English Abstract

Yu, A., et al. [Leprosy in children in Liaoning Province.] *China Lepr. J.* **10** (1994) 29–30. (in Chinese)

In Liaoning Province, there have been 145 cases of leprosy in children below the age of 14 at registration cumulatively, with the ratio of male-to-female being in 1.4-to-1 and the disease duration of 2.8 years as a mean. The ratios of MB and disabled among them at detection had decreased from 77.8% and 80% to zeros respectively; 80.7% among them had a history of contact with leprosy patients before their own disease was detected. In the last 7 years there were no leprosy patients in children in the province.—Authors' English Abstract

Rehabilitation

Hardy, M., Jimenez, S., Jabaley, M. and Horch, K. Evaluation of nerve compression with the Automated Tactile Tester. *J. Hand Sur.* **17A** (1992) 838–842.

The Automated Tactile Tester (ATT) was used to measure threshold values for trapézoidal skin indentation (light touch), low- and high-frequency vibration (50 and 150 Hz), pinprick (sharp-dull transition point), warming (temperature awareness), and two-point discrimination in 61 patients with symptoms of median nerve compression at the wrist. We compared these data with values obtained in the same patients with manual monofilament tests, manual two-point discrimination measurements, and electrophysiologic nerve conduction studies. The ATT detected abnormal sensation in 71% of the hands tested, nerve conduction velocity was abnormal in 44% of the cases, and the manual tests indicated abnormality in 42% of the hands. The most indicative single test among those included in the present study for detecting sensory abnormality in these patients was threshold to a 50-Hz vibration administered by the ATT. We conclude that the ATT is a sensitive tool for the diagnosis and evaluation of compressive peripheral neuropathy, and may allow objective documentation in a higher percent of patients than do more traditional testing methods.—Authors' Abstract

Horch, K., Hardy, M., Jimenez, S. and Jabaley, M. An Automated Tactile Tester for evaluation of cutaneous sensibility. *J. Hand. Surg.* **17A** (1992) 829–837.

The Automated Tactile Tester (ATT) is a computer-controlled device designed to measure patients' cutaneous perception of touch, vibration, temperature, and pain. The ATT provides repeatable and precise control of the amplitude, rate of application, and duration of stimuli. Threshold values for skin indentation (touch), high- and low-frequency vibration, pinprick (sharpness), warmth, and two-point discrimination were obtained with the ATT from the fingers of 62 normal subjects. Manual monofilament and two-point discrimination tests were also

performed on the same subjects. All the tests with the ATT, except pinprick, showed a statistically significant increase in threshold with age. There were no significant differences attributable to the hand or digit tested or the sex of the subject. These data were used to derive age-adjusted criteria for normal sensory function in the glabrous skin of the fingers. Thresholds were found to remain within normal limits when these subjects were retested at various time intervals. We conclude that the ATT provides repeatable and reliable measurements of sensory function in the skin and has potential application in the diagnosis and evaluation of compression and other peripheral neuropathies.—Authors' Abstract

Huang, X. [Effects of protective footwear on plantar ulcers.] *China Lepr. J.* **10** (1994) 26–28. (in Chinese)

Protective footwear with soft insole has been supplied and self-care taught to leprosy patients with plantar ulcers. Through observation of 2 years in 356 cases, it was observed that cracks and ulcers decreased by 25% and 54.6%, respectively, and through 1 year in 330 cases, cracks and ulcers lessened by 43.1% and 18.5%, respectively. In 204 persons with insensitve feet the annual incidence rate of plantar ulcers was 2.7% and the relapse rate in 8.4% as an average. Now the form and quality of the protective footwear are being improved.—Author's English Abstract

Kazen, R. O. Management of plantar ulcers—theory or practice? (Editorial) *Lepr. Rev.* **64** (1993) 188–198.

The editorialist discusses why, how and where plantar ulcers are formed in leprosy patients, how the ulcers heal, how they can be prevented, and how they can be cured and prevented from recurring. He then reviews treatment measures for simple and complicated ulcers, drawing from experience in the ALERT (All Africa Leprosy and Rehabilitation Training Centre) program in Ethiopia.—C. A. Brown (*Trop. Dis. Bull.*)

Leading Team. [Summarization of the Cooperation Project (1990–1992) of Chinese MOPH and TLMI in Leprosy Rehabilitation.] *China Lepr. J.* **10** (1994) 20–24. (in Chinese)

Chinese MOPH and TLMI had completed a pilot project for leprosy rehabilitation in eight provinces in China during 1990 to 1992. In the final report, it was generally concluded that the project had been well run and most of its items completed, apart from the production of protective footwear and corrective surgery, with efforts of leprosy workers in the project and the financial support of TLMI. The staff of the project had benefited by increased knowledge and skill as well as improved attitude to the preventing disability in leprosy. The patients had also benefited from the project by correction and prevention of their disability, and acquired skill for self-care. The result demonstrated that the prevention and treatment of disability of leprosy are feasible as well as necessary in China.—Authors' English Abstract

Stratford, C. J. and Owen, B. M. The effect of footwear on sensory testing in leprosy. *Lepr. Rev.* **65** (1994) 58–65.

The aim of this study was to identify the effect of footwear on sensory testing in leprosy. This was achieved by using three methods of sensory testing within one district of East Africa. We included 72 leprosy patients and 36 controls (nonleprosy patients) in the study, and these were subdivided into two groups, depending on whether they normally wore shoes or went barefoot. The methods used were the WHO sensory test, graded monofilaments and the biothesiometer. The results showed significant differences in the threshold levels between both groups of patients with the biothesiometer and monofilaments, demonstrating the importance of having separate values when screening for leprosy and assessing which patients are at the most risk of developing ulcers. The importance of having quantitative methods of testing was also demonstrated, as only then can the results be sufficiently standardized to identify the at-risk groups and also be sufficiently sensitive to differentiate between shoe wear-

ing and nonshoe wearing patients.—Authors' Summary

Tan, W., et al. [Amputations in 80 leprosy patients.] *China Lepr. J.* **10** (1994) 25–26. (in Chinese)

Since 1985, 359 disabled persons whose leprosy had been cured were admitted to the China Leprosy Center for correction of their deformities, of which 80 persons had amputations, making up 22% because of plantar ulcers with severe complication, including chronic osteomyelitis, serious deformity, suspicious change to cancer, etc; 75% of these cases have undergone amputation in the legs, 2 cases in the thighs and 3 in the feet. The incisions of 72 cases healed by first intention. After the operation artificial limbs or supports have been supplied. The authors emphasize that amputation absolutely must not be used unless there is definite indication and artificial limbs or supports should be supplied to amputees who should also be taught to do physical exercise so as to prevent ulceration on the end of the remaining limb.—Authors' English Abstract

Wang, K. [Comparison of DDS monotherapy with MDT in MB leprosy.] *China Lepr. J.* **10** (1994) 28. (in Chinese)

The authors compared 83 MB leprosy patients under MDT with 414 cases of MB leprosy with dapsone (DDS) monotherapy in Gannan Prefecture, Gansu Province. The course of DDS monotherapy was as long as 2.8 times that of MDT. MDT made the skin smears negative in 2 to 3 years in 72.3% of the patients, but the monotherapy did so in 84.8% in 5 to 8 years. The mean rate of the BI decline in the cases with MDT was as quick as 4.6 times that in those with the monotherapy. Leprosy reactions under MDT were less frequent and milder.—Author's English Abstract

Yan, L., et al. [Results of controlling plantar ulcers for three years.] *China Lepr. J.* **10** (1994) 8–11. (in Chinese)

Plantar ulcers in 97 cases of leprosy had been treated according to the regimen proposed by ILEP. In 3 years, the number of plantar ulcers in 50 cases with insensitive

feet had decreased by 95% and annual relapse rate of the healed ulcers was 10%, 3.5% and 2%, respectively. The measures for healing and preventing the ulcers include teaching the patients to form a habit of doing self-care, i.e., wearing protective foot-

wear with hard sole, higher upper, soft insole and suitable size, and avoiding over fatigue. If necessary, surgical operation should be done for them.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Alavi, M. R. and Affronti, L. F. Induction of mycobacterial proteins during phagocytosis and heat shock: a time interval analysis. *J. Leukoc. Biol.* **55** (1994) 633–641.

Mycobacterium tuberculosis survives macrophage bactericidal activities by mechanisms that may include induction of stress proteins. We sought to determine whether the synthesis of any mycobacterial proteins is increased during phagocytosis and whether any of these proteins are also up-regulated during heat shock. Protein synthesis by *M. tuberculosis* H37Ra during phagocytosis by the mouse macrophage cell line IC-21, and during heat shock at 45° and 48°, was monitored at various time intervals using S-35-labeled methionine/cysteine and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Our data suggest the existence of certain common elements in the stress response of mycobacteria to the three stress stimuli. This apparent similarity was best characterized by the up-regulation of a 25-kDa protein after exposure to each of the stress conditions. Furthermore, this 25-kDa protein and a 37-kDa protein that also was synthesized during phagocytosis appeared to be extracellular because they were preferentially solubilized when infected macrophages were lysed with 0.5% NP-40.—Authors' Abstract

Andersen, P. Effective vaccination of mice against *Mycobacterium tuberculosis* infection with a soluble mixture of secreted mycobacterial proteins. *Infect. Immun.* **62** (1994) 2536–2544.

An experimental vaccine that was based on secreted proteins of *Mycobacterium tuberculosis* was investigated in a mouse model

of tuberculosis. I used a short-term culture filtrate (ST-CF) containing proteins secreted from actively replicating bacteria grown under defined culture conditions. The immunogenicity of the ST-CF was investigated in combination with different adjuvants, and peak proliferative responses were observed when ST-CF was administered with the surface-active agent dimethyldioctadecylammonium chloride. The immunity induced by this vaccine was dose dependent and, in the optimal concentration, the vaccine induced a potent T-helper-1 response which efficiently protected the animals against a subsequent challenge with virulent *M. tuberculosis*. Antigenic targets for the T cells generated were mapped by employing narrow-molecular-weight fractions of ST-CF. The experimental vaccine primed a broadly defined T-cell repertoire directed to multiple secreted antigens present in ST-CF. A vaccination with viable *M. bovis* bacillus Calmette-Guerin (BCG), in contrast, induced a restricted T-cell reactivity directed to two secreted protein fractions with molecular masses of 5 to 12 and 25 to 35 kDa. The protective efficacy of the ST-CF vaccine was compared with that of a BCG standard vaccine, and both induced a highly significant protection of equal magnitude. The vaccination with ST-CF gave rise to a population of long-lived CD4 cells which could be isolated 22 weeks after the vaccination and could adoptively transfer acquired resistance to T-cell-deficient recipients. My results confirm the hypothesis that *M. tuberculosis* cells release protective antigens during growth. The high efficacy of a subunit vaccine observed in the present study is discussed as a possible alternative to a live recombinant vaccine carrier.—Author's Abstract

Anthony, L. S. D., Chatterjee, D., Brennan, P. J. and Nano, F. E. Lipoarabinomannan from *Mycobacterium tuberculosis* modulates the generation of reactive nitrogen intermediates by gamma-interferon-activated macrophages. *FEMS Immunol. Med. Microbiol.* **8** (1994) 299–305.

Lipoarabinomannan derived from the virulent Erdman strain and a rapidly growing, laboratory-attenuated strain of *Mycobacterium tuberculosis* were evaluated for their ability to modulate the production of nitric oxide (NO) by macrophages activated with IFN-gamma or IFN-gamma and LPS. It was observed that in macrophages pretreated with 100 µg ml⁻¹ LAM, the NO induced by IFN-gamma alone was augmented while the NO induced by IFN-gamma and LPS was reduced. LAM was also shown to synergize with IFN-gamma in the induction of NO, with AraLAM from the attenuated strain exhibiting greater potency than ManLAM from the Erdman strain. Despite the modulation of NO production, LAM did not affect the IFN-gamma-induced macrophage growth inhibition of *Francisella tularensis* LVS, an organism whose growth inhibition in activated macrophages is dependent.—Authors' Abstract

Arya, A., Batra, H. V., Sahai, P. and Mukherjee, R. Production and characterization of new murine monoclonal antibodies reactive to *Mycobacterium tuberculosis*. *Hybridoma* **13** (1994) 21–30.

Murine monoclonal antibodies were raised against *Mycobacterium tuberculosis* (H37Rv) employing conventional hybridoma procedure. The binding characteristics of the four selected monoclonal antibodies reactive to *M. tuberculosis* were assessed by enzyme-linked immunosorbent assay (ELISA) using sonic extracts. The immunofluorescence test (IFT) was done using intact *M. tuberculosis*, 16 other mycobacterial species, and 10 bacteria of other genera. Monoclonal antibody A30 reacted strongly to *M. tuberculosis* both in ELISA and IFT. Monoclonal antibody A25 showed restricted crossreactivity with mycobacterial species whereas two other monoclonal antibodies B6 and H2 appeared broadly

crossreactive. In Western blot analysis, A30 reacted with the 30-kDa protein antigen, A25 with the 18- and 28-kDa protein bands, H2 with the 18-kDa antigen alone, and B6 with three bands of 17–19, 22, and 28 kDa of *M. tuberculosis* H37Rv. A30 exhibited high reactivity with virulent *M. tuberculosis* H37Rv and a clinical South Indian strain and minimal reactivity with avirulent *M. tuberculosis* H37Ra strain.—Authors' Abstract

Benson, C. A. Treatment of disseminated disease due to the *Mycobacterium avium* complex in patients with AIDS. *Clin. Infect. Dis.* **18** Suppl. 3 (1994) S237–S242.

Perhaps the most important recent advance in the field of infections due to the *Mycobacterium avium* complex (MAC) is the identification and development of more effective agents for the treatment and prevention of disseminated disease. These agents include clarithromycin, azithromycin, rifabutin and other rifamycins, ethambutol, clofazimine, fluoroquinolones, amikacin, and liposome-encapsulated gentamicin. Most clinicians currently use multidrug therapy to maximize efficacy and to minimize the emergence of resistance. Prospective clinical trials of multidrug regimens suggest that MAC colony counts in blood decline during therapy, usually with alleviation of clinical symptoms. The small size and short duration of these trials have not permitted an evaluation of survival or quality of life. Because the contribution of any single agent to multidrug trials is difficult to assess, short-term trials of monotherapy have been conducted recently; clarithromycin, azithromycin, ethambutol, and liposome-encapsulated gentamicin have been most active. Rifabutin and rifampin, clofazimine, amikacin, and ciprofloxacin may contribute to the efficacy of multidrug regimens. Current recommendations include the following: (1) disseminated MAC disease should be treated in patients with AIDS; (2) initial treatment should consist of at least two agents; (3) oral clarithromycin or azithromycin is the preferred first agent; (4) ethambutol is the most rational choice for the second agent; and (5) in appropriate cases, additional agents (rifampin or rifabutin, clofazimine, ciprofloxacin, or

parenteral amikacin) may be added. Therapy should continue for life.—Author's Abstract

Bermudez, L. E. and Young, L. S. Factors affecting invasion of HT-29 and HEP-2 epithelial cells by organisms of the *Mycobacterium avium* complex. *Infect. Immun.* **62** (1994) 2021–2026.

Organisms of the *Mycobacterium avium* complex cause disseminated blood-borne infection in patients with AIDS, who acquire the infection mainly through the gastrointestinal tract. Prior to causing infection, *M. avium* must colonize and invade the intestinal mucosa. This study examined the ability of several serovars of the *M. avium* complex to bind to and invade the HT-29 intestinal mucosal cell line and the HEP-2 laryngeal cell line. Logarithmic-phase *M. avium* was more efficient in binding and invasion than organisms in the stationary phase of growth. Bacteria incubated at 37°C and 40°C adhered to and invaded HT-29 cells more efficiently than bacteria cultured at 30°C. The ability of *M. avium* to invade HT-29 and HEP-2 cells was inhibited when the cells were incubated with cytochalasin B prior to exposure to the bacterium, suggesting active participation of the mammalian cell in the process of internalization. Two protein kinase inhibitors, staurosporin and H7, blocked invasion of *M. avium*, and a specific tyrosine protein kinase inhibitor, genistein, also blocked the internalization but not the binding of bacteria. The findings suggest that *M. avium* binds to a specific receptor(s) on the epithelial cells and uses the cytoskeleton of the mammalian cell to become internalized.—Authors' Abstract

Collins, D. M. DNA fingerprinting of *Mycobacterium xenopi* strains. *Lett. Appl. Microbiol.* **18** (1994) 234–235.

Mycobacterium xenopi is an environmental bacterium that occasionally causes disease in humans. A method is described for DNA fingerprinting strains of this species. Seven of 10 strains from humans were clearly distinguishable from each other using DNA fingerprinting. This method will enable the investigation of possible environmental sources and human spread of disease due to this species.—Author's Abstract

Cynamon, M. H., Klemens, S. P. and Grossi, M. A. Comparative activities of azithromycin and clarithromycin against *Mycobacterium avium* infection in beige mice. *Antimicrob. Agents Chemother.* **38** (1994) 1452–1454.

The comparative activities of azithromycin (AZI) and clarithromycin (CLA) against eight *Mycobacterium avium* complex (MAC) isolates were evaluated in the beige mouse model of disseminated infection. Mice were infected intravenously with approximately 10^7 viable MAC isolate. AZI at 100 or 200 mg/kg of body weight or CLA at 200 mg/kg of body weight was given by gavage daily for 10 days starting at 7 days postinfection. In each study, groups of treated mice were compared with untreated control animals. A dose-related reduction in organism cell counts in the spleens between the groups receiving AZI at 100 and 200 mg/kg was observed. AZI at 200 mg/kg was more active than CLA at 200 mg/kg against 6 of 8 MAC isolates in the spleens. CLA at 200 mg/kg was more active than AZI at 200 mg/kg against 3 of 8 MAC isolates in the lungs. The difference between AZI at 200 mg/kg and CLA at 200 mg/kg against organisms in the lungs was not significant for the remaining five isolates. Clinical trials comparing the activities of AZI and CLA in combination with other agents in patients with disseminated MAC infection are necessary to ascertain any clinically significant differences in the efficacies of these agents.—Authors' Abstract

Dolin, P. J., Raviglione, M. C. and Kochi, A. Global tuberculosis incidence and mortality during 1990–2000. *Bull. WHO* **72** (1994) 213–220.

Forecasts of tuberculosis morbidity and mortality are presented for the decade 1990–1999. An estimate 88 million new cases of tuberculosis, of which 8 million will be attributable to HIV infection, will occur in the world during the decade; 30 million people are predicted to die of tuberculosis in the same period, including 2.9 million attributable to HIV infection.

The number of new tuberculosis cases occurring each year is predicted to increase from 7.5 million (143 cases per 100,000) in 1990 to 8.8 million (152 per 100,000) in

1995 and 10.2 million (163 per 100,000) in the year 2000. In 1990, 2.5 million persons were estimated to have died of tuberculosis; at the same level of availability of treatment, it is predicted that 3.0 million tuberculosis deaths will occur in 1995 and 3.5 million in 2000.

Demographic factors, such as population growth and changes in the age structure of populations, will account for 79.5% of the predicted increases in new cases. Age-specific incidence rates in sub-Saharan Africa are increasing due to the HIV epidemic and will account for the remaining 20.5% of the forecast increase in new cases. In WHO's South-East Asian Region and in Central and South America the age-specific incidence rates are expected to fall during 1990–2000, but at a slower rate than in previous years because of the expected increase in HIV seroprevalence. In the Western Pacific and Eastern Mediterranean Regions the age-specific incidence rates are expected to fall during 1990–2000 because of the effects of intervention strategies, but the total number of new cases will continue increase until the end of the decade because of population growth.—Authors' Abstract

Galleni, M., Franceschini, N., Quinting, B., Fattorini, L., Orefici, G., Oratore, A., Frere, J.-M. and Amicosante, G. Use of the chromosomal class A β -lactamase of *Mycobacterium fortuitum* D316 to study potentially poor substrates and inhibitory β -lactam compounds. *Antimicrob. Agents Chemother.* **38** (1994) 1608–1614.

Sixteen different compounds usually considered β -lactamase stable or representing potential β -lactam inhibitors and inactivators were tested against the β -lactamase produced by *Mycobacterium fortuitum*. The compounds exhibiting the most interesting properties were BRL42715, which was by far the best inactivator, and CGP31608 and ceftazidime, which were not recognized by the enzyme. These compounds thus exhibited adequate properties for fighting mycobacterial infections. Although cloxacillin, dicloxacillin, cefoxitin, and CP65207-2 exhibited poor inhibitory efficiency against the enzyme, they were also rather poor substrates and might be considered potential antimycobacterial agents. By contrast,

CGP31523A and ceftamet were good substrates.—Authors' Abstract

Gilot, P. and Misonne, M. C. *Mycobacterium paratuberculosis* and *Escherichia coli* share common antigenic determinants. *Vet. Microbiol.* **39** (1994) 353–360.

Paratuberculosis (Johne's disease) is a chronic enteritis of ruminants, which is due to infection by *Mycobacterium paratuberculosis*. By comparative analysis of Western blots of *M. paratuberculosis* components incubated with sera from paratuberculous cows (either pre-absorbed or not on an *Escherichia coli* sonicate), we have shown the presence of crossreactive antigens between *M. paratuberculosis* and *E. coli*. Components in the range of 22 to 75 kDa are recognized by sera from paratuberculous cows, but only some components in the range of 20 to 40 kDa are endowed with specific B-cell epitopes to *M. paratuberculosis* with respect to *E. coli*. Crossreactions were further stressed by the fact that rabbit immunoglobulins to *E. coli* recognized some ten *M. paratuberculosis* components (in the range of 45 to 66 kDa). The importance of crossreactivity between the two bacterial species was evaluated by enzyme-linked immunosorbent assay (ELISA) using soluble sonic-extract components from *M. paratuberculosis* as antigens. Pre-absorption of series of bovine sera with *E. coli* resulted in the diminution of ELISA mean optical density readings by 16.1% and 62.0% for paratuberculous or healthy animals, respectively.—Authors' Abstract

Grange, J. M. and Yates, M. D. Zoonotic aspects of *Mycobacterium bovis* infection. *Vet. Microbiol.* **40** (1994) 137–151.

The bovine tuberculosis eradication campaigns in many industrially developed countries have led to a huge reduction in the incidence of human tuberculosis caused by *Mycobacterium bovis*. Overt disease in man may, however, manifest decades after the initial infection and the occurrence of such disease raises several important questions. In particular, it is important to determine whether man-to-man transmission occurs, thereby rendering man a continuing reservoir of infection, and whether, if this is the case, man develops infectious forms

of tuberculosis that enable *M. bovis* to be transmitted back to cattle. Epidemiological studies in south-east England indicate that human tuberculosis due to *M. bovis* is rare and that the incidence is declining. In contrast to earlier days, the lung is now involved in many cases, raising the possibility of transmission of bacilli to other human beings and to cattle by the aerogenous route. No direct evidence of man-to-man transmission of overt disease was found but it is possible that inapparent primary pulmonary infections are occurring and these may proceed to overt post-primary disease in the future. The genito-urinary tract is now the most prevalent site of nonpulmonary lesions and there is firm evidence that this form of tuberculosis poses a hazard to cattle. Although uncommon, human tuberculosis due to *M. bovis* is still a public health problem of concern to both the medical and veterinary professions, and there is a need to maintain careful bacteriological surveillance.—Authors' Abstract

Grosset, J. H. Assessment of new therapies for infection due to the *Mycobacterium avium* complex—appropriate use of *in vitro* and *in vivo* testing. Clin. Infect. Dis. 18 Suppl. 3 (1994) S233–S236.

Laboratory testing is a prerequisite to predictions about the potential value of human clinical trials—the gold standard for the assessment of new therapies for infection with the *Mycobacterium avium* complex (MAC). These laboratory assessments must be made in the proper sequence, with appropriate models and methodology used to obtain data valid in determining whether clinical trials are warranted. *In vitro* testing permits measurements of minimal inhibitory and minimal bactericidal concentrations, identification of the synergism or antagonism of various agents, definition of an agent's pharmacokinetic properties (e.g., hydrophilicity or lipophilicity), and evaluation of a drug's intracellular penetration and activity against intracellular organisms. The most appropriate animal model for *in vivo* testing of activity against MAC is the beige mouse. Experiments in this model provide important data on an agent's minimal effective dose and on its optimal dose, dosing frequency, and route(s) of administration.

Evaluations in the beige mouse also document whether the agent is bactericidal or bacteriostatic, whether it selects drug-resistant mutants, and whether its use in combination with other agents is beneficial.—Author's Abstract

Hoge, C. W., Fisher, L., Donnell, H. D., Dodson, D. R., Tomlinson, G. V., Breiman, R. F., Bloch, A. B. and Good, R. C. Risk factors for transmission of *Mycobacterium tuberculosis* in a primary school outbreak—lack of racial difference in susceptibility to infection. Am. J. Epidemiol. 139 (1994) 520–530.

Recent data have suggested that there are racial differences in the susceptibility to infection by *Mycobacterium tuberculosis*. An opportunity to test this suggestion was afforded by an outbreak of tuberculosis in a racially mixed elementary school in St. Louis County, Missouri. A physical education teacher was discovered to have cavitary pulmonary tuberculosis. Of 343 students in the school, 176 (51%) were found to be tuberculin skin-test positive (≥ 5 mm induration by Mantoux method); 32 children had abnormal chest radiographs. More frequent contact with the physical education teacher was associated with infection ($p < 0.001$). Black children were no more likely to be infected than were white children [relative risk (RR) = 0.98, 95% confidence interval (CI) 0.78–1.22]. However, black children who were tuberculin positive had larger skin reactions than did white children (mean, 18.9 vs 16.6 mm, $p < 0.001$) and were more likely to have abnormal chest radiographs (RR = 2.76, 95% CI 1.44–5.27). Among tuberculin-positive children, low body mass index (< 10 th percentile) was associated with active disease (RR = 2.90, 95% CI 1.45–5.80). The analysis of race was unchanged after controlling for sex, body build, and level of contact with the physical education teacher. Widespread tuberculous infection resulted from contact with a highly infectious staff person. Thin body build was a risk factor for active disease. Black children were no more susceptible to infection than were white children, although they more commonly developed radiographic evidence of active disease.—Authors' Abstract

Husson, R. N., Ross, L. A., Sandelli, S., Inderlied, C. B., Venzon, D., Lewis, L. L., Woods, L., Conville, P. S., Witebsky, F. G. and Pizzo, P. A. Orally administered clarithromycin for the treatment of systemic *Mycobacterium avium* complex infection in children with acquired immunodeficiency syndrome. *J. Pediatr.* 124 5 Part 1 (1994) 807–814.

[This study was] to determine the safety, tolerance, pharmacokinetics, and antimycobacterial activity of orally administered clarithromycin in children with acquired immunodeficiency syndrome and disseminated *Mycobacterium avium* complex (MAC) infection. [This was a] Phase I study with a 10-day pharmacokinetic phase followed by a 12-week continuation therapy phase. Twenty-five patients with a median age of 8.3 years were enrolled. Ten were receiving zidovudine and 13 were receiving didanosine at the time of enrollment. Clarithromycin suspension was administered to each patient at one of three dose levels: 3.75, 7.5, and 15 mg/kg per dose every 12 hr. Clarithromycin and antiretroviral pharmacokinetics were measured during single-drug and concurrent-drug administration. Clinical and laboratory monitoring was performed biweekly. Clarithromycin was well tolerated at all dose levels. Plasma clarithromycin concentrations increased proportionately with increasing doses, and significant pharmacokinetic interactions were not observed during concurrent administration with zidovudine or didanosine. Decreases in mycobacterial load in blood were observed only at the highest clarithromycin dose level. Decreased susceptibility to clarithromycin developed rapidly (within 12 to 16 weeks) in the majority of MAC strains isolated from study patients.—Authors' Abstract

Ji, B. H., Truffot Pernot, C., Lacroix, C., Raviglione, M. C., O'Brien, R. J., Olliaro, P., Roscigno, G. and Grosset, J. Effectiveness of rifampin, rifabutin, and rifapentine for preventive therapy of tuberculosis in mice. *Am. Rev. Respir. Dis.* 148 (1993) 1541–1546.

To identify alternative regimens for preventive therapy of tuberculosis, the phar-

macokinetics and antimicrobial activities of rifampin (RMP), rifabutin (RBT), and rifapentine (RPT) were compared in BCG-vaccinated and *Mycobacterium tuberculosis*-infected immunocompetent mice. RPT showed the highest serum peak level (C_{max}) and the longest half-life (t_{1/2}); whereas RBT displayed the lowest C_{max} and the shortest t_{1/2}. On weight-to-weight basis, both RPT and RBT were more bactericidal than RMP. The activity of RMP was significantly reduced when the frequency of administration was reduced from six to three times weekly; whereas significant bactericidal activity was still observed in mice treated with RPT, 10 mg/kg up to once fortnightly, or RBT, 10 mg/kg twice weekly. Because the bactericidal activity of RBT, 10 mg/kg six times/wk for 6 wk, or RPT, 10 mg/kg two times/wk for 12 wk, was comparable to that of RMP, 10 mg/kg six times/wk for 12 wk in mice, the two regimens are appropriate for clinical trials of preventive therapy of tuberculosis.—Authors' Abstract

Johnson, B. J. and McMurray, D. N. Cytokine gene expression by cultures of human lymphocytes with autologous *Mycobacterium tuberculosis*-infected monocytes. *Infect. Immun.* 62 (1994) 1444–1450.

In order to better understand the immunoregulation following *Mycobacterium tuberculosis* infection, cytokine mRNA induction in response to *in vitro* infection of human monocytes with live virulent *M. tuberculosis* H37Rv co-cultured with autologous lymphocytes was quantitated by reverse transcriptase-PCR. Induced levels of interleukin 1-beta (IL-1-beta), IL-2, tumor necrosis factor-alpha, and gamma-interferon (IFN-gamma) were compared among groups of individuals representing three phases of immunity to infection with *M. tuberculosis*: naive normal control subjects, purified protein derivative (PPD)-reactive normal donors, and individuals with active tuberculosis (TB [diseased]). Levels of IL-1-beta and tumor necrosis factor-alpha mRNA in co-cultured cells from TB patients were 51% and 45%, respectively, of those obtained in cells from sensitized healthy volunteers and were comparable to those from naive normal donors. Lympho-

proliferative responses to *M. tuberculosis* and induction of the T-cell cytokine IL-2 were predictably high in the cells of PPD-sensitized donors, low in normal naive individuals, and variable among TB patients. In contrast, the induced level of another lymphokine, IFN-gamma, did not follow the pattern seen in IL-2 induction. Infection with live *M. tuberculosis* induced high levels of IFN-gamma mRNA in lymphocytes of both PPD-sensitized and normal naive donors compared with those of TB patients. Interestingly, polyclonal stimulation with the mitogen concanavalin A induced similar IFN-gamma levels in cells from all three donor groups. The high level of IFN-gamma induced by the infection of monocytes from naive normal donors suggests a role for natural killer (NK) cells in the production of IFN-gamma in this co-culture system. This response appears independent of the role performed by T cells.—Authors' Abstract

Jones, B. E., Young, S. M. M., Antoniskis, D., Davidson, P. T., Kramer, F. and Barnes, P. F. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. *Am. Rev. Respir. Dis.* **148** (1994) 1292–1297.

To evaluate the relationship between the clinical presentation of tuberculosis and the CD4 cell count in patients with human immunodeficiency virus (HIV) infection, we evaluated clinical and laboratory features of 97 HIV-infected patients with tuberculosis in whom CD4 cell counts were available. Extrapulmonary tuberculosis was found in 30 (70%) of 43 patients with ≤ 100 CD4 cells/ μ l, 10 (50%) of 20 patients with 101 to 200 CD4 cells/ μ l, 7 (44%) of 16 patients with 201 to 300 CD4 cells/ μ l, and 5 (28%) of 18 patients with > 300 CD4 cells/ μ l ($p = 0.02$). Mycobacteremia was found in 18 (49%) of 37 patients with ≤ 100 CD4 cells/ μ l, 3 (20%) of 15 patients with 101 to 200 CD4 cells/ μ l, 1 (7%) of 15 patients with 201 to 300 CD4 cells/ μ l, and 0 of 8 patients with > 300 CD4 cells/ μ l ($p = 0.002$). Acid-fast smears were more often positive in patients with low CD4 cell counts. Positive tuberculin skin tests were more common in patients with high CD4 counts. On chest roentgenograms, mediastinal adenopathy

was noted in 20 (34%) of 58 patients with ≤ 200 CD4 cells/ μ l and 4 (14%) of 29 patients with > 200 CD4 cells/ μ l ($p = 0.04$). Pleural effusions were noted in 6 (10%) of 58 patients with < 200 CD4 cells/ μ l and 8 (28%) of 29 patients with > 200 CD4 cells/ μ l ($p = 0.04$). The CD8 cell counts did not correlate with the manifestations of tuberculosis. We conclude that, in HIV-infected patients, markers of severe tuberculosis, such as mycobacteremia and positive acid-fast smears, are more common in those with low CD4 cell counts. Features dependent on delayed-type hypersensitivity responses, such as positive tuberculin skin tests and tuberculous pleuritis, are more common in patients with higher CD4 cell counts. These findings suggest that CD4 cells play a central role in limiting the severity of tuberculosis.—Authors' Abstract

Kauppinen, J., Mantylarvi, R. and Katila, M.-L. Random amplified polymorphic DNA genotyping of *Mycobacterium malmoeense*. *J. Clin. Microbiol.* **32** (1994) 1827–1829.

The suitability of random amplified polymorphic DNA-PCR for the detection of differences between *Mycobacterium malmoeense* strains was evaluated. With 2 of the 32 tested primers seven fingerprint patterns which proved excellent in distinguishing intraspecies variations of *M. malmoeense* were obtained. The combination of the results obtained with the two primers permitted a clear separation of the strains. This technique is useful for analyzing species whose DNA sequences are not known. It can easily be adapted to the any mycobacterial species.—Authors' Abstract

Kauppinen, J., Pelkonen, J. and Katila, M. L. RFLP analysis of *Mycobacterium malmoeense* strains using ribosomal RNA gene probes—an additional tool to examine intraspecies variation. *J. Microbiol. Methods* **19** (1994) 261–267.

To examine the intraspecies variation of *Mycobacterium malmoeense*, 29 clinical isolates, chemotypes by thin-layer chromatography of their surface glycolipids into five subgroups, were typed using RFLP analysis. Two (32) P-labelled ribosomal RNA (rRNA)

gene fragments were used as probes for chromosomal DNA digested with BanI. By comparison of the fingerprint patterns, the strains could be divided into five ribotypes, two major and three minor ones. The chemotypes were not connected to any of the ribotypes. Combination of the results of the two techniques produced a considerable resolution. This method is a useful additional tool when studying the epidemiological behavior of *M. malmoense*.—Authors' Abstract

Kikuchi, K., Bernard, E. M., Kiehn, T. E., Armstrong, D. and Riley, L. W. Restriction fragment length polymorphism analysis of clinical isolates of *Mycobacterium haemophilum*. *J. Clin. Microbiol.* **32** (1994) 1763–1767.

Mycobacterium haemophilum is an emerging opportunistic pathogen, and since 1989, infections caused by this organism have been identified more frequently in the New York City area than in any other region of the United States. A DNA fingerprinting method, based on restriction fragment length polymorphisms (RFLPs) was developed. A genomic library of *M. haemophilum* isolate 1A was constructed; screening the library yielded a recombinant strain that incorporated a genetic element present in multiple copies in the *M. haemophilum* genome. This clone was used to produce a probe for RFLP analyses of *Pvu*II digests of genomic DNA. We used this probe to determine the RFLP patterns of 43 clinical isolates of *M. haemophilum* from 28 patients. A total of six distinct patterns were observed. Two patterns, designated types 1 and 2, accounted for 91% of the infections in patients from the New York City area. Two isolates from Arizona had identical patterns but were distinct from those of New York isolates, and an isolate from Israel, the type strain, had another distinct pattern (type 6). The type 6 pattern was also seen in a recent isolate from Norway. All of the type 1 isolates and 60% of the type 2 isolates were recovered from patients with AIDS in the New York City area. This molecular subtyping method should provide a useful tool for epidemiological studies and may help identify the associated risk factors, vehicles, and pos-

sible reservoirs of this newly emerging pathogen.—Authors' Abstract

Klemens, S. P. and Cynamon, M. H. Activities of azithromycin and clarithromycin against nontuberculous mycobacteria in beige mice. *Antimicrob. Agents Chemother.* **38** (1994) 1455–1459.

The comparative activities of azithromycin (AZI) and clarithromycin (CLA) were evaluated against nontuberculous mycobacteria in a murine model of disseminated infection. Four-week-old beige mice (C57BL/6Jbgⁱ/bgⁱ) were infected intravenously with approximately 10⁷ viable *Mycobacterium kansasii*, *M. xenopi*, *M. simiae*, or *M. malmoense*. Treatment with AZI at 200 mg/kg, CLA at 200 mg/kg, ethambutol at 125 mg/kg, rifampin at 200 mg/kg, or clofazimine at 20 mg/kg of body weight was started 7 days postinfection, and the treatments were administered 5 days per week for 4 weeks. Control groups were sacrificed at the start and end of the treatments. Spleens and lungs were homogenized, and viable cell counts were determined by serial dilution and plating onto 7H10 agar. AZI and CLA had activities comparable to or better than that of rifampin ethambutol, or clofazimine against these nontuberculous mycobacteria in the beige mouse test system. AZI at 200 mg/kg was more active than CLA at 200 mg/kg against organisms in the spleens for *M. xenopi* and *M. malmoense*. The activities of AZI and CLA were comparable against organisms in the spleens for *M. kansasii* and *M. simiae*. The activities of these two agents were comparable against organisms in the lungs for all four nontuberculous mycobacterial species. AZI or CLA in combination with other agents may be useful for the therapy of nontuberculous mycobacterial infections in humans.—Authors' Abstract

Klemens, S. P., Sharpe, C. A., Rogge, M. C. and Cynamon, M. H. Activity of levofloxacin in a murine model of tuberculosis. *Antimicrob. Agents Chemother.* **38** (1994) 1476–1479.

The activity of levofloxacin (LEV) was evaluated in a murine model of tuberculosis. Approximately 10⁷ viable *Mycobacterium tuberculosis* ATCC 35801 were given

intravenously to 4-week-old female outbred mice. In a dose-response study, treatment with LEV at 100, 200, and 400 mg/kg of body weight was started 1 day after infection and was given daily for 28 days. Viable cell counts were determined from homogenates of spleens and lungs. A dose-related reduction in organism cell counts in organs was noted for LEV. The activities of LEV at 100, 200, and 300 mg/kg were compared with those of first-line antituberculosis agents. Both isoniazid and rifampin were more active than LEV. There was no difference in activity between LEV and either ethambutol or pyrazinamide against splenic organisms. The activities of ethambutol and LEV at the two higher doses were comparable against lung organisms. LEV at 300 mg/kg was more active than pyrazinamide against lung organisms. The activity of LEV was compared with those of two other quinolones, ofloxacin and sparflaxacin. LEV at 200 mg/kg had more than twofold greater activity than ofloxacin at the same dose. Sparflaxacin at 100 mg/kg was more active than LEV at 200 mg/kg; however, the activities of sparflaxacin at 50 mg/kg and LEV at 200 mg/kg were comparable. The promising activity of LEV in *M. tuberculosis*-infected mice suggests that it is a good candidate for clinical development as a new antituberculosis agent.—Authors' Abstract

Levee, G., Glaziou, P., Gicquel, B. and Chanteau, S. Follow-up of tuberculosis patients undergoing standard antituberculosis chemotherapy by using a polymerase chain reaction. *Res. Microbiol.* **145** (1994) 5–8.

We have analyzed the clearance of *Mycobacterium tuberculosis* in sputum specimens from pulmonary tuberculosis patients undergoing 6-month chemotherapy, using the polymerase chain reaction (PCR) and standard microbiological methods. In a group of 19 patients, 11 (58%) were smear- or culture-positive and 13 (74%) were PCR-positive before treatment. Of the 16 patients followed from 2 months after the start of treatment and thereafter, all became smear-negative and culture-negative; whereas, with PCR, 4 (27%), 2 (13%) and 1 (7%) of these patients remained positive after 2, 3 and 6 months, respectively. These results suggest

the possible usefulness of PCR in monitoring the efficacy of treatment when bacteriological tests are negative, so as to identify patients with a high risk of relapse.—Authors' Abstract

Lopez-Marin, L. M., Gautier, N., Laneelle, M.-A., Silve, G. and Daffe, M. Structures of the glycopeptidolipid antigens of *Mycobacterium abscessus* and *Mycobacterium chelonae* and possible chemical basis of the serological crossreactions in the *Mycobacterium fortuitum* complex. *Microbiology* **140** (1994) 1109–1118.

Mycobacterium abscessus and *M. chelonae*, two members of the *M. fortuitum* complex, contain five major glycolipids. A combination of NMR spectroscopy, fast atom bombardment mass spectrometry and chemical degradation was used to elucidate their structures. All the compounds belong to the family of glycopeptidolipids. A 6-deoxy- α -L-talosyl unit, which may bear one or two acetyl groups, invariably occupies the site of glycosylation on the threonine residue in the various compounds. A 3,4-di-*O*-methyl- or 2,3,4-tri-*O*-methyl- α -L-rhamnosyl unit modifies the alaninol end of the diglycosylated molecules. Both species also contain a multiglycosylated compound consisting of α -L-rhamnosyl-(1 \rightarrow 2)-3,4-di-*O*-methyl- α -L-rhamnosyl linked to alaninol, which belongs to the class of new variants of glycopeptidolipids recently described. Using an ELISA, the latter glycolipid as well as the diglycosylated ones (not previously reported to be antigenic), were shown to react with the serum raised against the whole lipid antigens of *M. chelonae*. A comparative serologic study of the native and chemically modified glycopeptidolipid antigens allowed the identification of their epitope as the 3,4-di-*O*-methyl- α -L-rhamnosyl residue. Similar experiments conducted on the glycopeptidolipids isolated from the serologically crossreacting species *M. peregrinum* led to the conclusion that the epitope identified in *M. chelonae* and *M. abscessus* was involved in the crossreactions and demonstrated the existence of a second haptenic moiety in the glycolipids of *M. peregrinum*, the 3-*O*-methyl- α -L-rhamnosyl unit. In addition to this latter non-shared epitope, the recently described

sulfated glycopeptidolipid antigen of *M. peregrinum* did not react with the *M. chelonae* serum, thus further explaining the difference in the seroreactivity within the complex.—Authors' Abstract

McNeil, M. R., Robuck, K. G., Harter, M. and Brennan, P. J. Enzymatic evidence for the presence of a critical terminal hexa-arabinoside in the cell walls of *Mycobacterium tuberculosis*. *Glycobiology* 4 (1994) 165–173.

A species of *Cellulomonas* was isolated from soil by enrichment culture and shown to secrete enzymes capable of degrading mycobacterial cell-wall arabinogalactan, both the insoluble peptidoglycan-bound and base-solubilized forms. The major degradation product was purified and characterized as a hexa-arabinofuranoside, [beta-D-Araf-(1→2)-alpha-Araf-(1→)] (2)→3, 5-alpha-D-Araf-(1→5)-D-Araf. The nonreducing ends of this unit are the sites of mycolic acid attachment and, as they also appear in lipoarabinomannan (LAM), the point of mannose capping in some mycobacteria. Thus, elaboration of the structure of this focal hexasaccharide is critical to our understanding of much of the physiology and pathogenesis of mycobacteria. The extracellular enzymes of *Cellulomonas* sp. also released the disaccharide alpha-D-Araf-(1→5)-D-Araf from internal linear regions of arabinan and, surprisingly, convert the linear galactan backbone into cyclic oligosaccharides of the structure [→5-D-Galf-(1→6)-beta-D-Galf(1→)] (n) where n is 2, 3 or 4. Thus, the preparation contains Scharinger-like enzyme activity. This group of enzymes are powerful tools for the dissection of the mycolylarabino-galactan-peptidoglycan (mAGP) complex of mycobacteria toward understanding its role in drug resistance, disease processes and mycobacterial physiology.—Authors' Abstract

Mor, N., Van der Kolk, J. and Heifets, L. Inhibitory and bactericidal activities of levofloxacin against *Mycobacterium tuberculosis* *in vitro* and in human macrophages. *Antimicrob. Agents Chemother.* 38 (1994) 1161–1164.

Levofloxacin exhibited twofold greater inhibitory and bactericidal activities than

ofloxacin against either extracellular or intracellular tubercle bacilli. The activities of both drugs against extracellular and intracellular bacteria were about the same, despite the accumulation of the drugs in macrophages at a level four- to fivefold greater than that in the extracellular medium. The activities of both drugs against intracellular bacteria were largely associated with the short, 2-hr pulsed exposures of the infected macrophages to the concentrations which correspond to those attainable in blood during the period of the maximum concentration of drug in serum.—Authors' Abstract

Myatt, N., Coghill, G., Morrison, K., Jones, D. and Cree, I. A. Detection of tumor necrosis factor alpha in sarcoidosis and tuberculosis granulomas using *in situ* hybridization. *J. Clin. Pathol.* 47 (1994) 423–426.

The aims were to determine the site of tumor necrosis factor alpha (TNF- α) product and mRNA in granulomas. *In situ* hybridization with digoxigenin labelled or biotinylated oligonucleotide probes was used to demonstrate the presence of total mRNA, and then the presence of TNF- α mRNA in the biopsy specimens of 37 granulomas (31 sarcoidosis, 6 tuberculosis). TNF- α mRNA was detected in epithelioid cells, giant cells, and lymphocytes in the granulomas. Some sarcoidosis specimens did not contain detectable mRNA for TNF, but did contain TNF peptide in the epithelioid or giant cells on immunostaining. This may have been due to stored TNF present in cells in which mRNA for TNF is no longer being produced. The results suggest that giant cells should not be regarded as effete cells, since they contain large amounts of mRNA and seem to be actively producing TNF- α .—Authors' Abstract

Pascopella, L., Collins, F. M., Martin, J. M., Lee, M. H., Hatfull, G. F., Stover, C. K., Bloom, B. R. and Jacobs, W. R. Use of *in vivo* complementation in *Mycobacterium tuberculosis* to identify a genomic fragment associated with virulence. *Infect. Immun.* 62 (1994) 1313–1319.

Novel molecular tools and genetic methods were developed to isolate genomic frag-

ments of *Mycobacterium tuberculosis* that may be associated with virulence. We sought to restore virulence, a characteristic of *M. tuberculosis* that is correlated with growth rate in mouse spleen and lung tissue, to the avirulent strain H37Ra by complementation. A representative library of the virulent *M. tuberculosis* strain H37Rv was constructed and transformed into H37Ra. Enrichment for individual faster-growing recombinants was achieved by passage of pools of H37Ra transformants harboring the H37Rv library through mice. A molecular strategy was devised to isolate and clone the H37Rv genomic DNA fragment *ivg*, which conferred a more rapid *in vivo* growth rate to H37Ra.—Authors' Abstract

Pastrana, J. and Espana, A. Erythema nodosum and abdominal tuberculosis. *Eur. J. Dermatol.* **4** (1994) 221–222.

Erythema nodosum (EN) is a form of septal panniculitis. No cause is found in up to 50% of cases. It is associated with primary tuberculosis in children. The association between EN and abdominal tuberculosis, a rare form of tuberculosis, is unusual. We report the case of a patient with EN and tuberculous mesenteric lymphadenitis. A diagnosis of *Mycobacterium tuberculosis* infection was made on the ground of EN because the abdominal tuberculosis was asymptomatic. In view of the rise in tuberculosis in recent years, we should alert ourselves to this possible association.—Authors' Abstract

Plikaytis, B. B., Marden, J. L., Crawford, J. T., Woodley, C. L., Butler, W. R. and Shinnick, T. M. Multiplex PCR assay specific for the multidrug-resistant strain W of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **32** (1994) 1542–1546.

In 1991, a multidrug-resistant strain of *Mycobacterium tuberculosis* was isolated from eight people with tuberculosis at a state correctional facility in New York. This strain, which is designated strain W (IS6110 restriction fragment length polymorphism type 212072), was resistant to isoniazid, rifampin, ethambutol, streptomycin, kanamycin, ethionamide, and rifabutin. Since that outbreak, the W strain has been associated with outbreaks in five hospitals in the

New York City area, and is a continuing public health problem in the area. To be able to identify this strain rapidly, we developed a multiplex PCR assay which targets a direct repeat of IS6110 with a 556-bp intervening sequence (NTF-1). The amplification generates two amplicons from strain W, which indicate the presence and orientation of the NTF-1 sequence between the direct repeat of IS6110, and a third amplicon, which serves as an internal PCR control. The assay was evaluated with 193 isolates of *M. tuberculosis*, and all 48 strain W isolates among those 193 isolates were correctly identified.—Authors' Abstract

Pollock, J. M., Douglas, A. J., Mackie, D. P. and Neill, S. D. Identification of bovine T-cell epitopes for three *Mycobacterium bovis* antigens: MPS700, 19,000 MW and MPB57. *Immunology* **82** (1994) 9–15.

Bovine tuberculosis remains a serious problem in several regions, partly due to a lack of specific diagnostic tests. The aim of this study was to identify bovine T-cell epitopes for defined *Mycobacterium bovis* antigens using an experimental model of the natural disease. Panels of synthetic peptides (16-mers with five residue overlaps) were produced from published amino acid sequences for MPB70, the 19,000 MW antigen and MPB57. *In vitro* lymphocyte proliferation assays were used to identify T-cell epitopes. Lymphocytes from experimentally infected cattle proliferated in response to five epitopes (residues 88–105 and 144–163 for MPB70; 1–16 and 67–84 for the 19,000 MW antigen; and 85–100 for MPB57). These epitopes were not recognized by control, noninfected animals, but were recognized by field reactors to intradermal tuberculin testing. All five epitopes were recognized by three different breeds of cattle (Friesian, Charolais and Simmental). In addition, the bovine T-cell epitopes identified for the 19,000 MW antigen in this study were similar to epitopes previously reported for man and mouse. Thus, as well as identifying candidate reagents for improved diagnostic tests and vaccination, this study provides evidence for genetic promiscuity in T-cell recognition of major mycobacterial epitopes.—Authors' Abstract

Pourshafie, M. R., Davis, S. A. and Sonnenfeld, G. Cellular interactions of murine immune cells exposed to live *Mycobacterium intracellulare*, its whole lipid extract, and its serovar-specific glycopeptidolipid. *Cell. Immunol.* **155** (1994) 11–26.

In this study we examined some of the immunological responses to *Mycobacterium intracellulare* and its lipid components. Our results indicate that infection with *M. intracellulare* can increase the expression of adhesion molecules, ICAM-1 and LFA-1, only at the site of injection (peritoneum). There was no change in the expression of these adhesion molecules in the lymphoid organs (thymus and spleen). Significant increases in the adhesion molecules were observed in the spleen cells incubated with the lipid derived from mycobacteria in the presence of concanavalin (ConA) compared to the ConA alone. The expression of the Thy 1.2 and Lyt-2 markers was not affected by the bacteria or their lipids. The results indicate a marked increase in the mitogenic response by the infected spleen cells removed at an early day. The blastogenic study also indicated that the lipids can reduce the mitogen-induced blastogenesis of spleen cells removed from *M. intracellulare*- and saline-injected mice; moreover, they suggested that the spleen cells removed from *Listeria monocytogenes*-infected mice can also be affected by mycobacterial lipids. This indicates a nonspecific effect by these lipids. The results suggest that the immunological response was contingent upon prior exposure of the mice to *M. intracellulare*, and also was dependent on whether the cells came from the peritoneal cavity or lymphoid organs.—Authors' Abstract

Prinzis, S., Rivoire, B. and Brennan, P. J. Search for the molecular basis of morphological variation in *Mycobacterium avium*. *Infect. Immun.* **62** (1994) 1946–1951.

Isolates of *Mycobacterium avium* exhibit three different colonial variations: smooth domed (SmD), smooth transparent (SmT), and rough (Rg). Because the discrimination between morphotypes is founded on morphological rather than molecular principles

and because of the absence of consensus over the relevance of morphology to pathogenesis and drug sensitivity, a comparative study at the protein level was undertaken. By direct immunization of BALB/c mice with the soluble sonicate of one of the morphotypes of *M. avium* serovar 2, eight monoclonal antibodies (MAbs) were identified, of which one was *M. avium* specific. Cross-immunization of syngeneic mice with serum-absorbed antigens allowed the generation of 15 further MAbs; 11 were *M. avium* or *M. avium*-complex specific, but none of them was morphotype specific. Subcellular fractions analyzed by electrophoresis showed similar profiles, with the exception of a cytosolic protein with a relative molecular mass of ca. 66 kDa (protein SmT 66), which was most highly expressed in SmT variants of *M. avium* serotypes 2 and 4. Because a well-known, ubiquitous stress-heat shock protein (hsp65) has a similar molecular mass, protein SmT 66 was compared with hsp65. Western blot (immunoblot) analyses using several crossreacting MAbs and N-terminal amino acid sequencing established that this protein was not the ubiquitous stress protein. Thus, SmT 66 is the first product to be described which might be associated with the SmT morphotype.—Authors' Abstract

Rastogi, N., Goh, K. S. and Bryskier, A. Activities of roxithromycin used alone and in combination with ethambutol, rifampin, amikacin, ofloxacin and clofazimine against *Mycobacterium avium* complex. *Antimicrob. Agents Chemother.* **38** (1994) 1433–1438.

Preliminary studies showed that roxithromycin possessed significant *in vitro* activity against a variety of atypical mycobacteria such as the *Mycobacterium avium* complex, *M. scrofulaceum*, *M. szulgai*, *M. malmoense*, *M. xenopi*, *M. marinum*, and *M. kansasii* and rare pathogens such as *M. chelonae* and *M. fortuitum*. In this investigation, radiometric MICs of roxithromycin, ethambutol, rifampin, amikacin, ofloxacin, and clofazimine for 10 clinical isolates of the *M. avium* complex (5 each from human immunodeficiency virus [HIV]-positive and HIV-negative patients) were determined. Roxithromycin MICs against all the isolates

were below the reported maximum concentration of drug in serum at the routine pH of 6.8, and the MICs were further lowered by 1 to 2 dilutions at a pH of 7.4. *In vitro* enhancement of roxithromycin activity against all strains was further investigated by the previously established BACTEC 460-TB method by combining the drugs at sub-MIC levels. Antibacterial activity of roxithromycin was enhanced in all 10 strains by ethambutol, in 3 strains each by rifampin and clofazimine, in 2 strains by amikacin, and in 1 strain by ofloxacin. *In vitro* screening of three-drug combinations showed that combinations of roxithromycin, ethambutol, and a third potential anti-*M. avium* drug (rifampin, amikacin, ofloxacin, or clofazimine) resulted in further enhancement of activity in 13 out of 20 drug combinations screened.—Authors' Abstract

Schlesinger, L. S., Hull, S. R. and Kaufman, T. M. Binding of the terminal mannosyl units of lipoarabinomannan from a virulent strain of *Mycobacterium tuberculosis* to human macrophages. *J. Immunol.* **152** (1994) 4070–4079.

Recent studies from this laboratory have demonstrated that macrophage phagocytosis of virulent strains (Erdman and H37Rv), but not the attenuated H37Ra strain of *Mycobacterium tuberculosis*, is mediated by phagocyte mannose receptors (MR) in addition to complement receptors (CR1 and the leukocyte integrins CR3 and CR4). Lipoarabinomannan (LAM) is a major surface lipoglycan of *M. tuberculosis*. LAM from the Erdman strain (ManLAM) contains mannose oligosaccharides at the terminal portions of the molecule. This study investigated the ability of ManLAM to serve as a microbial ligand in adherence to human monocyte-derived macrophages (MDM). Polystyrene microspheres were coated with known amounts of purified ManLAM, LAM without the terminal mannosyl units from an avirulent mycobacterium (AraLAM), lipomannan (LM), or buffer and incubated with MDM monolayers in the absence of serum. The presence of LAM on microspheres was confirmed by indirect immunofluorescence studies. Microspheres coated with ManLAM demonstrated a more than threefold increase in adherence to

MDM when compared with microspheres coated with AraLAM, LM, or buffer and the low levels of adherence of microspheres in the latter three groups were comparable. Compared with control monolayers, selective down-modulation of MDM MR on a mannan substrate abrogated the enhanced adherence of microspheres mediated by ManLAM. Adherence of microspheres coated with AraLAM, LM, or buffer was not influenced by MR modulation. To confirm the importance of the terminal mannosyl units of ManLAM in the enhanced adherence of ManLAM microspheres to MDM, these units were selectively removed by exomannosidase treatment. The structure of LAM products before and after enzyme treatment was confirmed by high performance anion exchange chromatography with pulsed amperometric detection. Removal of the terminal mannosyl units abolished the capacity of ManLAM to mediate enhanced adherence of microspheres to MDM. Finally, preincubation of Erdman *M. tuberculosis* with CS-40, a mAb directed against LAM, resulted in a consistent inhibition of adherence of the bacteria to MDM (up to 49% inhibition), confirming a role for ManLAM on intact bacteria in adherence to MDM. Thus, we provide evidence for a novel receptor-ligand pathway in phagocytosis of *M. tuberculosis* that consists of MR on macrophages and mannosyl units at the terminal end of ManLAM, a major microbial surface lipoglycan.—Authors' Abstract

Sepkowitz, K. A., Telzak, E. E., Recalde, S., Armstrong, D., Berger, J., Berkowitz, L., Blevins, A., Brassel, J., et al. Trends in the susceptibility of tuberculosis in New York City. *Clin. Infect. Dis.* **18** (1994) 755–759.

The annual number of cases of tuberculosis in New York City has increased since 1978. In addition, in 1991 a 1-month survey of cases of tuberculosis in New York City found that 33% of all cases were resistant to at least one drug. To determine susceptibility trends from 1987 to 1991, a period during which an unprecedented rise in resistant tuberculosis occurred in New York City, we reviewed the microbiology records of 44 New York City hospitals (comprising

> 14,000 cases). The percentage of cases resistant to at least one drug rose from 19% in 1987 to 28% in 1991, and the percentage of cases resistant to isoniazid rose from 13% in 1987 to 23% in 1991, while resistance to at least both isoniazid and rifampin rose from 6% to 14%. The rise of multidrug-resistant tuberculosis occurred in all four surveyed boroughs (counties) of New York City. These data demonstrate how rapidly multidrug-resistant tuberculosis can appear, and they suggest that initial empirical regimens should be broadened at certain hospitals.—Authors' Abstract

Shijubo, N., Imai, K., Nakanishi, F., Yachi, A. and Abe, S. Elevated concentrations of circulating ICAM-1 in far advanced and miliary tuberculosis. *Am. Rev. Respir. Dis.* **148** (1994) 1298–1301.

Intercellular adhesion molecule-1 (ICAM-1) plays an important role in inflammatory diseases. Cellular expression and shedding of ICAM-1 are up-regulated by cytokines, such as tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ). With an ELISA containing two monoclonal antibodies to human ICAM-1, we measured concentrations of circulating ICAM-1 in patients with tuberculosis. Prominently elevated values were found in miliary tuberculosis and in far advanced pulmonary tuberculosis but not in minimal or moderately advanced disease. The measurement of serum IFN- γ and TNF- α also revealed high concentrations of the cytokines in miliary tuberculosis and far advanced pulmonary tuberculosis. The circulating ICAM-1 values were significantly correlated to serum IFN- γ and TNF- α values. In the case of miliary tuberculosis and far advanced pulmonary tuberculosis treated with anti-tuberculous drugs, the circulating ICAM-1 concentrations were gradually decreased to correspond with improvement of clinical symptoms and chest X-ray findings. The measurement of circulating ICAM-1 is useful to evaluate the severity of tuberculosis and to monitor disease activity during antituberculous drug treatment.—Authors' Abstract

Singh, S. and Hogan, S. E. Isolation and characterization of sugar nucleotides from

Mycobacterium smegmatis. *Microbios* **77** (1994) 217–222.

The cell walls of *Mycobacterium smegmatis* contain a number of serologically active polysaccharides such as arabinomannan, mannan, and glucan in addition to arabinogalactan. The biosynthetic pathways of these polysaccharides are not well understood. Characterization of the sugar nucleotide pool of *M. smegmatis* showed the presence of (uridine diphosphate-) UDP-glucose, UDP-galactose, UDP-mannose, UDP-arabinose and UDP-hexuronic acid(s). It is suggested that these compounds may be intermediates in the biosynthesis of a number of mycobacterial polysaccharides.—Authors' Abstract

Slutsky, A. M., Arbeit, R. D., Barber, T. W., Rich, J., von Reyn, C. F., Pieciak, W., Barlow, M. A. and Maslow, J. N. Polyclonal infections due to *Mycobacterium avium* complex in patients with AIDS detected by pulsed-field gel electrophoresis of sequential clinical isolates. *J. Clin. Microbiol.* **32** (1994) 1773–1778.

Invasive infection with organisms of the *Mycobacterium avium* complex (MAC) is common among patients with advanced human immunodeficiency virus infection. In previous studies, we analyzed multiple individual colonies of MAC isolated from specimens obtained at the same time and observed that 14% to 20% of patient are simultaneously infected with more than one strain. In this study, we examined sequential isolates from 12 patients with AIDS who had two or more MAC isolates available from clinical specimens collected more than 1 week apart; the intervals between the first and last specimens ranged from 8 to 192 (median 46) days. For each isolate, restriction digests of genomic DNA were analyzed by pulsed-field gel electrophoresis; DNA was prepared by using a protocol, described here in detail, which had been optimized for conditions of bacteria growth and lysis. The pulsed-field gel electrophoresis analysis identified four patients (33%) infected with two different MAC strains. Both *M. avium* and *M. intracellulare* were cultured from blood specimens from two patients. In each of the four patients, the second strain was

identified from a culture taken within 14 days of the initial study isolate, and in three of these patients, the first strain was detected again in a subsequent culture. These observations suggest that the presence of two different strains among isolates from sequential cultures may reflect ongoing polyclonal infection. We conclude that polyclonal infection with MAC is common among patients with AIDS. The identification of such infections may be critical in the development of effective treatments.—Authors' Abstract

Sripathamathan, R. M., Plumb, J. A. and Fearon, K. C. H. Clofazimine alters the energy metabolism and inhibits the growth rate of human lung-cancer cell line *in vitro* and *in vivo*. *Int. J. Cancer* **6** (1994) 900–905.

The antileprosy drug clofazimine is known to inhibit respiratory function and, hence, energy metabolism in yeast and in transformed fibroblasts. The aim of this study was to examine the effect of clofazimine on the energy metabolism of a chemoresistant human non-small-cell bronchial-carcinoma cell line (WIL) and to determine whether this agent might inhibit the growth rate of this cell line *in vitro* and *in vivo*. Oxidative phosphorylation was estimated *in vitro* by measuring oxygen consumption polarographically and glycolysis was estimated from lactate production. In cells that had been pretreated with an ATP synthetase inhibitor (oligomycin), the addition of clofazimine resulted in an increase in oxygen consumption similar to that observed with 2,4-dinitrophenol, a classical inhibitor of oxidative phosphorylation. This inhibition of mitochondrial function was associated with an increase in lactate production. Cellular ATP levels were maintained, possibly indicating a compensatory increase in ATP production via glycolysis. Clofazimine was shown to have a direct cytotoxic effect *in vitro* with an ID₅₀ of 10.2 μ M. When clofazimine was administered to athymic mice bearing WIL as a subcutaneous xenograft, tumor growth rate was significantly reduced, so that after 3 weeks, tumor size was one third that of controls ($p < 0.01$). These results suggest that selective inhibition of tumor energy metabolism with agents such

as clofazimine is a potential novel approach to cancer treatment.—Authors' Abstract

Suster, S., Moran, C. A. and Blanco, M. Mycobacterial spindle-cell pseudotumor of the spleen. *Am. J. Clin. Pathol.* **101** (1994) 539–542.

A case of spindle-cell pseudotumor of the spleen due to nontuberculous mycobacteria in a patient with acquired immunodeficiency syndrome (AIDS) is described. The patient was a 55-year-old, human immunodeficiency virus-positive Haitian man who died of acute neurologic complications while on treatment for central nervous system toxoplasmosis. At autopsy, an enlarged multinodular spleen was noted. Histologic examination revealed coarse nodules of splenic parenchyma replaced by a dense spindle-cell proliferation, admixed with scattered inflammatory cells. Immunostains showed strong cytoplasmic positivity of the spindle cells with MAC 387, HAM 56, and alpha-1-antichymotrypsin antibodies and negative staining for actin, vimentin, and S-100 protein antibodies. Ziehl-Neelsen stains revealed numerous elongated acid-fast bacilli within the cytoplasm of the cells that were occasionally lying free within the interstitium. The organisms also had a strongly positive reaction with antibodies to desmin intermediate filaments. Mycobacterial spindle-cell pseudotumor should be included in the differential diagnosis of conditions affecting the spleen in patients with AIDS.—Authors' Abstract

Tomioka, H. and Saito, H. Phospholipids of *Mycobacterium intracellulare* inhibit T cell blastogenesis. *Microbiology* **140** (1994) 829–837.

A crude lipid fraction obtained from *Mycobacterium intracellulare* (MI whole lipids) suppressed concanavalin A (ConA)-induced blastogenesis of murine spleen cells (SPCs). Among three lipid fractions, the phospholipid fraction possessed the highest inhibitory activity, followed by the polar mycoside fraction, but the apolar mycoside fraction showed no activity. Since MI whole lipid and phospholipid fractions inhibited the ConA-induced proliferative response of SPCs which had been treated with ConA

before the addition of the lipid fractions, their action is not due to hindrance of ConA binding to T cells. These two MI lipid fractions reduced IL-2-producing ability, acquisition of IL-2 reactivity, and expression of IL-2 receptors in ConA-stimulated T cells. However, they did not affect IL-2-induced proliferation of an IL-2-dependent cytotoxic T cell line, CTLL-2. When SPCs were pretreated with either MI whole lipid or phospholipid fraction for 24 hr, an irreversible reduction in ConA responsiveness was seen only in the phospholipid-treated SPCs. The action of these MI lipid fractions was phase-dependent and they exhibited considerably decreased but still significant inhibitory activity against ConA blastogenesis of SPCs, even when they were added at 24 hr after the initiation of SPC culture with ConA. MI whole lipids and the three lipid fractions (polar mycoside, apolar mycoside, and phospholipid fractions) did not exhibit suppressor cell-inducing activity, while MI whole lipid fraction antagonized the ConA-mediated generation of suppressor cells. Silica gel thin-layer chromatography of the phospholipid fraction showed four spots containing phosphate and one spot without. SPC ConA blastogenesis-inhibitory activity was shared by the two least polar phosphate-containing substances. One of those apparently contained amino groups and carbohydrate moieties. The second component contained no such moieties.—Authors' Abstract

Tortoli, E., Simonetti, M. T., Lacchini, C., Penati, V. and Urbano, P. Tentative evidence of AIDS-associated biotype of *Mycobacterium kansasii*. *J. Clin. Microbiol.* **32** (1994) 1779–1782.

Previous studies revealed heterogeneous behavior within the species *Mycobacterium kansasii* against commercially available DNA probes (Accuprobe *M. kansasii* culture identification test; Gen-Probe); several isolates, conventionally identified as *M. kansasii*, failed in fact to hybridize. Looking for a possible association with phenotypic features, we tested a fully characterized panel of 69 clinical isolates of *M. kansasii* (19 of which were Accuprobe negative) with a semiquantitative micromethod which tests for 19 enzymatic activities (Api Zym;

BioMérieux). The strains were from 25 hospitals in 18 Italian towns; 20 isolates came from human immunodeficiency virus type 1-positive patients who fulfilled the Centers for Disease Control criteria for AIDS diagnosis. On the basis of the whole set of phenotypic traits, our strains clustered in two groups, allowing the differentiation of biotypes within the species. There was a perfect association between biotype 2 and hybridization failures with Accuprobe and a very significant association between this novel biotype 2 and AIDS status, which suggests that it differs in virulence.—Authors' Abstract

Turrett, G. S. and Telzak, E. E. Normalization of CD3 (+) T-lymphocyte depletion in patients without HIV infection treated for tuberculosis. *Chest* **105** (1994) 1335–1337.

A decrease in the number of circulating CD4(+) T-lymphocytes occurs in subjects infected with the human immunodeficiency virus (HIV). In those without HIV infection, depletion of T-lymphocytes in general and CD4(+) cells in particular has been reported in association with many underlying conditions, including tuberculosis. A low CD4(+) T-lymphocyte count at the time of diagnosis of tuberculosis does not clarify whether the low count is a predisposing factor for or a consequence of the disease. Our patients without HIV infection but with tuberculosis and CD4(+) T-lymphocyte depletion on presentation normalized their CD4(+) cell counts with tuberculosis treatment. This normalization strongly suggests that tuberculosis is a reversible cause of CD4(+) lymphocytopenia.—Authors' Abstract

van der Giessen, J. W. B., Haring, R. M. and van der Zeijst, B. A. M. Comparison of the 23S ribosomal RNA genes and the spacer region between the 16S and 23S rRNA genes of the closely related *Mycobacterium avium* and *Mycobacterium paratuberculosis* and the fast-growing *Mycobacterium phlei*. *Microbiology* **140** (1994) 1103–1108.

The 23S rDNA sequences of *Mycobacterium paratuberculosis*, *M. avium* and *M.*

phlei and the sequences of the spacer regions between the 16S and 23S rRNA genes were determined. The overall 23S rDNA sequence identity between *M. paratuberculosis* and *M. avium* was 99.7% (nine mismatches), showing the very close relatedness of these mycobacteria. Evolutionary distances between the five known mycobacterial 23S rRNA/rDNA sequences and those of other gram-positive G + C-rich bacteria were determined. The 23S rDNA sequences of mycobacteria showed two inserted regions compared to the other bacteria. A mycobacterial unique region contained one mismatch between *M. paratuberculosis* and *M. avium*. An *Actinomycetales*-specific insertion, consisting of 111 nucleotides, was completely identical for *M. paratuberculosis* and *M. avium*. The sequence of the intergenic spacer region between 16S and 23S rDNA had a length of 278 bp for *M. paratuberculosis* and *M. avium* with only two mismatches. The spacer region of the fast-growing *M. phlei* was 85-bp longer. No tRNA-encoding region was found in the spacer region.—Authors' Abstract

van Noort, J. M., Anderton, S. M., Wagenaar, J. P. A., Wauben, M. H. M., van Holten, C. and Boog, C. J. P. Differential rat T cell recognition of cathepsin D-released fragments of mycobacterial 65 kDa heat-shock protein after immunization with either the recombinant protein or whole mycobacteria. *Int. Immunol.* **6** (1994) 603–609.

T cells specific for the mycobacterial 65-kDa heat-shock protein (hsp65) play a pivotal role in the development of adjuvant arthritis (AA) in Lewis rats. Upon adoptive transfer, CD4(+) T cells recognizing a particular hsp65 epitope trigger the onset of disease. Activation of hsp65-reactive T cells can be achieved by immunization with heat-killed mycobacteria in mineral oil—complete Freund's adjuvant (CFA)—or with purified recombinant hsp65. Arthritis, however, will only develop after immunization with CFA. In fact, preimmunization with hsp65 protects against any subsequent attempt to induce AA. In this study, we examined polyclonal lymph node cell responses in Lewis rats, immunized with either CFA or purified recombinant hsp65 in in-

complete Freund's adjuvant, to a set of hsp65 fragments generated by a mild digestion with cathepsin D. Proliferative responses to several hsp65 fragments varied with the type of antigen used for immunization. A cathepsin D-released fragment, identified as residues 376–408, preferentially triggered proliferation of rat T cells after hsp65 immunization. Preimmunization of Lewis rats with this peptide delayed the onset and reduced the severity of AA. Preimmunization with another fragment which was preferentially recognized after CFA immunization, representing residues 40–60, did not have such a protective effect. Our findings suggest the presence of mycobacterial hsp65 determinants that selectively trigger AA-regulating T cells and illustrate that cathepsin D may be used as an experimental tool to generate such determinants.—Authors' Abstract

van Puyvelde, L., Ntawukiliyayo, J. D., Portaels, F. and Hakizamungu, E. *In vitro* inhibition of mycobacteria by Rwandese medicinal plants. *Phytother. Res.* **8** (1994) 65–69.

A series of 15 Rwandese medicinal plants used by traditional healers to treat pulmonary diseases were screened for anti-*Mycobacterium tuberculosis* activity. Three plant extracts showed activity at 1000 µg/ml: *Bidens pilosa* (leaves), *Pentas longiflora* (roots) and *Tetradenia riparia* (leaves). These plant extracts were studied further against several mycobacterial species: *M. tuberculosis*, *M. avium* complex, *M. simiae* and a new *simiae*-like *Mycobacterium* species (SLM). None of the plant extracts showed activity against *M. avium* and SLM at 1000 µg/ml. *M. simiae* was sensitive to *T. riparia* and *P. longiflora* extracts (1000 µg/ml) and *M. tuberculosis* toward *T. riparia* (500 µg/ml) and *B. pilosa* (100 µg/ml). The active principle of *T. riparia*, 8(14), 15-sandaracopimaradiene-7α, 18-diol, was also evaluated for its antimycobacterial activity and the concentration required for inhibiting *M. tuberculosis* ranged from 25 µg/ml to 100 µg/ml. The extract of the leaves of *T. riparia* can be used as a differentiation test (at 1000 µg/ml in the medium) for *M. simiae* and SLM.—Authors' Abstract

von Reyn, C. F., Maslow, J. N., Barber, T. W., Falkinham, J. O. and Arbeit, R. D. Persistent colonisation of potable water as a source of *Mycobacterium avium* infection in AIDS. *Lancet* **343** (1994) 1137–1141.

The source of *Mycobacterium avium* infection in AIDS has not been identified, and it is not known whether most patients with AIDS acquire the organism from recent infection or by reactivation of previous infection. As part of a prospective epidemiological study, we isolated multiple colonies of *M. avium* from patients with AIDS and from potable water to which they had been exposed. All isolates were analyzed with pulsed field gel electrophoresis (PFGE). As judged by PFGE, 29 (81%) of 36 patients were infected with one or more unique clinical strains of *M. avium*; 7 patients (19%) were infected with three groups of common strains. Group 1 included 3 patients who lived in separate rural areas and had no common exposures apart from treatment at hospital A. The same strain was isolated repeatedly during 41 months from a recirculating hot water system at hospital A; residential water cultures were negative. Group 2 included 2 patients with no common exposures apart from treatment at hospital B; the same strain was isolated repeatedly over a period of 24 months from a recirculating hot water system at hospital B. Patients in

groups 1 and 2 had numerous possible exposures to hospital hot water. Group 3 included 2 patients treated at the same methadone treatment facility. In an institution the hot water system may be persistently colonized with a particular strain of *M. avium*. HIV-infected patients exposed to these water sources can develop disseminated *M. avium* infection.—Authors' Abstract

Weglicki, W. B., Stafford, R. E., Dickens, B. F., Mak, I. T., Cassidy, M. M. and Phillips, T. M. Inhibition of tumor necrosis factor-alpha by thalidomide in magnesium deficiency. *Mol. Cell. Biochem.* **129** (1993) 195–200.

The effect of thalidomide on circulating cytokines and myocardial lesion formation was investigated in Mg-deficient rats. After 2 weeks on a Mg-deficient diet, rats show an increase in circulating levels of tumor necrosis factor-alpha and interleukin 1 (IL-1). Thalidomide (1 mg/day) caused a complete inhibition of the increase in circulating tumor necrosis factor-alpha levels, without having an effect on IL-1. However, a marked increase in cardiomyopathic lesion formation was observed in Mg-deficient animals treated with thalidomide; possible mechanisms for thalidomide's enhancement of myocardial injury are discussed.—Authors' Abstract