# CURRENT LITERATURE

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

## General and Historical

**Jacob, M. S., Amar, D., Christopher, A. and Keystone, J. S.** Transmission of health information on leprosy from children to their families in an urban centre. Lepr. Rev. **65** (1994) 272–278.

A health education study utilizing a homework assignment was carried out in a private secondary school in Bangalore, South India, to determine whether health information about leprosy would be transferred from children to their families. After a pretest questionnaire on knowledge and attitude about leprosy was administered to three Standard VII classes and their family members, a different comprehensive health education session was given to each class: (i) leprosy plus a homework assignment; (ii) leprosy alone; and (iii) tuberculosis alone. A post-test questionnaire was administered to all participants 1 month later.

Of the 118 children and 229 family members who entered the study, almost 80% of the participants completed it. The children in the leprosy-educated groups showed significant improvement in knowledge compared with controls, but no change in their attitude toward leprosy. Although post-test responses of household members showed modest improvement in knowledge about leprosy, attitudes remained the same or worsened. The homework assignment did not appear to improve the transmission of health information to household members. This study showed that the knowledge level of family members in South India could be improved modestly by educating their children about leprosy. However, attitudes toward leprosy were unaffected or worsened.—Authors' Summary

**Murray, C. J. L.** Quantifying the burden of disease: the technical basis for disability-adjusted life years. Bull. WHO **72** (1994) 429–445.

Detailed assumptions used in constructing a new indicator of the burden of disease, the disability adjusted life year (DALY), are presented. Four key social choices in any indicator of the burden of disease are carefully reviewed. First, the advantages and disadvantages of various methods of calculating the duration of life lost due to a death at each age are discussed. DALYs use a standard expected-life lost based on model life-table West Level 26. Second, the value of time lived at different ages is captured in DALYs using an exponential function which reflects the dependence of the young and the elderly on adults. Third, the time lived with a disability is made comparable with the time lost due to premature mortality by defining six classes of disability severity. Assigned to each class is a severity weight between 0 and 1. Finally, a 3% discount rate is used in the calculation of DALYs. The formula for calculating DALYs based on these assumptions is provided.—Authors' Abstract

**Murray, C. J. L. and Lopez, A. D.** Global and regional cause-of-death patterns in 1990. Bull. WHO **72** (1994) 447–480.

Demographic estimation techniques suggest that worldwide about 50 million deaths occur each year, of which about 39 million are in the developing countries. In countries with adequate registration of vital statistics, the age at death and the cause can be reliably determined. Only about 30%-35% of all deaths are captured by vital registration (excluding sample registration schemes); for the remainder, cause-of-death estimation procedures are required. Indirect methods which model the cause-of-death structure as a function of the level of mortality can provide reasonable estimates for broad cause-of-death groups. Such methods are generally unreliable for more specific causes. In

this case, estimates can be constructed from community-level mortality surveillance systems or from epidemiological evidence on specific diseases. Some check on the plausibility of the estimates is possible in view of the hierarchical structure of cause-of-death lists and the well-known age-specific patterns of diseases and injuries.

The results of applying these methods to estimate the causes of death for over 120 diseases or injuries, by age, sex and region, are described. The estimates have been derived in order to calculate the years of life lost due to premature death, one of the two components of overall disability-adjusted life years (DALYs) calculated for the 1993 World development report. Previous attempts at cause-of-death estimation have been limited to a few diseases only, with little age-specific detail. The estimates reported in detail here should serve as a useful reference for further public health research to support the determination of health sector priorities.—Authors' Abstract

**Murray, C. J. L. and Lopez, A. D.** Quantifying disability: data, methods and results. Bull. WHO **72** (1994) 481–494.

Conventional methods for collecting, analyzing and disseminating data and information on disability in populations have relied on cross-sectional censuses and surveys which measure prevalence in a given period. While this may be relevant for defining the extent and demographic pattern of disabilities in a population, and thus indicating the need for rehabilitative services, prevention requires detailed information on the underlying diseases and injuries that cause disabilities. The Global Burden of Disease methodology described in this paper provides a mechanism for quantifying the health consequences of the years of life lived with disabilities by first estimating the age-sex-specific incidence rates of underlying conditions, and then mapping these to a single disability index which collectively reflects the probability of progressing to a disability, the duration of life lived with the disability, and the approximate severity of the disability in terms of activity restriction. Detailed estimates of the number of disability-adjusted life years (DALYs) lived are provided in this paper, for eight geographical regions. The results should be useful to those concerned with planning health services for the disabled and, more particularly, with determining policies to prevent the underlying conditions which give rise to serious disabling sequelae.—Authors' Abstract

**Murray, C. J. L., Lopez, A. D. and Jamison, D. T.** The global burden of disease in 1990: summary results, sensitivity analysis and future directions. Bull. WHO **72** (1994) 495–509.

A basic requirement for evaluating the cost-effectiveness of health interventions is a comprehensive assessment of the amount of ill health (premature death and disability) attributable to specific diseases and injuries. A new indicator, the number of disability-adjusted life years (DALYs), was developed to assess the burden of disease and injury in 1990 for over 100 causes by age, sex and region. The DALY concept provides an integrative, comprehensive methodology to capture the entire amount of ill health which will, on average, be incurred during one's lifetime because of new cases of disease and injury in 1990. It differs in many respects from previous attempts at global and regional health situation assessment which have typically been much less comprehensive in scope, less detailed, and limited to a handful of causes.

This paper summarizes the DALY estimates for 1990 by cause, age, sex and region. For the first time, those responsible for deciding priorities in the health sector have access to a disaggregated set of estimates which, in addition to facilitating cost-effectiveness analysis, can be used to monitor global and regional health progress for over a hundred conditions. The paper also shows how the estimates depend on particular values of the parameters involved in the calculation.—Authors' Abstract

**Talhari, S.** [Hanseniasis: actual situation.] An. Bras. Dermatol. **69** (1994) 209–215. (in Portuguese)

This is a review of the main aspects related to the etiology, transmission, classification and clinical aspects of leprosy and an analysis of the effectiveness and side ef-

fects of multidrug therapy and its operational problems. The world distribution of this disease and the goal of its elimination by the year 2000 are discussed.—Author's English Summary

**Turk, J. L.** Sir James Simpson: leprosy and syphilis. J. R. Soc. Med. **87** (1994) 549–551.

Sir James Simpson (1811–1870) had a long-standing interest in antiquarian studies. In 1841 he published *Antiquarian Notices of Leprosy and Leprosy Hospitals in Scotland and England.* Scotland was severely affected by leprosy, and the disease lingered there long after it had almost vanished from England. King Robert the Bruce contracted leprosy in 1339. Simpson lists the Scottish leprosy hospitals and records the history of their foundation and organization. He mentioned the role of the Knights of St. Lazarus who supervised a number of leprosy hospitals in England and Scotland. Simpson points out that leprosy was known in France as early as the 8th century and in Britain from the early 10th century, thus before the Crusades. During the 17th and 18th centuries it was believed that leprosy was introduced into northern Europe from the East by those returning from the Crusades.—From the Article

**Walter, C. S.** Changing role of voluntary organizations in the context of elimination of leprosy. Indian J. Lepr. **66** (1994) 37–43.

Elimination of leprosy as a disease still leaves hundreds and thousands of affected who require care and assistance. Hence decades of opportunities and challenges are there and the voluntary organizations will continue to play a major role in cooperation with the government of India and WHO until the disease is eliminated and finally eradicated. The main opportunities lie in the areas of expansion of MDT coverage, rehabilitation of cured patients, and spreading the knowledge and awareness about leprosy to general public.—Author's Conclusion

## Chemotherapy

**Altagracia, M., Monroy Noyola, A., Osoriorico, L., Kravzov, J., Alvarado Calvillo, R., Manjarrez Marmolejo, J. and Rios, C.** Dapsone attenuates kainic acid-induced seizures in rats. Neurosci. Lett. **176** (1994) 52–54.

We tested the ability of dapsone (4,4′-diamino-diphenyl sulfone) to attenuate kainic acid-induced seizures. We observed that 9.375 and 12.5 mg/kg doses of dapsone administered 30 min before a single kainic acid (10 mg/kg) i.p. injection were able to decrease the time of electroencephalographic seizures by 52% and 82%, respectively, as compared with rats administered 10 mg/kg kainic acid only. The 12.5 mg/kg dose of dapsone was also able to diminish both kainic acid-evoked body and head shakes (58%) and kainic acid-induced mortality (75%). These results suggest that dapsone could be used in clinical trials as an anticonvulsant.—Authors' Abstract

**Bwire, R. and Kawuma, H. J. S.** Type 1 reactions in leprosy, neuritis and steroid therapy: the impact of the human immunodeficiency virus. Trans. R. Soc. Trop. Med. Hyg. **88** (1994) 315–316.

The incidence of type 1 reactions and neuritis among HIV seronegative and HIV seropositive leprosy patients was investigated. HIV seropositivity was associated with an increased incidence of type 1 reactions among multibacillary (MB) patients, which were observed in 9 of 12 seropositive MB patients and in 8 of 40 HIV seronegative MB patients ($p < 0.0005$). Similarly, the incidence of neuritis was significantly increased among the HIV seropositive MB patients, of whom 8 developed acute neuritis compared to 3 of the HIV seronegative patients ($p < 0.0005$). There was no significant difference between the numbers of paucibacillary HIV seropositive and HIV seronegative patients who devel-

oped these complications. Both the HIV seronegative and HIV seropositive patients showed a similar response to steroid therapy for the management of acute neuritis.—Authors' Abstract

**Dietrich, M., Gaus, W., Kern, P. and Meyers, W. M.** An international randomized study with long-term follow-up of single versus combination chemotherapy of multibacillary leprosy. Antimicrob. Agents Chemother. **38** (1994) 2249–2257.

A total of 307 patients with lepromatous leprosy and borderline lepromatous leprosy were randomized to dapsone monotherapy or to one of two types of drug combinations. A 3-year treatment phase was followed by a 5-year observation phase. The evaluation included 233 patients for whom together there were 1404 years of observation. A total of 1956 blinded histopathological specimens were processed centrally. When entering the trial isolates from 13 patients (5.6%) showed dapsone resistance in the mouse foot pad test, and these patients were evaluated separately. Dapsone monotherapy (68 patients) had the same frequency of cure as the combination of dapsone and rifampin (77 patients) or the four-drug regimen consisting of dapsone, rifampin, isoniazid, and prothionamide (75 patients). We did not find a significant difference in the clearance of bacteria either between the monotherapy and the two-drug combination or the monotherapy and the four-drug combination. Six months after the initiation of treatment, disease in 15% of the patients who received dapsone monotherapy but none of the patients who received combined treatment were clinically progressive: After another 1 to 9 months of treatment the disease in all patients was stable or regressive. There was no difference in the type or frequency of reactions. Only after the end of the scheduled observation phase were three relapses reported. All three treatment regimens were well tolerated. Dapsone monotherapy is highly effective in the treatment of multibacillary leprosy under the conditions of well-controlled treatment. Combination regimens seem only to accelerate the regression of active disease when they are compared with monotherapy with dapsone. The mouse foot pad test does not reflect the clinical resistance and cannot be recommended for use in making therapeutic decisions.—Authors' Abstract

**dos Santos, S. N. M. B., Araujo, M. G., Patrus, O. A. and Samaha, D.** [Variation in the concentration of hemoglobin in the dosage of sulfone used to treat hanseniasis.] An. Bras. Dermatol. **69** (1994) 281–284. (in Portuguese)

Hemolysis has been observed frequently as one of sulfone's effects. This seems to happen also in doses applied to the treatment of leprosy patients. One-hundred-four leprosy patients using sulfones were studied and 33 were statistically analyzed. There was no significant statistical correlation with hemoglobin (hb) variation and the initial level of hb, interval between laboratory tests, age and weight in the male. In the female there was moderate correlation with weight. Eighty-four percent of patients had a fall in hb concentration of 1g/dl or more, confirming reports in the literature. It is very important to observe this fact carefully. This may have serious clinical implications, especially in endemic areas where, owing to nutrition, malaria, and intestinal parasitism, the hb concentration is already compromised.—Authors' English Summary

**Herbert, D., Paramasivan, C. N., Prabhakar, R. and Swaminathan, G.** *In vitro* experiments with *Centella asiatica*: investigation to elucidate the effect of an indigenously prepared powder of this plant on the acid-fastness and variability of *M. tuberculosis*. Indian J. Lepr. **66** (1994) 65–68.

The herb *Centella asiatica* (Linn.), found throughout India, is acclaimed to have medicinal properties and has been used in leprosy patients from very early times. It is considered that the active compound of this herb, called asiaticoside, probably acts on the waxy covering of *Mycobacterium leprae*. The *in vitro* effect of an indigenously produced dry powder of *Centella asiatica* (CA) on the acid-fastness and viability of *M. tuberculosis* was investigated in the present study. The results indicate that CA may not have any direct action on the acid-fastness or viability of *M. tuberculosis* H37Rv *in vitro*. Further studies using purified asiati-

coside of the plant or *in vivo* studies are required.—Authors' Abstract

**Matz, J., Borish, L. C., Routes, J. M. and Rosenwasser, L. J.** Oral desensitization to rifampin and ethambutol in mycobacterial disease. Am. J. Respir. Crit. Care Med. **149** (1994) 815–817.

The incidence of disease caused by *Mycobacterium tuberculosis* (including drug-resistant strains) and *M. avium* complex (MAC) is increasing. Hypersensitivity reactions to antimycobacterial agents are relatively uncommon, but when they occur they may result in cessation of therapeutic medications. We report our experience with rapid oral desensitization to ethambutol and rifampin in a group of 10 patients with mycobacterial disease who had experienced cutaneous hypersensitivity reactions to these drugs. An adaptation of the rapid oral desensitization protocol for penicillin was used, with the dosing intervals increased to account for the different kinetics of these drugs. Adverse reactions were few and easily treated without necessitating cessation of therapy. We conclude that oral desensitization to rifampin and ethambutol by our protocol is safe and effective, allowing these patients to proceed with an optimal antimycobacterial regimen.—Authors' Abstract

**Nau, R., Kinzig, M., Dreyhaupt, T., Kolenda, H., Sorgel, F. and Prange, H. W.** Kinetics of ofloxacin and its metabolites in cerebrospinal fluid after a single intravenous infusion of 300 milligrams of ofloxacin. Antimicrob. Agents Chemother. **38** (1994) 1849–1853.

Ofloxacin has been reported to diffuse readily into the cerebrospinal fluid (CSF) in subjects with both inflamed and uninflamed meninges. However, with moderately susceptible bacteria, ofloxacin concentrations in CSF may be subtherapeutic after administration of an intravenous (i.v.) dose of 200 mg. For this reason, the kinetics of a higher dose of ofloxacin in CSF was studied with humans. Six patients with occlusive hydrocephalus caused by cerebrovascular diseases who had undergone external ventriculostomy received 400 mg of ofloxacin i.v. over 30 min. Serum and CSF samples were drawn repeatedly. Serum from 12 healthy volunteers was sampled repeatedly after they had received 400 mg of ofloxacin i.v. over 60 min. Ofloxacin, ofloxacin-*N*-oxide, and *N*-desmethyl-ofloxacin concentrations were determined by high-pressure liquid chromatography with fluorescence detection. The maximum ofloxacin concentrations in the serum of the patients ranged from 7.36 to 11.6 mg/liter (mean, 9.55 mg/liter), the apparent volume of distribution/body weight was 0.96 to 1.19 liters/kg (mean, 1.11 liters/kg), and the total body clearance was 115 to 280 ml/min (mean 192 ml/min). In healthy volunteers, the volume of distribution/body weight and the total body clearance were higher and amounted to 1.27 $\pm$ 0.18 liters/kg and 217 $\pm$ 43 ml/min (means $\pm$ standard deviations), respectively. These differences were attributed to the older ages of the patients than the volunteers. In the CSF of patients, maximum concentrations of 1.00 to 2.85 mg/liter (mean 2.04 mg/liter) were observed 0.5 to 4 hr following the completion of the ofloxacin infusion. Ofloxacin elimination from CSF was slightly slower than that from serum (half-lives, 4.33 to 10.02 versus 4.27 to 9.14 hr). The overall penetration of ofloxacin into CSF, as expressed by the ratios of the areas under the concentration-time curves, amounted to 0.59 to 0.81 (mean 0.65). The more hydrophilic metabolites ofloxacin-*N*-oxide and *N*-desmethyl-ofloxacin passed less readily than ofloxacin into the CSF. In conclusion, the concentrations of CSF attained after a single i.v. infusion of 400 mg of ofloxacin in the absence of meningeal inflammation appear to be high enough to inhibit the growth of most staphylococci and members of the family *Enterobacteriaceae*, which are often involved in CSF shunt infections. Yet, in view of pharmacodynamic studies suggesting a peak concentration of CSF of at least 10-fold the MIC, the use of ofloxacin for central nervous system infections is optimal only with highly susceptible pathogens (MIC $\leq$ 0.12 mg/liter).—Authors' Abstract

**O'Reilly, J. R., Corrigan, O. I. and O'Driscoll, C. M.** The effect of mixed micellar systems, bile salt/fatty acids, on the solubility and intestinal absorption of clofazimine (B663) in the anaesthetised rat. Int. J. Pharmaceut. **109** (1994) 147–154.

Clofazimine (B663) is a highly lipophilic drug used in the treatment of leprosy. The solubility and gastrointestinal membrane permeability (P-app) of B663 in mixed micellar systems were examined. Membrane permeability was determined using a rat gut perfusion model and, in addition, these studies incorporated the hydrophilic marker PEG 4000. The mixed micellar systems studied contained the bile salt, sodium cholate (NaC), in association with different fatty acids including caprylic acid, oleic acid and linoleic acid. At a set concentration of NaC (40 mM) the solubility of B663 increased with increasing concentration of each fatty acid. Relative to NaC, the maximum enhancement in solubility (16-fold) was obtained with the NaC/linoleic acid (40:40 mM) system. An optimum bile salt/fatty acid ration of 1:1 existed for maximum solubility enhancement. All mixed micellar systems enhanced the absorption of B663 relative to the simple micelle. The P-app tended to increase with increasing fatty acid concentration, maximum enhancement being obtained with the NaC/linoleic acid 40:40 mM system. With each mixed micellar system a higher P-app was obtained with lower drug loading. The effects of the mixed micellar systems on the absorption of PEG 4000 varied with fatty acid loading. These results have shown that mixed micelles can enhance the absorption of B663 to a greater extent relative to non-micellar and simple micellar systems. Maximum enhancement (> 800-fold) in the rate of B663 absorption was obtained with the NaC/linoleic acid 40:40 mM system. These results offer a possible explanation for the reported enhancement in gastrointestinal absorption of B663 when co-administered with fatty materials.—Authors' Abstract

**Pertel, P. and Hirschtick, R.** Adverse reactions to dapsone in persons infected with human immunodeficiency virus. Clin. Infect. Dis. **18** (1994) 630–632.

Dapsone is used in prophylaxis for and treatment of *Pneumocystis carinii* pneumonia. We present a case of Stevens-Johnson syndrome that was likely induced by administration of dapsone. A review of charts at the HIV Treatment Center of Northwestern University (Chicago, Illinois, U.S.A.) revealed that 40.3% of patients treated with trimethoprim-sulfamethoxazole could not tolerate the medication, while 25.2% of those treated with dapsone were intolerant of the drug. We also found a higher rate of adverse reactions to dapsone among patients with prior intolerance to trimethoprim-suflamethoxazole than among patients without such a history; however, the difference was not significant.—Authors' Summary

**Rao, P. S., Ramachandran, A., Sekar, B., Ravi, S. and Subramanian, M.** Ofloxacin-containing combined drug regimens in the treatment of lepromatous leprosy. Lepr. Rev. **65** (1994) 181–189.

A total of 26 clinically diagnosed adult patients, with active untreated lepromatous leprosy, with a bacterial index of 4+ or more, were admitted to the hospital of the Central Leprosy Teaching and Research Institute, Chengalpattu, India, between 1989 and 1991. After prescribed investigations, the patients were randomly allocated in groups of 3 to three treatment regimens, namely: 1) clofazimine 50 mg daily and 300 mg once in 4 weeks + dapsone 100 mg daily (AA); 2) (AA)+ofloxacin 400 mg daily (BB); and 3) (AA)+ofloxacin 800 mg daily (CC). The drugs were administered for 56 days continuously under supervision. Sequential biopsy results on days 0, 7, 14, 28 and 56 in normal mouse foot pad revealed no growth by days 28 and 56 in all patients treated with CC and BB regimens, respectively. Calculation of the proportion of viable *Mycobacterium leprae* through analysis of median infectious dose ($ID_{50}$) showed significant differences on day 7 in the percentage of kill between the ofloxacin-containing regimens and the other. Moderate-to-marked clinical improvement has been observed in a significantly higher proportion of patients treated with ofloxacin-containing regimens. All three regimens were well tolerated. No severe complications or side effects to the drugs were noticed with any of the regimens that required any suspension of treatment or the administration of steroids. Addition of ofloxacin to the standard WHO recommended MDT regimen for multibacillary patients may reduce the present duration of therapy. Ofloxacin may also be considered

as an alternative drug in rifampin-resistant cases or where rifampin is contraindicated.—Authors' Summary

**Reepmeyer, J. C., Rhodes, M. O., Cox, D. C. and Silverton, J. V.** Characterization and crystal structure of two polymorphic forms of racemic thalidomide. J. Chem. Soc. Perkin **2** (1994) 2063–2067.

There are two polymorphic forms of racemic thalidomide. The alpha-polymorph was formed by crystallization from 2-ethoxyethanol, methanol or dichloromethane, while the beta-polymorph was formed by crystallization from a supersaturated solution in refluxing 2-ethoxyethanol. The two polymorphs were characterized by IR spectra, differential scanning calorimetry (DSC), melting points, powder X-ray diffraction patterns, and X-ray crystallography. They were easily differentiated by IR (KBr) in which the alpha-polymorph absorbed at 3196, 3098 and 859 $cm^{-1}$ and the beta at 3276 and 755 $cm^{-1}$. DSC scans of the alpha and beta forms showed endothermic peaks at 272.3°C and 275.7°C, respectively. During this measurement the alpha form was partially or fully converted into the beta form if the rate of temperature change was slow or if the alpha form contained some beta which provided seed crystals for the interconversion. Upon remelting both forms gave one peak corresponding to the beta-polymorph. The powder X-ray diffraction patterns for the two forms differ significantly. The crystal structures of the two polymorphs differ primarily in their mode of hydrogen bonding. In the alpha-polymorph the molecules form dimers, while in the beta the dimers form infinite linear strings linked by bifurcated hydrogen bonds along the b-axis.—Authors' Abstract

**Rege, V. L., Shukla, P. and Mascarenhas, M. F.** Dapsone syndrome in Goa. Indian J. Lepr. **66** (1994) 59–64.

Dapsone syndrome was noted within 6 weeks of starting treatment in 1.3% of about 700 leprosy patients on MDT reporting to the skin department of Goa Medical College. Skin rash, photosensitivity, fever, lymphadenopathy, sore throat, hepatosplenomegaly, abnormal liver function tests and raised reticulocyte counts were consistent features in all the patients. Other drugs, infectious mononucleosis and viral exanthemata were considered in a differential diagnosis. Withdrawal of dapsone and administration of prednisolone controlled the condition within 3 to 4 weeks in majority of the patients. One patient died of ischemic heart disease unrelated to dapsone syndrome.—Authors' Summary

**Vage, C. and Svensson, C. K.** Evidence that the biotransformation of dapsone and monoacetyldapsone to their respective hydroxylamine metabolites in rat liver microsomes is mediated by cytochrome P450 2C6/2C11 and 3A1. Drug Metab. Dispos. **22** (1994) 572–577.

The formation of dapsone hydroxylamine (DDS-NOH) and monoacetyldapsone hydroxylamine (MADDS-NOH) was found to be greater in male vs. female rat liver microsomes, suggesting a role for either CYP2C11 or CYP3A2. Preincubation with cimetidine (selective for inhibition of CYP2C11), but not troleandomycin (selective for inhibition of CYP3A1/2), inhibited metabolite formation. Furthermore, incubation with monoclonal antibodies (Mabs) to CYP2C6/2C11 reduced metabolite formation to below the level of detection. Together, these data indicate that N-hydroxylation of DDS and MADDS in rat liver microsomes from untreated male rats is catalyzed by CYP2C6/2C11. Interestingly, dexamethasone pretreatment increased the hydroxylation of both metabolites. Preincubation with cimetidine or Mabs to CYP2C6/2C11 (at an antibody:protein ratio of 26:1) in microsomes from dexamethasone pretreated animals did not reduce the N-hydroxylation of DDS; whereas preincubation with troleandomycin reduced metabolite formation by $\leq$ 50%. Collectively, these data indicate that the constitutive enzymes CYP2C6 and/or CYP2C11, as well as CYP3A1 (nonconstitutive), are capable of catalyzing the hydroxylation of DDS and MADDS.—Authors' Abstract

## Clinical Sciences

**Breen, G. A., Brocavich, J. M., Etzel, J. V., Shah, V., Schaefer, P. and Forlenza, S.** Evaluation of effects of gastric pH on absorption of dapsone in healthy volunteers. Antimicrob. Agents Chemother. **38** (1994) 2227–2229.

A prospective, randomized, crossover study was performed with seven healthy volunteers to address the effect of increased gastric pH on dapsone absorption. Subjects were randomized to receive a single 100-mg dose of dapsone or a single 100-mg dose of dapsone in addition to 30 ml of a high potency antacid 1 hr before dapsone administration and hourly thereafter for a total of 10 doses. Dapsone concentrations in serum were measured periodically for 48 hr. No statistical differences between the two regimens were noted when mean dapsone maximal initial concentrations, times to peak, and areas under the curve were compared. These data suggest that an increase in gastric pH has little or no effect on the absorption of dapsone in healthy subjects.—Authors' Abstract

**Courtright, P., Hu, L. F., Li, H. Y. and Lewallen, S.** Multidrug therapy and eye disease in leprosy; a cross-sectional study in the People's Republic of China. Int. J. Epidemiol. **23** (1994) 835–842.

Factors associated with leprosy-related eye disease in a multidrug therapy (MDT) treated population in China were assessed to determine if status prior to inclusion in the MDT program (newly diagnosed leprosy patient or leprosy patient on prior dapsone monotherapy) contributed to the prevalence of ocular pathology. Trained leprosy paramedical workers in Sichuan Province examined 974 leprosy patients in a standardized fashion. Univariate analyses and multiple logistic regression were used to assess the contribution of demographic and clinical parameters to leprosy-related eye disease. In both groups (prior dapsone and new MDT) leprosy-related eye disease was associated with a longer distance to leprosy health worker or health center. Among patients with a history of prior dapsone monotherapy, age and duration on dapsone monotherapy were also associated with leprosy-related ocular morbidity. Among newly diagnosed leprosy patients the prevalence of ocular morbidity remained between 8% and 11% regardless of when the patient started MDT. Our findings suggest that, even when case detection is good, ocular pathology will still occur in MDT-treated leprosy patients. There remains an important role for health workers in the prevention of ocular morbidity. Our data also demonstrated that pooling of results from all patients (newly diagnosed and on prior dapsone monotherapy) in a leprosy control program will likely give rise to inadequate estimates of risk of ocular disease due to variable clinical disease histories in these groups.—Authors' Abstract

**Daniel, A. E., Arunthathi, S., Bhat, L. and Rao, P. S. S.** Intraocular pressure in leprosy patients without clinically apparent anterior segment pathology. Indian J. Lepr. **66** (1994) 165–172.

A widely prevalent notion is that intraocular pressures are generally lower in leprosy patients than in normal individuals. Applanation intraocular pressures were recorded in 166 leprosy patients who had no clinically visible anterior segment pathology and in 111 healthy controls. Mean (S.D.) intraocular pressures in leprosy patients (13.6 (3.0) mm Hg) did not differ significantly from that of controls (13.1 (2.7) mm Hg). Eyes of only 1.5% of the leprosy patients had pressures of 7 mm Hg or less. Correlation coefficients (r) between age, sex and intraocular pressures were not statistically significant both in leprosy patients and in controls. No statistically significant differences in mean intraocular pressures were noted when leprosy patients were grouped according to the Ridley and Jopling classification. Duration of disease also did not affect the intraocular pressures. Neither did smear positivity or differing bacterial indices. This study questions the widely held belief that low intraocular pressures are a common feature in leprosy and contends that in the era of MDT where ocular complications associated with low intraocular

pressures are thought to be less, the occurrence of low intraocular pressure may not be as common a phenomenon as it is believed to be.—Authors' Abstract

**Frommel, D., Tekle Haimanot, R., Verdier, M., Negesse, Y., Bulto, R. and Denis, F.** HIV infection and leprosy: a four-year survey in Ethiopia. Lancet **344** (1994) 165–166.

Between 1988 and 1992 three cross-sectional surveys for antibodies to HIV were done in 644 Ethiopian patients with histologically proven leprosy. Whereas the frequency of HIV-1 infection gradually increased from 3.2% to 6.5%, the clinical presentation, number of new patients, and frequency of relapse did not differ between HIV-negative and -positive patients. Thus, HIV appears unlikely to have a significant impact on the incidence of leprosy in sub-Sahelian populations.—Authors' Abstract

**Glaser, J. B., Levis, W. R., Gruber, T., Cabrera, A. and Poiesz, B. J.** Prevalence of human T cell lymphotrophic virus (HTLV) types I and II and human immunodeficiency virus type 1 infections among persons with Hansen's disease in New York City. J. Infect. Dis. **170** (1994) 1007–1009.

One-hundred-seven consecutive patients attending a New York Hansen's disease clinic from November 1990 through June 1991 were tested for retroviruses. This cohort included 58 patients diagnosed with Hansen's disease after the onset of the AIDS epidemic, 54 of whom immigrated to the United States before diagnosis of Hansen's disease (median, 7 years). The overall rate (1.9%) of human T-cell lymphotrophic virus (HTLV) type I infection was low. Two (3.6%) of 55 Caribbean-born patients had polymerase chain reaction (PCR)-documented HTLV-I infection, but this incidence was not higher than expected in persons without Hansen's disease. No patient had PCR-documented evidence of either HTLV-II or human immunodeficiency virus (HIV) type 1 infection. The low rate of HIV-1 among those studied was likely related to an absence of classic HIV risk behavior because about half of the cohort could have incubated *Mycobacterium leprae* for a prolonged period while infected with HIV-1.—Authors' Abstract

**Hiran, S., Pande, T. K., Pani, S. and Vishwanathan, K. A.** Dapsone agranulocytosis in a leprosy patient. Lepr. Rev. **65** (1994) 279–281.

Dapsone-induced agranulocytosis is a rare adverse effect. There are various reports of agranulocytosis in patients treated with dapsone for malaria prophylaxis and other dermatological diseases. However, this adverse reaction in leprosy is not often encountered. We describe agranulocytosis in a young patient who was taking dapsone (100 mg) for borderline tuberculoid leprosy in a rural environment.—Authors' Summary

**Lora Robles, M. and Gonzalez, K.** [Dimorphous leprosy with a lesion of the radial nerve; a case report.] Rev. Domin. Republ. **20** (1993) 55–57. (in Spanish)

The authors study a case of dimorphous leprosy with damage to the radial nerve in a 9-year-old male patient.—Authors' English Summary

**Lubbers, W. J., Schipper, A., Hogeweg, M. and de Soldenhoff, R.** Eye disease in newly diagnosed leprosy patients in eastern Nepal. Lepr. Rev. **65** (1994) 231–238.

To determine the magnitude of eye lesions in newly diagnosed leprosy patients we examined their eyes. The Eastern Leprosy Control Project was supported by The Netherlands Leprosy Relief Association; we used the regional clinic in Biratnagar and 5 mobile clinics in surrounding districts as our survey area. All patients who presented at the clinics over 10 weeks, diagnosed as having untreated leprosy were included. Of the 260 examined patients 97 (37.3%, 95% confidence interval 28.3–40.3%) had an eye lesion; 12/260 patients (4.6%, 95% confidence interval 2.0–7.2%) had sight-threatening lesions (lagophthalmos, iris involvement, corneal anesthesia), directly related to leprosy; 46 (17.7%) patients were diagnosed as having some degree of cataract; 2 patients were aphakic; 3 patients (1.2%) were blind according to the WHO definition.

In this series of new and untreated leprosy patients many eye lesions found are not relevant or leprosy related. There were 9 new patients with lagophthalmos, some too longstanding to treat with steroids. We found 3 patients with iris involvement. The figures we found for eye lesion, sight-threatening lesions and blindness are low when compared to other studies. The number of patients with any grade of cataract is high. The average total of leprosy patients who were blind can be compared with the average total who are blind in the general population.—Authors' Summary

**Mahajan, P. M., Jadhav, V. H., Patki, A. H., Jogaikar, D. G. and Mehta, J. M.** Oral zinc therapy in recurrent erythema nodosum leprosum: a clinical study. Indian J. Lepr. **66** (1994) 51–57.

The effect of oral zinc as an immunomodulator was studied clinically in patients with recurrent erythema nodosum leprosum (ENL) over a period of 1 year. In this study, 40 leprosy patients with chronic ENL, requiring more than 30–40 mg of prednisolone/day for the control of their reactions, were given oral zinc sulfate for a period of 4 months, and marked improvement in the frequency, duration and severity of reactions was observed after zinc therapy. Also evident was marked reduction in the steroid requirement after oral zinc therapy. It appears that zinc may be a good substitute for the present day antireaction treatment which is not free from disadvantages. Further investigations to know the precise action of zinc on the immune system may help to understand the role of zinc therapy and its optimum duration.—Authors' Abstract

**Pavithran, K.** Palatal palsy in a case of lepromatous leprosy. Lepr. Rev. **65** (1994) 248–252.

A male patient with lepromatous leprosy developed nasal regurgitation of food due to palatal palsy during type 2 reaction. Early high-dose administration of corticosteroid achieved a prompt therapeutic response and he completely recovered from palatal palsy. The associated lagophthalmos, foot drop and ulnar paralysis persisted.—Author's Summary

**Saran, B. R., Maguire, A. M., Nichols, C., Frank, I., Hertle, R. W., Brucker, A. J., Goldman, S., Brown, M. and Vanuitert, B.** Hypopyon uveitis in patients with acquired immunodeficiency syndrome treated for systemic *Mycobacterium avium* complex infection with rifabutin. Arch. Ophthalmol. **112** (1994) 1159–1165.

Iridocyclitis has been identified as a dosage-dependent side effect in patients with the acquired immunodeficiency syndrome (AIDS) who are treated for *Mycobacterium avium* complex (MAC) infection with systemic rifabutin. We reviewed cases of acute hypopyon uveitis occurring in patients with AIDS to establish whether there was an association. The cases came from an outpatient clinic and inpatient hospital-based ophthalmology referral practice and infectious disease specialty service. Seven patients with AIDS were studied, aged 10 to 40 years, presenting with acute unilateral hypopyon mimicking infectious endophthalmitis. At the time of presentation, all 7 patients were receiving treatment for MAC infection with rifabutin (dosage range 300 to 600 mg/d) and clarithromycin. Results of microbiological investigations in 5 patients were negative. Iridocyclitis became bilateral in all 7 patients, and hypopyon developed in the contralateral eye in 5 of 7 patients. Hypopyon resolved rapidly with intensive topical corticosteroid therapy. Residual inflammation responded to topical corticosteroids with or without reduction of the rifabutin dosage. Concomitant use of rifabutin, clarithromycin, and fluconazole may precipitate hypopyon uveitis in patients with AIDS being treated for MAC infection.—Authors' Abstract

**Schipper, A., Lubbers, W. J., Hogeweg, M. and de Soldenhoff, R.** Disabilities of hands, feet and eyes in newly diagnosed leprosy patients in eastern Nepal. Lepr. Rev. **65** (1994) 239–247.

The objective of the study was to determine the magnitude of hand/feet/eye disabilities in newly diagnosed leprosy patients by examining all newly diagnosed leprosy patients who presented at the Eastern Leprosy Control Project (supported by The Netherlands Leprosy Relief Association),

made up of a regional clinic in Biratnagar and 5 mobile clinics in surrounding districts. The study comprised of all new and previously untreated patients who presented at the clinics over a 10-week period who were diagnosed as leprosy sufferers. Of the 260 leprosy patients examined 12 (4.6%) had sight-threatening lesions (lagophthalmos, iris involvement, corneal anesthesia); 3 patients were blind due to cataract; 96/260 patients (37.0%, 95% confidence interval 35.0–43.0%) had 1 or more disabilities of their hands and/or feet. The most frequently found disabilities were sensory loss of the hands and feet, claw hand and plantar ulcers. According to the WHO disability grading 60% had no disabilities, 19% had grade 1, and 21% had grade 2 disability. Disability assessment is very important not only to evaluate the effectiveness of the control program but also for the patient, whose most important worry is the stigmatizing deformities leprosy patients suffer. The earlier detection of sensory loss might reduce these secondary deformities.—Authors' Summary

**Sekar, B., Sharma, R. N., Anandan, D., Vasanthi, B. and Jayasheela, M.** Indeterminate leprosy: a seroimmunological and histochemical evaluation. Lepr. Rev. **65** (1994) 167–174.

An effort was made to differentiate indeterminate (IND) leprosy from other types of the paucibacillary (PB) group of leprosy and to identify among indeterminate leprosy cases those which may evolve to multibacillary (MB) leprosy, using serological, immunological and histochemical parameters. A total of 92 untreated, histologically classified (TT-19, BT-30, IND-32) patients, including 11 cases diagnosed as nonspecific dermatitis (NSD), which were clinically strongly suspected to be leprotic, were screened for antibodies against PGL-I, 35-kDa and LAM antigens. Lepromin tests and antigen demonstration in tissue by indirect immunoperoxidase staining were also carried out. Though a qualitative analysis did not differentiate, a quantitative analysis in terms of a cumulative index (CI) showed a higher antibody level among the indeterminate group of patients than the other groups included in PB leprosy. Also, the lepromin-negative indeterminate group patients showed a higher CI than the lepromin-positive cases, indicating that perhaps these may be the cases which may develop into MB leprosy. Thus, the semiquantification of antibody levels in the form of a CI may be a useful parameter to predict the possible evolution of a given case of indeterminate leprosy. Interestingly 64% of NSD cases had either antigen or antibody, which indicated that they were probably cases of leprosy.—Authors' Summary

**Soni, N. K.** Eustachian tube functions in lepromatous leprosy: a tympanometric study. Indian J. Lepr. **66** (1994) 45–49.

Tympanometry was performed in 20 patients with lepramatous leprosy. About 30% showed tympanogram B type, indicating middle-ear pathology which was shown to be related to the stage of lepramatous rhinitis. The pathogenesis of middle-ear malfunction in lepromatous leprosy is discussed.—Author's Abstract

**Subramaniam, K., Nah, S. H. and Marks, S. C.** A longitudinal study of alveolar bone loss around maxillary central incisors in patient with leprosy in Malaysia. Lepr. Rev. **65** (1994) 137–142.

The loss of alveolar bone supporting the maxillary central incisors and the general periodontal conditions were evaluated after 14 years in the 12 patients remaining from an original group of 47 under treatment in Malaysia. Alveolar bone loss was minimal during this period even in the presence of periodontal inflammation. These data suggest that treatment protects patients with leprosy from alveolar bone loss and suggests that other skeletal deformities might respond similarly.—Authors' Summary

**Turkof, E., Tambwekar, S., Mansukhani, K., Millesi, H. and Mayr, N.** Intraoperative spinal root stimulation to detect most proximal site of leprous ulnar neuritis. Lancet **343** (1994) 1604–1605.

In 10 borderline leprosy patients with leprous ulnar neuritis, we investigated the most proximal site of lesion in the affected nerves. Spinal roots C8 and T1 were stimulated intraoperatively to evoke efferent mixed com-

pound nerve action potentials which were recorded from the exposed ulnar nerves. The site at which amplitudes reached a maximum was considered the most proximal site of lesion. Nerve damage was found far proximally from the thickened segments in otherwise inconspicuous sections. The most proximal site of lesion was finally localized at the proximal third of the upper arm in 9 cases and further centrally in 1 case. Epineuriotomy within these apparently unaffected segments revealed fibrosis of the interfascicular epineurium in 9 patients, which is an indication for microsurgical interfascicular neurolysis.—From the report

**van Brakel, W. H. and Khawas, I. B.** Nerve damage in leprosy: an epidemiological and clinical study of 396 patients in west Nepal—Part 1. Definitions, methods and frequencies. Lepr. Rev. **65** (1994) 204–221.

An historic cohort study was performed to determine the prevalence and incidence rates of nerve function impairment (NFI) as demonstrated by sensory testing with a nylon monofilament and standard tests of motor function. The records of 396 new leprosy patients registering at Green Pastures Hospital, Pokhara, West Nepal, between January 1988 and January 1992 were analyzed. The mean follow-up period was 21 months.

In all, 36% (141/396) of patients had either sensory or motor function impairment at their initial examination. For each nerve the prevalence of sensory and motor impairment is reported separately. The posterior tibial nerve was the most frequently affected (sensory) nerve (21%). Sensory impairment of the ulnar nerve was found in 17% of the patients; 8.8% had sensory impairment of the median nerve. The overall incidence rate of motor function impairment was 7.5 (5.4–10) per 100 person years at risk (PYAR). Sensory impairment had a significantly higher rate of 13 (10–17)/100 PYAR (rate ratio [1.8 (1.2–2.7), p = 0.0076]. BL patients had a significantly higher incidence rate of nerve function impairment than BT patients (rate ratio 2.3 (1.4–3.7), p = 0.006). Altogether 152/396 (39%) of the patients required corticosteroid treatment for "recent" or "acquired" impairment, and 78 of the patients (20%) developed severe nerve function impairment during or after antileprosy treatment. Analysis of potential risk factors for nerve function impairment showed a significant association with the extent of clinical disease expressed as the number of body areas (out of 9) with primary or secondary signs of leprosy (rate ratio 5.0 (1.5–17), p = 0.0091).

It was concluded that nerve function impairment is a serious problem, often occurring during or after multidrug therapy. The extent of clinical disease expressed as a count of body areas involved, or of skin or nerve lesions, may identify patients who are at increased risk of nerve damage.—Authors' Summary

**van Brakel, W. H., Khawas, I. B. and Lucas, S. B.** Reactions in leprosy: an epidemiological study of 386 patients in west Nepal. Lepr. Rev. **65** (1994) 190–203.

This paper presents epidemiological data on reversal reaction (RR) and erythema nodosum leprosum reaction (ENL) from a retrospective study of 386 leprosy patients newly registered at Green Pastures Hospital, Pokhara, West Nepal. The average follow-up time was 21 months. The prevalence of RR at first examination was 28% (23–32), and the prevalence of ENL reaction was 5.7% (2.3–9.2). The overall incidence rates among the 335 patients that were available for follow up were 8.7 (6.5–12)/100 person years at risk (PYAR) for RR and 3.2 (1.5–6.7)/100 PYAR for ENL. Relapse of RR was common (1.4/patient). In all, 52% of RR were complicated by new nerve function impairment, against 59% of ENL reactions. The finding of other investigators that most RRs occur during the first year of treatment was confirmed by this study. The most significant risk factor for RR was extent of clinical disease measured by a count of body areas with clinical signs of leprosy. The risk of developing a RR for patients with "extensive disease" (3 or more out of 9 body areas involved) was 10 times that of patients with limited disease (rate ratio 10 (1.3–76), p = 0.026).

The study indicated that the following categories of patients in Nepal are at high or increased risk of developing a RR: 1)

borderline patients during their first year of MDT; and 2) patients with more extensive clinical disease as described above.—Authors' Summary

## Immuno-Pathology

**Adams, E., Britton, W., Morgan, A., Sergeantson, S. and Basten, A.** Individuals from different populations identify multiple and diverse T-cell determinants on mycobacterial HSP70. Scand. J. Immunol. **39** (1994) 588–596.

The 70-kDa heat-shock protein (HSP) of *Mycobacterium leprae* stimulates both cellular and antibody responses in leprosy patients and subclinically infected individuals despite partial homology with host HSP70. Furthermore, mycobacterial HSP70 can act as a carrier protein in unprimed mice, suggesting the presence of widely shared T-cell determinants on this protein. In order to elucidate the frequency and genetic restriction of these T-cell epitopes, we have undertaken a systematic analysis of the proliferative responses to 20mer peptides encompassing the whole protein in different populations. Caucasian BCG vaccinees who responded to recombinant *M. leprae* HSP70 identified multiple scattered T-cell determinants, four of which were recognized by 60% of subjects in association with a variety of HLA-DR haplotypes. When a group of Nepali leprosy and tuberculosis patients were tested, significant differences in the pattern of peptide recognition were observed. The dominant peptides recognized by Caucasian subjects were infrequently reactive and other peptides were stimulatory, again in association with a variety of HLA-DR phenotypes. The C-terminal 70 residues of the *M. leprae* HSP70 are specific to *M. leprae* and sera from lepromatous leprosy patients bind to this region. However, few T-cell determinants were identified in these residues, indicating that this region is unhelpful as a diagnostic tool for detecting *M. leprae*-specific T-cell responses. When compared with the equivalent regions of the human HSP70, the commonly recognized peptides showed significant differences in amino-acid sequence. When taken in conjunction with the failure of human HSP70 to stimulate *M. leprae* HSP70-reactive T-cell clones (E. Adams *et al.*, unpublished observations), this finding indicates that the human T-cell response to this protein is largely directed at mycobacterial-specific determinants. The presence of multiple T-cell epitopes on *M. leprae* HSP70 with varied patterns of HLA-DR association suggests that the whole protein is required for stimulating effective T-cell responses in genetically diverse populations.—Authors' Abstract

**Anand, P., Pandya, S., Ladiwala, U., Singhal, B., Sinicropi, D. V. and Williams Chestnut, R. E.** Depletion of nerve growth factor in leprosy. (Letter) Lancet **344** (1994) 129–130.

Recent advances have led to the identification of neurotrophic factors that rescue nerve fibers after injury and disease in animal models of neuropathy and help nerve regeneration. The best established of these, nerve growth factor (NGF), is normally produced in skin and taken up by nerve fibers via receptors on nerve terminals. We report that NGF levels are depleted in skin and nerve from patients with leprosy.—From the letter

**Appelberg, R., Castro, A. G., Pedrosa, J. and Minoprio, P.** Role of interleukin-6 in the infection of protective T cells during mycobacterial infections in mice. Immunology **82** (1994) 361–364.

Interleukin-6 (IL-6) has been shown to regulate numerous functions of the immune system, including the differentiation of T-cell subpopulations. Here we examined the involvement of this cytokine in the *in vivo* generation of a population of T cells able to protect mice against mycobacterial infections. BALB/c mice were infected intravenously with *M. avium* 2447 and anti-IL-6 monoclonal antibodies were administered

intraperitoneally throughout the course of the infection. Control mice were able to control the mycobacterial proliferation 1 month after inoculation; whereas mice whose IL-6 had been blocked showed progressive bacterial growth. To distinguish a role for IL-6 associated to the induction or expression of immunity mediated by T cells, we immunized mice with *M. bovis* bacillus Calmette-Guérin (BCG) Pasteur and challenged them 2 months later with *M. avium*. One group of mice received anti-IL-6 during the BCG vaccination and another during the *M. avium* challenge. When *M. avium* proliferation was assessed at day 30 of the challenge, it was found that the administration of anti-IL-6 during vaccination reduced the protection afforded by BCG compared to administration of the isotype control antibody. No difference in bacterial proliferation was observed at day 30 of challenge when antibodies were administered during *M. avium* challenge. Our results show that protective T cells arise during *M. avium* infections in mice after differentiating in the presence of IL-6.—Authors' Abstract

**Barbosa, A. D., Silva, T. C., Patel, B. N., Santos, M. I. R., Wakamatsu, A. and Alves, V. A. F.** Demonstration of mycobacterial antigens in skin biopsies from suspected leprosy cases in the absence of bacilli. Pathol. Res. Prac. **190** (1994) 782–785.

Skin-biopsies from 56 patients suspected of early leprosy from Bahia State, Brazil, were examined histopathologically. The Fite-Faraco staining failed to demonstrate acid-fast bacilli in this material. The prominent features of the lesions were inflammation of the neurovascular bundles and sometimes inflammation of the skin appendages. The nonspecific infiltrate was predominantly composed of histiocytes and lymphocytes. In 41 cases (73.2%) epidermal atrophy was also present. The avidin-biotin peroxidase technique was used with primary antibodies to detect bacillary antigens (anti-BCG serum) and nerve branches (anti-S-100 protein serum). Immunohistochemical detection of bacillary antigens using the anti-BCG serum was positive in 28 cases (50%). A positive staining for S-100 protein was observed in 40 cases (71.4%) in dendritic antigen-presenting cells of the skin. The detection of bacillary antigens, together with the clear demonstration of nerve bundles enhanced our capacity to fulfill morphologic criteria for the diagnosis of early leprosy. Our observations indicate that the use of immunohistochemical methods represent a useful tool for the early diagnosis of leprosy.—Authors' Abstract

**Bharadwaj, V. P.** The present status of serological techniques and future perspectives in the study of epidemiology of leprosy. Jpn. J. Lepr. **62** Suppl. (1993) 152–172.

Several seroassays are now available for detection of *Mycobacterium leprae* infection. It appears that at present FLA-ABS is the most sensitive test for detection of *M. leprae* infection in the community and, besides estimating the prevalence of infection it is likely to be quite useful for monitoring the transmission of the disease. Great strides in the serological techniques and their application to epidemiology of leprosy have been made. Traditionally, the presence of antigen/antibody has been investigated in sera/leprous tissues but it might be worthwhile to investigate other body fluids/excretory fluids as well. The field of serology of leprosy is still evolving. It appears that in coming years there would be more information available on the combined application of various serological assays (which may be further modified by the newer advances about antigens/epitopes) and molecular techniques for better understanding of the epidemiology of the disease.—From the Article

**Brown, D. H. and Zwilling, B. S.** Activation of the hypothalamic-pituitary-adrenal axis differentially affects the antimycobacterial activity of macrophages from BCG-resistant and -susceptible mice. J. Neuroimmunol. **53** (1994) 181–187.

The effect of hypothalamic-pituitary-adrenal (HPA) axis activation and exogenous glucocorticoids on the ability of splenic macrophages to control the growth of *Mycobacterium avium* was evaluated. We found that activation of the HPA axis by restraint stress or the addition of corticosterone in-

creased the susceptibility of macrophages from mice that are innately susceptible to the *in vivo* growth of *M. avium*. In contrast, the ability of macrophages from innately resistant, congenic mice to control the growth of *M. avium* was not affected by HPA activation or the addition of corticosterone. The effect of restraint and of corticosterone on macrophage function was abrogated by either treating mice with the glucocorticoid receptor antagonist RU486 or the addition of the drug to cultures of macrophages. Activation of the HPA axis as well as the addition of corticosterone to cultures of macrophages resulted in a suppression of the production of tumor necrosis factor (TNF)-alpha and of reactive nitrogen intermediates by macrophages from both strains of mice. The lack of effect of HPA activation and of corticosterone on the mycobacterial resistance of macrophages from BCG-resistant mice, while at the same time suppressing the production of reactive nitrogen intermediates, appears to rule out a role for this antimicrobial pathway in innate resistance to mycobacterial growth.—Authors' Abstract

**Castells Rodellas, A., Garcia-Patos Briones, V., Repiso Montero, T. and Terencio de las Aguas, J.** [The immunology of leprosy 1993.] Rev. Leprol. Fontilles **19** (1994) 477–532. (in Spanish)

The macrophage immune response, humoral and cellular immunity against *Mycobacterium leprae* and its different antigens are reviewed (270 references). Total and partial defects of the cellular immunity exist with disturbed secretion patterns of cytokines while the humoral immunity is unaltered. The immunodeficiency is not satisfactorily explained.—Authors' English Summary

**Chui, D. H., Tabira, T., Izumi, S., Koya, G. and Ogata, J.** Decreased beta-amyloid and increased abnormal tau deposition in the brain of aged patients with leprosy. Am. J. Pathol. **145** (1994) 771–775.

We examined the brains of 37 leprosy patients (mean age 76.3 ± 7.8 years), 5 patients with Alzheimer-type dementia (mean age 79.0 ± 9.5 years), and 23 age-matched nondementia controls (mean age 77.6 ± 5.4 years). The frequency of beta-amyloid (Abeta)-positive cases was lower (27.0%) in leprosy patients (N = 37) than in controls (47.8%; $p = 0.05$, $Z = 1.49$). When senile plaque subtypes were examined, type III (classical) plaques were significantly fewer ($p < 0.05$) in leprosy subjects compared with controls. Interestingly, neurofibrillary tangles in the temporal cortex were much more frequent in leprosy patients than in controls ($p < 0.05$). However hippocampal CA3 pyramidal neurons in leprosy patients were well preserved. These data indicate that: 1) leprosy patients have a low risk of Abeta deposition but a high risk of abnormal tau deposition, 2) abnormal tau deposition is unrelated to AP deposition in leprosy, and 3) neuronal loss is unrelated to abnormal tau deposition. It is not clear at present whether the results is related to the disease process itself, antileprosy treatment, environmental factors, or the genetic background in leprosy patients.—Authors' Abstract

**Denis, M.** Interleukin-12 (IL-12) augments cytolytic activity of natural killer cell toward *Mycobacterium tuberculosis*-infected human monocytes. Cell. Immunol. **156** (1994) 529–536.

We tested the impact of interleukin-12 (IL-12) and of cytotoxic leukocytes [particularly natural killer (NK) cells] from normal and HIV-1-infected subjects on lysis of human monocytes infected with *Mycobacterium tuberculosis*. Nylon-wool-nonadherent cells stimulated with interleukin-2 (IL-2) or with IL-12 developed significant killing activity against infected monocytes, with IL-12 being a superior stimulant on a molar basis. Cells of the CD16+ phenotype mediated most of the cytotoxicity against infected monocytes, and this lytic activity was associated with a significant decrease in mycobacterial numbers. When peripheral blood mononuclear cells (PBMC) from HIV-1-infected subjects were examined, it was also found that IL-12 very significantly increased lytic activity against *M. tuberculosis*-infected cells. Moreover, purified NK cells from normal volunteers or from HIV-1-infected subjects were shown to have elevated lytic activity against *M. tuberculosis*-

infected monocytes after IL-2 or IL-12 stimulation. These data suggest an important involvement of NK cells and their activating stimuli (particularly IL-12) in host resistance to tuberculosis.—Authors' Abstract

**Desikan, P., Parkash, O. and Narang, P.** The role of antiperipheral nerve antibodies in nerve damage in leprosy. Lepr. Rev. **65** (1994) 222–230.

The objective of this study was to determine the role of antineural antibodies in leprosy. Indirect ELISA using antigen prepared from normal human peripheral nerves was carried out on the sera from 100 leprosy patients and 18 normal controls. In total, 9% of the patients had demonstrable levels of IgG antineural antibodies and 11% had demonstrable levels of IgM antibodies. There was no correlation with the type of leprosy, bacteriological index, treatment taken, the presence of a reactional state, the presence of enlarged nerves or active neuritis.—Authors' Summary

**Dumarey, C. H., Labrousse, V., Rastogi, N., Vargaftig, B. B. and Bachelet, M.** Selective *Mycobacterium avium*-induced production of nitric oxide by human monocyte-derived macrophages. J. Leuk. Biol. **56** (1994) 36–40.

Infection with a virulent strain of *Mycobacterium avium,* but not with virulent *M. tuberculosis* or avirulent *M. smegmatis,* induced the formation of nitric oxide by human monocyte-derived macrophages. This process was not affected by lipopolysaccharide or cytokines such as interferon-gamma or tumor necrosis factor alpha. *M. avium*-induced nitric oxide production was significantly decreased by N-G-monomethyl-L-arginine, a potent inhibitor of nitric oxide synthase activity, without any significant enhancement of intramacrophagic mycobacterial growth. Infection with all the three mycobacterial species induced a significant activation of phospholipase A(2) activity of macrophages as evidenced by the increased release of thromboxane A(2). Finally, nitric oxide production by human monocyte-derived macrophages required infection with live *M. avium,* as neither gamma-irradiated *M. avium* nor the subcellular fractions of this microorganism (cell wall, cytosol) were able to trigger nitric oxide synthesis.—Authors' Abstract

**Flesch, I. E. A., Hess, J. H., Oswald, I. P. and Kaufmann, S. H. E.** Growth inhibition of *Mycobacterium bovis* by IFN-gamma stimulated macrophages—regulation by endogenous tumor necrosis factor-alpha and by IL-10. Int. Immunol. **6** (1994) 693–700.

Murine bone marrow-derived macrophages (BMM) are able to inhibit the intracellular growth of *Mycobacterium bovis* and *M. tuberculosis* H37Rv after activation with recombinant (r) IFN and growth inhibition is mediated by reactive nitrogen intermediates (RNI) derived from L-arginine. We now demonstrate that tumor necrosis factor (TNF)-alpha acts as an endogenous cofactor in the induction of mycobacterial growth inhibition. TNF-alpha was produced by BMM stimulated with rIFN-gamma and infected with mycobacteria, and a specific antiserum to TNF-alpha inhibited rIFN-gamma-induced production of RNI as well as growth inhibition of *M. bovis.* IL-10, a cytokine which suppresses antimycobacterial macrophage functions, was also produced by BMM activated with rIFN-gamma and infected with *M. bovis.* IFN-gamma-induced production of TNF-alpha and of reactive nitrogen intermediates as well as mycobacterial growth inhibition were inhibited by exogenous IL-10, but only when given prior to IFN-gamma stimulation. We conclude that the outcome of mycobacterial infection is regulated by a coordinate interplay between IFN-gamma, TNF-alpha and IL-10.—Authors' Abstract

**Gelber, R. H., Mehra, V., Bloom, B. R., Murray, L. P., Siu, P., Tsang, M. and Brennan, P. J.** Vaccination with pure *Mycobacterium leprae* proteins inhibits *M. leprae* multiplication in mouse foot pads. Infect. Immun. **62** (1994) 4250–4255.

In this study, we evaluated vaccination with a number of purified, as well as recombinant, *Mycobacterium leprae* proteins for protective efficacy in mice. BALB/c mice were immunized intradermally with vari-

ous native somatic (purified) or recombinant *M. leprae* proteins and their synthetic polypeptides emulsified in Freund's incomplete adjuvant. The protective efficacy of these preparations was assessed by enumeration of bacilli in the foot pads of mice challenged with viable *M. leprae* 1 to 2 months following immunization. Protection was afforded by the purified and recombinant 10-kDa *M. leprae* cytoplasmic heat shock protein, the recombinant cell wall-associated 65-kDa *M. leprae* heat shock protein and, to a lesser extent, the purified 28-kDa *M. leprae* cytoplasmic protein (superoxide dismutase). Vaccination with either the purified or recombinant 35-kDa *M. leprae* cell membrane protein, the synthetic 27-amino-acid N-terminal peptide of the 10-kDa protein, the recombinant 18-kDa *M. leprae* protein, or the purified 22-kDa cell membrane protein was ineffective. When the interval between immunization and challenge was increased to 6 months, the purified 10-kDa *M. leprae* protein and the recombinant 65-kDa *M. leprae* protein lost vaccine efficacy, while a sodium dodecyl sulfate-soluble protein fraction of the *M. leprae* cell wall (soluble proteins), as had been found previously, continued to protect, suggesting that multiple *M. leprae* protein epitopes are critical for solid vaccine protection.—Authors' Abstract

**Gheorghiu, M., Lagranderie, M. R. R., Gicquel, B. M. E. and LeClerc, C. D.** *Mycobacterium bovis* BCG priming induces a strong potentiation of the antibody response induced by recombinant BCG expressing a foreign antigen. Infect. Immun. **62** (1994) 4287–4295.

Several recent studies have demonstrated that strong cellular or humoral immune responses can be induced against foreign antigens expressed by recombinant *Mycobacterium bovis* BCG. It has therefore been suggested that BCG could represent one of the best candidate vectors for live recombinant vaccines. However, a large percentage of the human population has been immunized by BCG, and this priming could modify the immune response to future recombinant BCG vaccines. In the present study, we have therefore compared the immune responses induced in naive and BCG-primed mice by two recombinant BCG vaccines expressing either beta-galactosidase or human immunodeficiency virus type 1 Nef antigens. Our results demonstrated that BCG priming limits the growth of recombinant BCG in mouse spleen or lymph nodes. This reduction in BCG growth was associated with decreased proliferative responses against Nef or beta-galactosidase antigens. This suppression, however, never exceeded 50%. Interestingly, in contrast to these reduced T-cell responses, BCG-primed mice developed high levels of anti-beta-galactosidase antibodies after immunization with recombinant BCG expressing this antigen. This stimulation of antibody responses was not due to polyclonal stimulation or to a nonspecific adjuvant effect of BCG. The isotypic patterns of anti-beta-galactosidase antibody responses induced by the recombinant BCG were similar in naive and BCG-primed mice. These results indicate that priming with BCG will not be a limitation for the use of recombinant BCG vaccines in humans.—Authors' Abstract

**Haftel, H. M., Chang, Y., Hinderer, R., Hanash, S. M. and Holoshitz, J.** Induction of the autoantigen proliferating cell nuclear antigen in T lymphocytes by a mycobacterial antigen. J. Clin. Invest. **94** (1994) 1365–1372.

Mycobacteria have been implicated in the pathogenesis of autoimmunity. To determine the potential effect of mycobacterial antigens on peripheral blood mononuclear cells (PBMC), we analyzed PBMC incubated with the acetone-precipitable fraction of *Mycobacterium tuberculosis* (AP-MT) for changes in cellular induction of a 36-kDa polypeptide identified as proliferating cell nuclear antigen (PCNA), a known autoantigen, after incubation with AP-MT. PCNA plays a role in cell proliferation and is expressed as a late growth-regulated factor. However, its synthesis in response to AP-MT was induced as an early event. The early induction of PCNA was regulated at a post-transcriptional level and was restricted to T cells. Treatment of PBMC with known T-cell mitogens, namely PHA, anti-CD3 antibodies, and staphylococcal superantigens, failed to induce an early PCNA increase. The distinct characteristics of the AP-MT effect on

PCNA expression suggest a separate mechanism of induction in response to AP-MT, compared with the late increase observed in response to mitogens. The induction of PCNA in response to mycobacterial antigens may represent a pathogenically relevant mechanism in autoimmunity.—Authors' Abstract

**Hetland, G. and Wiker, H. G.** Antigen 85C on *Mycobacterium bovis*, BCG and *M. tuberculosis* promotes monocyte-CR3-mediated uptake of microbeads coated with mycobacterial products. Immunology **82** (1994) 445–449.

The uptake in monocytes of monodispersed latex microbeads precoated with whole bacillus Calmette-Guérin (BCG) cells, *Mycobacterium tuberculosis* sonicate or culture fluid, or antigen (Ag) from the culture fluid was examined by microscopy. There was a significantly higher cell association of beads coated with whole BCG cells, the secreted 85C component of the Ag85 complex or *M. tuberculosis* sonicate than of phosphate-buffered saline (PBS)-treated control beads. Antibodies (Ab) to Ag85 inhibited the uptake of BCG- and Ag85C-treated beads. A monoclonal antibody (mAb) to complement receptor type 3 (CR3), but not mAb to CR1, inhibited the uptake of Ag85C-coated beads, indicating that the mycobacterial Ag-dependent uptake of particles was mediated via CR3 on the monocytes. This points to the existence of a ligand on Ag85C which may promote monocyte uptake of *M. bovis*, BCG and *M. tuberculosis*.—Authors' Abstract

**Ilangumaran, S., Narayan, N. P. S., Ramu, G. and Muthukkaruppan, V.** Cellular and humoral immune responses to recombinant 65-kD antigen of *Mycobacterium leprae* in leprosy patients and healthy controls. Clin. Exp. Immunol. **96** (1994) 79–85.

Cellular and humoral immune responses to recombinant 65-kDa antigen of *Mycobacterium leprae* (rML65) were studied in leprosy patients and healthy contacts from a leprosy-endemic population. Peripheral blood mononuclear cells from a considerable proportion of tuberculoid leprosy patients, healthy contacts and noncontacts showed proliferative response to rML65 *in vitro*. A strong positive correlation was observed between the responses to rML65 and bacille Calmette-Guérin (BCG) or leprosin A. Addition of recombinant IL-2 (rIL-2) enhanced the proportion of responders to rML65 considerably in all groups of leprosy patients, healthy contacts and noncontacts. Among lepromatous patients this enhancement was more pronounced in the bacterial index (BI)-negative group. These results indicate that the 65-kDa antigen of *M. leprae* is a dominant T-cell immunogen in our study population. Though lepromatous patients showed poor lymphoproliferative response to rML65, their IgG antibody levels to the same antigen were markedly high. Most of the BI-positive lepromatous patients with elevated anti-rML65 IgG levels did not show T-cell reactivity even with the addition of rIL-2. On the other hand, tuberculoid leprosy patients, healthy contacts and noncontacts showed good T-cell reactivity but low levels of IgG antibodies to rML65, thus indicating the presence of an inverse relationship between cell-mediated and humoral immune responses to a defined protein antigen of *M. leprae* in humans. A significant proportion of individuals among tuberculoid leprosy patients, healthy contacts and noncontacts showed neither T-cell reactivity nor elevated levels of IgG antibody to rML65. However, in most of these subjects, a T-cell response to rML65 was demonstrable with the addition of rIL-2. These results are discussed with reference to the immunoregulatory mechanisms occurring during *M. leprae* infection on the basis of differential activation of Th1 and Th2 subsets.—Authors' Abstract

**Kawamura, I., Yang, J. F., Takaesu, Y., Fujita, M., Nomoto, K. and Mitsuyama, M.** Antigen provoking gamma interferon production in response to *Mycobacterium bovis* BCG and functional difference in T-cell responses to this antigen between viable and killed BCG-immunized mice. Infect. Immun. **62** (1994) 4396–4403.

It has been shown that gamma interferon (IFN-γ)-producing CD4+ T cells, which are generated only by immunization with viable bacteria, exert a significant role in protective immunity against mycobacteria in

mice. In this study, we have tried to determine the antigen recognized by the T cells in search of a possible protective antigen. T cells from viable *Mycobacterium bovis* BCG-immunized mice were stimulated with several antigens, and IFN-γ production was measured. Purified protein derivative and viable and killed BCG lysates caused significant IFN-γ production, and almost the same level of IFN-γ activity was detected in both groups stimulated with viable end killed BCG lysates. However, heat shock protein (HSP) 65 and HSP 70 were not a major antigen for IFN-γ production. The antigen provoking IFN-γ production is localized mainly in the membrane fraction of BCG cells, and the approximate molecular size was 18 kDa. On the other hand, T cells from killed BCG-immunized mice never responded to this antigen for IFN-γ production; whereas they could mount a delayed-type hypersensitivity response. These results showed that the antigen provoking IFN-γ production was present in killed as well as viable BCG. In addition to the antigen presentation by antigen-presenting cells, some kinds of differentiation factor (such as monokines) that are produced only by stimulation with viable cells seemed to be necessary for the development of IFN-γ-producing T cells.—Authors' Abstract

**Launois, P., Deleys, R., Niang, M. N., Drowart, A., Andrien, M., Dierckx, P., Cartel, J.-L., Sarthou, J. L., Van Vooren, J. P. and Huygen, K.** T-cell-epitope mapping of the major secreted mycobacterial antigen Ag85A in tuberculosis and leprosy. Infect. Immun. **62** (1994) 3679–3687.

Lymphoproliferation and gamma interferon (IFN-γ) secretion in response to 28 overlapping 20-mer synthetic peptides covering the complete sequence of the mature (295-amino-acid) 85A component of the major secreted, fibronectin-binding antigen 85 complex from *Mycobacterium tuberculosis* and *M. bovis* BCG (MTAg85A) was examined by using peripheral blood mononuclear cell (PBMC) cultures from healthy tuberculin- and lepromin-positive volunteers and from patients with tuberculosis and leprosy. Peptide recognition was largely promiscuous, with a variety of human leukocyte antigen haplotypes reacting to the same peptides. PBMC from all tuberculin-positive subjects reacted to Ag85, and the majority proliferated in response to peptide 6 (amino acids 51 to 70), peptides 13, 14, and 15 (amino acids 121 to 160), or peptides 20 and 21 (amino acids 191 to 220). PBMC from tuberculosis patients demonstrated a variable reactivity to Ag85 and its peptides, and the strongest proliferation was observed against peptide 7 (amino acids 61 to 80). MTAg85A peptides were also recognized by PBMC from healthy lepromin-positive volunteers and paucibacillary leprosy patients (again in a promiscuous manner), but despite a 90% homology between the 85A proteins of *M. leprae* and *M. tuberculosis,* the peptides recognized were different. PBMC from lepromin-positive healthy contacts reacted against peptide 2 (amino acids 11 to 30), peptide 5 (amino acids 41 to 60), and peptides 25 and 26 (amino acids 241 to 270). PBMC from paucibacillary patients reacted preferentially against peptide 1 (amino acids 1 to 20) and peptide 5. Multibacillary patients were not reactive to Ag85 or the MT85A peptides. IFN-γ production was generally detected simultaneously with positive lymphoproliferative responses, although peptide 1 mostly stimulated proliferation and peptides 27 and 28 mostly elicited an IFN-γ response. In conclusion, regions 41 to 80 and 241 to 295 demonstrated powerful and promiscuous T-cell-stimulatory properties, resulting in proliferative responses and IFN-γ secretion, respectively, in the majority of reactive subjects tested in this study. These results could be of value in the development of a subunit vaccine for tuberculosis and leprosy.—Authors' Abstract

**Launois, P., Ndiaye, M. N., Sarthou, J. L., Drowart, A., Van Vooren, J. P., Cartel, J. L. and Huygen, K.** T-cell reactivity against antigen-85 but not against the 18-kD and 65-kD heat shock proteins in the early stages of acquired immunity against *Mycobacterium leprae.* Clin. Exp. Immunol. **96** (1994) 86–90.

T cell proliferation and interferon-gamma (IFN-γ) production of peripheral blood mononuclear cells (PBMC) from 20 household contacts were tested against the 18-

and 65-kDa heat shock proteins from *Mycobacterium leprae* (ML18 and ML65, respectively) and antigen 85 from *M. bovis* bacille Calmette-Guérin (BCG) (Ag 85) during a 12-months follow-up study. Among the eight contacts that became positive, eight showed positive reactivity against Ag 85, 5/8 against ML65 and 4/8 against ML18 at the end of the study. Of the 16 contacts who were lepromin-positive either at first or second testing, all responded to Ag 85, 11 to ML65, but only eight reacted to ML18 antigen. Contacts who were lepromin-positive at first testing developed responses to ML18 only at second testing. In contrast, among the four contacts that remained lepromin-negative during the follow up, three proliferated to Ag 85 either at first or second testing, but only one produced IFN-$\gamma$ against Ag 85 at the end of the study. These results demonstrated that T-cell reactivity and particularly IFN-$\gamma$ secretion against Ag 85, but not against ML18 and ML65, might be a predominant mechanism in the early stages of acquired protective immunity against *M. leprae*.—Authors' Abstract

**Modlin, R. L.** Th1-Th2 paradigm: insights from leprosy. J. Invest. Dermatol. **102** (1994) 828–832.

The mechanism by which T cell and cytokines regulate immune processes in skin can be investigated by studying patients with leprosy. The disease, caused by the obligate intracellular bacterium *Mycobacterium leprae*, forms a spectrum. At one pole, patients with tuberculoid leprosy are able to restrict the growth of the pathogen and their skin lesions are characterized by a predominance of CD4+ T cells and type 1 cytokines including interleukin 2 and interferon gamma. At the opposite pole, patients with lepromatous leprosy are unable to contain the infection and their skin lesions are characterized by a predominance of CD8+ T cells and type 2 cytokines including interleukins 4 and 10. A key determinant of the T-cell cytokine response may be interleukin 12, which selectively favors expansion of CD4+ T cells producing interferon gamma. By understanding the factors that regulate T-cell and cytokine responses in leprosy, it should be possible to devise specific immunologic interventions in diseases of skin.—Authors' Abstract

**Nogueira, A. C., Neubert, R., Helge, H. and Neubert, D.** Thalidomide and the immune system. 3. Simultaneous up- and down-regulation of different integrin receptors on human white blood cells. Life Sci. **55** (1994) 77–92.

Time-dependent changes in the surface receptor expression of various maturational and integrin receptors on peripheral blood cell were studied in two healthy human volunteers following oral applications of thalidomide (Thd). In each measurement the receptor density was quantified by prior calibration of the flow cytometer with latex beads bearing a determined number of fluorescence molecules. The effects observed in the course of the Thd-treatment were practically identical or at least very similar in both of the volunteers during four different trials, and were in accord with previous results obtained in large-scale studies (68 treated animals) with nonhuman primates. It should be stressed that no clear-cut changes were observed in the percentage or absolute numbers of primary lymphocyte subsets such as CD3, CD4 and CD20. After the first two doses of 7 mg Thd/kg body wt the CD18 (the common beta-chain of the beta 2-integrins) marker already decreased in surface density or was no longer detectable on granulocytes, monocytes and lymphocytes. This effect persisted throughout the treatment period and slowly subsided after discontinuation of treatment. With a few days lag phase, the surface density of CD54 (ICAM-1) on granulocytes increased and many cells previously not bearing this receptor newly acquired such surface markers. On monocytes, however, the CD54 receptor was lost on many cells. Within the lymphocyte fraction a loss of the CD54 marker could be noted on CD4 cells but not on CD8 cells, where an increase of the receptor expression could be observed. Other markers, such as the alpha chains of the beta 1 integrins CD49b (VLA alpha 2) and CD49d (VLA alpha 4) showed contrasting reactions to the Thd-treatment. Whereas a pronounced loss of the receptor density of CD49d was observed and only few cells with high epitope density were left in the blood at the end of the complete dosing schedule, no such effect was observable on cells bearing the CD49b epitope. A distinct reduction of the number of receptors was also notice-

able on L-selectin (Leu8) bearing cells. On CD4 positive lymphocytes, the majority of the described effects on the integrin and adhesion receptors was seen on cells bearing the CD45RO maturational epitope. This functional receptor is strongly downregulated and the pathway of CD45RA to CD45RO maturation is apparently altered by Thd-treatment. These multiple changes we observed may explain the large variety of therapeutic effects experienced in the treatment with Thd.—Authors' Abstract

**Ohmen, J. D., Barnes, P. F., Grisso, C. L., Bloom, B. R. and Modlin, R. L.** Evidence of a superantigen in human tuberculosis. Immunity **1** (1994) 35–43.

T cells are not only required for resistance to tuberculosis, but they likely contribute to the tissue damage characteristic of the disease. To define better the T-cell populations that contribute to the immunopathogenesis of human tuberculosis, we investigated the T-cell receptor (TCR) beta chain repertoire expressed in patients with tuberculous pleuritis. Analysis by polymerase chain reaction and flow cytometry indicated an expansion of V beta 8(+) T cells at the site of disease in some donors, suggesting the possibility that *Mycobacterium tuberculosis* contains a superantigen. *M. tuberculosis* induced strong T-cell proliferative responses in tuberculin-negative healthy donors *in vitro,* with preferential expansion of V beta 8(+) T cells, independent of the CDR3 region. T-cell stimulation was MHC class II-dependent and did not require antigen processing by the antigen-presenting cells. These findings are consistent with the presence of a superantigen in *M. tuberculosis,* aspects of which may contribute to the immunopathology of tuberculosis and to the adjuvant properties of *M. tuberculosis.*—Authors' Abstract

**Ortega, V. V., Diaz, F. M., Pacheco, G. O. and Rubiales, F. C.** Ultrastructural study across the leprosy spectrum. Ultrastruct. Pathol. **18** (1994) 423–432.

We have carried out a systematic ultrastructural study of the bacilli, the cell-mediated response in the host, and the dermal microvasculature in lepromatous (LL), borderline lepromatous (BL), and borderline tuberculoid (BT) types of active leprosy (eight cases). In the types of least resistance (LL and BL), macrophages with large cytoplasmic processes were observed; in addition, numerous peripheral vacuoles were found in BL. Mast cells were abundant and vascular alterations constant. BT macrophages showed more regular outlines and multivacuolated cytoplasms with plentiful rough endoplasmic reticulum. Giant cells were scarce. Bacilli, both isolated and in globi, were contained within the vacuoles and appeared constantly in macrophages and endothelial and Schwann cells in LL and BL. Conversely, in BT they were found singly, infrequently in the endothelial cells, and not at all in Schwann cells. Forms in the process of destruction or degradation were more common than intact forms, in which the symmetric outline of the membrane could be seen clearly.—Authors' Abstract

**Peetermans, W. E., Raats, C. J. I., Langermans, J. A. M. and Van Furth, R.** Mycobacterial heat-shock protein 65 induces proinflammatory cytokines but does not activate human mononuclear phagocytes. Scand. J. Immunol. **39** (1994) 613–617.

The 65-kDa heat-shock protein (Hsp65), a well-conserved and immunodominant antigen which elicits a cellular and humoral immune response, may play a role in host defense against invading microorganisms and autoimmune disorders. The aim of the present study was to assess the effects of Hsp65 on the functional activities of human mononuclear phagocytes in the absence of lymphocytes. Incubation with Hsp65 resulted in an enhanced release of TNF-alpha and IL-1 beta by human monocytes and monocyte-derived macrophages (MDM). The amount of cytokines released by these cells in response to Hsp65 was similar to that released in response to IFN-gamma together with LPS. Incubation with ovalbumin did not stimulate the release of these cytokines. *In vitro* stimulation of monocytes with Hsp65 enhanced the membrane expression of complement receptor III but did not influence either the expression of Fc gamma-receptor I and HLA class-II antigens or the release of reactive oxygen intermediates. Therefore, Hsp65-stimulated monocytes cannot be considered to be activated according to classical criteria. The release of the proinflammatory cytokines

TNF-alpha and IL-1 beta by human mononuclear phagocytes in response to Hsp65 indicates that this protein can contribute to both host defense and tissue damage in inflammatory lesions characterized by an abundant expression of Hsp65.—Authors' Summary

**Roche, P. W., Peake, P. W., Davenport, M. P. and Britton, W. J.** Identification of a *Mycobacterium leprae*-specific T-cell epitope on the 70 kDa heat shock protein. Immunol. Cell Biol. **72** (1994) 215–221.

A major antigen of the leprosy bacillus, *Mycobacterium leprae,* is the 70-kDa heat-shock protein (Hsp70), which has significant sequence homology with Hsp70 from other mycobacterial species as well as Hsp70 from eukaryotes. A unique region of 70 amino acids at the C-terminus of the *M. leprae* Hsp70 has been previously identified. This study investigated whether mice immunized with the C-terminal fragment of *M. leprae* Hsp70 recognize T-cell epitopes in this species-specific portion of the molecule. Murine lymphoproliferative responses to overlapping peptides spanning the C-terminal 70 amino acids were restricted to mice of an H-2 (b) haplotype and identified the presence of a determinant in sequence 567–591. Lymph-node cells from mice immunized with this peptide recognized both the C-terminal fragment and the whole Hsp70 molecule. Moreover, mice immunized with the same peptide responded to the whole Hsp70 molecule in a delayed-type hypersensitivity reaction. The significance of *M. leprae*-specific T-cell epitopes in the host response to mycobacterial infection is discussed.—Authors' Abstract

**Santos, D. O., Suffys, P. N., Moreira, A. L., Bonifacio, K., Salgado, J. L., Esquenazi, D., Bertho, A. L. and Sarno, E. N.** Evaluation of chemiluminescence, procoagulant activity and antigen presentation by monocytes from lepromatous leprosy patients with or without reactional episodes. Lepr. Rev. **65** (1994) 88–99.

In this study, we evaluated the activity of peripheral blood mononuclear cells (PBMC), isolated from treated and untreated lepromatous leprosy patients, from lepromatous leprosy patients during and after reactional episodes [erythema nodosum leprosum (ENL) and reversal reaction (RR)], and from normal healthy individuals. We determined reactive oxygen intermediate (ROI) production, procoagulant activity (PCA) and HLA-DR antigen expression of monocytes, besides lymphoproliferation, both in the presence and absence of various stimulatory agents. Phorbol myristate acetate (PMA) stimulated ROI production by monocytes from all the groups studied, with patients during reactional episodes (ENL and RR) showing a significantly higher response ($p < 0.009$ and $p < 0.00001$). Irradiated *Mycobacterium leprae,* although having little effect when added alone, strongly suppressed PMA-stimulated ROI production. Muramyl dipeptide (MDP) had no influence on either basal or on PMA-induced ROI production. Basal monocyte PCA, as well as *M. leprae* or concanavalin A (ConA)-induced monocyte PCA, was comparable in monocytes from all the groups studied. ConA was able to induce mitogenic activity in mononuclear cells isolated from all the groups studied. *M. leprae,* although stimulatory for normal individuals, did not induce lymphoproliferation in lepromatous leprosy patients, except for cells from patients during RR, which responded equally to *M. leprae* and to ConA. The absence of *M. leprae*-induced lymphoproliferation in lepromatous leprosy patients is not caused by the lack of basal HLA-DR expression, as PBMC from all individuals studied showed the same level of this antigen. Our results suggest an increase of spontaneous or PMA-induced monocyte activity, as detected by ROI production, during the reactional episode; addition of *M. leprae* suppressed this response. The increase in monocyte activity could be correlated with the increase of lymphoproliferation response to *M. leprae* during RR, but not during ENL. The importance of a possible immune suppressive action of *M. leprae* is discussed.—Authors' Summary

**Schoel, B., Sprenger, S. and Kaufmann, S. H. E.** Phosphate is essential for stimulation of V gamma 9V delta 2 T lymphocytes by mycobacterial low molecular weight ligand. Eur. J. Immunol. **24** (1994) 1886–1892.

T lymphocytes are divided into two subsets which express different T-cell receptor heterodimers. In the peripheral blood of healthy individuals, the majority of T cells express the alpha/beta T-cell receptor (> 90%) while a minority have the gamma/delta T-cell receptor (< 10%). The gamma/delta T cells of adults use preferentially the V gamma 9V delta 2 chain combination. Although the stimulation requirements for gamma/delta T lymphocytes are still undetermined, it has been reported that gamma/delta T cells are not only stimulated, like alpha/beta T cells, by conventional protein antigens and superantigens, but also by unusual ligands. Mycobacteria selectively stimulate V gamma 9V delta 2 T cells, and a nonproteinacious low molecular weight fraction of 1–3 kDa has been identified as the tentative active component. Here, we confirm the nonproteinacious nature of this ligand, and show that it is comprised of unusual carbohydrate and phosphate. Importantly, cleavage of the terminal phosphate by alkaline phosphatase completely abrogates the stimulatory activity of the low molecular weight ligand for V gamma 9V delta 2 T cells. Even mycobacterial whole lysate loses its stimulatory activity, for this T-cell subset, after dephosphorylation with alkaline phosphatase. These findings identify phosphocarbohydrates as a novel molecular entity with selective stimulatory activity for a defined T-cell subset.—Authors' Abstract

**Shetty, V. P., Uplekar, M. W. and Antia, N. H.** Immunohistological localization of mycobacterial antigens within the peripheral nerves of treated leprosy patients and their significance to nerve damage in leprosy. Acta Neuropathol. **88** (1994) 300–306.

The presence and distribution of *Mycobacterium leprae*-specific and crossreacting antigens within the peripheral nerves of multidrug-treated patients with leprosy were investigated to gain a better understanding of the mechanism of nerve damage and the effect of multidrug therapy (MDT) on it. There was no specific qualitative difference in the type of antigens in the leprosy spectrum. However, our results indicate that there may be differential handling of antigens by the macrophages as compared to Schwann cells. This could play a key role in the pathogenesis of the disease. Multidrug treatment is effective in arresting the progression of the disease process as well as in reducing the viable bacterial load, both in borderline lepromatous and lepromatous (MB) and borderline tuberculoid and tuberculoid (PB) cases. However, the presence of *M. leprae* antigens in all the multidrug-treated MB nerve lesions and 87% of PB nerve lesions suggest that the antigens persist for a prolonged period. Hence, the risk of immunological reaction and antigen-associated silent nerve damage may continue even after the majority of *M. leprae* were killed. The findings give further support to the view that most of the nerve damage is due to bacterial antigens.—Authors' Abstract

**Torres, M., Mendez Sampeiro, P., Jimenez Zamudio, L., Teran, L., Camarena, A., Quezada, R., Ramos, E. and Sada, E.** Comparison of the immune response against *Mycobacterium tuberculosis* antigens between a group of patients with active pulmonary tuberculosis and healthy household contacts. Clin. Exp. Immunol. **96** (1994) 75–78.

The mycobacterial antigens and the factors related to protection for the development of active tuberculosis are not known. In a natural model of tuberculosis, we studied 10 patients with active pulmonary tuberculosis (nonprotective immune response) and 38 healthy household contacts (protective immune response). We tested the lymphocyte proliferative response by T-cell Western blotting to eight different antigen fractions and to two purified mycobacterial antigens of 30 and 64 kDa. Patients with active tuberculosis recognized fractions with molecular weights of 80–114, 60–80, 28–41 and 14–19 kDa. Household contacts recognized the same fractions except the 14–19 kDa. The response to the 64-kDa antigen was not significantly different between groups. In contrast, 10% of the patients with active tuberculosis and 73% of the household contacts responded to the 30-kDa antigen. The humoral response against the 30-kDa antigen by ELISA showed a significantly higher production of antibodies in tuberculosis patients compared with house-

hold contacts. We conclude that patients with active pulmonary tuberculosis develop an immune response characterized by poor proliferative response to the 30-kDa antigen with a strong humoral response; whereas the opposite occurs in healthy subjects infected by *Mycobacterium tuberculosis.*—Authors' Abstract

**Wadee, A. A., Sussman, G., Kuschke, R. H. and Reddy, S. G.** Suppression of cytokine production by supernatants from CD8+ lymphocytes activated by mycobacterial fractions: the role of interleukins 4 and 6. Biotherapy **7** (1994) 125–136.

Supernatants derived from CD8+ lymphocytes treated with mycobacterial components, or the partially purified carbohydrates from these supernatants, increased the production of IL-4 and IL-6 by mononuclear cells. The addition of anti-IL4 or anti-IL6 antibodies to LPS-stimulated MN cells incubated with supernatants from CD8+ lymphocytes or carbohydrates resulted in the restoration of other cytokine production by these MN cells. Recombinant IL-4 and IL-6 on their own suppressed the production of IL-1 beta, TNF-alpha, IL-2, and IFN-gamma by mononuclear cells. Such suppression could be reversed with antibodies to IL-4 and IL-6. The addition of rIL-4 and rIL-6 did not increase the suppression of cytokine production induced by suppressor supernatants or carbohydrates. IL-4 decreased the production of IL-6 by MN cells; IL-6 suppressed IL-4 production in a dose-dependent manner. Both effects could be reversed with the appropriate antisera. Our results suggest that mycobacteria could evade host immunity by inducing the production of IL-4 and IL-6 by host mononuclear cells. These cytokines, in turn, would suppress the production of other cytokines necessary for effective cellular immunity.—Authors' Abstract

**Wheeler, P. R., Raynes, J. G. and McAdam, K. P. W. J.** Autoantibodies to cerebroside sulphate (sulphatide) in leprosy. Clin. Exp. Immunol. **98** (1994) 145–150.

Sera from 40 leprosy patients were screened for autoantibodies to cerebroside sulfate (sulfatide). Anti-sulfatide IgM in groups of patients with lepromatous (LL) and borderline (BL + BB + BT), but not with tuberculoid (TT) disease, were significantly elevated above the levels found in endemic control subjects. Eight-six percent (18 out of 21; mean 1.59 OD units) of LL, 33% (four out of 12; mean 1.59 OD units) of borderline and 13% (one out of eight; mean 0.69 OD units) of tuberculoid patients had anti-sulfatide IgM in their sera above a cut-off value of 2 S.D. above the mean value (0.66 OD units) for control sera. Elevated anti-sulfatide IgG was detected in only one patient's serum, an individual with LL disease. The level of anti-sulfatide IgM was strongly correlated to expression of the TH3 idiotype, an idiotype previously defined by a human MoAb that bound *Mycobacterium leprae* phenolic glycolipid, *Klebsiella* capsular polysaccharide, polynucleotides and human tissues. The purified, TH3 MoAb was found in this study to bind sulfatide, but not cholesterol-3-sulfate or cerebroside. It is suggested that antisulfatide IgM is elevated in leprosy, in relation to the bacterial load. Anti-sulfatide IgM fell at the onset of erythema nodosom leprosum (ENL) reaction, consistent with the deposition of serum antibodies, and thus may play a part in pathology during periods of inflammation, particularly in multibacillary patients.—Authors' Abstract

**Xu, S. M., Cooper, A., Sturgill Koszycki, S., Van Heyningen, T., Chatterjee, D., Orme, I., Allen, P. and Russell, D. G.** Intracellular trafficking in *Mycobacterium tuberculosis* and *Mycobacterium avium*-infected macrophages. J. Immunol. **153** (1994) 2568–2578.

Despite the potential role of the macrophage in the eradication of invading microbes, *Mycobacterium* species have evolved mechanisms to ensure their survival and replication inside the macrophage. Particles phagocytosed by macrophages normally will be delivered into acidic lysosomal compartments for degradation. Mycobacteria must, in some way, avoid this fate by modulation of their phagosome. Immunoelectron microscopy of macrophages infected with *Mycobacterium avium* or *M. tuberculosis* indicates that the vacuolar membrane surrounding the bacilli possesses the late endosomal/lysosomal marker, LAMP-1 (lysosomal-associated membrane protein-1),

but lacks the vesicular proton-ATPase. Analysis of the intersection of the bacteria-containing vacuoles with the endocytic network of the macrophage supports previous studies indicating that these bacilli restrict the fusion capability of their intracellular compartments. The occurrence of vesicles containing lipoarabinomannan, discrete from those containing mycobacteria, indicate that material does traffic out from the mycobacterial vacuole. To compensate for this loss of membrane, the vacuole must remain dynamic and fuse with LAMP-1-containing vesicles to maintain the density of this marker.—Authors' Abstract

**Yassin, R. J. and Hamblin, A. S.** Altered expression of CD11/CD18 on the peripheral blood phagocytes of patients with tuberculosis. Clin. Exp. Immunol. **97** (1994) 120–125.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis,* is characterized by granulomatous lesions made up of epithelioid cells, giant cells and mononuclear leukocytes. Cell-cell adhesion is important in granuloma formation and in the leukocyte migration which accompanies it. We have recently shown increased expression of the adhesion molecules CD11/CD18 (LeuCAMs, beta (2) integrins) on peripheral blood leukocytes from patients with sarcoidosis (Shakoor and Hamblin, 1992). Here we have studied the expression of CD11/CD18 and CD29 (VLA beta (1) integrin) on the peripheral blood leukocytes of 10 TB patients by flow cytometry. The density (expressed as mean fluorescence intensity) of CD11b on monocytes and polymorphs was increased ($p < 0.005$), as was CD11c ($p < 0.005$) and CD18 ($p < 0.05$) on polymorphs. CD11a expression was significantly reduced on polymorphs ($p < 0.05$). No differences were found in the expression of CD29, the percentages of cells expressing any molecule and, in contrast to sarcoidosis, the density of any molecule on lymphocytes. Although the cytokine tumor necrosis factor (TNF) has been implicated in the process of up-regulation, an ELISA for TNF failed to detect significant levels in plasma. The results suggest increased peripheral phagocyte CD11/CD18 expression is a feature of TB, which may contribute to the pathological processes involved.—Authors' Abstract

## Microbiology

**Baelden, M. C., Bouckaert, A. E., Gregoire, D., Vandepoele, M. and Coene, M.** Comparison of thermostable macromolecular antigens from leprosy-associated bacteria. Can. J. Microbiol. **40** (1994) 508–512.

Three types of bacteria are associated with leprosy: *Mycobacterium leprae,* leprosy-derived corynebacteria (LDC), and armadillo-derived mycobacteria (ADM). The immunological relationships between these three types of bacteria and *M. bovis* BCG, used as a reference, were determined by cross-immunoelectrophoresis. When compared with the reference, crossreactions were observed with a variable number of antigens: 2 in the case of strain LDC 15, 4 with *M. leprae,* and from 1 to 10 in the case of the ADM, depending on their subgroup. Next, thermostable macromolecular antigens (TMAs), the major crossreactive antigens of leprosy-associated bacteria, were compared by anti-TMA antibody ELISA tests. The LDC TMAs displayed high crossreactivity between the subgroups and lower crossreactivity with the TMAs of *M. bovis* BCG. Evidence for the presence of a species-specific moiety in TMA of the different LDC was obtained by using depleted anti-TMA antisera. Western blot analysis revealed the presence of many proteins in the TMAs of LDC and *M. bovis* BCG, some of them being species-specific and other crossreactive.—Authors' Abstract

**Bhaduri, T. and Nagaraja, V.** DNA topoisomerase I from *Mycobacterium smegmatis.* Indian J. Biochem. Biophys. **31** (1994) 339–343.

DNA topoisomerase I has been purified from *Mycobacterium smegmatis* to near ho-

mogeneity using different column chromatographic techniques. The enzyme activity relaxes form I DNA into form IV DNA, requiring Mg2+, but not ATP or any other cofactors for its activity. Several properties of the enzyme were found to be similar to that of the prototype enzyme, *Escherichia coli* topoisomerase I.—Authors' Abstract

**Bhatia, V. N.** Repeated isolation of dermatophilus-like organism from leprosy material. Indian J. Lepr. **66** (1994) 149–156.

An organism having actinomyatoid-type colonies has been grown repeatedly in pure culture from leprosy material using a solid medium. The isolates obtained from different biopsies, skin scrapes and mouse footpad harvests were found to be similar to each other on the routine taxonomical tests and had many characters common with *Dermotophilus congolensis*. Similar growth could be obtained from a strain of *Mycobacterium leprae* from armadillo and also from a DOPA-positive mycobacterium isolated previously from the blood of a leprosy patient.—Author's Abstract

**Cirillo, J. D., Weisbrod, T. R., Banerjee, A., Bloom, B. R. and Jacobs, W. R., Jr.** Genetic determination of the *meso*-diaminopimelate biosynthetic pathway of mycobacteria. J. Bacteriol. **176** (1994) 4424–4429.

The increasing incidence of multiple-drug-resistant mycobacterial infections indicates that the development of new methods for treatment of mycobacterial diseases should be a high priority. *meso*-Diaminopimelic acid (DAP), a key component of a highly immunogenic subunit of the mycobacterial peptidoglycan layer, has been implicated as a potential virulence factor. The mycobacterial DAP biosynthetic pathway could serve as a target for design of new antimycobacterial agents as well as the construction of *in vivo* selection systems. We have isolated the *asd, dapA, dapB, dapD,* and *dapE* genes involved in the DAP biosynthetic pathway of *Mycobacterium bovis* BCG. These genes were isolated by complementation of *Escherichia coli* mutations with an expression library of BCG DNA. Our analysis of these genes suggests that BCG may use more than one pathway for biosynthesis of DAP. The nucleotide sequence of the BCG *dapB* gene was determined. The activity of the product of this gene in *E. coli* provided evidence that the gene may encode a novel bifunctional dihydrodipicolinate reductase and DAP dehydrogenase.—Authors' Abstract

**Colston, M. J.** The molecular biology of *Mycobacterium leprae*. (Editorial) Lepr. Rev. **64** (1993) 289–294.

This editorial is a modified version of a presentation made to the Royal Society of Tropical Medicine and Hygiene at a meeting to mark the 30th anniversary of the Ridley-Jopling classification for leprosy. The full paper was to be published in the *Transactions of the Royal Society of Tropical Medicine and Hygiene*. Topics covered here include the taxonomic position of *Mycobacterium leprae* based on nucleic acid analysis, cloning and expression of *M. leprae* genes, molecular methods for rapid detection and identification of *M. leprae*, the *M. leprae* genome map, and the molecular basis of rifampin resistance and rapid detection of resistant isolates.—C. A. Brown (Trop. Dis. Bull.)

**de Wit, M. Y. L. and Klatser, P. R.** *Mycobacterium leprae* isolates from different sources have identical sequences of the spacer region between the 16S and 23S ribosomal RNA genes. Microbiology **140** (1994) 1982–1987.

To test for genotypic variations between different isolates of *Mycobacterium leprae*, the causative agent of leprosy, the 282-bp spacer region between the 16S and 23S rRNA genes was amplified using PCR, and submitted to single-strand conformation polymorphism (SSCP) anlaysis. The procedure was optimized using four modified spacer fragments, containing mutations at one, three, four and six positions, respectively. Seventy-five *M. leprae* isolates from different sources, including isolates from leprosy patients, healthy individuals, armadillos and mouse foot pads, were identical in the SSCP analysis. DNA sequencing and restriction enzyme analysis performed on four and 40 samples, respectively, con-

firmed the results obtained with SSCP analysis.—Authors' Abstract

**Griggs, D. J., Hall, M. C., Jin, Y. F. and Piddock, L. J. V.** Quinolone resistance in veterinary isolates of salmonella. J. Antimicrob. Chemother. **33** (1994) 1173–1189.

Twenty-seven nalidixic acid-resistant (MIC ≥ 256 mg/L) isolates of salmonella from veterinary sources were also less susceptible to fluoroquinolones (range of MICs of ciprofloxacin, 0.12–2 mg/L). Six isolates were crossresistant to one or more chemically unrelated antibacterial agents. The concentrations of enrofloxacin that inhibited DNA synthesis by 50% were similar to the MIC values for 23 of 27 isolates, suggesting a mutation in *gyrA*. Insertion of pNJR3-2 (*gyrA*) in 9 of 20 isolates increased susceptibility to quinolones, suggesting that resistance was due to mutation in *gyrA*. Five of 27 isolates had reduced levels of accumulation of enrofloxacin. Two of the five also had increased susceptibility to quinolones when pNJR3-2 was introduced. None of the outer membrane protein profiles of the resistant isolates differed from those of sensitive control strains. Three of 27 isolates had differences in lipopolysaccharide profiles compared to control strains. Although the MIC of ciprofloxacin was less than the recommended U.K. break point concentrations for most isolates, the increasing incidence of quinolone-resistance in salmonella from veterinary sources is a matter of concern.—Authors' Abstract

**Ji, Y., Kempsell, K. E., Colston, M. J. and Cox, R. A.** Nucleotide sequences of the spacer-1, spacer-2 and trailer regions of the *rrn* operons and secondary structures of precursor 23S rRNAs and precursor 5S rRNAs of slow-growing mycobacteria. Microbiology **140** (1994) 1763–1773.

The single ribosomal RNA (*rrn*) operons of slow-growing mycobacteria comprise the genes for 16S, 23S and 5S rRNA, in that order. PCR methodology was used to amplify parts of the *rrn* operons, namely the spacer-1 region separating the 16S rRNA and 23S rRNA genes and the spacer-2 region separating the 23S rRNA and 5S rRNA genes of *Mycobacterium avium, M. intracellulare, "M. lufu"* and *M. simiae*. The amplified DNA was sequenced. The spacer-2 region, the 5S rRNA gene, the trailer region and the downstream region of the *rrn* operon of *M. tuberculosis* were cloned and sequenced. These data, together with those obtained previously for *M. leprae*, were used to identify putative antitermination signals and RNase III processing sites within the spacer-1 region. Notable features include two adjacent potential Box B elements and a Box A element. The latter is located within a sequence of 46 nucleotides which is very highly conserved among the slow-growers which were examined. The conserved sequence has the capacity to interact through base-pairing with part of the spacer-2 region. Secondary structures for mycobacterial precursor 23S rRNA and for precursor 5S rRNA were devised, based on sequence homologies and homologous nucleotide substitutions. All the slow-growers, including *M. leprae*, conform to the same scheme of secondary structure. A putative motif for the intrinsic termination of transcription was identified approximately 33 bp downstream from the 3'-end of the 5S rRNA gene. The spacer-1 and spacer-2 sequences may prove a useful supplement to 16S rRNA sequences in establishing phylogenetic relationships between very closely related species.—Authors' Abstract

**Linton, C. J., Jalal, H., Leeming, J. P. and Millar, M. R.** Rapid discrimination of *Mycobacterium tuberculosis* strains by random amplified polymorphic DNA analysis. J. Clin. Microbiol. **32** (1994) 2169–2174.

Investigations of the epidemiology of tuberculosis have been hampered by the lack of strain-specific markers that can be used to differentiate isolates of *Mycobacterium tuberculosis*. We report the development of a rapid protocol for random amplified polymorphic DNA analysis which included the use of a commercially available DNA extraction kit (GeneReleaser). This was applied to 14 strains of *M. tuberculosis*, including strains associated with temporal and geographical clusters of tuberculosis in the United Kingdom and those from India, Africa, and Saudia Arabia. Strains of *M. tu-*

*berculosis* could be discriminated in about 8 hr by this method, which is, therefore, a rapid and simple alternative to restriction fragment length polymorphism analysis.—Authors' Abstract

**Lopez Marin, L. M., Quesada, D., Lakhdarghazal, F., Tocanne, J. F. and Laneelle, G.** Interactions of mycobacterial glycopeptidolipids with membranes: influence of carbohydrate on induced alterations. Biochemistry **33** (1994) 7056–7061.

Glycopeptidolipids (GPLs) are specific constituents of mycobacteria known as opportunistic pathogens. The influence of the carbohydrate moiety on GPL-induced membrane alterations was examined with GPLs bearing 1–5 sugar residues (GPL-1 to GPL-5) and a sulfated GPL (S-GPL-2). GPLs decreased the ADP/O ratio and increased controlled respiration of isolated mitochondria. The more polar GPLs were the less active, with the following order of efficiency: GPL-1 > GPL-2 > S-GPL-2 = GPL-3 = GPL-5. GPL-1 and GPL-2 increased passive permeability of liposomes to carboxyfluorescein (GPL-1 > GPL-2), while GPL-3 and GPL-5 were inactive. GPL-2 and GPL-3 decreased the transmembrane electrical potential (Delta Psi) in isolated mitochondria (GPL-2 > GPL-3). These results suggest that GPLs uncouple oxidative phosphorylation by increasing the passive permeability of the mitochondrial membrane to protons. Compression isotherms of GPL-2 monolayers showed that, at low surface pressure, the area per GPL-2 molecule was about 5 times that of an acyl chain: it is likely that the peptide moiety was at the air/water interface. With an increase in the surface pressure, its area decreased, down to that of a tightly packed acyl chain. It is postulated that the glycopeptidic moiety can be either at the interface or dipping into the water. GPL-2 insertion in liposomes rendered the acyl-chain part of the bilayer more accessible to ions, since a fluorescent probe located deep in the bilayer was much more quenched by Cu2+ ions in liposomes containing GPL-2 than in control liposomes, suggesting a disturbance of the bilayer interface. A model is proposed to explain the influence of the polarity of GPLs on their activity toward membrane properties.—Authors' Abstract

**Pessolani, M. C. V., Smith, D. R., Rivoire, B., McCormick, J., Hefta, S. A., Cole, S. T. and Brennan, P. J.** Purification, characterization, gene sequence, and significance of a bacterioferritin from *Mycobacterium leprae*. J. Exp. Med. **180** (1994) 319–327.

The study of tissue-derived *Mycobacterium leprae* provides insights to the immunopathology of leprosy and helps identify broad molecular features necessary for mycobacterial parasitism. A major membrane protein (MMP-II) of *in vivo*-derived *M. leprae* previously recognized (Hunter, S. W., B. Rivoire, V. Mehra, B. R. Bloom, and P. J. Brennan. 1990. J. Biol. Chem. 265: 14065) was purified from extracts of the organism and partial amino acid sequence obtained. This information allowed recognition, within one of the cosmids that encompass the entire *M. leprae* genome, of a complete gene, bfr, encoding a protein of subunit size 18.2 kD. The amino acid sequence deduced from the major membrane protein II (MMP-II) gene revealed considerable homology to several bacterioferritins. Analysis of the native protein demonstrated the iron content, absorption spectrum, and large native molecular mass (380 kD) of several known bacterioferritins. The ferroxidase-center residues typical of ferritins were conserved in the *M. leprae* product. Oligonucleotides derived from the amino acid sequence of *M. leprae* bacterioferritin enabled amplification of much of the MMP-II gene and the detection of homologous sequences in *M. paratuberculosis, M. avium, M. tuberculosis, M. intracellulare,* and *M. scrofulaceum.* The role of this iron-rich protein in the virulence of *M. leprae* is discussed.—Authors' Abstract

**Rastogi, N., Goh, K. S., Wright, E. L. and Barrow, W. W.** Potential drug targets for *Mycobacterium avium* defined by radiometric drug-inhibitor combination techniques. Antimicrob. Agents Chemother. **38** (1994) 2287–2295.

Previously established radiometric techniques were used to assess the effectiveness

of combined antimicrobial drug-inhibitory drug (drug-inhibitor) treatment on two clinical isolates of the *Mycobacterium avium* complex representing three colony variants: smooth opaque (dome) (SmO), smooth transparent (SmT), and rough (Rg). All variants were identified as members of the *M. avium* complex; however, only the SmT colony type of strain 373 possessed characteristic serovar-specific glycopeptidolipid (GPL) antigens. MICs, determined radiometrically, of drugs with the potential to inhibit the biosynthesis of GPL antigens or other cell-envelope constituents were similar for all strains. These drugs included cerulenin, N-carbamyl-DL-phenylalanine, N-carbamyl-L-isoleucine, trans-cinnamic acid, ethambutol, 1-fluoro-1-deoxy-beta-D-glucose, 2-deoxy-D-glucose, and m-fluorophenylalanine. The MICs of the antimicrobial drugs amikacin, sparfloxacin, and clarithromycin varied, but overall the MICs for the SmO variant were the lowest. Radiometric assessment of drug-inhibitor combinations by using established x/y determinations revealed enhanced activity when either ethambutol or cerulenin were used in combination with all antimicrobial agents for all variants except the Rg variant of strain 424, for which ethambutol was not effective. Enhanced activity with amino acid analogs was observed with the Rg colony variants of strains 373 and 424. Two potential sites for drug targeting were identified: fatty acid synthesis, for all strains assayed, and peptide biosynthesis, particularly for Rg colony variants that possess previously identified phenylalanine-containing lipopeptides as potential targets for future drug development.—Authors' Abstract

**Revel, V., Cambau, E., Jarlier, V. and Sougakoff, W.** Characterization of mutations in *Mycobacterium smegmatis* involved in resistance to fluoroquinolones. Antimicrob. Agents Chemother. **38** (1994) 1991–1996.

Fluoroquinolone-resistant mutants of *Mycobacterium smegmatis* have been obtained *in vitro* by using ofloxacin as a selecting agent. Two types of mutants were identified according to their quinolone resistance patterns. Type 1 showed a low level of resistance to ofloxacin (MIC of 8 μg/ml); whereas a high level of resistance to this drug (MICs of 32 to 64 μg/ml) characterized type 2. By using two oligonucleotide primers homologous to DNA sequences flanking the quinolone resistance-determining region (QRDR) in the *gyrA* gene of *Escherichia coli* and *Staphylococcus aureus*, a 150-bp DNA fragment was obtained by PCR amplification from total DNA of two wild-type and five mutant strains of *M. smegmatis*. The nucleotide sequences of the amplified fragments were determined. The deduced amino acid sequence from the wild-type strains showed ca. 79% similarity with the QRDR in the gyrase A subunit from other gram-positive and gram-negative bacteria. The DNA sequences obtained from the fluoroquinolone-resistant mutants of *M. smegmatis* exhibited nucleotide modifications compared with the wild-type QRDR. The QRDR from type 1 mutants had a C-T or an A-G transition leading to a change from Ala-83 to Val or Asp-87 to Gly, respectively. The QRDR from type 2 mutants had a Val-83 mutation or both Val-83 and Gly-87 mutations detected in the type 1 mutants. These results suggest that point mutations in the QRDR of the mycobacterial *gyrA* gene are responsible for acquired quinolone resistance in *M. smegmatis*.—Authors' Abstract

**Shannon, E. J., Frommel, D., Guebre-Xabier, M. and Haile-Mariam, H. S.** Titration of numbers of human-derived *Mycobacterium leprae* required to progressively oxidize $^{14}$C-palmitic acid and release $^{14}CO_2$. Lepr. Rev. **65** (1994) 100–105.

*Mycobacterium leprae* was isolated from skin-punch biopsies of two untreated lepromatous leprosy patients. The bacteria were enumerated, diluted 10-fold and cultured in Middlebrook 7H9 medium supplemented with albumin, dextrose, catalase and $^{14}$C-palmitic acid. The cultures were incubated at 33°C in a modified Buddemeyer radiorespiratory detection vessel. Those cultures containing at least $10^7$ mycobacteria demonstrated a progressive evolution of $^{14}CO_2$.—Authors' Summary

**Takano, M., Ohara, N., Mizuno, A. and Yamada, T.** Cloning, sequencing and ex-

pression in *Escherichia coli* of the gene for alpha antigen from *Mycobacterium scrofulaceum*. Scand. J. Immunol. **40** (1994) 165–170.

The gene for the extracellular $\alpha$ antigen of *Mycobacterium scrofulaceum* (S-$\alpha$) was cloned by using the $\alpha$ antigen gene fragments of *M. bovis* BCG as probes. The complete nucleotide sequence was determined. The gene was expressed in *Escherichia coli*. The gene encodes 330 amino acids, including 40 amino acids for the signal peptide, followed by 290 amino acids for the mature protein. The deduced amino acid sequences were highly homologous to the $\alpha$ antigen of the species of other mycobacteria. Interestingly, the antigens of MAIS complex (*M. avium-M. intracellulare-M. scrofulaceum*) were closely homologous even at the C-terminal regions which were variable among those of *M. bovis* BCG, *M. leprae* and *M. kansasii*.—Authors' Abstract

**Trias, J. and Benz, R.** Permeability of the cell wall of *Mycobacterium smegmatis*. Mol. Microbiol. **14** (1994) 283–290.

The cell wall of *Mycobacterium smegmatis* mc$^2$155 was shown to be an effective permeability barrier to hydrophilic compounds. Permeability coefficients to $\beta$-lactams ranged from $10 \times 10^{-7}$ to $0.5 \times 10^{-7}$cm sec$^{-1}$. Cell-wall proteins were solubilized with EDTA and Genapol and were tested for channel-forming activity by reconstitution into lipid bilayers. Proteins were able to induce a voltage-gated cation-selective channel. The mycobacterial porin channel appeared to be water-filled since the single-channel conductance followed the mobility sequence of hydrated ions in the aqueous phase. On the basis of the Renkin equation and the single-channel conductance, the channel diameter was estimated to be around 3 nm. Model calculations showed that cation selectivity may be caused by four negative point-charges at the channel mouth. The permeability properties of the cell wall of intact cells were in good agreement with those of the reconstituted channel. Negatively charged cephalosporins, cefamandole and cephalothin, diffused at a 10- to 20-fold lower rate than the zwitterionic cephaloridine. The mycobacterial porin represents a major hydrophilic pathway of the cell wall of *M. smegmatis*.—Authors' Summary

**van der Vliet, G. M. E., Schepers, P., Schukkink, R. A. F., van Gemen, B. and Klatser, P. R.** Assessment of mycobacterial viability by RNA amplification. Antimicrob. Agents Chemother. **38** (1994) 1959–1965.

We investigated whether the presence of intact RNA is a valuable indicator of viability of mycobacteria with *Mycobacterium smegmatis*. *M. smegmatis* was exposed to various concentrations of rifampin and ofloxacin suspended in broth for different periods of time. The nucleic acid-sequence based amplification (NASBA) nucleic acid amplification system was used because of its rapid, sensitive, and specific detection of 16S rRNA. During drug exposure, the viability of the mycobacteria, expressed by the number of cfu, was compared with the presence of 16S rRNA as determined by NASBA and with the presence of DNA coding for 16S rRNA as determined by PCR. Both NASBA and PCR were shown to have a detection limit of approximately $5 \times 10^2$ cfu/ml. The intensity of the NASBA signal corresponded well with the number of cfu, and the lack of NASBA signal coincided with a loss of viability, which was reached after 3 days of exposure to bactericidal concentrations of both drugs. The presence of mycobacterial DNA, as determined by the intensity of the PCR signal, and the viability of *M. smegmatis* were not related, but an increase in the number of cells and intensity of PCR signal correlated well. Bacterial viability may thus be assessed by a rapid, sensitive, and specific, and semiquantitative technique by using NASBA. This system of viability testing provides the potential for rapid evaluation of drug susceptibility testing.—Authors' Abstract

**Verma, A., Rattan, A. and Tyagi, J. S.** Development of a 23S rRNA-based PCR assay for the detection of mycobacteria. Indian J. Biochem. Biophys. **31** (1994) 288–294.

The partial nucleotide sequence of a recombinant plasmid containing the 23S

rRNA gene of *Mycobacterium tuberculosis* was determined and an assay was developed for amplifying 23S rRNA gene sequences of mycobacteria. The PCR-based non-radioactive test enabled us to distinguish *Mycobacterium* from other closely related genera and was sensitive enough to detect two bacterial genome equivalents. The assay was extended to the detection of mycobacterial DNA in uncultured clinical specimens; 23S rRNA sequences were detected in 34 of 48 (70.8%) sputum and cerebrospinal fluid (CSF) specimens by the PCR assay; whereas direct smear examination and culture methods demonstrated a positivity rate of 29.2% and 16.7%, respectively, for the same specimens. A RNA-based PCR assay with a detection limit of 1 genome equivalent was also developed. These PCR assays should prove useful for early and rapid detection of mycobacterial infection in uncultured clinical specimens.—Authors' Abstract

**Walker, G. T., Nadeau, J. G., Spears, P. A., Schram, J. L., Nycz, C. M. and Shank, D. D.** Multiplex strand displacement amplifications (SDA) and detection of DNA sequences from *Mycobacterium tuberculosis* and other mycobacteria. Nucl. Acids Res. **22** (1994) 2670–2677.

Strand Displacement Amplification (SDA) is an isothermal, *in vitro* method of amplifying a DNA target sequence prior to detection [Walker, *et al.* (1992) Nucleic Acids Res., 20, 1691–1693]. Here we describe a multiplex form of SDA that allows two target sequences and an internal amplification control to be co-amplified by a single pair of primers after common priming sequences are spontaneously appended to the ends of target fragments. Multiplex SDA operates at a single temperature, under the same simple protocol previously developed for single-target SDA. We applied multiplex SDA to co-amplification of a target sequence (IS*6110*) that is specific to members of the *Mycobacterium tuberculosis*-complex and a target (16S ribosomal gene) that is common to most clinically relevant species of mycobacteria. Both targets are amplified $10^8$-fold during a 2 hr, single temperature incubation. The relative sensitivity of the system was evaluated across a number of clinically relevant mycobacteria and checked for crossreactivity against organisms that are closely related to mycobacteria.—Authors' Abstract

**Wayne, L. G. and Sramek, H. A.** Metronidazole is bactericidal to dormant cells of *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **38** (1994) 2054–2058.

Very abrupt exposure to anaerobic conditions has a lethal effect on actively growing cultures of *Mycobacterium tuberculosis.* However, incubation under conditions in which oxygen is depleted gradually causes *M. tuberculosis* to shift down from active replication to dormancy. The dormant bacilli are resistant to the bactericidal effects of anaerobiosis and also exhibit partial or complete resistance to the bactericidal effects of isoniazid and rifampin. On the other hand, metronidazole, a drug specific for anaerobes, kills dormant tubercle bacilli under anaerobic conditions, but it has no effect on actively growing aerobic cultures. The lethal effect of metronidazole under anaerobic conditions is enhanced by rifampin. The possible implications of these findings on the phenomenon of latency in tuberculosis are discussed.—Authors' Abstract

**Williams, D. L., Waguespack, C., Eisenach, K., Crawford, J. T., Portaels, F., Salfinger, M., Nolan, C. M., Abe, C., Sticht-Groh, V. and Gillis, T. P.** Characterization of rifampin resistance in pathogenic mycobacteria. Antimicrob. Agents Chemother. **38** (1994) 1380–1386.

The emergence of rifampin-resistant strains of pathogenic mycobacteria has threatened the usefulness of this drug in treating mycobacterial diseases. Critical to the treatment of individuals infected with resistant strains is the rapid identification of these strains directly from clinical specimens. It has been shown that resistance to rifampin in *Mycobacterium tuberculosis* and *M. leprae* apparently involves mutations in the *rpoB* gene encoding the $\beta$-subunit of the RNA polymerases of these species. DNA sequences were obtained from a 305-bp fragment of the *rpoB* gene from 110 rifampin-resistant and 10 rifampin-susceptible strains of *M. tuberculosis* from diverse

geographical regions throughout the world. In 102 of 110 rifampin-resistant strains 16 mutations affecting 13 amino acids were observed. No mutations were observed in rifampin-susceptible strains. No association was found between particular mutations in the *rpoB* gene and drug susceptibility patterns of multidrug-resistant *M. tuberculosis* strains. Drug-resistant *M. tuberculosis* strains from the same outbreak and exhibiting the same IS*6110* DNA fingerprint and drug susceptibility pattern contained the same mutation in the *rpoB* gene. However, mutations are not correlated with IS*6110* profiling outside of epidemics. The evolution of rifampin resistance as a consequence of mutations in the *rpoB* gene was documented in a patient who developed rifampin resistance during the course of treatment. Rifampin-resistant strains of *M. leprae, M. avium,* and *M. africanum* contained mutations in the *rpoB* gene similar to that documented for *M. tuberculosis.* This information served as the basis for developing a rapid DNA diagnostic assay (PCR-heteroduplex formation) for the detection of rifampin susceptibility of *M. tuberculosis.*—Authors' Abstract

**Zwadyk, P., Jr., Down, J. A., Myers, N. and Dey, M. S.** Rendering of mycobacteria safe for molecular diagnostic studies and development of a lysis method for strand displacement amplification and PCR. J. Clin. Microbiol. **32** (1994) 2140–2146.

Two criteria must be met before mycobacterial specimens can be tested by DNA amplification methods: (i) the samples must be rendered noninfectious, and (ii) the organisms must be lysed to free the DNA. Previous publications reporting DNA amplification of mycobacteria have concentrated on lysis and amplification procedures and have not addressed the issue of sample safety. We have shown that heating of samples below 100°C may not consistently kill mycobacteria; however, heating at 100°C in a boiling-water bath or a forced-air oven for a minimum of 5 min kills mycobacteria, including *Mycobacterium thermoresistibile.* Furthermore, heating at 100°C for 30 min consistently lyses mycobacteria to produce short fragments of DNA that are suitable for amplification by PCR and strand displacement amplification. This procedure works with clinical samples digested by the *n*-acetyl cysteine-NaOH method as well as with suspensions of organisms in phosphate buffer. This paper also demonstrates the feasibility of using strand displacement of amplification with clinical specimens.—Authors' Abstract

## Experimental Infections

**De Blaquiere, G. E., Santamaria, L., Curtis, J., Terenghi, G., Polak, J. M. and Turk, J. L.** A morphological and functional assessment of *Mycobacterium leprae*-induced nerve damage in a guinea-pig model of leprous neuritis. Neuropathol. Appl. Neurobiol. **20** (1994) 261–271.

Nerve damage, resembling that caused by *Mycobacterium leprae* in man, was created by the injection of cobalt-irradiated *M. leprae* organisms into the tibial nerve of guinea pigs. Assessment of nerve damage was made by clinical, electrophysiological and morphometric means that intervals up to 13 weeks after injection. Quantitative immunohistochemical analysis of neuropeptide-containing fibers in the skin of the foot was also carried out. Significant nerve damage occurred 3 weeks after injection of *M. leprae* organisms. Motor and sensory functional loss peaked at 5 weeks after injection, and there was a significant decrease of peptide-immunoreactive nerves in all skin compartments. The nerve damage was self-limiting and functional recovery had occurred by 13 weeks. The model shows that many of the features found in the nerve damage of treated leprosy patients.—Authors' Abstract

**Gelber, R. H., Hunter, S. W., Murray, L. P., Siu, P., Tsang, M. and Brennan, P. J.** Effective vaccination of mice against *Mycobacterium leprae* with density-gradient

subfractions of soluble *M. leprae* proteins: clues to effective protein epitopes. Lepr. Rev. **65** (1994) 175–180.

It had previously been discovered that intradermal mouse vaccination with a protein fraction of *Mycobacterium leprae* (called soluble proteins) in Freund's incomplete adjuvant (FIA) resulted in consistent and long-lived protection against *M. leprae* multiplication from subsequent viable foot pad challenges. In this study certain density-gradient subfractions of this soluble protein, but not others, in FIA afforded vaccine protection. The results of this study suggest which *M. leprae* proteins may be involved in protective immunity, particularly 1–3 kD, 10 kD, 65 kD, and those of higher molecular weight.—Authors' Summary

**Ramakrishnan, L. and Falkow, S.** *Mycobacterium marinum* persists in cultured mammalian cells in a temperature-restricted fashion. Infect. Immun. **61** (1994) 3222–3229.

We have explored the relatively rapidly growing animal and human pathogen *Mycobacterium marinum* as an experimental model for mycobacterial pathogenesis. *M. marinum,* which has a lower temperature for optimal growth than does *M. tuberculosis,* has a much shorter generation time and can be safely studied in ordinary laboratory facilities and examined in multiple animal infection models. We have established an *in vitro* essay for its interaction with eukaryotic cells and shown that it persists in these cells in a temperature-specific fashion that correlates with its ability to cause disease *in vivo* at lower temperatures. Additionally, preliminary evidence that *M. marinum* causes a chronic disease with some features resembling tuberculosis in frogs of the species *Rana pipiens* is presented.—Authors' Summary

## Epidemiology and Prevention

**Gupte, M. D.** Elimination of leprosy: forecasts and projections. Indian J. Lepr. **66** (1994) 19–35.

It is possible to diagrammatically represent the pattern of prevalence and incidence under dapsone and MDT interventions observed in various endemic regions. A substantial decline in prevalence following dapsone was observed in several regions. Though a substantial proportion of this decline was attributable to cure on account of dapsone, it was also on account of some other factors such as patients found to be not in need of treatment, migration and deaths. Prevalence of leprosy was getting stabilized after the initial 10 to 15 years of the dapsone-based program. Interventions with the MDT campaigns have produced a dramatic decline in leprosy prevalence in a very short period, again followed by stabilization at a much lower level. Decline in prevalence was again attributable partly to therapy and partly to other factors. New case detection did not show a downward trend that could be attributed to dapsone or MDT *per se.* New case detection rates in children have not shown signs of reduction. It is also important to note that despite the occurrence of dapsone resistance or irregular intake of dapsone or possible shortcomings in the program, no recrudescence of leprosy on a large scale was ever recorded. There is a definite perceptible change in the profile of new cases of leprosy for the better. The essential effect of MDT campaigns has been a phenomenal decline in leprosy prevalence, providing opportunities to deliver disability care services to leprosy patients and to expand coverage of the program. There is a definite need to continue vigorously the MDT implementation and provide curative services and ensure availability of those services to all leprosy patients in the country. Available data do not indicate any immediate effect of MDT on transmission. It is quite likely that persons already infected will keep on developing leprosy lesions for some years. But then one would expect an increase in the proportion of cases occurring in adults as compared to children. This is not observed. There will

be several questions like this and more. Monitoring leprosy trends may provide some clues and development of sentinel centers would be an essential requirement to generate the necessary data. It is expected that MDT implementation would become operationally easier over a period of time after devising shorter and effective drug regimens. There is a distinct need to improve the operational performance in terms of case detection. The need for diagnosing single-lesion evanescent forms of leprosy is being doubted, at least in some quarters. Emphasis on diagnosing leprosy patients mainly responsible for transmission or those at risk of deformities cannot be questioned. Perhaps there is a need to optimize the program by adopting some of these kinds of suggestions. Will these modifications lead to recrudescence? Based on earlier experiences with dapsone therapy program such a scenario is an unlikely event. It is thus possible to adopt some of these reforms while keeping a close watch on the situation through the sentinel centers. These centers would provide the kind of data that can be effectively used for predictions of trends, corrections of strategies and developing rational programs.—Authors' Conclusions

**Isa Isa, R. A. and Castellazzi, Z.** [Scope of the Action Plan for the Elimination of Leprosy in the Dominican Republic.] Rev. Dominica Republ. **30** (1993) 19–22. (in Spanish)

The principal features of the Action Plan for the Elimination of Leprosy as a Public Health Problem in the Dominican Republic are presented. The essential components of the epidemiological situation, projections, intervention strategy, support and epidemiological observation are reviewed. An outline of their projection into the near future is presented.—Authors' English Summary

**Krishnamurthy, P., Rao, P. S., Subramanian, M. and Inderparkash.** The influence of operational factors in the profile of monolesional leprosy cases in South India. Lepr. Rev. **65** (1994) 130–136.

A comparison of the profile of monolesional cases among new PB cases detected in a Governmental Leprosy Control Unit (GLCU) and the field area of a Central Leprosy Teaching and Research Institute (CLTRI), both located in South India, demonstrates that the proportion of monolesional cases among new cases detected between 1987 and 1991 was higher in children than adults, higher in females than males (only in the CLTRI)—over 95% were the tuberculoid type. A significantly increasing trend in this proportion could be seen in the GLCU but not in the CLTRI; an explanation of this is based on the difference in operational aspects in case detection methodology adopted by the two areas—e.g., intersurvey interval and mode of case detection. Such studies, focusing on single skin lesions, help us in understanding the role of various possible operational factors in influencing the behavior of the disease.—Authors' Summary

**Sansarricq, H.** Some points on the elimination of leprosy. (Editorial) Lepr. Rev. **65** (1994) 81–87.

Is the elimination strategy an improvement compared to the control strategy? It would appear that, if compared to the control strategy, the elimination strategy is likely to result in increased case detection, increased MDT coverage and better monitoring of progress. The reasons for which the elimination strategy should be more effective than the control strategy are its specific components: a "time limit" and a "defined target."

What is the importance of the drawbacks of the elimination strategy? (a) The need for additional resources does not appear a serious constraint. (b) There will be important operational difficulties: (1) related to activities (case detection, MDT delivery, treatment compliance, etc.); and (2) related to monitoring of progress as we do not have an adequate indicator for measuring progress in low prevalence/incidence situations.

Is it timely to engage in an elimination strategy? It seems that if the elimination strategy is implemented as a further step and natural expansion of the control strategy, there can be only advantages, such as: (a) It can be built on the infrastructure and facilities already established for the control strategy, and can take advantage of the experience gained by its implementation. (b) In terms of caseload, a better operational and possibly epidemiological (concerning

incidence) impact than with the control strategy; in any case, the greatest impact which could be obtained from an MDT-based strategy.

It is important to note that these advantages will become effective only if the two following conditions are met. (a) Operational methods are developed to overcome operational difficulties. This should be feasible, although unconventional solutions to some problems should be accepted. (b) Methods for monitoring progress in low prevalence/incidence situations will have to be developed. This may cause real difficulties. However, full use should be made of the ratio prevalence/case detection rate. This indicator may prove to have a greater significance than expected.

Altogether, it seems that the advantages of implementing the elimination strategy in sequence with the control strategy, and as a natural expansion of the latter, outweighs the disadvantages. In contrast, if implementing the elimination strategy was delayed, there is no certainty that the methods required to facilitate case-detection and case-holding, and to monitor progress in low prevalence/incidence situations, would be developed without the commitment of national authorities to eliminate leprosy as a public health problem.—From the Editorial

**Suite, M., Edinborough, N. B., Lewis, M. and Tollefson, J.** A survey to determine the prevalence of leprosy in a community in East Trinidad. Lepr. Rev. **65** (1994) 122–129.

A house-to-house survey was conducted in a community in East Trinidad, where a clustering of cases has been observed. There were 1355 residents, of whom 73.5% had a complete visual skin examination. No new cases of leprosy were found but a variety of skin disorders were diagnosed. The most common disorder was pityriasis versicolor, which is one of the differential diagnoses of hypopigmented skin lesions. This has serious implications for the delayed diagnosis of leprosy. In all, 5 of the 9 old cases residing in the survey area suffered from paucibacillary disease, and had a history of contact with a lepromatous case. They were not listed initially as contacts of this index case. Contact lists should therefore include non-familial persons having frequent contact with an index case. The definition of "frequent" should be determined by each program. It may also be necessary to review the duration of surveillance of contacts. The survey was estimated to have cost about US $2500 and was not considered to be cost-effective.—Authors' Summary

## Rehabilitation

**Birke, J. A., Foto, J. G., Deepak, S. and Watson, J.** Measurement of pressure walking in footwear used in leprosy. Lepr. Rev. **65** (1994) 262–271.

Pressure measurements were made on 10 leprosy patients while walking barefoot and while using 6 sample shoes. The sample shoes, which represented footwear currently used worldwide in leprosy programs, included: 1. a U.S.A. extradepth shoe without insole; 2. a U.S.A. extradepth shoe with insole; 3. a Chinese tennis shoe; 4. a Mozambique sandal; 5. a Bombay sandal; 6. a Bombay sandal with rigid sole; and 7. the patients prescribed footwear. Peak pressure was significantly lower while walking in all footwear, except with the extradepth shoe without an insole, when compared to barefoot walking. Peak pressure was significantly lower walking in the Bombay sandals, the Chinese tennis shoe, the extradepth shoe with an insert and the patient's prescribed shoe when compared to the extradepth shoe without an insert. Regression analysis showed a significant inverse relationship between pressure and insole thickness.—Authors' Summary

**Kuipers, M. and Schreuders, T.** The predictive value of sensation testing in the development of neuropathic ulceration on the hands of leprosy patients. Lepr. Rev. **65** (1994) 253–261.

The early detection of the loss of protective sensation in leprosy patients is vital if neuropathic ulceration and subsequent dis-

abilities are to be avoided. The aim of this study was to find the protective value of sensory thresholds in the hands of leprosy patients. Thresholds for touch-pressure, vibration and temperature were assessed in areas on leprosy-affected hands near ulcers or ulcer scars (LU-group), in areas without lesions (LN-group), and in controls (N-group). Semmes-Weinstein monofilaments (SWF) were used for testing the touch-pressure threshold (PST), a biothesiometer for the vibration threshold (VST) and a Thermo Sensation Tester for the temperature threshold (TST). The distribution of ulcers was about equal on both palmar and dorsal aspects of the hands. In the LU-group there was a negative response to SWF of 2.0 g in all patients, while 74% could feel the 2.0 g in LN-areas and in N-areas 100% could detect the 2.0 g SWF. In the LU-group about 11% felt 8 V VST, in the LN-group about 60% and in the N-group 89%. Testing temperature sensation was given up prematurely because the results in controls were unsatisfactory. Both palmar and dorsal sides of the hands should be tested for sensation. The thresholds for protective sensation are 2.0 g SWF and 8 V for vibration sense. It is recommended that Semmes-Weinstein monofilaments should always be used for early detection of loss of protective sensation.—Authors' Summary

**Malaviya, G. N., Husain, S., Girdhar, A. and Girdhar, B. K.** Sensory functions in limbs of normal persons and leprosy patients with peripheral trunk damage. Indian J. Lepr. **66** (1994) 157–164.

The threshold to touch was tested in the hands and feet of normal persons using Semmes-Weinstein graded monofilament nylons. The minimum stimulus to which response could be elicited was nylon number 3.61 in palms and 4.31 in soles. These numbers relate to the logarithm of the force applied, 3.61 corresponding to 0.217 gm force and 4.31 to 2.35 gm force, respectively. The area of pain insensitivity complained by the patient more or less corresponds to that revealed by objective testing. It was interesting to observe that loss of pain sensitivity was confined to a smaller area compared to touch and thermal insensibility in the part innervated by the same nerve trunk.—Authors' Abstract

**Terencio de las Aguas, J.** [Pathology of the feet in leprosy.] Rev. Leprol. Fontilles **19** (1994) 543–555. (in Spanish)

The affinity of leprosy for the skin and peripheral nervous system leads to situations that frequently affect the foot regarding skin and nerve lesions. The different lesions are explained, the most important, being neurotrophic and plantar ulcers, dissociated tarsus, and bone and joint lesions; all are causes of disabilities and need hospitalization, and are an important cause of difficulty for social reinsertion.—Authors' English Summary

**van Brakel, W. H., Shute, J., Dixon, J. A. and Arzet, H.** Evaluation of sensibility of leprosy—comparison of various clinical methods. Lepr. Rev. **65** (1994) 106–121.

In order to determine whether various sensibility tests, not in common use at our hospital, are appropriate for the neurological screening of leprosy patients, an extended nerve function assessment (NFA) was done on 50 in- and outpatients who had been diagnosed as suffering from leprosy (100 hands and feet). The nerve function assessment battery consisted of Semmes-Weinstein monofilament testing (SWMT), moving 2-point discrimination (M2PD), pinprick (PP), position sense (PS), vibration sense (VS) and voluntary muscle testing (VMT). In addition the SWMT was performed on 637 hands and 634 feet of "field patients" in order to get a better indication of the prevalence of sensory impairment as measured with the SWMT. The SWMT has been shown to be a sensitive test of peripheral nerve function; therefore the other tests were compared with the SWMT. Results were reported separately for the ulnar, median and posterior tibial nerve. Test sites were the pulp of the distal phalanx of the index finger, the little finger and the big toe. Correlation between the SWMT and each of the other tests proved statistically significant; the closest correlations were between the SWMT, M2PD and PP for both ulnar and median nerves ($r > 0.7$, F test $> 100$, $p < 0.0001$). It is argued that the first tests

to show nerve function impairment (NFI) are the M2PD and the SWMT. VS and PS were also absent in a significant proportion of patients. Arguments are presented that this may indicate advanced nerve function impairment. Results are compared with other data currently available in the literature.—Authors' Summary

## Other Mycobacterial Diseases and Related Entities

**Adal, K. A., Anglim, A. M., Palumbo, C. L., Titus, M. G., Coyner, B. J. and Farr, B. M.** The use of high-efficiency particulate air-filter respirators to protect hospital workers from tuberculosis—a cost-effectiveness analysis. N. Engl. J. Med. **331** (1994) 169–173.

After outbreaks of multidrug-resistant tuberculosis (TB), the Centers for Disease Control and Prevention proposed the use of respirators with high-efficiency particulate air filters (HEPA respirators) as part of isolation precautions against TB, along with a respiratory-protection program for health care workers that includes medical evaluation, training, and tests of the fit of the respirators. Each HEPA respirator costs between $7.51 and $9.08, about 10 times the cost of respirators currently used.

We conducted a cost-effectiveness analysis using data from the University of Virginia Hospital on the exposure to patients with TB and rates at which the purified-protein-derivative (PPD) skin test became positive in hospital workers. The costs of a respiratory-protection program were based on those of an existing program for workers dealing with hazardous substances.

During 1992, 11 patients with documented TB were admitted to our hospital. Eight of 3852 workers (0.2%) had PPD tests that became positive. Five of these conversions were believed to be due to the booster phenomenon; one followed unprotected exposure to a patient not yet in isolation; the other two occurred in workers who had never entered a TB isolation room. These data suggest that it will take more than 1 year for the use of HEPA respirators to prevent a single conversion of the PPD test. Assuming that one conversion is prevented per year, however, it would take 41 years at our hospital to prevent one case of occupationally acquired TB, at a cost of $1.3 million to $18.5 million.

Given the effectiveness of currently recommended measures to prevent nosocomial transmission of TB, the addition of HEPA respirators would offer negligible protective efficacy at great cost.—Authors' Abstract

**Adams, J. L. and Czuprynski, C. J.** Mycobacterial cell wall components induce the production of TNF-alpha, IL-1, and IL-6 by bovine monocytes and the murine macrophage cell line RAW 264.7. Microb. Pathogen. **16** (1994) 401–411.

Johne's disease is characterized by a chronic enteritis that results in granulomatous inflammation, cachexia, and eventual death of cattle infected with *Mycobacterium paratuberculosis*. The cytokines tumor necrosis factor-alpha (TNF-$\alpha$), interleukin-1 (IL-1), and interleukin-6 (IL-6) have been associated with granuloma formation and wasting in other disease syndromes. The potential role of these cytokines in the development and progression of Johne's disease has not been investigated. Freshly isolated bovine peripheral blood monocytes and the murine macrophage cell line RAW 264.7 were examined for their ability to release inflammatory cytokines in response to mycobacterial cell wall components. Bovine monocytes and RAW 264.7 cells incubated with *M. paratuberculosis* lipoarabinomannan (LAM), muramyl dipeptide (MDP), or lipopolysaccharide (LPS) released INF-$\alpha$, IL-1 $\beta$, and IL-6 as detected by appropriate bioassays. Using the RAW 264.7 cells, cytokine mRNA levels were elevated after *in vitro* incubation with live *M. paratuberculosis* or LPS as determined using a reverse-transcriptase polymerase chain reaction procedure.—Authors' Abstract

**Altamirano, M., Marostenmaki, J., Wong, A., Fitzgerald, M., Black, W. A. and Smith, J. A.** Mutations in the catalase-peroxidase gene from isoniazid-resistant *Mycobacterium tuberculosis* isolates. J. Infect. Dis. **169** (1994) 1162–1165.

Isoniazid resistant in *Mycobacterium tuberculosis* has been associated with total deletion of the katG gene, which codes for catalase-peroxidase production. To determine whether this is a common mechanism of drug resistance, 9 isolates of isoniazid-resistant and 1 of isoniazid-sensitive *M. tuberculosis* were analyzed by polymerase chain reaction amplification of a 237-bp sequence of the katG gene. Amplification was observed in the isoniazid-sensitive isolate and in 8 resistant isolates; in only 1 isoniazid-resistant isolate was there no amplification of the expected band, suggesting gene deletion. DNA sequencing showed that 8 of the 9 isolates had point mutations, deletions, or insertions of 1–3 bases. Evidence corroborating the presence of mutations in the katG gene was obtained by single-strand conformation polymorphism analysis in these 8 isolates. Thus, mutations as well as insertions and deletions in the katG gene can account for inactive catalase peroxidase, leading to isoniazid resistance; gene deletion occurs only infrequently, similar to 11% of cases.—Authors' Abstract

**Appelberg, R., Castro, A. G., Pedrosa, J., Silva, R. A., Orme, I. M. and Minoprio, P.** Role of gamma interferon and tumor necrosis factor alpha during T-cell-independent and -dependent phases of *Mycobacterium avium* infection. Infect. Immun. **62** (1994) 3962–3971.

To design an effective immunotherapy for *Mycobacterium avium* infections, the protective host response to the infection must be known. Here were analyzed the role of gamma interferon (IFN-$\gamma$) and tumor necrosis factor alpha (TNF-$\alpha$) in the innate and acquired responses to *M. avium* infections in mice. T-cell depletion studies showed that CD4+ T cells were required for control of the infection. CD4+-dèpleted mice showed enhanced bacterial proliferation and at the same time showed a reduction in the level of expression of both IFN-$\gamma$ and TNF-$\alpha$ mRNAs in spleen cells. In contrast, *M. bovis* BCG immunization restricted *M. avium* proliferation and at the same time promoted expression of the mRNAs for the two cytokines. *In vivo* depletion studies using specific monoclonal antibodies showed that both IFN-$\gamma$ and TNF-$\alpha$ are involved in an early protection possibly involving NK cells, and furthermore, IFN-$\gamma$ is involved in the later T-cell-protective response to infection. *In vivo* neutralization of IFN-$\gamma$ during *M. avium* infection also blocked the priming for enhanced TNF-$\alpha$ secretion triggered by endotoxin. Both cytokines were found to be involved in the resistance expressed in BCG-immunized animals and exhibited additive bacteriostatic effects *in vitro* on bone marrow-derived macrophages infected with different strains of *M. avium*. These data suggest that both cytokines act in an additive or synergistic fashion in the induction of bacteriostasis and that IFN-$\gamma$ is also involved in priming TNF-$\alpha$ secretion.—Authors' Abstract

**Bermudez, L. E., Kolonoski, P., Young, L. S. and Inderlied, C. B.** Activity of KRM 1648 alone or in combination with ethambutol or clarithromycin against *Mycobacterium avium* in beige mouse model of disseminated infection. Antimicrob. Agents Chemother. **38** (1994) 1844–1848.

Rifamycins are active against slowly growing mycobacteria, such as *Mycobacterium tuberculosis* and *M. kansasii,* but the majority of rifamycins thus far investigated both *in vitro* and *in vivo* are inactive or have only modest activity against the *M. avium* complex (MAC). We investigated the activity of three doses of semisynthetic benzoxazinorifamycin KRM 1648, alone or in combination with ethambutol or clarithromycin, in beige mice challenged with the MAC strain 101. Our results show the following: (i) KRM 1648 was significantly effective against MAC infection as determined by the reduction of the number of bacteria in the blood, liver, and spleen when administered at doses of 20 and 40 mg/kg of body weight per day but not at 10 mg/kg/day, compared with untreated controls.

(ii) KRM 1648 (40 mg/kg/day) administered in combination with ethambutol (100 mg/kg/day) resulted in significant reduction in bacteremia compared with values for untreated controls ($p < 0.001$), KRM 1648 alone ($p = 0.019$), and ethambutol alone ($p = 0.003$). Furthermore, the combination of KRM 1648 and ethambutol was associated with a significant decrease in the number of bacteria in the spleen and the liver compared with values for both untreated controls and each drug alone ($p < 0.001$ for all comparisons). (iii) KRM 1648 (40 mg/kg/day) administered in combination with clarithromycin (200 mg/kg/day) resulted in a significant decrease in the number of bacteria in the blood and the spleen compared with the number for untreated controls ($p < 0.001$ for all comparisons). In our experience, using MAC 101 as the challenging organism, KRM 1648 is the first rifamycin with significant activity *in vivo* against MAC infection in beige mice.—Authors' Abstract

**Bila, V. and Kren, V.** Evidence for teratogenicity of thalidomide using congenic and recombinant inbred rat strains. Folia Biol. **40** (1994) 161–171.

Teratogenic properties of thalidomide were tested in two systems of laboratory rat strains carrying the mutant lx allele that determines the polydactyly-luxate syndrome. In agreement with our previous experiments, we have confirmed that the response of fetuses basically depends on their genotype. Fetuses of LEW/BN, +/+ genotype remained unaffected following 500 or 3 × 500 mg/kg thalidomide doses (43 and 56 fetuses, respectively). In LEW/BN, +/lx fetuses these doses elicited 24% and 87% hind feet polydactyly (14/59 and 53/61 fetuses, respectively), which was highly significant when compared with 84 vehiculum-treated and 235 untreated controls ($p < 0.001$). However, in 48 SHR/RI 2, lx/lx fetuses both pairs of limbs were affected in an opposite way after the 500 mg/kg thalidomide dose: hind feet oligodactyly (94/96 limbs) and increased front feet polydactyly occurred (in comparison with 70 controls, $p < 0.001$). The mutant lx allele as well as modifying genes are involved in the response to thalidomide.—Authors' Abstract

**Cambau, E., Sougakoff, W., Besson, M., Truffot-Pernot, C., Grosset, J. and Jarlier, V.** Selection of a gyrA mutant of *Mycobacterium tuberculosis* resistant to fluoroquinolones during treatment with ofloxacin. J. Infect. Dis. **170** (1994) 479–483.

A strain of *Mycobacterium tuberculosis* resistant to ofloxacin was selected in a patient with a long history of multidrug-resistant tuberculosis eventually treated by ofloxacin combined with other second-line drugs. A mutation in the gyrA gene was hypothesized to be the mechanism of acquired resistance to ofloxacin in this strain. Chromosomal DNA of strains MTB1, isolated before treatment and susceptible to ofloxacin (MIC, 1 μg/mL), and MTB2, isolated during treatment and resistant to ofloxacin (MIC, 32 μg/mL), was amplified by polymerase chain reaction (PCR) using two oligonucleotide primers highly homologous to DNA sequences flanking the quinolone resistance-determining region in gyrA of mycobacteria. Comparison of the nucleotide sequences of the 150-bp fragments obtained by PCR revealed a point mutation in MTB2 leading to the substitution of histidine for aspartic acid at a position corresponding to residues involved in quinolone resistance in *Escherichia coli* (Asp87), *Staphylococcus aureus* (Glu88), and *Campylobacter jejuni* (Asp90).—Authors' Abstract

**Cauthen, C. M., Snider, D. E. and Onorato, I. M.** Boosting of tuberculin sensitivity among Southeast Asian refugees. Am. J. Respir. Crit. Care Med. **149** (1994) 1597–1600.

Following an initial negative Mantoux tuberculin skin test, a second test, given as soon as 1 wk later, has been shown to elicit markedly larger reactions (boosting) in 20% to 40% of refugees tested in the United States. We conducted a study to determine the explanation for this phenomenon. Using the Mantoux method of intradermal skin testing, 2469 refugees from Southeast Asia were initially tested with tuberculin followed by sequential retesting 7 and/or 90 d later. They were also tested initially with nontubuculous mycobacterial antigens. A high proportion (35.5%) of Southeast Asian

refugees had reactions ≥ 10-mm induration to an initial tuberculin test, and 30.9% of the nonreactors exhibited boosting on a subsequent tuberculin test. Boosting, unlike reactivity to the initial tuberculin test, was not associated with exposure to a person with tuberculosis. However, boosting was associated with reactivity to nontuberculous mycobacterial antigens and a history of bacille Calmette-Guérin (BCG) vaccination. Boosting in this population is therefore attributable to environmental exposure to nontuberculous mycobacteria that are endemic in Southeast Asia or to BCG vaccination, rather than to remote infection with *Mycobacterium tuberculosis*. Sequential tuberculin screening and preventive therapy of persons with boosted reactions is not recommended as a tuberculosis prevention strategy in this population.—Authors' Abstract

**Chin, D. P., Reingold, A. L., Stone, E. N., Vittinghoff, E., Horsburgh, C. R., Simon, E. M., Yajko, D. M., Hadley, W. K., Ostroff, S. M. and Hopewell, P. C.** The impact of *Mycobacterium avium* complex bacteremia and its treatment on survival of AIDS patients—a prospective study. J. Infect. Dis. **170** (1994) 578–584.

It is currently recommended that patients with AIDS and *Mycobacterium avium* complex (MAC) bacteremia receive antimycobacterial treatment. However, no study has prospectively evaluated the impact of this infection and its treatment on survival. This study prospectively followed a cohort of 367 AIDS patients with ≤50 CD4+ cells/μL and found that MAC bacteremia was independently associated with an increased risk of death (relative hazard [RH] = 1.8, 95% confidence interval [CI] = 1.3–2.4, $p < 0.001$). Patients with MAC bacteremia who were treated had a longer median survival than those who were not (263 vs 139 days, $p < 0.001$); treatment was independently associated with a lower risk of death (RH = 0.45, 95% CI = 0.23–0.89, p , 0.001). However, 23% of patients with bacteremia died within 28 days of that diagnosis; few were treated. MAC bacteremia contributes to the death of patients with AIDS, and treatment increases survival. However, many patients will not survive long enough to receive treatment. These results underscore the importance of early diagnosis and chemoprophylaxis for MAC bacteremia.—Authors' Abstract

**Curcic, R., Dhandayuthapani, S. and Deretic, V.** Gene expression in mycobacteria: transcriptional fusions based on *xylE* and analysis of the promoter region of the response regulator *mtrA* from *Mycobacterium tuberculosis*. Mol. Microbiol. **13** (1994) 1057–1064.

Understanding promoter regulation and signal-transduction systems in pathogenic mycobacteria is critical for uncovering the processes that govern interactions of these bacteria with the human host. In order to develop additional genetic tools for analysis of mycobacterial promoters, the *xylE* gene from *Pseudomonas* was tested as a transcriptional fusion reporter in fast- and slow-growing mycobacteria. Initially, its utility was demonstrated by expression behind the hsp60 promoter in *Mycobacterium smegmatis* and *M. bovis* BCG. The presence of an active promoter in front of the promoterless *xylE* cassette on a plasmid was scored by development of a bright yellow color upon spraying of mycobacterial colonies on plates with a solution of catechol. The gene product of *xylE*, catechol 2,3 dioxygenase, was measurable in sonic extracts and whole cells, permitting quantitative determination of promoter activity in both fast- and slow-growing mycobacteria. The *xylE*-based mycobacterial transcriptional fusion plasmid pRCX3 was constructed and used to assess promoter activity within the sequences located upstream of the newly characterized *M. tuberculosis* H37Rv response regulator *mtrA*, a member of the superfamily of bacterial signal-transduction systems.—Authors' Summary

**Darling, T. N., Sidhumalik, N., Corey, G. R., Allen, N. B., Kamino, H. and Murray, J. C.** Treatment of *Mycobacterium haemophilum* infection with a antibiotic regimen including clarithromycin. Br. J. Dermatol. **131** (1994) 376–379.

A patient with rheumatoid arthritis developed ulcerated nodules predominantly on his legs. Skin biopsy and culture dem-

onstrated rheumatoid vasculitis and infection with *Mycobacterium haemophilum.* Improvement was not seen until clarithromycin was added to his treatment regimen.—Authors' Abstract

**Denis, M.** Tat protein from HIV-1 binds to *Mycobacterium avium* via a bacterial integrin—effects on extracellular and intracellular growth. J. Immunol. **153** (1994) 2072–2081.

We examined the interaction between HIV-1 Tat protein and the opportunistic pathogen *Mycobacterium avium.* AIDS-associated strains of *M. avium* were shown to bind Tat protein quite avidly in an attachment assay. The attachment of *M. avium* to Tat was shown to occur via the integrin alpha(5) beta(1) present on the mycobacterial cell surface. *M. avium* strains ere shown to bind to viral Tat protein with high affinity in a specific fashion (600 binding sites with a Kd of 1 to 5 nM). *M. avium* coated with Tat protein were shown to be more infective for human alveolar macrophages than untreated *M. avium.* Other HIV-1 Ags had no such effects (e.g., p24, p17). Examination of the cytokine profile of infected macrophages showed that *M. avium*-Tat complexes induced higher levels of TGF beta-1 [TGF beta(1)] than *M. avium* alone or *M. avium* that had been in contact with other viral proteins. Conditioned media from HIV-1-infected H9 cells released a factor that enhanced *M. avium* intramacrophage growth, and was partially neutralized by an anti-Tat Ab. Finally, Tat protein (purified or present in conditioned media from infected cells) moderately enhanced the growth of *M. avium* strains in extracellular media, and exposure of *M. avium* to Tat protein in the presence of IL-6 enhanced the growth of AIDS-associated strains. These data argue for an interaction between the Tat viral product an the opportunistic pathogen *M. avium* which may contribute to the exquisite susceptibility of AIDS subjects to this pathogen.—Authors' Abstract

**de Wit, M. Y. L., Palou, M. and Content, J.** Nucleotide sequence of the 85B-protein gene of *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis.* DNA Seq. **4** (1994) 267–270.

We have cloned and sequenced the genes coding for the 85B antigen from *M. bovis* BCG and *M. tuberculosis.* Within this gene, the only difference in sequence between *M. bovis* BCG and *M. tuberculosis* corresponds, respectively, to a C → T yielding a Leu → Phe replacement at position 100 of the mature 85B protein. Therefore, as we described previously for the 85A gene, there is also very little variation between these two species within the 85B gene.—Authors' Abstract

**Elsaatari, F. A. K., Engstrand, L., Hachem, C. Y., Graham, D. Y. and Naser, S. A.** Identification and characterization of *Mycobacterium paratuberculosis* recombinant proteins expressed in *E. coli.* Curr. Microbiol. **29** (1994) 177–184.

*Mycobacterium paratuberculosis* is the causative agent of Johne's disease, a chronic enteritis in ruminants, and it has also been isolated and identified from patients with Crohn's disease, an inflammatory bowel disease. The control of Johne's disease has been hampered by the lack of a reliable diagnostic test because of the large degree of antigenic crossreactivity between mycobacterial and nonmycobacterial species. To help identify specific antigen(s) or epitope(s), an *M. paratuberculosis* expression library was screened with antibodies and DNA probes. In total, 54 clones were randomly picked, purified, and characterized by DNA probes and monoclonal antibodies with known specificity to individual mycobacterial antigens. Four clones carrying the heat shock protein 65K-, two representing the secreted protein 32K-, three representing the 21K-, and 20 clones representing the specific insertion element of *M. paratuberculosis* (IS900)-encoding genes and their gene products were identified and characterized. Well-defined recombinant antigens and/or epitopes representing *M. paratuberculosis* may facilitate the development of specific diagnostic tests and the investigation of their role in these chronic diseases.—Authors' Abstract

**Gevaudan, M. J., Bollet, C. and Demicco, P.** *In vitro* evaluation of clarithromycin alone and in combination against *Myco-*

*bacterium chelonae* complex. Pathol. Biol. **42** (1994) 412–418.

The *in vitro* activity of clarithromycin alone and in combination with amikacin, ethambutol and rifabutin was tested against 12 strains of *Mycobacterium chelonae abscesses* and eight strains of *M. chelonae chelonae* isolated from patients. Extracellular activity of clarithromycin was assessed by determining MICs using the 1% proportion method in Middlebrook 7H11 agar media compared to the radiometric methodology in 7H12 broth at two pHs, 6.8 and 7.4. The MICs obtained at pH 7.4 were 2 to 4 more dilutions lower than those obtained at pH 6.8. By both methods, clarithromycin appeared more active against isolates of *M. chelonae chelonae* than against isolates of *M. chelonae abscessus*. Clarithromycin-amikacin combination demonstrated the most important additive effect. The use of three drugs in association resulted in a synergistic effect. Studies of intracellular bacteria showed that the most effective bactericidal combination was clarithromycin, amikacin and ethambutol together.—Authors' Abstract

**Ghossein, R. A., Ross, D. G., Salomon, R. N. and Rabson, A. R.** A search for mycobacterial DNA in sarcoidosis using the polymerase chain reaction. Am. J. Clin. Pathol. **101** (1994) 733–737.

The etiology of sarcoidosis is unknown, but mycobacteria have been considered as a possible etiologic agent. The authors used the polymerase chain reaction (PCR) to search for mycobacterial DNA in paraffin-embedded granulomatous tissues from patients with sarcoidosis. The target sequence used for PCR amplification is a 383-base pair segment of the gene encoding the 65-kDa mycobacterial surface antigen. This assay can detect *Mycobacterium tuberculosis* and atypical mycobacteria in archival material. Its sensitivity, which is superior to Ziehl-Nielsen staining for acid-fast bacilli, is 1 bacterium per 2500 cells. Ten sarcoidosis blocks and 10 normal controls were negative with mycobacterial PCR but positive with beta-actin PCR, indicating the presence of amplifiable DNA. Mycobacterial PCR gave positive results for six acid-fast bacilli stain/culture-positive blocks from patients with tuberculosis. These results indicate that sarcoidosis probably does not represent an active mycobacterial infection. These data also suggest that mycobacterial PCR is helpful in differentiating tuberculosis and sarcoidosis.—Authors' Abstract

**Harris, D. P., Vordermeier, H.-M., Brett, S. J., Pasvol, G., Moreno, C. and Ivanyi, J.** Epitope specificity and isoforms of the mycobacterial 19-kilodalton antigen. Infect. Immun. **62** (1994) 2963–2972.

The topography and specificity of B- and T-cell stimulatory epitopes from the 19-kDa protein of *Mycobacterium tuberculosis* were investigated by using overlapping synthetic peptides. Murine antisera identified two cryptic epitopes (residues 11 to 30 and 61 to 80) and one species-specific immunodominant epitope (residues 140 to 159). Immunoglobulins G1 and G2a antibody isotypes varied for the respective peptide immunogens but without relationship to the T-cell cytokine profiles which were characterized by high gamma-interferon and low interleukin 5 levels. Antisera to recombinant *M. tuberculosis* 19-kDa protein (rGST-19) crossreacted with homologous proteins of similar size from organisms of the *M. avium-intracellulare* complex. Two-dimensional gel electrophoresis revealed differences in the number, relative mobility, and charge of isoforms of the 19-kDa protein, possibly reflecting post-translational modifications. The immunodominant T-cell epitope from the *M. tuberculosis* 19-kDa protein (residues 61 to 80) and the corresponding peptide sequence from *M. avium* subsp. *intracellulare* (residues 64 to 83), differing at five residues, were both recognized in a genetically permissive manner. Peptides 61–80 and 64–83 stimulated crossreactive responses in BALB/c (H-$2^d$) mice, while in the C57BL/10 (H-$2^b$) strain, responses to peptide 61–80 were species specific. In purified protein derivative-positive healthy individuals, the *M. avium* subsp. *intracellulare* peptide stimulated stronger responses than did the *M. tuberculosis* peptide; whereas patients with active tuberculosis had enhanced *in vitro* T-cell responses to both peptides.—Authors' Abstract

**Harth, G., Clemens, D. L. and Horwitz, M. A.** Glutamine synthetase of *Mycobacterium tuberculosis*: extracellular release and characterization of its enzymatic activity. Proc. Natl. Acad. Sci. U.S.A **91** (1994) 9342–9346.

We have investigated the activity and extracellular release of glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] of *Mycobacterium tuberculosis*. The purified, homogeneous *M. tuberculosis* glutamine synthetase appears to consist of 12 most likely identical subunits of M(r) 58,000, arranged in two superimposed hexagons. In the catalysis of L-glutamine, the enzyme has an apparent Km for L-glutamate of approximately 3 mM at the pH optimum of 7.5. *M. tuberculosis* releases a large proportion (approximately 30%) of its total measurable enzyme activity into the culture medium, a feature that is highly specific for pathogenic mycobacteria. Immunogold electron-microscopy revealed that *M. tuberculosis* also releases the enzyme into its phagosome in infected human monocytes. Two potentially important roles for glutamine synthetase in the pathogenesis of *M. tuberculosis* infection are: (i) the synthesis of L-glutamine, a major component of the cell wall of pathogenic but not nonpathogenic mycobacteria, and (ii) the modulation of the ammonia level in the *M. tuberculosis* phagosome, which may in turn influence phagosomal pH and phagosome-lysosome fusion.—Authors' Abstract

**Harth, M., Ralph, E. D. and Faraawi, R.** Septic arthritis due to *Mycobacterium marinum*. J. Rheumatol. **21** (1994) 957–960.

We describe a patient with septic arthritis and osteomyelitis of the ring finger due to *Mycobacterium marinum*. A review of the literature shows fewer than 40 reported cases of joint infection with this organism. Most of the patients reported had been previously in good health, and had been in contact with fish or otherwise involved in aquatic activities. The arthritis affects mainly the hands or wrists and is insidious in onset. Delay in diagnosis, and initial inappropriate treatment with intraarticular steroids are frequent. Therapy with antibiotics and/or surgical debridement is usually successful.—Authors' Abstract

**Heger, W., Schmahl, H. J., Klug, S., Felies, A., Nau, H., Merker, H. J. and Neubert, D.** Embryotoxic effects of thalidomide derivatives in the non-human primate *Callithrix jacchus*. 4. Teratogenicity of μg/kg doses of the EM12 enantiomers. Teratogen. Carcinogen. Mutagen. **14** (1994) 115–122.

The dose-response of the teratogenic potency of the thalidomide (Thd) derivative EM12 was evaluated in the common marmoset (*Callithrix jacchus*). The smallest daily dose found to be effective was 30 μg EM12/kg body wt. This is the lowest dose of a Thd derivative ever reported to induce severe skeletal abnormalities. Ten micrograms EM12/kg body wt may be considered the no-observed-adverse-effect-level (NOAEL) under the experimental conditions chosen. The teratogenic potencies of the two EM12 enantiomers were tested at 100 μg/kg body wt, the dose which just induces an almost 100% effect in the case of the racemate. The S(−)-EM12 was found to induce typical severe limb abnormalities such as amelia, phocomelia, and radius aplasia, and none of the exposed fetuses were devoid of skeletal defects. In contrast, only few and minor skeletal defects were observed after application of the R(+) enantiomer. Although a pronounced teratogenic potency of the R(+)-EM12 can now largely be excluded, these low-dose studies are not sufficient to completely rule out any teratogenic potential of this enantiomer, since racemization to small amounts of the S(−) form may occur *in vivo*. Further studies with Thd derivatives which are unable to racemize are necessary to prove the assumed complete ineffectiveness of the R(+) enantiomers.—Authors' Abstract

**Henriques, B., Hoffner, S. E., Petrini, B., Juhlin, I., Wahlen, P. and Kallenius, G.** Infection with *Mycobacterium malmoense* in Sweden; report of 221 cases. Clin. Infect. Dis. **18** (1994) 546–600.

*Mycobacterium malmoense* was first described in 1977 and today is second only to the *M. avium* complex as a cause of atypical

mycobacterial infection in Sweden. We retrospectively studied the records of 221 patients from whom *M. malmoense* was isolated during 1968–1989. *M. malmoense* was recovered from the respiratory tract of 171 patients (170 adults and one child) and from cervical lymph nodes of 36 patients (35 children and one adult). In addition, the organism was isolated from the urine of six patients, one of whom had disseminated disease and two of whom had abscesses caused by *M. malmoense.* A majority of the patients with pulmonary infection as well as the patient with disseminated disease had other underlying diseases.—Authors' Abstract

**Heym, B., Honore, N., Truffot-Pernot, C., Banerjee, A., Schurra, C., Jacobs, W. R., van Embden, J. D., Grosset, J.-H. and Cole, S. T.** Implications of multidrug resistance for the future of short-course chemotherapy of tuberculosis: a molecular study. Lancet **344** (1994) 293–298.

Tuberculosis-control programs are compromised by the increased frequency of multidrug-resistant strains of *Mycobacterium tuberculosis.* We used the polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) analysis techniques to establish the molecular basis of resistance in 37 drug-resistant isolates of *M. tuberculosis,* and correlated these findings with clinical and antibiotic-sensitivity data. Resistance to isoniazid was found in 36 strains, 16 of which were also resistant to ethionamide. Of the 36 isoniazid-resistant strains, 23 had mutations in the katG gene, and 5 of these also had mutations in the inhA gene. A further 5 strains had alterations in the inhA locus without the katG gene being mutated. Rifampin resistance was less frequent (13 strains) and usually associated with isoniazid resistance (11 of 13 strains). Mutations in the rpoB gene were detected for all these rifampin-resistant isolates. Mutations in the rpsL and rrs genes, associated with streptomycin resistance, were found in 13 of 25 and 2 of 25 streptomycin-resistant strains, respectively. The same chromosomal mutations, or combinations of mutations, were found in strains displaying single or multidrug resistance, from cases of both primary and secondary resistance, and from patients infected with human immunodeficiency virus. Thus, multidrug resistance is not due to a novel mechanism and tuberculosis chemotherapy is not subject to a new threat.—Authors' Abstract

**Hill, A. R., Mateo, F. and Hudak, A.** Transient exacerbation of tuberculous lymphadenitis during chemotherapy in patients with AIDS. Clin. Infect. Dis. **19** (1994) 774–776.

We describe three men with disseminated, drug-sensitive tuberculosis and advanced human immunodeficiency virus disease (CD4+ lymphocyte count, $<50/mm^3$) who had flares of tuberculous lymphadenitis with suppuration during the initial weeks of successful chemotherapy. Bactericidal drugs may kindle these transient exacerbations, which involve neutrophils but apparently do not require normal helper T-cell function. In patients with AIDS, as in immunocompetent individuals, treatment-related flares of lymphadenitis are usually not an adverse sign, provided that drug resistance and nonadherence have been excluded.—Authors' Abstract

**Hoffner, S. E., Klintz, L., Olsson-Liljequist, B. and Bolstrom, A.** Evaluation of Etest for rapid susceptibility testing of *Mycobacterium chelonae* and *M. fortuitum.* J. Clin. Microbiol. **32** (1994) 1946–1949.

Etest is a new concept for MIC determinations for antimicrobial agents that is based on a predefined antibiotic gradient on a plastic strip calibrated with a continuous logarithmic MIC scale covering 15 twofold dilutions. Etest was compared with a reference agar dilution method for susceptibility testing of clinical isolates of *Mycobacterium chelonae* and *M. fortuitum.* Results read after 3 days showed good agreement between MICs obtained with Etest and those obtained with the reference method within $\pm 2$ dilutions for 90% of all test combinations. All but one of the strains were inhibited by low concentrations of ciprofloxacin or amikacin. Susceptibility to clarithromycin, erythromycin, imipenem, rifampin, doxycycline, and fusidic acid was variable, and all strains were resistant to

ceftazidime and trimethoprim. The results suggest that Etest is well suited for studies of drug resistance in rapidly growing mycobacteria.—Authors' Abstract

**Horsburgh, C. R., Chin, D. P., Yajko, D. M., Hopewell, P. C., Nassos, P. S., Elkin, E. P., Hadley, W. K., Simon, E. M., Gonzalez, P., Ostroff, S. and Reingold, A. L.** Environmental risk factors for acquisition of *Mycobacterium avium* complex in persons with human immunodeficiency virus infection. J. Infect. Dis. **170** (1994) 362–367.

A case-control study was done to determine risk factors for *Mycobacterium avium* complex (MAC) disease in persons infected with human immunodeficiency virus (HIV) with <50 CD4+ cells/mm$^3$. In univariate analysis, cases (N = 83) had lower CD4+ cell counts than controls (N = 177) (median, 10 vs 17/mm$^3$; $p < 0.001$) and were more likely to have consumed hard cheese (odds ratio [OR], 5.44; 95% confidence interval [CI], 1.61–18.4) but were less likely to have taken daily showers (OR, 0.55; 95% CI, 0.33–0.94). In multivariate analysis, CD4+ cell count < 25/mm$^3$ (OR, 3.58; 95% CI, 1.71–7.49) and consumption of hard cheese (OR, 5.63; 95% CI, 1.58–20.1) remained associated with disease, while daily showering (OR, 0.58; 95% CI, 0.28–0.88) remained protective. Increased risk for MAC disease in persons with HIV infection and low CD4+ cell counts is not associated with exposure to water or a variety of other environmental sources but may be associated with the consumption of hard cheese.—Authors' Abstract

**Horsburgh, C. R., Metchock, B., Gordon, S. M., Havlik, J. A., McGowan, J. R. and Thompson, S. E.** Predictors of survival in patients with AIDS and disseminated *Mycobacterium avium* complex disease. J. Infect. Dis. **170** (1994) 573–577.

Patients with AIDS and disseminated *Mycobacterium avium* complex disease (DMAC), as defined by the presence of a positive blood culture for MAC, were studied retrospectively to define the natural history of DMAC. All patients had fevers, severe anemia (hematocrit <26%), or both. Eighty-seven (76%) had signs, symptoms, or laboratory findings related to the gastrointestinal tract, but no distinct syndrome was identified. Sixty-nine patients received antimycobacterial therapy; assignment to therapy was not randomized. In a proportional hazards analysis, shorter survival was associated with higher initial level of mycobacteremia (relative risk [RR], 1.86; 95% confidence interval [CI], 1.49–2.31; $p < 0.001$), while administration of antimycobacterial chemotherapy (RR, 0.42; 95% CI, 0.26–0.70; $p < 0.001$) and antiretroviral therapy (RR, 0.40; 95% CI, 0.22–0.73; $p < 0.01$) had protective effects. Thus, the initial level of mycobacteremia of patients with DMAC may have prognostic value, and administration of antimycobacterial and antiretroviral agents may be associated with prolonged survival.—Authors' Abstract

**Hunt, J. M., Roberts, G. D., Stockman, L., Felmlee, T. A. and Persing, D. H.** Detection of a genetic locus encoding resistance to rifampin in mycobacterial cultures in clinical specimens. Diagn. Microbiol. Infect. Dis **18** (1994) 219–227.

The polymerase chain reaction (PCR) and automated DNA sequencing were used to detect a genetic locus, rpoB, associated with rifampin resistance in *Mycobacterium tuberculosis* (TB) in clinical isolates and directly in clinical specimens. Primers derived from the sequence of a TB rpoB gene fragment were used to amplify DNA from bacterial and mycobacterial isolates. An rpoB-specific PCR product was obtained for five of five TB, seven of eight other mycobacterial species, *Nocardia* sp., *Corynebacterium* sp., *Streptomyces* sp., *Actinomyces* sp., and *Rhodococcus* sp., but not for 15 isolates (eight genera) representing usual bacterial flora. Sequence comparison of the amplified rpoB region revealed the occurrence of TB-specific "signature nucleotides" at three positions. PCR yielded amplification products for seven of 16 clinical specimens. Five of the seven contained TB-specific DNA, as well as sequences that predicted rifampin susceptibility in accord with agar dilution results. None of ten specimens that were culture negative for TB yielded TB-specific PCR products. These results with a limited number of clinical specimens

demonstrate the feasibility of direct detection by PCR of rifampin-resistant TB in clinical specimens. Such testing may serve as a rapid surrogate test for multidrug-resistant TB in laboratories with PCR and automated sequencing capability.—Authors' Abstract

**Inderlied, C. B., Barbara-Burnham, L., Wu, M., Young, L. S. and Bermudez, L. E. M.** Activities of the benzoxazinorifamycin KRM 1648 and ethambutol against *Mycobacterium avium* complex *in vitro* and in macrophages. Antimicrob. Agents Chemother. **38** (1994) 1838–1843.

KRM 1648 is a 4-aminobenzoxazine derivative of rifamycin S with potent *in vitro* activity against the *Mycobacterium avium* complex (MAC); MIC for 90% of 24 MAC isolates from AIDS patients was 0.25 μg/ml as determined by a radiometric broth macrodilution assay. KRM 1648 was bactericidal for MAC isolates in Middlebrook 7H9 broth, with a reduction in viability of 1 to 4 orders of magnitude over 72 hr. In human macrophages, KRM 1648 also was bactericidal, with a reduction of 3 to 4 orders of magnitude in CFU per ml of macrophage lysate at a concentration of 1 μg/ml; however, the bactericidal activity varied approximately 10-fold among the three MAC serovars tested. In growth medium, ethambutol potentiated the effect of KRM 1648, but this potentiation was modest when tested against MAC in macrophages and also varied between MAC strains. KRM 1648 has potential as an antimycobacterial agent for MAC disease, perhaps in combination with other agents so that the use of lower dosages of KRM 1648 than are needed with other rifamycins may be possible.—Authors' Abstract

**Ji, B., Lounis, N., Truffot-Pernot, C. and Grosset, J.** Effectiveness of various antimicrobial agents against *Mycobacterium avium* complex in the beige mouse model. Antimicrob. Agents Chemother. **38** (1994) 2521–2529.

The results of five chemotherapeutic experiments in beige mice infected with organisms of the *Mycobacterium avium* complex are presented. After monotherapy with various antimicrobial agents for 4 weeks, only clarithromycin, amikacin, and ethambutol displayed definite bactericidal effects; sparfloxacin and clofazimine showed modest bacteriostatic effects; and rifampin and rifabutin were totally inactive against the isolate tested. After treatment for 4 weeks, the large quantities of clofazimine that had accumulated in the organs of mice seriously interfered with the enumeration of the CFU and assessment of the efficacy of the treatment. The *in vitro* synergistic effects of drug combinations against *M. avium* complex were not confirmed in beige mice. In combination with clarithromycin, amikacin could prevent the selection of clarithromycin-resistant mutants, whereas minocycline could not.—Authors' Abstract

**Kemper, C. A., Havlir, D., Haghighat, D., Dube, M., Bartok, A. E., Sison, J. P., Yao, Y. Z., Yagco, B., Leedom, J. M., Tilles, J. G., McCutchan, J. A. and Deresinski, S. C.** The individual microbiologic effect of three antimycobacterial agents, clofazimine, ethambutol and rifampin, on *Mycobacterium avium* complex bacteremia in patients with AIDS. J. Infect. Dis. **170** (1994) 157–164.

The individual antibacterial activities of clofazimine, ethambutol, and rifampin in the treatment of *Mycobacterium avium* complex bacteremia in patients with AIDS were determined. Sixty human immunodeficiency virus 1-infected patients who had at least one blood culture positive for *M. avium* complex were randomized to receive either clofazimine (200 mg), ethambutol (15 mg/kg), or rifampin (600 mg) once daily for 4 weeks. Only ethambutol resulted in a statistically significant reduction in the level of mycobacteremia. The median change in individual baseline colony counts was $-0.60$ $\log_{10}$ cfu/ml after 4 weeks of ethambutol ($p = 0.046$). In contrast, median changes in individual baseline colony counts were $-0.2$ $\log_{10}$ cfu/ml and $+0.2$ $\log_{10}$ cfu/ml for clofazimine and rifampin, respectively (both, $p > 0.4$). Ethambutol had greater antibacterial activity, as determined by changes in the level of mycobacteremia, than either rifampin or clofazimine, supporting its continued use in combination with other agents in the treatment of *M. avium* infection.—Authors' Abstract

**Kennedy, N., Gillespie, S. H., Saruni, A. O. S., Kisyombe, G., McNerney, R., Ngowi, F. I. and Wilson, S.** Polymerase chain reaction for assessing treatment response in patients with pulmonary tuberculosis. J. Infect. Dis. **170** (1994) 713–716.

The use of the polymerase chain reaction (PCR) for assessing treatment response in tuberculosis was investigated. Serial sputum samples were analyzed from 10 Tanzanian patients treated for smear-positive pulmonary tuberculosis, including 4 who relapsed after initially successful treatment. A one-tube nested PCR with a colorimetric detection system was compared with microscopy and culture. Samples were found to be negative by microscopy before they were by PCR or culture, often remaining positive 1–2 months longer by PCR than by culture. For the 76 samples available for both culture and PCR, there was a 76% (58/76) agreement between the methods. Nine samples were negative by culture but positive by PCR; 7 were either negative (5) or equivocal (2) by PCR despite being positive by culture. Two of the 4 relapse cases were detected earlier by PCR than by culture. These results demonstrate that PCR is a promising method for assessing treatment response in pulmonary tuberculosis.—Authors' Abstract

**Kenney, T. J. and Churchward, G.** Cloning and sequence analysis of the *rpsL* and *rpsG* genes of *Mycobacterium smegmatis* and characterization of mutations causing resistance to streptomycin. J. Bacteriol. **176** (1994) 6153–6156.

The *Mycobacterium smegmatis rpsL* and *rpsG* genes, encoding the ribosomal proteins S12 and S7, were cloned, and their DNA sequence was determined. The third nucleotide of the S12 termination codon overlapped the first nucleotide of the S7 translation initiation codon. A collection of 28 spontaneous streptomycin-resistant mutants of *M. smegmatis* was isolated. All had single-base-pair substitutions in the *rpsL* gene which were changed to a streptomycin-sensitive phenotype by complementation with a low-copy-number plasmid carrying the wild-type *M. smegmatis rpsL* gene. A total of eight different mutations were found in two specific regions of the *rpsL* gene. Fifty-seven percent (16 of 28) altered the Lys codon at position 43. Forty-six percent of the mutations (13 of 28) were due to a transition changing an AAG Lys codon to an AGG Arg codon, with eight changes at codon 43 and five at codon 88.—Authors' Abstract

**Kikuchi, K., Fukumoto, M., and Takahashi, H.** Iron storage in *Mycobacterium smegmatis* grown under iron-sufficient and iron-overload conditions. Biosci. Biotechnol. Biochem. **58** (1994) 885–888.

Two kinds of iron-containing proteins the molecular masses of which were about 10 kDa and 24 kDa were isolated from cytoplasmic fractions of *Mycobacterium smegmatis* grown under iron-sufficient (50 $\mu$M Fe) and iron-overload (500 $\mu$M Fe) conditions. Based on the elution profiles in two chromatographic systems, spectrophotometric analysis, and ESR spectrum measurement, the protein of 10 kDa met the criteria for classification as a ferredoxin. Another protein of 24 kDa showed no enzymatic activity, though its detailed structure was unknown. The ferredoxin and the protein of 24 kDa contained about 30% and 50% of the total cellular iron, respectively, when cells were grown under the above conditions. The synthesis of the protein of 24 kDa was, however, completely repressed in cells grown under iron-deficient (0.5 $\mu$M Fe) conditions, although the ferredoxin was still synthesized to some extent even in iron-deficient cells. These results suggested that both ferredoxin and the protein of 24 kDa could be synergistically involved in iron storage in this organism.—Authors' Abstract

**Klemens, S. P. and Cynamon, M. H.** Intermittent azithromycin for treatment of *Mycobacterium avium* infection in beige mice. Antimicrob. Agents Chemother. **38** (1994) 1721–1725.

The activity of azithromycin (AZI) was evaluated in the beige mouse model of disseminated *Mycobacterium avium* infection. Mice were infected intravenously with approximately $10^7$ viable *M. avium* ATCC 49601. AZI at 50, 100, or 200 mg/kg of body weight or clarithromycin (CLA) at 200 mg/kg was given by gavage 5 days per week

for 4 weeks. Groups of treated mice were compared with untreated control animals. A dose-related reduction in cell counts in organs was observed with AZI treatment. AZI at 200 mg/kg was more active than CLA at 200 mg/kg against organisms in spleens. The activities of these two agents at 200 mg/kg were comparable against organisms in lungs. In a second study, AZI at 200 mg/kg was given daily for 5 days; this was followed by intermittent AZI treatment for the next 3 weeks. The activities of AZI given on a three-times- and five-times-per-week basis in the continuation phase were comparable. AZI given on a once-weekly basis was less active. The regimen of AZI given in combination with rifapentine on a once-weekly basis for 8 weeks showed promising activity. Clinical evaluation of AZI and rifapentine will help to define the roles of these agents in the treatment of disseminated *M. avium* complex infection.—Authors' Abstract

**Klemens, S. P., Grossi, M. A. and Cynamon, M. H.** Activity of KRM-1648, a new benzoxazinorifamycin, against *Mycobacterium tuberculosis* in a murine model. Antimicrob. Agents Chemother. **38** (1994) 2245–2248.

The activity of KRM-1648 was evaluated in a murine model of tuberculosis. Approximately $10^7$ viable *Mycobacterium tuberculosis* ATCC 35801 organisms were given intravenously to 4-week-old female outbred mice. Treatment was started 1 week post-infection and given by gavage for 4 weeks. Viable-cell counts were determined from homogenates of spleen and lung tissues. The activity of KRM-1648 was compared with those of rifampin and rifabutin at 20 mg/kg of body weight. KRM-1648 was more active than either rifampin or rifabutin against organisms in spleens and lungs. KRM-1648 alone and in combination with either isoniazid, ethambutol, pyrazinamide, or levofloxacin was evaluated. Other treatment groups received isoniazid, ethambutol, pyrazinamide, or levofloxacin as single agents. KRM-1648 was the most active single agent evaluated. KRM-1648-pyrazinamide and KRM-1648-isoniazid were the most active combinations. These combinations were more active than KRM-1648 alone. The promising activity of KRM-1648 in *M. tuberculosis*-infected mice suggests that it is a good candidate for clinical development as a new antituberculosis agent.—Authors' Abstract

**Klopman, G., Li, J.-Y., Wang, S., Pearson, A. J., Chang, K., Jacobs, M. R., Bajaksouzian, S. and Ellner, J. J.** *In vitro* anti-*Mycobacterium avium* activities of quinolones: predicted active structures and mechanistic considerations. Antimicrob. Agents Chemother. **38** (1994) 1794–1802.

The relationship between the structures of quinolones and their anti-*Mycobacterium avium* activities has been previously derived by using the Multiple Computer-Automated Structure Evaluation program. A number of substructural constraints required to overcome the resistance of most of the strains have been identified. Nineteen new quinolones which qualify under these substructural requirements were identified by the program and subsequently tested. The results show that the substructural attributes identified by the program produced a successful *a priori* prediction of the anti-*M. avium* activities of the new quinolones. All 19 quinolones were found to be active, and 4 of them are as active or better than ciprofloxacin. With these new quinolones, the updated multiple computer-automated structure evaluation program structure-activity relationship analysis has helped to uncover additional information about the nature of the substituents at the C5 and C7 positions needed for optimal inhibitory activity. A possible explanation of drug resistance based on the observation of suicide inactivation of bacterial cytochrome P-450 by the cyclopropylamine moiety has also been proposed and is discussed in this report. Furthermore, we confirm the view that the amount of the uncharged form present in a neutral pH solution plays a crucial role in the drug's penetration ability.—Authors' Abstract

**Mabilat, C., Desvareene, S., Panteix, G., Machabert, N., Bernillon, M.-H., Guardiola, G. and Cros, P.** Routine identification of *Mycobacterium tuberculosis*

complex isolated by automated hybridization. J. Clin. Microbiol. **32** (1994) 2702–2705.

Methodologies for biochemical identification of mycobacteria isolated from clinical samples are still cumbersome, taking skilled technicians 3 to 6 weeks. We describe here a 2-hr identification system for mycobacterial isolates belonging to *Mycobacterium tuberculosis* complex using a DNA probe. After 30 min of hand-off sample preparation, the 1.5-hr hybridization test is totally automated in the newly developed VIDAS system (bioMérieux, Marcyl'Etoile, France), which performs solid-phase specific hybridization of 16S rRNA at 37°C. The strain collection of actinomycetes tested was composed of 662 isolates from 27 species: 461 members of the *M. tuberculosis* complex (443 *M. tuberculosis, 10 M. bovis,* and 8 *M. bovis* BCG isolates) and 201 isolates of other species, including 55 *M. avium-intracellulare* isolates). They were identified by traditional methods: growth rate, colonial morphology, pigmentation, and biochemical profiles. The automated probe assay displayed an excellent correlation with the reference results. The four members of the *Nocardia* and *Rhodococcus* genera tested did not cross-hybridize. This flexible random-access and automated technology was shown to suit the routine context of the laboratory by rapidly delivering the results.—Authors' Abstract

**Majumder, S. and Gupta, R.** Effect of interleukin-1 alpha on the growth of *Mycobacterium microti* within J774A.1 cells. FEMS Microbiol. Lett. **120** (1994) 329–334.

The effect of mouse recombinant interleukin-1 alpha (IL-1$\alpha$) on the intracellular growth of *Mycobacterium microti* in a murine macrophage cell line J774A.1 was investigated. IL-1$\alpha$ added after infection to the *M. microti*-infected macrophage monolayers enhanced the growth of *M. microti* in a concentration-dependent manner and this growth enhancement was abrogated by neutralization of IL-1$\alpha$ with anti-IL-1$\alpha$ antibody. Cyclic adenosine monophosphate level in J774A.1 cells was increased by the addition of IL-1$\alpha$. Addition of dibutyryl cyclic adenosine monophosphate to infected J774A.1 cells increased the number of intracellular bacteria in a concentration-dependent manner. These results suggest that IL-1$\alpha$ acts as a growth enhancer for intracellular *M. microti* and the growth-enhancing effect of IL-1$\alpha$ may be due to enhanced cellular cyclic adenosine monophosphate level.—Authors' Abstract

**McCormick, P. A., Scott, F., Epstein, O., Burroughs, A. K., Scheuer, P. J. and McIntyre, N.** Thalidomide as therapy for primary biliary cirrhosis: a double-blind placebo controlled pilot study. J. Hepatol. **21** (1994) 496–499.

Thalidomide has been reported to be effective in treating graft-versus-host disease, a condition with many clinical and pathological similarities to primary biliary cirrhosis. We performed a double-blind, placebo-controlled pilot study to assess the efficacy of thalidomide in 18 patients with biopsy-proven primary biliary cirrhosis (10 thalidomide, 8 placebo). Each patient was treated for 6 months and had a liver biopsy before and after treatment. Side effects, particularly sedation and fatigue, were more common on thalidomide and two patients were withdrawn from this group. There were no improvements in liver function tests or in liver histology, assessed morphometrically. A number of patients treated with thalidomide reported an improvement in pruritus. This study suggests that thalidomide is unlikely to be effective in altering the natural history of primary biliary cirrhosis.—Authors' Abstract

**Mills, J. A., McNeil, M. R., Belisle, J. T., Jacobs, W. R., Jr. and Brennan, P. J.** Loci of *Mycobacterium avium ser2* gene cluster and their functions. J. Bacteriol. **176** (1994) 4803–4808.

The highly antigenic glycopeptidolipids present on the surface of members of the *Mycobacterium avium* complex serve to distinguish these bacteria from all others and to define the various serovars that compose this complex. Previously, the genes responsible for the biosynthesis of the disaccharide hapten [2,3-di-*O*-methyl-$\alpha$-L-fucopyranosyl-(1→3)-$\alpha$-L-rhamnopyranose] of

serovar 2 of the *M. avium* complex were isolated, localized to a contiguous 22- to 27-kb fragment of the *M. avium* genome, and designated the *ser2* gene cluster (J. T. Belisle, L. Pascopella, J. M. Inamine, P. J. Brennan, and W. R. Jacobs, Jr., J. Bacteriol. 173:6991–6997, 1991). In the present study, transposon saturation mutagenesis was used to map the specific genetic loci within the *ser2* gene cluster required for expression of this disaccharide. Four essential loci, termed *ser2A,-B,-C,* and *-D,* constituting a total of 5.7 kb within the *ser2* gene cluster, were defined. The *ser2B* and *ser2D* loci encode the methyltransferases required to methylate the fucose at the 3 and 2 positions, respectively. The rhamnosyl-transferase was encoded by *ser2A*; whereas either *ser2C* or *ser2D* encoded the fucosyltransferase. The *ser2C* and *ser2D* loci are also apparently involved in the *de novo* synthesis of fucose. Isolation of the truncated versions of the hapten induced by the transposon insertions provides genetic evidence that the glycopeptidolipids of *M. avium* serovar 2 are synthesized by an initial transfer of the rhamnose unit of the peptide core followed by fucose and finally O methylation of the fucosyl unit.—Authors' Abstract

**Montano, L. F., Masso, F., Paez, A., Sandoval, S., Vazquez, L., Sanchez, L., Fournet, B. and Zenteno, E.** Isolation of a 32 kDa *Mycobacterium tuberculosis* protein by lectin affinity chromatography. Compar. Biochem. Physiol. [B] **108** (1994) 265–272.

A 32-kDa antigen from delipidated *Mycobacterium tuberculosis* H37Rv culture filtrate protein extract (CFPE) was purified by affinity chromatography on immobilized *Lens culinaris* lectin and electroelution. This antigen represents 0.4% of the total CFPE carbohydrate content and possesses galactose, xylose, mannose and GlcNAc (5:2:3:1 mol. ratio). A monoclonal antibody against the purified antigen reacted with the 32-kDa as well as a 30-kDa antigen in H37Rv CFPE, thus suggesting that both antigens represent closely related allelomorphic forms of the same antigen.—Authors' Abstract

**Newman, G. W., Guarnaccia, J. R., Vance, E. A., Wu, J. Y., Remold, H. G. and Kazanjian, P. H.** Interleukin-12 enhances antigen-specific proliferation of peripheral blood mononuclear cells from HIV-positive and -negative donors in response to *Mycobacterium avium.* AIDS **8** (1994) 1413–1419.

Objective: To determine if the addition of recombinant (r) human interleukin (IL)-12 enhances *in vitro* proliferative responses to *Mycobacterium avium* of peripheral blood mononuclear cells (PBMC) from HIV-positive donors with CD4 cell counts $<100 \times 10^6$/l.

Design and methods: PBMC proliferative responses to virulent and avirulent serovars of *M. avium* in the presence and absence of exogenously added IL-12 were determined in 24 HIV-positive and 11 HIV-negative donors by H-3-thymidine uptake assay. Changes in CD4 and CD8 cell populations after IL-12 treatment and *M. avium* stimulation were analyzed by FACS.

Results: IL-12 significantly enhanced proliferation of PBMC to both virulent and avirulent *M. avium* from all 24 HIV-positive donors ($p = 0.0001$) although the magnitude varied for each donor. In contrast, addition of IL-12 to PBMC from HIV-negative donors only increased the proliferative responses to the virulent *M. avium* serovar 4 ($p = 0.0044$). PBMC from HIV-positive donors in the presence of IL-12 responded better to the avirulent serovar of *M. avium* than the virulent serovar 4. Proliferative responses of HIV-positive donors to *M. avium* alone, however, were significantly less ($p = 0.0013$) than that of HIV-negative donors. Increased proliferative responses of HIV-positive donors were independent of CD4 counts. No significant changes in the ratio of CD4+ to CD8+ T cells occurred in either HIV-positive or negative donors under any culture conditions.

Conclusion: *In vitro* proliferative responses of PBMC from HIV-positive donors to *M. avium* were significantly enhanced by the addition of human rIL-12, which was not dependent on their CD4 cell counts. The use of IL-12 as an enhancer of cell-mediated immunity in AIDS patients against *M. avium* infections deserves further study.—Authors' Abstract

**Nibbering, P. H., Yoshida, S. I., Van den Barselaar, M. T. and Van Furth, R.** Bacteriostatic activity of BCG/PPD-activat-

ed macrophages against *Mycobacterium fortuitum* does not involve reactive nitrogen or oxygen intermediates. Scand. J. Immunol. **40** (1994) 187–194.

Mycobacteria preferentially reside in resident macrophages whereas activated macrophages are presumed to eliminate the bacteria effectively. The aim of the present study was to determine the antibacterial activities of resident and activated murine peritoneal macrophages against *Mycobacterium fortuitum* and the intracellular mechanisms involved. After phagocytosis *M. fortuitum* could not be killed by either BCG/PPD-activated and IFN-gamma-activated macrophages and resident macrophages. The mycobacteria did not multiply in BCG/PPD-activated macrophages and the rate of proliferation of *M. fortuitum* in IFN-gamma-activated macrophages was only slightly inhibited compared to that in resident macrophages. Experiments with selective inhibitors of the production of reactive nitrogen intermediates (RNI) and reactive oxygen intermediates (ROI) demonstrated that these factors are not essential for the mycobacteriostatic activity of BCG/PPD-activated macrophages. After phagocytosis of *M. fortuitum*, BCG/PPD-activated and IFN-gamma-activated macrophages produced substantial amounts of both RNI and ROI. No correlation was found between the levels of these intermediates and the proliferation of *M. fortuitum* in the macrophages. In conclusion, BCG/PPD-activated macrophages are bacteriostatic, but not bacteriocidal for *M. fortuitum* and the former does not involve reactive nitrogen and oxygen intermediates.—Authors' Abstract

**Perez, M. H., Fomukong, N. G., Hellyer, T., Brown, I. N. and Dale, J. W.** Characterization of IS1110 a highly mobile genetic element from *Mycobacterium avium*. Mol. Microbiol. **12** (1994) 717–724.

A highly mobile insertion sequence designated IS1110 was detected in *Mycobacterium avium* strain LR541 following an observed increase in size of the plasmid pLR20. Genomic libraries of *M. avium* strains carrying either parental pLR20 or the modified plasmid (pLR20′) were constructed and the sequence of the relevant clones was determined to characterize the insertion sequence and the target region. IS1110 is a 1457-bp element lacking terminal inverted repeats, and is related to IS900 (from *M. paratuberculosis*), IS901 and IS902 (from *M. avium*) and to IS116 (from *Streptomyces clavuligerus*). LR541 carries several copies of IS1110. Individual colonies from the same plate show differences in Southern blot patterns when tested with an IS1110-derived probe; the ability to detect transposition events in random colonies, without any selection pressure, indicates an exceptionally high degree of mobility, which will be invaluable for transposon mutagenesis. Analyses of *M. avium* isolates from human, veterinary, and environmental sources showed that IS1110-hybridizing sequences are present in some *M. avium* isolates but they were not detected in strains of other mycobacterial species. The polymorphism exhibited in *M. avium* isolates suggests that this element may be useful for molecular epidemiological studies of *M. avium* infections.—Authors' Summary

**Petty, R. E., Hunt, D. W. C., Mathers, D. M., McCormick, A. Q., Barker, H., Southwood, T. R. and Corson, L.** Experimental arthritis and uveitis in rats associated with *Mycobacterium butyricum*. J. Rheumatol. **21** (1994) 1491–1496.

The objective was to determine if the anterior uveitis associated with adjuvant arthritis (AA) in the rat can be passively transferred with arthritis to syngeneic recipients using spleen cells or T-cell lines prepared from animals given complete Freund's adjuvant (CFA) and *Mycobacterium butyricum* (*M. butyricum*) in incomplete Freund's adjuvant (IFA). Spleen cells from Lewis or Lewis SsN rats given IFA, CFA, type I collagen in IFA (CI-IFA), or type II collagen in IFA (CII-IFA) were administered to naive rats or rats treated with pertussis toxin or bacterial endotoxin. Three CD4+ T-cell lines, propagated from CFA injected rats and maintained *in vitro* with *M. butyricum* (M-1), bovine proteoglycan (PR-1) or an extract of *M. butyricum* (MBE-1) were administered to naive or immunosuppressed rats. The arthritogenic and uveitogenic properties of these cell preparations and intradermal MBE-IFA, CII-IFA and intraperitoneal (i.p.) *M. butyricum* without adjuvant were evaluated. Uveitis was ob-

served in 15/69 (22%) arthritic rats given CFA. Spleen cells prepared from CFA-injected rats caused arthritis in 55 (85%) and uveitis in 2 (3%) of 67 cell recipients. Uveitis occurred in 2/6 cell recipients pretreated with bacterial endotoxin. Neither uveitis nor arthritis was observed in rats given IFA (0/6) or spleen cells prepared from rats given IFA (0/27), CI-IFA (0/6), or CII-IFA (0/28). CII-IFA produced polyarthritis in 5/6 rats, but no uveitis. CII-IFA induced arthritis associated uveitis in 1/15 animals receiving spleen cells from rats given CII-IFA, but not those given CI-IFA (0/3) or IFA (0/13). Uveitis was observed in one recipient of the M-1 T-cell line and in two recipients of the PR-1 T-cell line. Immunization with 400 μg of MBE-IFA induced uveitis but not arthritis in 3/11 animals. The MBE-specific T-cell line was neither arthritogenic nor uveitogenic. A high frequency (5/6) of uveitis accompanied arthritis in male Lewis rats given i.p. *M. butyricum*. Arthritis occurred in 4/10 female Lewis rats given i.p. *M. butyricum* and 2 arthritic animals also developed uveitis. Uveitis occurs infrequently in arthritic rats given spleen cells from CFA-injected animals. The i.p. administration of *M. butyricum* constitutes a novel disease model in which the immunopathological relationships between arthritis and uveitis may be more reliably studied.—Authors' Abstract

**Popper, H. H., Winter, E. and Hofler, G.** DNA of *Mycobacterium tuberculosis* in formalin-fixed, paraffin-embedded tissue in tuberculosis and sarcoidosis detected by polymerase chain reaction. Am. J. Clin. Pathol. **101** (1994) 738–741.

Infection with *Mycobacterium tuberculosis* is a major cause of death worldwide. Identification of mycobacteria in tissue sections is usually easily achieved by acid-fast stains, but this method sometimes gives unsatisfactory results. The authors therefore compared conventional staining techniques and polymerase chain reaction (PCR) for mycobacterial DNA sequences in 24 selected tissue samples from patients with tuberculosis. In all samples, either positive or negative with acid-fast stain, mycobacterial DNA fragments were detected. In addition, tissue samples from patients with clinically proven sarcoidosis were included as controls. Surprisingly, strong signals for mycobacterial DNA were found in 2 of 15 cases. PCR is a useful technique in the demonstration of mycobacterial DNA fragments in patients with clinically suspected tuberculosis who have acid-fast stain-negative histology. An epithelioid granulomatous reaction in the lung, negative by acid-fast stain and positive for mycobacterial DNA by PCR, however, does not permit the diagnosis of tuberculosis, because a positive result can also be obtained in cases of sarcoidosis. In some cases of sarcoidosis, the causal agent might be either cell-wall defective mycobacteria or persistent intracellular DNA from mycobacteria.—Authors' Summary

**Sanchez Navarro, A., Martinez Cabarga, M. and Dominguez-Gil Hurle, A.** Oral absorption of ofloxacin administered together with aluminum. Antimicrob. Agents Chemother. **38** (1994) 2510–2512.

A clinical study was carried out to establish the influence of aluminum on the oral absorption of ofloxacin. Ten healthy volunteers were included in a crossover study based on a Latin square design. The treatments that all volunteers received were A (consisting of 400 mg of ofloxacin) and B (consisting of 400 mg of ofloxacin plus 11 g of colloidal aluminum phosphate). The absorption constant and other ofloxacin parameters were calculated from data on levels in plasma by using model-independent calculation methods. There were no statistically significant differences between the mean values of the areas under the curve corresponding to the administration of ofloxacin alone and those of ofloxacin with aluminum. Regarding the other pharmacokinetic parameters, a significant difference between the absorption constants was found. The presence of aluminum reduces the absorption rate of this quinolone but does not modify the percentage of the absorbed dose.—Authors' Abstract

**Sathe, S. S., Sarai, A., Tsigler, D. and Nedunchezian, D.** Pentoxifylline aggravates impairment in tumor necrosis factor-alpha secretion and increases mycobacterial load in macrophages from AIDS pa-

tients with disseminated *Mycobacterium avium-intracellulare* complex infection. J. Infect. Dis. **170** (1994) 484–487.

Pentoxifylline, which inhibits tumor necrosis factor-alpha (TNF-$\alpha$), decreases human immunodeficiency virus replication in peripheral blood mononuclear cells. However, TNF-$\alpha$ is important in cellular defense against *Mycobacterium avium-intracellulare* complex (MAC), a common infection in advanced AIDS. The effect of pentoxifylline on mycobacterial colony counts in macrophages with *in vivo* MAC infection was evaluated, and differences in lipopolysaccharide (LPS)-induced TNF release in infected and uninfected macrophages were determined. Macrophages with *in vivo* MAC infection released much less TNF-$\alpha$ in response to LPS ($p = 0.01$). The response was partially restored after antimycobacterial therapy. Pentoxifylline, in a concentration that inhibited LPS-induced TNF-$\alpha$ by 52.4%, increased MAC counts by 2.5- to 50.0-fold. Thus, macrophages from AIDS patients with disseminated MAC infection are deficient in their ability to release TNF-$\alpha$ and further inhibition by pentoxifylline may be detrimental.—Authors' Abstract

**Schluger, N. W., Condos, R., Lewis, S. and Rom, W. N.** Amplification of DNA of *Mycobacterium tuberculosis* from peripheral blood of patients with pulmonary tuberculosis. Lancet **344** (1994) 232–233.

Sputum examination for rapid diagnosis of pulmonary tuberculosis is not always satisfactory. We examined peripheral blood with the polymerase chain reaction (PCR). Blood samples were collected from 8 consecutive patients with suspected pulmonary tuberculosis and from 18 healthy controls, half of whom were tuberculin skin-test positive. All 8 patients had evidence of circulating *Mycobacterium tuberculosis* DNA in the lymphocyte fraction of peripheral blood, and positive sputum cultures indicating active pulmonary tuberculosis. None of the healthy controls had positive PCR results. This PCR technique may prove useful for the rapid diagnosis of tuberculosis.—Author's Abstract

**Sekosan, M., Cleto, M., Senseng, C., Farolan, M. and Sekosan, J.** Spindle cell pseudotumors in the lungs due to *Mycobacterium tuberculosis* in a transplant patient. Am. J. Surg. Pathol. **18** (1994) 1065–1068.

A rare spindle-cell pseudotumor in the skin, lymph nodes, and bone marrow has been previously reported in immunosuppressed transplant patients and patients with acquired immunodeficiency syndrome. All reported cases were caused by *Mycobacterium avium-intracellulare* or other nontuberculous mycobacteria. We are reporting spindle-cell pseudotumors in the lungs caused by *M. tuberculosis.* The patient had insulin-dependent diabetes mellitus and was status post-cadaveric renal and pancreatic transplants. His hospital course was complicated by pulmonary tuberculosis due to *M. tuberculosis.* At autopsy, the lungs showed numerous, bilateral gray nodules ranging from 0.2 to 2.5 cm. Microscopic examination uncovered a cellular proliferation composed of spindle cells arranged in fascicles. There were no granulomata. An acid-fast stain showed numerous acid-fast bacilli within the spindle cells. To our knowledge, this is the first case of spindle-cell pseudotumor caused by *M. tuberculosis* of the lungs. Awareness of this unusual manifestation of mycobacterial infection in immunosuppressed patients underscores the need for acid-fast staining of biopsies with spindle-cell proliferation even in the absence of overt granulomatous lesions in order to prevent misdiagnosis.—Authors' Abstract

**Sepulveda, R. L., Heiba, I. M., King, A., Gonzalez, B., Elston, R. C. and Sorensen, R. U.** Evaluation of tuberculin reactivity in BCG-immunized siblings. Am. J. Respir. Crit. Care Med. **149** (1994) 620–624.

The purpose of the present study was to determine the BCG-immunized sibships $\leq 14$ yr of age whether the correlations of intensity of tuberculin reactivity support a genetic regulation of the response to BCG immunization. The study population consisted of 659 healthy children living in 265 households exposed to an adult with tuberculosis: 38 children did not have a BCG scar, 327 children had one BCG scar, and 294 had two BCG scars from vaccinations at birth and at 6 yr of age. There were 603

full-siblings, 16 half-siblings, and 40 unrelated children. Tuberculin testing was performed by one trained nurse. Sibling correlations of the intensity of the tuberculin response were calculated after adjusting for various nongenetic covariates that could be important in predicting it. The sibling correlations were significant at the 1% significance level. There was no significant correlation of tuberculin reactivity among unrelated children in the same household. These results are consistent with genetic regulation of the development and persistence of tuberculin reactivity after BCG immunization.—Authors' Abstract

**Silva, S. R. B., Viana, P. C. F., Lugon, N. V., Hoette, M., Ruzany, F. and Lugon, J. R.** Thalidomide for the treatment of uremic pruritus: a crossover randomized double-blind trial. Nephron **67** (1994) 270–273.

Our observation that thalidomide administration to a dialysis patient with leprosy alleviated his pruritus led us to conduct this short-term study to assess the efficacy of the drug in this regard. From 210 hemodialysis patients, 29 cases of refractory uremic pruritus were entered into the study. Patients were instructed to score their symptoms from 0 to 3, three times a day, and assigned to receive thalidomide or placebo at bedtime for 7 days. After a washout period of 7 days, drugs were crossed over. Response was defined as a reduction of at least 50% in the pruritus scoring. Eighteen patients finished the study. In the first phase, 55% of patients responded showing a mean reduction in the pruritus scoring of 78% ($p < 0.05$ vs placebo); no response to placebo was observed. A similar proportion of patients responded to thalidomide in the second phase with a mean reduction in their pruritus scoring of 81%. In conclusion, thalidomide can be a precious tool in the handling of uremic pruritus unresponsive to available therapy.—Authors' Abstract

**Skuce, R. A., Brittain, D., Hughes, M. S., Beck, L.-A. and Neill, S. D.** Genomic fingerprinting of *Mycobacterium bovis* from cattle by restriction fragment length polymorphism analysis. J. Clin. Microbiol. **32** (1994) 2387–2392.

Two insertion sequences, IS*6110* and IS*1081*, specific to the tuberculosis complex mycobacteria and a highly reiterated DNA element (pTBN12) cloned from *Mycobacterium tuberculosis* were systematically used to identify restriction fragment length polymorphism (RFLP) types among bovine isolates of *M. bovis* in Northern Ireland. In a sample of 109 isolates, probes IS*6110*, IS*1081*, and pTBN12 identified 10, 2, and 12 distinct patterns, respectively. By combining the patterns generated by the three probes it was possible to identify 28 distinct RFLP types. The standard protocol advocated for RFLP analysis of *M. tuberculosis* was used and would facilitate computer-based gel documentation and image analysis to establish a database of *M. bovis* types for large-scale epidemiological studies. These procedures will facilitate interlaboratory comparisons of *M. bovis* isolates and will help to elucidate the precise epidemiology of bovine tuberculosis in different countries.—Authors' Abstract

**Spies, H. S. C. and Steenkamp, D. J.** Thiols of intracellular pathogens—identification of ovothiol A in *Leishmania donovani* and structural analysis of a novel thiol from *Mycobacterium bovis*. Eur. J. Biochem. **224** (1994) 203–213.

*Leishmania donovani*, the causative agent of visceral leishmaniases, is an intracellular pathogen which proliferates within the host macrophages. Analysis of the thiol composition of *L. donovani* by means of the thiol-specific reagent, 7-diethylamino-3-(4′-maleimidylphenyl)-4 indicated that this organism produces substantial amounts of ovothiol A. This observation was further substantiated by HPLC of extracts of *L. donovani* after derivatization with bromobimane. *L. donovani* extracts contained a thiol, the bimane derivative of which had identical retention time and fluorescence quenching to a thiol from *Crithidia fasciculata*, which had previously been identified as ovothiol A. By comparison, the intracellular bacterial pathogen, *Mycobacterium bovis*, contained only one major low-molecular-mass thiol, which was assigned a trivial name mycothiol. The structure of bimane derivative of mycothiol was solved by a combination of one- and two-dimen-

sional H-1 and C-13 NMR spectroscopy. Spatial relationships in the molecule were further refined by NOE experiments and allowed identification of mycothiol as 1-D-myo-inositol-2-(N-acetyl-L-cysteinyl)amino-2-deoxy-alpha-D-glucopyranoside. This assignment was confirmed by positive-ion fast-atom-bombardment mass spectrometry which gave m/z = 677.6 Da and a sodiated species at 699.6 Da. Analysis of the dansylated hydrolysis products of performic-acid-oxidized mycothiol indicated the presence of 0.85 mol glucosamine and 1.02 mol cysteic acid/mol sulfhydryl groups. Crude extracts of *M. bovis* contained an enzyme which catalysed the NAD(P)$H_2$-dependent reduction of mycothiol disulfide to the free thiol. Analysis of perchloric acid extracts of *M. tuberculosis* H37Rv indicated the presence of a thiol which comigrated with mycothiol, both as the free thiol and as the 7-diethylamino-3-(4′-maleimidylphenyl)-4 and bimane derivates, on reverse-phase HPLC. The significance of these findings in terms of the evasion of the host defense mechanisms by leishmania parasites and mycobacteria is considered.—Authors' Abstract

**Tomiyama, T., Asano, S., Suwa, Y., Morita, T., Kataoka, K., Mori, H. and Endo, N.** Rifampicin prevents the aggregation of neurotoxicity of amyloid beta protein *in vitro*. Biochem. Biophys. Res. Comm. **204** (1994) 76–83.

The aggregation and cerebral deposition of amyloid beta protein (AP), which is a major component of senile plaques in Alzheimer's disease (AD) brains, is believed to be involved in the pathogenesis of AD. Inhibition of A beta aggregation would seem to be a promising strategy for the treatment of AD. Here, we show that rifampin, which is an antibiotic widely used in the treatment of tuberculosis and leprosy, inhibited the aggregation and fibril formation of synthetic A beta 1-40 peptide in a dose-dependent manner at reasonable concentrations. Furthermore, rifampin was found to prevent A beta 1-40-induced neurotoxicity on rat pheochromocytoma PC12 cells. Rifampin may have therapeutic potential as an agent for inhibiting the initial step of amyloid formation in AD.—Authors' Abstract

**Valero, G., Moreno, F. and Graybill, J. R.** Activities of clarithromycin, ofloxacin, and clarithromycin plus ethambutol against *Mycobacterium simiae* in murine model of disseminated infection. Antimicrob. Agents Chemother. **38** (1994) 2676–2677.

After 2 weeks of intravenous challenge with *Mycobacterium simiae,* ICR outbred mice were treated with clarithromycin, ofloxacin, or clarithromycin plus ethambutol for 4 weeks. All three therapy groups demonstrated a decrease in the level of infection in both the lungs and the spleen. There were no significant differences among the three treated groups in decreasing mycobacterial counts in the lungs; however, both ofloxacin and clarithromycin plus ethambutol were superior to clarithromycin alone in reducing the level of infection in the spleen. Results of the study suggest a potential role for these agents in the treatment of human *M. simiae* infection.—Authors' Abstract

**Valway, S. E., Richards, S. B., Kovacovich, J., Greifinger, R. B, Crawford, J. T. and Dooley, S. W.** Outbreak of multi-drug-resistant tuberculosis in a New York state prison, 1991. Am. J. Epidemiol. **140** (1994) 113–122.

In the summer of 1991, four inmates from prison A in Upstate New York [U.S.A.] died of multidrug-resistant tuberculosis. To determine the extent of resistant tuberculosis at prison A and transmission patterns, the authors interviewed staff and reviewed medical records and inmate movement histories. Contact investigation results were examined to determine tuberculin skin-test conversions and to estimate risk of infection and disease for inmates who were seropositive for human immunodeficiency virus (HIV). Eight HIV-positive inmates and one HIV-negative guard, who was immunocompromised with cancer, had multidrug-resistant tuberculosis. Eight died a median of 28 days after the first culture-positive specimen was collected. All isolates had identical seven-drug resistance and DNA fingerprint patterns. Of exposed inmates, 92 out of 306 (30%) had skin-test conversions. HIV infection was not associated with becoming infected with drug-resistant tuber-

culosis (active disease or skin-test conversion), but once infected, HIV-positive inmates were significantly more likely to develop disease than were HIV-negative inmates ($p < 0.001$). The source case transferred to prison A in February 1991, was ill with undiagnosed multidrug-resistant tuberculosis, refused medical care, and lived in the general prison population where he transmitted the disease to other inmates. Lapses in infection control and laboratory delays contributed to this outbreak. Prisons should fully implement infection control guidelines to prevent tuberculosis transmission.—Authors' Abstract

**van Rensburg, C. E. J., Anderson, R., Myer, M. S., Joone, G. K. and O'Sullivan, J. F.** The riminophenazine agents clofazimine and B669 reverse acquired multidrug resistance in a human lung cancer cell line. Cancer Lett. **85** (1994) 59–63.

The potential of the riminophenazine agents clofazimine and B669, at therapeutically relevant concentrations, to reverse P-glycoprotein-mediated multidrug-resistance (MDR) in a human lung cancer cell line (H69/LX4) has been investigated *in vitro*. Cyclosporin A, a well-documented MDR-modifying agent, was included for comparison. Clofazimine, B669 and cyclosporin A at minimally cytotoxic concentrations of 1, 0.5 and 5 pg/ml, respectively, were equally effective in restoring sensitivity to vinblastine, doxorubicin, daunorubicin and mitomycin C in the H69-LX4 cell line. All three chemosensitizing agents also increased the accumulation of [C-14]vinblastine by H69/LX4 cells. Riminophenazines, which are relatively nontoxic, noncarcinogenic and non-myelosuppressive agents, are promising contenders for evaluation in experimental and clinical oncology as modulators of acquired MDR.—Authors' Abstract

**van Soolingen, D., de Haas, P. E. W., Haagsma, J., Eger, T., Hermans, P. W. M., Ritacco, V., Alito, A. and van Embden, J. D. A.** Use of various genetic markers in differentiation of *Mycobacterium bovis* strains from animals and humans and for studying epidemiology of bovine tuberculosis. J. Clin. Microbiol. **32** (1994) 2425–2433.

One-hundred-fifty-three *Mycobacterium bovis* strains from cattle, various animal species from zoos and wild parks, and humans were analyzed for three different genetic markers for use in the epidemiology of bovine tuberculosis. *M. bovis* strains isolated from cattle were found to carry a single IS*6110* element; whereas the majority of strains from other animals, such as antelopes, monkeys, and seals, harbored multiple IS*6110* elements, suggesting that the reservoirs in cattle and wild animals are separated. Because the single IS*6110* element in cattle strains is located at the same chromosomal position, strain differentiation by insertion sequence fingerprinting was hampered. Therefore, we investigated the usefulness of the direct repeat and polymorphic GC-rich repeat elements for strain differentiation. Both markers allowed sufficient strain discrimination for epidemiological purposes. Evidence is presented that in Argentina, most human *M. bovis* infections are due to transmission from cattle; whereas *M. bovis* infections among humans in the Netherlands are mainly contracted from animals other than cattle. Various outbreaks of *M. bovis* among animals and humans are described, including a small one which likely involved transmission from human to human.—Authors' Abstract

**Vila, J., Ruiz, J., Marco, F., Barcelo, A., Goni, P., Giralt, E. and Jimenez de Anta, T.** Association between double mutation in *gyrA* gene of ciprofloxacin-resistant clinical isolates of *Escherichia coli* and MICs. Antimicrob. Agents Chemother. **38** (1994) 2477–2479.

The mutations in the quinolone resistance-determining region of the *gyrA* and *gyrB* genes from 27 clinical isolates of *Escherichia coli* with a range of MICs of ciprofloxacin from 0.007 to 128 μg/ml and of nalidixic acid from 2 to >2000 μg/ml were determined by DNA sequencing. All 15 isolates with ciprofloxacin MICs of ≥1 μg/ml showed a change in Ser-83 to Leu of GyrA protein; whereas in clinical isolates with a MIC of ≥8 μg/ml (11 strains), a double change in Ser-83 and Asp-87 was found. All

isolates with a MIC of nalidixic acid of ≥128 μg/ml showed a mutation at amino acid codon Ser-83. Only 1 of the 27 clinical isolates of *E. coli* analyzed showed a change in Lys-447 of the B subunit of DNA gyrase. A change in Ser-83 is sufficient to generate a high level of resistance to nalidixic acid; whereas a second mutation at Asp-87 in the A subunit of DNA gyrase may play a complementary role in developing the strain's high levels of ciprofloxacin resistance.—Authors' Abstract

**Vilagut, L., Vila, J., Vinas, O., Pares, A., Gines, A., Deanta, M. T. J. and Rodes, J.** Cross-reactivity of anti-*Mycobacterium gordonae* antibodies with the major mitochondrial autoantigens in primary biliary cirrhosis. J. Hepatol. **21** (1994) 673–677.

Primary biliary cirrhosis is a chronic cholestatic liver disease associated with autoimmune disorders. Antimitochondrial autoantibodies and granulomatous portal lesions are characteristic in primary biliary cirrhosis. Since granuloma may be induced by mycobacteria, and there is evidence implicating mycobacteria as infectious agents capable of initiating autoimmunity, a study was performed to determine the presence of antibodies against 10 atypical mycobacteria in 19 patients with primary biliary cirrhosis, and in 35 controls (25 patients with other chronic liver diseases and 10 healthy subjects). All primary biliary cirrhosis sera and none of the controls reacted with the extract from *Mycobacterium gordonae,* showing identical recognition profiles with two polypeptides of 70–65 and 55 kDa. No other reaction was found in primary biliary cirrhosis patients and in controls with the extracts from the other nine atypical mycobacteria tested. Eluted immunoglobulins which reacted with the 70–65 and 55 kDa polypeptides from *M. gordonae,* bound to the mitochondrial antigens PDH-E2 and BCKDH-E2. Furthermore, when the extract from *M. gordonae* was tested with eluted immunoglobulins from recognized PDH-E2 and BCKDH-E2 by primary biliary cirrhosis patients, cross observed both 70–65 and 55 kDa polypeptides. These data indicate that antibodies to *M. gordonae,* found in all primary biliary cirrhosis patients, crossreact with the major mitochondrial targets of the disease. We suggest that *M. gordonae* may play a potential pathogenic role in primary biliary cirrhosis.—Authors' Abstract

**Villar, I., Hernandez, E., Cozzi, J., Paletta, C. and Mathurin, S.** [Glomerulonephritis due to immune complexes associated with pulmonary tuberculosis.] Medicina (B. Aires) **54** (1994) 237–240. (in Spanish)

A 32-year-old man was admitted for dyspnea, hemoptysis, macroscopic hematuria, hypertension (140/100), peripheral edema and hemodynamic decompensation. Lung X-rays revealed pulmonary edema and a cavity in the left apex. Laboratory determinations revealed an altered renal function with increased creatinine and urea levels and nephrotic syndrome. There was leucocyturia, hematuria and cylindruria. The sputum showed a large number of acid-fast bacilli. The patient began anti-tuberculosis treatment with three drugs (isoniazid, rifampin, pyrazinamide). On ultrasonography, both kidneys revealed ecogenic lesions with size, shape and cortico-medular relationship preserved. The patient persisted with altered renal function, steady levels of urea nitrogen, creatinine and potassium, preserved diuresis and hypertension. Bidimensional echocardiogram: LVDD 55 mm, hypoquinetic septum, pericardic effusion, thickened pericardium, pleural effusion, shortening fraction decreased. He received treatment for this congestive cardiac failure and hypertension with enalapril, nifedipine and furosemide. A percutaneous renal biopsy was performed with anatomopathologic diagnosis of diffuse encocapillar proliferative glomerulonephritis with crescents (15%) and total glomerular sclerosis (33%). Immunofluorescence: positive, immunecomplexes with IgM and C3. The patient gradually recovered his normal renal function, improved his pleural effusions and normalized his cardiac function. He was discharged in good clinical condition on the 69th day of anti-tuberculosis treatment. An association between pulmonary tuberculosis and glomerulonephritis is discussed. It is proposed that renal lesions might be the

consequence of the tuberculosis due to the sedimentation of circulating immune complexes.—Authors' English Abstract

**Wallace, R. J., Brown, B. A., Griffith, D. E., Girard, W. M., Murphy, D. T., Onyi, G. O., Steingrube, V. A. and Mazurek, G. H.** Initial clarithromycin monotherapy for *Mycobacterium avium-intracellulare* complex lung disease. Am. J. Respir. Crit. Care Med. **149** (1994) 1335–1341.

Sputum conversion rates in *Mycobacterium avium-intracellulare* (MAI) complex lung disease have ranged from only 50% to 80% despite the use of three to five antituberculosis agents. We initiated a prospective, open, noncomparative trial of initial clarithromycin monotherapy at 500 mg twice a day for 4 months in HIV-negative patients with MAI lung disease. The primary study end point was microbiologic improvement. Of 30 patients enrolled, 20 completed therapy. This latter group was predominantly male (60%), smokers (70%), older than 45 yr of age (90%), infected with *M. intracellulare* (70%) and with bilateral disease (85%). Of 19 patients with pretreatment minimum inhibitory concentrations (MIC) for clarithromycin <16 μg/ml, 58% became sputum-negative, and 21% showed significant reductions in sputum positivity. Heavily positive sputum cultures (>200 colonies) were reduced from 30 to 47 samples pretherapy (64%) to three of 54 (6%) post-therapy ($p < 0.0001$); 18 of 19 patients (95%) showed an improvement in sputum cultures, chest radiographs, or both. Only two patients (7%) discontinued the drug because of adverse events. Only three (16%) of 19 isolates developed clarithromycin resistance (MIC > 32 μg/ml). Clarithromycin-susceptible and -resistant MAI isolates from the same patient had identical DNA large-restriction fragment patterns. Clarithromycin is the first single agent to be shown efficacious in the treatment of MAI lung disease.—Authors' Abstract

**Wormser, G. P., Horowitz, H. and Dworkin, B.** Low-dose dexamethasone as adjunctive therapy for disseminated *Mycobacterium avium* complex infections in AIDS patients. Antimicrob. Agents Chemother. **38** (1994) 2215–2217.

Five, human immunodeficiency virus-infected patients with disseminated *Mycobacterium avium* complex infection had progressive weight loss and persistent fever despite multidrug antimycobacterial therapy. These patients were given daily low-dose oral dexamethasone (typically 2 mg/day) as adjunctive therapy. All had substantial and sustained weight gain (12% to 50% of presteroid treatment body weight [$p < 0.03$]), reduction in fever, and an improved sense of well-being. The serum albumin level increased during dexamethasone therapy (from 3.06 ± 0.59 g/dl [mean ± standard deviation] to 3.9 ± 0.22 g/dl [$p < 0.01$]), while the serum alkaline phosphatase level fell (from 368 ± 247 U/liter to 128 ± 43.6 U/liter [$p < 0.04$]). Further studies of the potential role for corticosteroids in the management of disseminated *M. avium* complex infections in human immunodeficiency virus-infected patients are warranted.—Authors' Abstract

**Yew, W. W., Piddock, L. J. V., Li, M. S. K., Lyon, D., Chan, C. Y. and Cheng, A. F. B.** *In-vitro* activity of quinolones and macrolides against mycobacteria. J. Antimicrob. Chemother. **34** (1994) 343–351.

The activities of eight quinolones (ciprofloxacin, clinafloxacin, levofloxacin, ofloxacin, A-80556, sparfloxacin, temafloxacin and tosufloxacin) and three macrolides (azithromycin, clarithromycin, and erythromycin) against 98 clinical isolates of *Mycobacterium tuberculosis* and 120 isolates of five different atypical mycobacterial species including 20 *M. kansasii*, 25 *M. scrofulaceum*, 25 *M. avium/intracellulare*, 25 *M. chelonae* and 25 *M. fortuitum* were determined with the Middlebrook 7H9 broth macro-dilution method. Sparfloxacin, clinafloxacin, levofloxacin, ciprofloxacin and ofloxacin were active against *M. tuberculosis* ($MIC_{90}$ 0.06–0.5 mg/L; $MBC_{90}$ 0.125–2.0 mg/L). However, higher $MIC_{90}$s and $MBC_{90}$s of these quinolones were obtained for strains of multidrug-resistant *M. tuberculosis*. The macrolides tested had poor activity against *M. tuberculosis* isolates ($MIC_{90}$ > 8.0 mg/L). Furthermore, high $MIC_{90}$s of the quinolones and macrolides (2.0 to 8.0 mg/L) were obtained for clinical isolates of atypical mycobacteria, with the exception

of clarithromycin against *M. kansasii* ($MIC_{90}$ = 1.0 mg/L) and sparfloxacin against *M. scrofulaceum* ($MIC_{90}$ = 1.0 mg/L).—Authors' Abstract

**Yuan, S. N., Tan, P. L. J. and Skinner, M. A.** The effect of prostaglandin E(2) and indomethacin on the cytotoxic response to mycobacterial antigens. Int. J. Immunopharmacol. **16** (1994) 525–531.

The effect of prostaglandin E(2) and indomethacin on the generation of cytotoxic T-lymphocytes in response to *Mycobacterium tuberculosis* (MTB) antigens was compared between health controls and rheumatoid arthritis (RA) patients. Peripheral blood mononuclear cells (PBMC) from 16 healthy individuals and 15 RA patients were stimulated for 7 days with an irradiated, sonicated preparation of MTB in the presence or absence of PGE(2) or indomethacin and assayed for cytotoxic activity on autologous target cells prepulsed with MTB. The mean cytotoxic activity generated was lower in patients than in controls. Exogenous PGE(2) suppressed the cytotoxicity directed against MTB-pulsed targets in 12 of 16 controls, but in only 1 of 11 patients. Indomethacin enhanced this cytotoxicity in only 2 of 16 controls but in 6 of 10 RA patients. When effector cells were derived from the synovial fluid, PGE(2) again had no effect and indomethacin enhanced the cytotoxicity. Our data suggest that the depressed cytotoxic response of RA patients to MTB may be due to the production of endogenous PGE(2). Cyclooxygenase inhibitors commonly used in the treatment of RA may influence MTB-induced cytotoxicity in patients. In addition to their antiinflammatory effects within the joint, nonsteroidal antiinflammatory drugs may potentially enhance cytotoxic reactions which are induced by antigens, such as MTB crossreactive heat-shock proteins.—Authors' Abstract

**Zhang, Y. and Young, D.** Molecular genetics of drug resistance in *Mycobacterium tuberculosis*. (Review) J. Antimicrob. Chemother. **34** (1994) 313–319.

Tuberculosis (TB) is the single largest killer among infectious diseases. The recent resurgence of TB together with outbreaks of multidrug-resistant tuberculosis has focused attention on understanding the mechanisms of such drug resistance. Because of the relative neglect of TB research in the past and late arrival of mycobacterial genetic tools, the molecular mechanisms of drug resistance in TB remained largely unknown until very recently. In this paper we review recent progress on the mechanisms of resistance to three major anti-TB drugs: isoniazid, rifampin and streptomycin. While in resistance mechanisms for rifampin and streptomycin are similar to those found in other bacteria, isoniazid susceptibility and resistance is unique to *Mycobacterium tuberculosis*. So far, mutations in two chromosomal loci, *katG* and *inhA* have been found to be involved in isoniazid resistance in TB. Identification and characterization of mutations responsible for resistance opens up new possibilities for rapid detection of drug-resistant strains. Molecular understanding of drug resistance and drug action in *M. tuberculosis* may eventually lead to rational design of new anti-TB drugs.—Authors' Abstract

**Zolg, J. W. and Philippi-Schulz, S.** The superoxide dismutase gene, a target for detection and identification of mycobacteria by PCR. J. Clin. Microbiol. **32** (1994) 2801–2812.

The superoxide dismutase gene has been identified as a target in screening for the presence of mycobacteria on the genus level and differentiating relevant mycobacterial species from one another by PCR. Consensus primers deduced from known superoxide dismutase gene sequences allowed the amplification of DNAs from a variety of bacteria, fungi, and protozoa. Selected amplicons from *Actinomyces viscosus, Corynebacterium diphtheriae, Corynebacterium pseudodiphtheriticum, Mycobacterium avium, M. Fortuitum, M. gordonae, M. intracellulare, M. kansasii, M. scrofulaceum, M. simiae, M. tuberculosis, M. xenopi,* and *Nocardia asteroides* were subsequently cloned and sequenced. The alignment of those sequences facilitated the selection of primers targeting conserved regions present in mycobacterial species but absent in nonmycobacterial species and thus allowed the genus-specific amplification of all 28 different

mycobacterial species tested. A pool of genus-specific probes recognized 23 of the 28 mycobacterial species and did not crossreact with any of the 96 nonmycobacterial species tested. In addition, probes recognizing species-specific variable regions within the superoxide dismutase genes of *M. avium, M. fortuitum, M. gordonae, M. intracellulare, M. kansasii, M. scrofulaceum, M. simiae,* the *M. tuberculosis* complex, and *M. xenopi* were identified. All probes recognized only the species from which they were derived and did not crossreact with any other mycobacterial species or with any of the nonmycobacterial species tested. We conclude that the superoxide dismutase gene is a suitable target for amplifying mycobacteria by PCR on the genus level, confirming correct amplification by genus-specific probes, and differentiating relevant species from one another by a set of species-specific probes.—Authors' Abstract