CURRENT LITERATURE

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

General and Historical

Grodos, D., Francois, I. and Tonglet, R. Health information systems for leprosy control programmes; a case for quality assessment. Lepr. Rev. **67** (1996) 171–182.

A qualitative study was carried out aimed at checking the level of understanding and the actual use of the indicators recommended in leprosy control programs by either the World Health Organization or the International Federation of Anti-Leprosy Associations. Two successive questionnaires were sent to 268 leprosy control program managers. The first one concerned information about the main characteristics of the program, the information system in operation, and the data regarded as indispensable or useful for program monitoring. The respondents to the first questionnaire (N = 64) proposed an extraordinarily wide range of indicators, mainly ill-defined. The respondents to the second questionnaire (N = 37), to whom a limited list of precisely defined indicators was submitted, did not succeed in reaching a complete agreement on any of these indicators. Although the question of program monitoring has been dealt with at an international level for years, there is an urgent need for a real agreement of international agencies and managers of leprosy control programs on the indicators to be used. Program managers in the field are obviously open to the idea of greater intervention by international organizations to improve data collection and to encourage standardization of computerized information systems.—Authors' Summary

Mao, Z., *et al.* [Utilization of medical service by leprosy patients.] China Lepr. J. **12** (1996) 78–81. (in Chinese)

To adapt leprosy control to socialist market system and changed way of raising health funds, the utilization of health service in 67 patients with leprosy was surveyed in Weifang City, Shandong Province. Among them, mean disease duration was 9.97 ± 13.1 months because 57.14% considered the disease not to be worth notice and 35.71% feared their own disease to be known by others. Medical expenditure was 503 yuan on the average before being diagnosed as leprosy in county leprosy stations where 86.6% of them were diagnosed, and since then 97.01% of them could regularly accept treatment if the medical care was free and 62.7% would give up if it was at their own expense; 82.1% want to be treated in secret. On diagnosing 22.39% had disability with WDI (weighted disability index) of 0.84 and the survey showed 52.% with a WDI of 2.3%. The authors appeal to strengthen health education and county leprosy stations, to renew knowledge and to reform the current system of accepting fees for medical care.—Authors' English Abstract

Mao, Z., *et al.* [Utilization of medical service by persons cured of leprosy.] China Lepr. J. **12** (1996) 82–85. (in Chinese)

The survey showed that the degree of utilizing health service by leprosy patients and cures was significantly limited by their low income, discrimination in the community and their self-abasement. The authors put some suggestion to improve the situation.—Authors' English Abstract

Tan, Y., *et al.* [Survey on socio-economic states of leprosy patients and ex-patients.] China Lepr. J. **12** (1996) 88–92. (in Chinese)

The survey showed that the illiterate rate in the group of leprosy patients was 51.1%,

being much higher than the 19.8% in local general population. The per capita income in the patients was 852 yuan, much lower than 1433 yuan in the population. Marriage rate in the patients was 56.9% while it was 68.2% in the population. The divorce rate in the patients was 17.6% after diagnosis and it was 13.86% on surveying, higher than 0.29% in the population. Many patients with leprosy could not marry or have been isolated from their family.—Authors' English Abstract

Tiendrebeogo, A., Blanc, L., Sylla, P. M. and Bobin, P. [Training of health personnel by the Marchoux Institute from 1979 to 1995.] Acta Leprol. **10** (1996) 37–43. (in French)

The Marchoux Institute, an OCCGE center for leprosy research, has provided training for more than a thousand health workers between 1979 and 1995. Formerly, this training was offered entirely at the Marchoux Institute. It was aimed at leprosy control workers administering dapsone monotherapy within the framework of vertically integrated programs. With the introduction of treatment programs using multidrug therapy, leprosy control was integrated into the comprehensive health services. This change in approach dramatically increased the need for training and made it necessary to adapt the training offered by the Marchoux Institute. Since 1990, the Marchoux Institute has targeted doctors in training and health care staff at the supervisory level. The rise in the number of health agents to be trained has led to the arrangement of short-term training courses in the states concerned, with the participation of facilitators from the Marchoux Institute.—Authors' English Summary

Tomimori-Yamashita, J., Maeda, S. M., Jabur, R. and Rotta, O. [Hanseniasis: new methods for leprosy diagnosis.] An. Bras. Dermatol. **71** (1996) 343–349. (in Portuguese)

The authors present a review about the new methods for leprosy diagnosis and their perspectives for their future use in routine investigation. The complementary methods used on routine investigation nowadays are: Mitsuda reaction, bacteriological exam (slit-skin smear technique) and histopathology. Some new methods are presented as: specific serology, antigen detection in organic fluids, PCR (polymerase chain reaction) and immunohistochemical techniques. Other complementary methods useful in clinical evaluation of the patient are also presented, such as: peripheral nerves ultrasound study, electromyography, and the use of Semmes-Weinstein monofilaments.—Authors' English Summary

Xu, Z., *et al.* [Social, economic and clinical benefit of case finding in leprosy.] China Lepr. J. **12** (1966) 93–95. (in Chinese)

To study how to raise social, economic and clinical effectiveness of case findings in leprosy control is very important. On the basis of comparison of the data between the period of 1955 to 1985 and in the last years, the authors think that beside examination of patients' families, case finding should mainly be among outpatients at dermatological clinics if the prevalence was very low, but some survey could be used only for some population groups among which the prevalence was higher in the warm season and, in addition, to strengthen health education on leprosy is essential.—Authors' English Abstract

Zheng, D. *et al.* [On the use of rehabilitation service by persons cured of leprosy.] China Lepr. J. **12** (1996) 85–88. (in Chinese)

The study of influence of the changed way of raising health funds for leprosy control in 362 persons cured of leprosy showed that 61.88% of them had disability grade II or III on diagnosing, and since then among them 33.43% got worse and 48.9% have indications for orthopedic operation of which 55.9% should be willing to take the operation if it were free of charge. In 54 out of 57 persons who had taken the operation it was free, but 24.4% of those who needed the operation could not do it. The authors put some ways to make it better.—Authors' English Abstract

Chemotherapy

Coleman, M. D., Ogg, M. S., Holmes. J. L., Gardiner, J. M. and Jacobus, D. P. Studies on the differential sensitivity between diabetic and non-diabetic human erythrocytes to monoacetyl dapsone hydroxylamine-mediated methaemoglobin formation *in vitro.* Environ. Toxicol. Pharmacol. **1** (1996) 97–102.

Methemoglobin generation by monoacetyl dapsone hydroxylamine in nondiabetic and diabetic erythrocytes was investigated *in vitro.* Methemoglobin formation in purified hemoglobin isolated from both types of erythrocytes as well as hemolysates from both diabetic and nondiabetic erythrocytes did not differ. Prior to an 18-hr incubation with 10 and 20 mM glucose, diabetic erythrocytes were significantly less sensitive to monoacetyl dapsone-induced methemoglobinemia. After pre-incubation the differential was lost, although significant change in glutathione concentrations could not be shown between the two cell types. NADH-diaphorase levels measured in diabetics and nondiabetics did not significantly differ. It is possible that diabetic cells display reduced hydroxylamine-mediated methemoglobin generation due to differences in glutathione metabolism.—Authors' Abstract

DeBruyn, E. E., Steel, H. C., van Rensburg, C. E. J. and Anderson, R. The riminophenazines, clofazimine and B669, inhibit potassium transport in gram-positive bacteria by a lysophospholipid-dependent mechanism. J. Antimicrob. Chemother. **38** (1996) 349–362.

The effects of the riminophenazine antimicrobial agents clofazimine and B669, as well as those of lysophosphatidylcholine (LPC), on microbial K+-transporting systems were investigated in a range of gram-positive and gram-negative bacteria using K-42 and ^{86}rubidium (Rb-86) as tracers. Exposing the gram-positive bacteria to 0.1–10 mg/l of the drugs resulted in a dose-related inhibition of uptake of both radiolabeled cations due primarily to the inhibition of their influx which was prevented by pretreating the microorganisms with 25 mg/l alpha-tocopherol (vitamin E) which forms a complex with lysophospholipids. In contrast, gram-negative bacteria were resistant to the riminophenazine-mediated inhibition of K+-transport, with only one of four well-characterized K+-transport system mutants of *Escherichia coli,* namely, Kup, being affected by the antimicrobial agents. The selective antimicrobial activity of riminophenazines against gram-positive bacteria is probably achieved by the lysophospholipid-mediated inactivation of K+-transport, while gram-negative microorganisms possess several K+-transport systems which are either inaccessible and/or insensitive to lysophospholipids. Thus, K+-transport systems may represent novel targets for antimicrobial agents.—Authors' Abstract

Fromm, M. F., Busse, D., Kroemer, H. K. and Eichelbaum, M. Differential induction of prehepatic and hepatic metabolism by verapamil by rifampin. Hepatology **24** (1996) 796–801.

Cytochrome P450 (CYP) enzymes, which metabolize numerous drugs, are expressed both in liver and in extrahepatic tissues. CYP3A4, for example, is present and inducible by rifampin in epithelial cells of the gastrointestinal tract. It has been shown that such prehepatic metabolism contributes substantially to total clearance of CYP3A4 substrates (e.g., cyclosporine) before and even more pronounced during enzyme induction. We examined the effect of enzyme induction on prehepatic and hepatic metabolism of the model compound R/S-verapamil after simultaneous oral and intravenous administration using a stable isotope technology. This approach allows us to exclude intra-individual day-to-day variability and is, therefore, suitable to quantitatively assess prehepatic extraction of high-clearance drugs. Moreover, because verapamil is administered as a racemate with the S-enantiomer being preferentially metabolized, we investigated the influence of induction on stereoselectivity of prehepatic and hepatic metabolism. Eight male volunteers received 120 mg of racemic ve-

rapamil b.i.d. for 24 days. Rifampin (600 mg daily) was given from day 5 to day 16. Systemic clearance and bioavailability of the verapamil enantiomers were determined by coadministering deuterated verapamil intravenously on day 4, on day 16, and on day 24. Effects of verapamil on atrioventricular conduction after oral and intravenous (i.v.) administration were assessed by measuring the maximum PR-interval prolongation. Rifampin increased the systemic clearance of the active S-verapamil 1.3-fold ($p < 0.001$). In contrast, rifampin increased the apparent oral clearance of S-verapamil 32-fold ($p < 0.001$) and decreased its bioavailability 25-fold ($p < 0.001$), with partial recovery after rifampin withdrawal ($p < 0.01$). With rifampin, the effect of oral verapamil on atrioventricular conduction was nearly abolished ($p < 0.01$); whereas no significant changes were observed after intravenous administration. Induction caused a considerable reduction of stereoselectivity after both intravenous and oral administration ($p < 0.001$). Rifampin altered the pharmacokinetics and the pharmacological effects of verapamil to a much greater extent after oral administration compared with intravenous administration. These data clearly indicate that prehepatic metabolism of verapamil (presumably in the gut wall) is preferentially induced compared with hepatic metabolism and that stereoselectivity of verapamil metabolism is affected by induction.—Authors' Abstract

Gallo, M. E. N., Alvin, M. F. S., Nery, J. A. C., Albuquerque, E. C. A. and Sarno, E. N. Two multidrug fixed-dosage treatment regimens with multibacillary leprosy patients. Indian J. Lepr. **68** (1996) 235–245.

This study compares the clinical, bacilloscopic, and histopathological evolution of 140 patients classified as having multibacillary leprosy with no previous specific treatment who were submitted to two multidrug treatment regimens with a fixed dose. Regimen I—Group I: 70 cases received 600 mg rifampin (RMP) + 100 mg dapsone (DDS) daily for 3 consecutive months followed by 100 mg DDS daily, self-administered doses for 21 months. Regimen II—Group II: 70 cases received 600 mg RMP + 300 mg clofazimine (CLO) once a month under supervision plus self-administered doses of 50 mg CLO + 100 mg DDS daily for 24 months. The bacilloscopic, histopathological and neuromotor evaluation parameters showed no statistically meaningful differences ($p > 0.05$) between the two groups except for reaction frequency ($p < 0.05$) in that Group II patients presented the least number of reactional episodes during the treatment and in the dermatological examination at discharge. Follow up after treatment was carried out for a consecutive 4-year period. During routine clinical examination one case submitted to Regimen I developed a nodular skin lesion over the right arm. Skin biopsy was done for histopathological examination and mouse foot pad experiment by the Shepard technique. The drug-susceptibility test with DDS and RPM showed that the *Mycobacterium leprae* strain isolated was susceptible to both the drugs.—Authors' Abstract

Herbert, D., Paramasivan, C. N., Venkatesan, P., Kubendiran, G., Prabhakar, R. and Mitchison, D. A. Bactericidal action of ofloxacin, sulbactam-ampicillin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **40** (1996) 2296–2299.

The bactericidal actions of ofloxacin and sulbactam-ampicillin, alone and in combination with rifampin and isoniazid, on exponential-phase and stationary-phase cultures of a drug-susceptible isolate of *Mycobacterium tuberculosis* were studied *in vitro.* In exponential-phase cultures, all drugs were bactericidal, with the higher concentrations of ofloxacin (5 μg/ml) and sulbactam-ampicillin (15 μg of ampicillin per ml) being as bactericidal as 1 μg of isoniazid per ml or 1 μg of rifampin per ml. In two-drug combinations, both drugs increased the levels of activity of isoniazid and rifampin and were almost as bactericidal as isoniazid-rifampin; they also appeared to increase the level of activity of isoniazid-rifampin in three-drug combinations. In contrast, ofloxacin and sulbactam-

ampicillin had little bactericidal activity against stationary-phase cultures and were less active than isoniazid or rifampin alone. Furthermore, in two-drug or three-drug combinations, they did not increase the level of activity of isoniazid, rifampin, or isoniazid-rifampin. These findings suggest that ofloxacin and sulbactam-ampicillin are likely to be most useful in the early stages of treatment and in preventing the emergence of resistance to other drugs, but are unlikely to be effective as sterilizing drugs helping to kill persisting lesional bacilli—Authors' Abstract

Ji, B., Jamet, P., Perani, E. G., Sow, S., Lienhardt, C., Petinon, C. and Grosset, J. H. Bactericidal activity of single dose of clarithromycin plus minocycline, with or without ofloxacin, against *Mycobacterium leprae* in patients. Antimicrob. Agents Chemother. **40** (1996) 2137–2141.

Fifty patients with newly diagnosed lepromatous leprosy were allocated randomly to one of five groups and treated with either a month-long standard regimen of multidrug therapy (MDT) for multibacillary leprosy, a single dose of 600 mg of rifampin, a month-long regimen with the dapsone (DDS) and clofazimine (CLO) components of the standard MDT, or a single dose of 2000 mg of clarithromycin (CLARI) plus 200 mg of minocycline (MINO), with or without the addition of 800 mg of ofloxacin (OFLO). At the end of 1 month, clinical improvement accompanied by significant decreases of morphological indexes in skin smears was observed in about half of the patients of each group. A significant bactericidal effect was demonstrated in the great majority of patients in all five groups by inoculating the foot pads of mice with organisms recovered from biopsy samples obtained before and after treatment. Rifampin proved to be a bactericidal drug against *Mycobacterium leprae* more potent than any combination of the other drugs. A single dose of CLARI-MINO, with or without OFLO, displayed a degree of bactericidal activity similar to that of a daily regimen of doses of DDS-CLO for 1 month, suggesting that it may be possible to replace the DDS and CLO components of the MDT with a monthly dose of CLARI-MINO, with or without OFLO. However, gastrointestinal adverse events were quite frequent among patients treated with CLARI-MINO, with or without OFLO, and may be attributed to the higher dosage of CLARI or MINO or to the combination of CLARI-MINO plus OFLO. In future trials, therefore, we propose to reduce the dosages of the drugs to 1000 mg of CLARI, 100 mg of MINO, and 400 mg of OFLO.—Authors' Abstract

Lai, Z., *et al.* [Surveillance of five years after MDT in 667 cases of leprosy.] China Lepr. J. **12** (1996) 104–105. (in Chinese)

WHO's MDT of 24 months was completed in 667 cases of MB leprosy, of which 608 had taken some antileprosy drugs and 59 were newly detected cases. Their skin lesions subsided all or basically and BI values decreased by 0.6 and 0.84 in both groups, respectively, when the treatment was stopped. During surveillance, BI and clinical conditions were decreasing and bettering continuously. By the fifth year of surveillance, the BI became negative in 603 and 58 in both the groups, accounting for 99.2% and 98.3%, respectively. Two cases relapsed only in the group treated before MDT, being 0.3%.—Authors' English Abstract

O'Connor, R., O'Sullivan, J. F. and O'Kennedy, R. Determination of serum and tissue levels of phenazines including clofazimine. J. Chromatogr. B Biomed. Appl. **681** (1996) 307–315.

A rapid and sensitive HPLC method is described for the analysis of synthetic phenazines, including clofazimine, from a variety of biological samples. Phenazines were extracted from serum, tissue and fat using a mixture of dichloromethane and sodium hydroxide. The drugs were then quantified on a reversed-phase C-18 column using a mobile phase consisting of 594 ml of water, 400 ml of tetrahydrofuran, 6 ml of concentrated acetic acid and 0.471 g of hexanesulfonic acid. In this mobile phase, each phenazine tested had its own

retention time. This allowed one phenazine to be used as an internal standard for the analysis of other phenazines. The method was validated for clofazimine [3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine] and B4090 [7-chloro-3-(4-chloranilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(2,2,6,6-tetramethylpiperid-4-ylimino)phenazine] (VI) and shown to be accurate and precise across a broad concentration range from 0.01 to 50 μg/g (μg/ml). Extraction was 100% for each agent across this range. This system was used to measure clofazimine and VI levels following their administration to rats. The pharmacokinetic profile of VI was different to that of clofazimine, with high tissue concentrations but lower fat levels.—Authors' Abstract

Patnaik, P. K. B. and McDougall, A. C. The contribution of "cure by dapsone monotherapy" to the reduction of prevalence of leprosy in the state of Orissa, India, 1983–1993. Indian J. Lepr. **68** (1996) 223–226.

The implementation of multiple drug therapy (MDT) in the state of Orissa, India, started in early 1983 and was extended in a phased manner to 9 out of the then total of 13 districts by 1993. As part of a program to bring the remaining four districts under MDT, an intensive screening of the registers was carried out in early 1993. From a total of 28,855 cases registered in these districts, 26,113 (90.5%) were examined and 18,008 (69.9%) deleted. The deleted included patients who had died, emigrated, double (or occasionally treble) entries for the same patient, and those in whom careful re-assessment suggested that the original diagnosis of leprosy had been wrong. In addition, however, 8260 (45.8%) of the 18,008 cases deleted were considered to have been cured by dapsone monotherapy. This figure, from districts with a relatively poor development of services for leprosy control, prompted a retrospective examination of data from the other (more privileged) 9 districts. This revealed that from a total of 264,000 patients screened, prior to the implementation of MDT from 1983 onward, 75,590 (28.6%) were removed from the registers and that of these, 63,562 (84.0%) were considered to have been cured by dapsone monotherapy. Thus, from a total of 93,598 patients removed from registers in this state since 1983, 71,822 (76.7%) have been assessed as cured by dapsone monotherapy. The significance of this finding and its apparently considerable contribution to the overall reduction in the prevalence of leprosy in Orissa, 1983–1993, is discussed.—Authors' Abstract

Renau, T. E., Gage, J. W., Dever, J. A., Roland, G. E., Joannides, E. T., Shapiro, M. A., Sanchez, J. P., Gracheck, S. J., Domagala, J. M., Jacobs, M. R. and Reynolds, R. C. Structure-activity relationships of quinolone agents against mycobacteria: effect of structural modifications of the 8 position. Antimicrob. Agents Chemother. **40** (1996) 2363–2368.

A series of quinolones with substitutions at the 8 position has been prepared as part of a study to examine the relationship between structural modifications at this position and activity against mycobacteria. The compounds were prepared by procedures described in the literature and were evaluated for their activities against *Mycobacterium fortuitum* and *M. smegmatis*. The activities of the compounds against these two organisms were used as a measure of *M. tuberculosis* activity. The results demonstrate that the contribution of the 8 position to antimycobacterial activity was dependent on the substituent at N-1 and was in the order (i) COMe ≈ CBr > CCl > CH ≈ CF ≈ COEt > N > CCF_3 when N-1 was cyclopropyl; (ii) N ≈ CH > CF > COMe when N-1 was 2,4-difluorophenyl; (iii) N ≥ CH when N-1 was *tert*-butyl; and (iv) N > CH when N-1 was ethyl. In general, derivatives with piperazine substitutions at C-7 were slightly less active against mycobacteria than the analogs with pyrrolidine substitutions, regardless of the pattern of substitution at the 8 position. Several of the best compounds were evaluated for their potential side effects as well as their activities against *M. aurum*, *M. avium-M. intracellulare*, and *M. tuberculosis*. These agents exhibited biological profiles similar to or better than

those of the positive controls ciprofloxacin and sparfloxacin.—Authors' Abstract

Richter, H. G. F., Angehorn, P., Hubschwerlen C., Kania, M., Page, M. G. P., Specklin, J. L. and Winkler, F. K. Design, synthesis, and evaluation of 2 beta-alkenyl penam sulfone acids as inhibitors of beta-lactamases. J. Med. Chem. **39** (1996) 3712–3722.

A general method for synthesis of 2 beta-alkenyl penam sulfones has been developed. The new compounds inhibited most of the common types of beta-lactamase. The level of activity depended very strongly on the nature of the substituent in the 2 beta-alkenyl group. The inhibited species formed with the beta-lactamase from *Citrobacter freundii* 1205 was sufficiently stable for X-ray crystallographic studies. These, together with UV absorption spectroscopy and studies of chemical degradation, suggested a novel reaction mechanism for the new inhibitors that might account for their broad spectrum of action. The (Z)-2 beta-acrylonitrile penam sulfone Ro 48–1220 was the most active inhibitor from this class of compound. The inhibitor enhanced the action of, for example, ceftriaxone against a broad selection of organisms producing beta-lactamases. The organisms included strains of *Enterobacteriacea* that produce cephalosporinases, which is an exceptional activity for penam sulfones.—Authors' Abstract

Wiese M., Schmalz, D. and Seydel, J. K. New antifolate 4,4′-diaminodiphenyl sulfone substituted 2,4-diamino-5-benzylpyrimidines; proof of the dual mode of action and autosynergism. Arch. Pharm. (Weinheim) **329** (1996) 161–168.

New 4,4′-diaminodiphenylsulfone substituted 2,4-diamino-5-benzylpyrimidines were synthesized. These compounds are highly active inhibitors of both bacterial dihydrofolate reductase (DHFR) and dihydropteroic acid synthase (SYN). The simultaneous inhibition of both enzymes leads to autosynergism in whole cells in the same way as known for combinations of sulfonamides with trimethoprim. The inhibitory activity is demonstrated in cell-free systems of DHFR and SYN derived from various species (*Mycobacterium lufu, Escherichia coli, Candida albicans*) and in whole cell systems of the mycobacterial strain *M. lufu*. The compounds are rare examples for the combination of two mechanisms of action in one molecule.—Authors' Abstract

Clinical Sciences

Chauhan, S. L., Girdhar, A., Mishra, B., Malaviya, G. N., Venkatesan, K. and Girdhar, B. K. Calcification of peripheral nerves in leprosy. Acta Leprol. **10** (1996) 51–56.

A study conducted in 74 TT/TB patients, with gross thickening of nerves together with nerve abscess, showed calcification in eight patients. Calcification was most common in the ulnar nerve followed by the lateral popliteal nerve. All eight patients were males with a significantly longer duration of illness before start of treatment. Patients with late onset of nerve abscess were found to be more prone to calcium deposition in the nerves. Caseous pus of the abscess had high lipid content with raised cholesterol and cholesterol ester ratio to total lipids, suggesting a dystrophic nature of calcification.—Authors' Summary

Hoetelmans, R. M. W., Otten, J. M. M. B., Koks, C. H. W., Soesan, M. and Beijnen, J. H. Combined dapsone and clofazimine intoxication. Hum. Exp. Toxicol. **15** (1996) 625–628.

We report clinical findings and pharmacokinetic data regarding a combined dapsone and clofazimine intoxication in a man who ingested 50 tablets of dapsone (100 mg), 20 capsules of clofazimine (100 mg) and two tablets of rifampin (600 mg). Oral administration of activated charcoal (50 g)

and sodium sulfate (20 g) after gastric lavage resulted in an elimination half-life in plasma of 11.1 and 10.8 hr for dapsone and its main metabolite, monoacetyldapsone, respectively. A rapid initial decrease of the plasma concentration of clofazimine was observed after gastric lavage and administration of activated charcoal and sodium sulfate. Fifteen hours after this treatment, clofazimine plasma levels remained relatively constant. Dapsone-induced methemoglobinemia (48% at admission) was treated successfully with methylene blue.—Authors' Abstract

Prussick, R. and Shear, N. H. Dapsone hypersensitivity syndrome. J. Am. Acad. Dermatol. **35** (1996) 346–349.

We describe a 22-year-old woman with cutaneous polyarteritis nodosa in whom dapsone hypersensitivity syndrome (DHS) developed 5 weeks after initiation of dapsone therapy. She had fever and cervical lymphadenopathy, and later a widespread erythematous eruption studded with pustules developed. She also had liver involvement with mixed hepatocellular and cholestatic features. The patient was treated with prednisone 60 mg daily. Once the patient's liver function normalized, prednisone dosage was reduced by 5 mg weekly. The clinical features and treatment of DHS are reviewed. We encourage immediate discontinuation of the drug in a patient in whom a fever or flu-like illness develops, especially 4 or more weeks after the treatment is started. We also suggest routine thyroid function testing 3 months after recovery because of the possible risk of hypothyroidism.—Authors' Abstract

Ramesh Kumar, G., Ramana, P. V., Vasundhara, N. and Kumaraswamy Reddy, M. Two unusual nerve abscesses—lepromatous leprosy and pure neural leprosy: case reports. Lepr. Rev. **67** (1996) 217–221.

We report two cases of nerve abscesses, one suffering from lepromatous leprosy (LL) and the other from tuberculoid neural leprosy. Neither had any signs of reactions. Both were untreated cases. Surgical nerve decompression and systemic prednisolone had resolved the nerve abscess in the first case; whereas the second one responded only to surgical nerve decompression. The unusual nature of clinical presentation of nerve abscess has been outlined.—Authors' Summary

Rosa, H., Martins, R. and Vanderborght, B. Association between leprosy and hepatitis C infection: a survey in a region of central Brazil. Am. J. Trop. Med. Hyg. **55** (1996) 22–23.

Prevalence of antibodies to hepatitis C virus (HCV) was determined in 216 Brazilian lepromatous patients (83 outpatients and 133 institutionalized). The overall prevalence was 1.8% after confirmatory tests. No difference in the HCV infection was found between outpatients and institutionalized ones. Our results from this region of central Brazil are lower than those found in leprosy patients in Africa and in Japan.—Authors' Abstract

Salafia, A. and Chauhan, G. Nerve abscess in children and adult leprosy patients: analysis of 145 cases and review of the literature. Acta Leprol. **10** (1996) 45–50.

The authors report on their experience of nerve abscess in leprosy. They have found that in the last 5 years there is a significant increase in this type of pathology, at a time when the total number of patients has decreased in adults. Nerve abscesses are, recently, noticed in a large number of children and teenagers. This was not the case 7–9 years ago. Abscesses were excised from 145 nerves in 116 patients between May 1985 and May 1994, out of which, 14 patients (12.6%) were operated on during the period May 1985 to December 1989 and 102 (87.93%) in the period January 1990 to May 1994. Children and teenagers account for 47% of all cases of nerve abscess in this series. The incidence of abscess in multiple nerves is high too in these groups. Abscess of cutaneous nerves is very common too (35% of cases) though rarely reported in the literature. There is a higher incidence of nerve abscess in male adults as compared to

females. The authors believe that this sudden increase in neural pathology can be attributed, in part, to the extension of multidrug therapy (MDT) programs without adequate infrastructure to detect and treat early neuritis.—Authors' Summary

Schoeman, B. J. [Squamous cell carcinoma in neuropathic plantar ulcers in leprosy.] S. Afr. Med. J. **86** (1996) 966–969. (in Afrikaans)

Seven cases of squamous cell carcinoma (SCC) arising in chronic neuropathic plantar ulcers of leprosy are described. These patients (average age 59 years) presented over a 5-year period. The mean duration of neuropathic ulceration until diagnosis of SCC was 24.5 years. Six patients required limb amputations and 3 underwent lymphadenectomy for involved nodes. One patient died of disseminated disease. The history of the eponym "Marjolin's ulcer" is traced and a case put forward for recognition of malignant change in neuropathic ulcers as yet another example of Marjolin's ulcer. A plea is made for an increased awareness of the possibility of malignant transformation in chronic neuropathic ulcers in order to effect an early diagnosis of a potentially aggressive cancer. This is the first report of its kind in South Africa. Similar cases, however, have been reported from other parts of the world where leprosy is endemic.—Author's English Abstract

Thacker, A. K., Chandra, S., Mukhija, R. D. and Sarkari, N. B. S. Electro-physiological evaluation of nerves during reactions in leprosy. J. Neurol. **243** (1996) 530–535.

Forty-two patients with leprosy (7 with tuberculoid type, 30 borderline, 5 lepromatous) were studied electrophysiologically during reactions. Thirty-three had type 1 reactions while 9 had type 2 reactions. Each patient received 60 mg/day prednisolone tapered over a 6-week period. Motor conduction studies were performed on one clinically affected and one unaffected nerve and were repeated 12 weeks after the beginning of steroid therapy. Significant motor conduction abnormalities were observed in 14 affected (33.3%) and 8 unaffected nerves (19.1%). The majority of these nerves were in patients with borderline leprosy having type 1 reactions. Following steroid therapy, nerve function improved in 14 affected (33.3%) and 20 unaffected nerves (47.66%). However, 5 affected (10.2%) and 12 unaffected nerves (28.5%) showed a worsening of nerve function following steroid therapy. The majority of the nerves that showed improvement with steroid therapy had type 1 reactions, while those that showed deterioration had type 2 reactions. Steroids improved nerve function mainly in patients with type 1 reactions. Their role in patients with type 2 reactions remains debatable.—Authors' Abstract

Yang, J., *et al.* [Survey on leprosy ophthalmopathy and its relevant factors.] China Lepr. J. **12** (1996) 106–108. (in Chinese)

A survey in 2145 cases of leprosy who are active or cured in Liangshan and Panzhihua, Sichuan, showed that 1570 persons (73.2%) have various ophthalmopathy, of them those caused by leprosy are in 465 cases (21.7%) and the commonest are numbness of the cornea and lagophthalmos, relating closely to their ages and education levels, and to ages, duration and type of the disease, BI and lepra reaction on being diagnosed. It is found that cataract has no significant relation to leprosy in these persons.—Authors' English Abstract

Zheng, D. *et al.* [Socio-medicine and treatment of leprosy.] China Lepr. J. **12** (1996) 96–99. (in Chinese)

As their effect is known up to now, multidrug therapy for leprosy, except for shorter duration of treatment and possibly less relapse, is similar to dapsone (DDS) monotherapy clinically and bacteriologically, so it is essential to develop some new and better drugs or therapies. The authors combined MDT with mental hygiene, rational nutrition, suitable labor and rehabilitation, and achieved good results, making the duration of treatment shorten from 141.87 months before 1984 and 68 months in 1987 to 44 months.—Authors' English Abstract

Immuno-Pathology

Beckman, E. M., Melian, A., Behar, S. M., Sieling, P. A., Chatterjee, D., Furlong, S. T., Matsumoto, R., Rosat, J. P., Modlin, R. L. and Porcelli, S. A. CD1c restricts responses of mycobacteria-specific T cells—evidence for antigen presentation by a second member of the human CD1 family. J. Immunol. **157** (1996) 2795–2803.

Previous studies suggest that CD1 is a family of antigen (Ag)-presenting molecules distantly related to those encoded by the MHC. However, of the four known human CD1 proteins, only CD1b has been shown to restrict Ag-specific T-cell responses. In this study, we have shown that a second member of the human CD1 family, CD1c, could also mediate Ag presentation to T cells. Three T-cell lines recognizing mycobacterial Ags in a CD1c-restricted manner were isolated from normal donor blood. These T cells were MHC unrestricted, and their recognition of Ag was independent of the products of the transporter associated with Ag presentation-1/2 and DMA/B genes that are generally required for Ag presentation by MHC-encoded Ag-presenting molecules. Furthermore, unlike MHC-restricted responses to peptides, the CD1c-restricted T-cell lines recognized protease-resistant mycobacterial lipid Ags. These T-cell lines also showed significant cytotoxicity toward CD1c-expressing target cells even in the absence of mycobacterial Ags, which was shown by clonal analysis to be mediated by a subpopulation of T cells directly reactive to CD1c molecules. Our findings establish the ability of a second member of the CD1 family to restrict responses of Ag-specific T cells, and thus support the general hypothesis that the CD1 family comprises a third lineage of Ag-presenting molecules that presents a novel class of foreign and self Ags to MHC-unrestricted T cells.—Authors' Abstract

Beimnet, K., Soderstrom, K., Jindal, S., Gronberg, A., Frommel, D. and Kiessling, R. Induction of heat shock protein 60 expression in human monocytic cell lines infected with *Mycobacterium leprae*. Infect. Immun. **64** (1996) 4356–4358.

Monocytic cell lines (HL-60 and THP-1) were infected with viable *Mycobacterium leprae*. Levels of human hsp60 were estimated by Western blot (immunoblot) assay and a sandwich enzyme-linked immunosorbent assay. The results showed that infection of both of the cell lines induced the synthesis of human hsp60, which may be of significance in relation to autoimmune manifestations associated with mycobacterial infections.—Authors' Abstract

Dockrell, H. M., Young, S. K., Britton, K., Brennan, P. J., Rivoire, B., Waters, M. F. R., Lucas, S. B., Shahid, F., Dojki, M., Chiang, T. J., Ehsan, Q., McAdam, K. P. W. J. and Hussain, R. Induction of Th1 cytokine responses by mycobacterial antigens in leprosy. Infect. Immun. **64** (1996) 4385–4389.

Twelve mycobacterial antigens were compared for induction of gamma interferon (IFN-γ) secretion by human blood mononuclear cells of patients with leprosy. Fractionated *Mycobacterium leprae* antigens containing cell-wall proteins or cytosolic and membrane proteins induced good IFN-γ responses in tuberculoid leprosy patients. Lipoarabinomannan from *M. tuberculosis* Erdman and *M. leprae* mycolylarabinogalactan peptidoglycan were the poorest IFN-γ inducers.—Authors' Abstract

Hewish, M. J., Miekle, A. M., Hunter, S. D. and Crowe, S. M. Quantifying phagocytosis of *Mycobacterium avium* complex by human monocytes in whole blood. Immunol. Cell Biol. **74** (1996) 306–312.

Studies of phagocytic efficiency in cells of the macrophage lineage have assumed additional importance since the discovery that HIV infection of these cells impairs their immune function. A rapid method has been developed for measuring phagocytosis of the opportunistic pathogen *Mycobac-*

terium avium complex by human monocytes. Fluoresceinated *M. avium* complex (F-MAC) was incubated with whole blood at 37°C and the fluorescence of extracellular F-MAC was quenched using a vital blue stain. Monocytes were then stained with a monoclinal antibody (mAb) to human CD14 conjugated to phycoerythrin (PE), red cells were lysed, and the percentage of monocytes which had phagocytosed F-MAC was measured by flow cytometry. The results were reproducible in samples of blood taken from individual donors over a period of 1 or 2 weeks, and optimum F-MAC concentrations and an optimum incubation time were determined by experiment. This method has the advantages of requiring only a small volume of blood, not necessitating manipulation of cells before testing, and using a phagocytic target relevant to the pathogenesis of HIV infection.—Authors' Abstract

Huygen, K., Content, J., Denis, O., Montgomery, D. L., Yawman, A. M., Deck, R. R., Dewitt, C. M., Orme, I. M., Baldwin, S., D'Souza, C., Drowart, A., Lozes, E., Vandenbussche, P., van Vooren, J. P., Liu, M. A. and Ulmer, J. B. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. Nat. Med. **2** (1996) 893–898.

Tuberculosis is the most widespread and lethal infectious disease affecting humans. Immunization of mice with plasmid DNA constructs encoding one of the secreted components of *Mycobacterium tuberculosis,* antigen 85 (Ag85), induced substantial humoral and cell-mediated immune responses and conferred significant protection against challenge with live *M. tuberculosis* and *M. bovis* bacille Calmette-Guerin (BCG). These results indicate that immunization with DNA encoding a mycobacterial antigen provides an efficient and simple method for generating protective immunity and that this technique may be useful for defining the protective antigens of *M. tuberculosis* leading to the development of a more effective vaccine.—Authors' Abstract

Jeevan, A., Ullrich, S. E., de Gracia, M., Shah, R. and Sun, Y. Mechanism of UVB-induced suppression of the immune response to *Mycobacterium bovis* bacillus Calmette-Guerin: role of cytokines on macrophage function. Photochem. Photobiol. **2** (1996) 259–266.

Previously, we demonstrated that treatment of mice with either WB radiation or supernatants derived from UVB-irradiated PAM 212 keratinocytes decreased the induction of the delayed-type hypersensitivity (DTH) response to *Mycobacterium bovis* bacillus Calmette-Guerin (BCG), impaired the clearance of bacteria from their lymphoid organs and also altered macrophage functions. In order to characterize the cytokines involved in these phenomena, UV-irradiated mice were injected with antibodies to interleukin-10 (IL-10), transforming growth factor-beta 1 (TGF-β 1), or tumor necrosis factor-alpha (TNF-α). Injection of UVB-irradiated mice with anti-IL-10 immediately after UV irradiation restored the DTH response and reversed the UV-induced inhibition of bacterial clearance. Injection of UV-irradiated mice with anti-TGF-β only partially restored the DTH response although it allowed a better clearance of BCG than injection of mice with the control antibody. In contrast, injection of anti-TNF-α did not affect the UVB-induced suppression of DTH or impaired bacterial clearance. Similarly, the ability of macrophages to phagocytose BCG and kill the intracellular organisms was restored to almost normal levels after injecting UV-irradiated mice with antibodies specific for IL-10 or TGF-β. Injection of mice with either recombinant IL-10 or TGF-β mimicked the effect of whole-body UV irradiation on immune function. These results suggest that IL-10 has a major role in UV-induced suppression of both DTH to BCG and impairment in the clearance of bacteria and that TGF-β has a more significant role in blocking bacterial clearance. Furthermore, these cytokines seem to modulate immune responses by altering macrophage functions in UVB-irradiated mice.—Authors' Abstract

Kusner, D. J., Hall, C. F. and Schlesinger, L. S. Activation of phospholipase D is tightly coupled to the phagocytosis of *Mycobacterium tuberculosis* or opson-

ized zymosan by human macrophages. J. Exp. Med. **184** (1996) 585–595.

Phagocytosis of *Mycobacterium tuberculosis* by human mononuclear phagocytes is mediated primarily by complement receptors (CRs) but the transmembrane signaling mechanisms that regulate phagocytosis of the bacterium are unknown. We have analyzed the activation of phospholipase D (PLD) during phagocytosis of the virulent Erdman and attenuated H37Ra strains of *M. tuberculosis* by human monocyte-derived macrophages (MDMs), radiolabeled with [H-3]-lyso-phosphatidylcholine. Phagocytosis of either Erdman or H37Ra *M. tuberculosis* in the presence of autologous nonimmune serum was associated with a 2.5–3-fold increase in phosphatidic acid (PA). Definitive evidence for activation of PLD by *M. tuberculosis* was provided by markedly increased generation of the PLD-specific product phosphatidylethanol (PEt) (9.9-fold increases in [H-3]-PEt for both Erdman and H37Ra strains compared to control, $p < 0.001$, $N = 12$), in the presence of 0.5% ethanol. Phagocytosis of opsonized zymosan (OZ), which is also mediated by CRs, was similarly associated with activation of PLD (12.2-fold increase in PEt, $p < 0.001$, $N = 12$). The competitive PLD inhibitor 2,3-diphosphoglycerate (2,3-DPG) produced concentration-dependent inhibition of PLD activity stimulated by either *M. tuberculosis* (–78 ± 8%) or OZ (–73 ± 6%). Inhibition of PLD by 2,3-DPG was associated with concentration-dependent reductions in phagocytosis of *M. tuberculosis* (–74 ± 4%) and OZ (–68 ± 5%). Addition of purified PLD from *Streptomyces chromofuscus* to 2,3-DPG-treated macrophages restored phagocytosis of *M. tuberculosis* to control levels. Inhibition of *M. tuberculosis*- or OZ-stimulated PA generation by ethanol was associated with concentration-dependent reductions in phagocytosis of both particles.

Incubation of MDMs with either Erdman or H37Ra *M. tuberculosis,* or OZ resulted in rapid (onset 1 min) and sustained (60 min) increases in the tyrosine phosphorylation (Tyr-P) of multiple MDM proteins. Prominent Tyr-P was noted in proteins of 150, 95, 72, 56, and 42 kD. The protein tyrosine kinase (PTK) inhibitors genistein and herbimycin A reduced *M. tuberculosis*-stimulated PLD activity by 66%–84%. Inhibition of PLD activity by genistein or herbimycin A was associated with inhibition of phagocytosis of *M. tuberculosis* and OZ. These data demonstrate that PLD is activated during macrophage phagocytosis of *M. tuberculosis* or OZ, that PTKs are involved in this stimulation of PLD, and that the extent of phagocytosis of these particles is tightly coupled to activation of PLD.—Authors' Abstract

Lagrange, P. H. and Abel, L. [Human genetic susceptibility to leprosy.] Acta Leprol. **10** (1996) 11–27. (in French)

The capacity of certain individuals to resist certain diseases, including leprosy, has for a long time been considered as being influenced by genetic factors. The clinical and pathological spectrum of leprosy, epidemiological heterogeneity, both geographic and ethnic, in the prevalence of polar forms may be explained by genetic differences in host resistance. While the specific genes in question have not been identified, recent studies suggest a genetic basis for differences in the capacity of macrophages in the host to reduce bacterial multiplication.

Experimental models analyzing the reactions of antimycobacterial defense have underscored existing differences in resistance or vulnerability to infection. *M. bovis,* BCG, *M. lepraemurium, M. tuberculosis* were guided by a dominant gene which exists in two allelic forms, bcg^r and bcg^s. The bcg^r allele confers resistance and is more dominant than the bcg^s allele which represents greater vulnerability to infection. The murine candidate gene for the bcg gene has been named NRAMP (natural resistance-associated macrophage protein). Even though the exact function of NRAMP is not currently known, it has been demonstrated that this gene is expressed mainly in macrophages and that it brings about increased bacteriostatic capacity in these cells. NRAMP is structurally homologous to the family of membranous proteins having a transport function linking ATP. NRAMP is similar to the membranous bacterial system transporting nitrites. The NRAMP protein

is also involved as a signal of transduction during the activation of macrophages. It is therefore possible to conceive of genetic polymorphism at this locus intervening in specific and nonspecific immune responses to infection. Apart from such potential polymorphism during the initial phase of infection, immunogenetic studies suggest that the polymorphism of class II HLA molecules could intervene in the evolution of secondary immune response to *M. leprae*. Knowing that HLA molecules are expressed in a co-dominant form, and attributing extraordinary allelic polymorphism to this locus, there may be a rather wide range of immune responses to the *M. leprae* antigens in subjects with discordant HLA and in populations which have varied genetic profiles. In general, it has been acknowledged that HLA-DR isotypes are associated with protective response, while HLA-DQ isotypes are said to be associated with multibacillary lepromatous forms. The chief role of the HLA systems controlling cell-mediated immunity leads to the probability that differences in HLA haplotypes could contribute to the wide spectrum of immune responses observed in leprosy. Genetic determinants of resistance to leprosy cannot be described in a straightforward manner using a classic approach because the complex mechanisms of resistance, yet to be clarified and for which at least two loci are believed to be contributory, may be re-assessed like a multifactorial, multigenetic complex in which environmental events linked to the transmission of *M. leprae,* its duration, intensity and host factors, varying as a function of time, intervene. A close study of each element and better understanding of the physiological and pathological mechanisms of infection and disease are necessary in order to state the influence of genetic factors on each of them with greater precision.—Authors' English Abstract

Miyachi, H., Azuma, A., Hioki, E., Iwasaki, S., Kobayashi, Y. and Hashimoto, Y. Cell type-/inducer-specific bidirectional regulation by thalidomide and phenylphthalimides of tumor necrosis factor-alpha production and its enantiodependence. Biochem. Biophys. Res. Com. **226** (1996) 439–444.

Regulation by thalidomide and phenylphthalimide analogs (FPP-33 and PPS-33) of TNF-alpha (TNF-α) production is specific to cell type and to inducer, i.e., (i) the compounds enhance TPA-induced TNF-α production by human leukemia HL-60 cells, while they inhibit TPA-induced TNF-α production by another human leukemia cell line, THP-1, and (ii) the compounds inhibit TNF-α production by both HL-60 and THP-1 cells when the cells are stimulated with okadaic acid (OA). The structure-activity relationships of these compounds are similar in the four assay systems (TPA/HL-60, TPA/THP-1, OA/HL-60, and OA/THP-1). However, optically active analogs, (S)- and (R)-alpha-methylthalidomides, show distinct bidirectional regulatory effects on TNF-α production, i.e., only the (S)-form shows TNF-α production-enhancing activity in the TPA/HL-60 assay system, while the (R)-form shows much more potent TNF-α production-inhibiting activity than the (S)-form in the other assay systems.—Authors' Abstract

Rao, S. P., Ratnakar, P. and Catanzaro, A. Macrophage release of tumor necrosis factor-alpha by *Mycobacterium avium* antigens. FEMS Immunol. Med. Microbiol. **15** (1996) 27–34.

Mycobacterium avium complex (MAC) infection is the most common disseminated opportunistic infection encountered in patients with AIDS. We have studied the ability of specific *Mycobacterium avium* (MA) antigens to stimulate human monocyte-derived macrophages (MDM) to produce tumor necrosis factor-alpha (TNF-α). MDM stimulated with MA sonicate, MA 68 kDa and MA 48–52 kDa antigens were found to produce TNF-α in a dose-dependent manner. Reverse transcriptase-polymerase chain reaction analysis of mRNA extracts from antigen-stimulated MDM indicated that TNF-α mRNA expression was of brief duration and the time point of peak TNF-α mRNA levels was found to be antigen-specific. A significant difference in TNF-α production in response to MA 48–52-kDa antigen and *M. bovis* 65-kDa antigen was observed between MDM from normal and HIV-positive individuals.—Authors' Abstract

Rieckmann, P., Scholze, G., Weichselbraun, I., Ganapati, R. and Prange, H. W. Soluble adhesion molecules in sera of patients with leprosy: levels of soluble intercellular adhesion molecule-1 (sICAM-1) rapidly decrease during multi-drug therapy. Clin. Exp. Immunol. **105** (1996) 65–68.

The clinicopathological spectrum of leprosy is associated with an altered immunological reaction. The expression of adhesion molecules on endothelial cells directs the cellular traffic to sites of local skin and nerve inflammation. Soluble forms of adhesion molecules, which are released upon cytokine activation, can be detected in the circulation and may reflect ongoing tissue inflammation. We determined the serum levels of sICAM-1, sE-selectin and sL-selectin in 74 patients with leprosy (tuberculoid form, N = 23; lepromatous form, N = 36; acute leprous reaction, N = 16) and 15 healthy age- and sex-matched control donors. Patients with lepromatous leprosy had significantly higher levels of sICAM-1 (564 ± 174 versus 450 ± 92 versus 334 ± 57 ng/ml) and E-selectin (90 ± 31 versus 74 ± 29 versus 50 ± 10 ng/ml) than patients with tuberculoid leprosy and normal donors ($p < 0.01$). No differences between groups were detected for L-selectin. Patients with leprous reactions had similar high levels to lepromatous patients. Twenty lepromatous patients were re-examined after 4 weeks of therapy. A significant decrease in sICAM-1 serum levels was observed after 1 month of anti-mycobacterial treatment, which was accompanied by a reduction of mycobacteria in skin biopsies ($p < 0.01$). Patients with leprous reactions (N = 13) also demonstrated a drop in sICAM-1 after antiinflammatory therapy. SE-selectin and sl-selectin serum values decreased only in lepromatous patients after therapy. It can be concluded that soluble adhesion molecules like sICAM-1 and sE-selectin are promising activity markers in patients with leprosy, which may be useful for treatment monitoring.—Authors' Abstract

Suneetha, L. M., Korula, R. J. and Balasubramanian, A. S. Protein phosphorylation in human peripheral nerve: altered phosphorylation of a 25-kDa glycoprotein in leprosy. Neurochem. Res. **21** (1996) 707–712.

Protein phosphorylation in a low-speed supernatant of human peripheral nerve (tibial and sural) homogenate was investigated. The major phosphorylated proteins had molecular mass in the range of 70, 55, 45, and 25 kDa. Mg2+ or Mn2+ was essential for maximum phosphorylation although Zn2+, Co2+, and Ca2+ could partially support phosphorylation. External protein substrates casein and histone were also phosphorylated. The protein phosphatase inhibitor orthovanadate enhanced the phosphorylation of the 45 and 25 kDa proteins significantly. Concanavalin A-Sepharose chromatography of the phosphorylated peripheral nerve proteins showed that the 25-kDa protein was a glycoprotein. Protein phosphorylation of peripheral nerves from leprosy-affected individuals was compared with normals. The phosphorylation of 25-kDa protein was decreased in most of the patients with leprosy.—Authors' Abstract

Tantawichien, T., Young, L. S. and Bermudez, L. E. Interleukin-7 induces anti-*Mycobacterium avium* activity in human monocyte-derived macrophages. J. Infect. Dis. **174** (1996) 574–582.

To examine the modulatory role of interleukin-7 (IL-7) on intracellular growth of *Mycobacterium avium* complex (MAC), human macrophages were treated either before or after MAC infection with different concentrations of IL-7. At 100 pg/ml, 1 ng/ml, and 10 ng/ml, treatment with IL-7 before infection stimulated secretion of tumor necrosis factor-alpha (TNF-α) from MAC-infected macrophages (increase up to 40%) and resulted in dose-dependent reduction in the number of intracellular bacteria. Pretreatment with IL-7 did not inhibit the secretion of transforming growth factor-beta (') (TGF-β'). IL-7 added to the macrophage monolayer 4 hr after infection resulted in both the secretion of TNF-α from MAC-infected macrophages (up to 90% increase, $p < 0.05$) and antimycobacterial activity (up to 50% reduction in bacteria, $p < 0.05$); however, TGF-β' production was not inhibited. IL-7-dependent anti-

MAC activity of macrophages was inhibited by anti-human TNF-α antibody. These results suggest that IL-7 may contribute to the regulation of the immune response against MAC.—Authors' Abstract

Tascon, R. E., Colston, M. J., Ragno, S., Stavropoulos, E., Gregory, D. and Lowrie, D. B. Vaccination against tuberculosis by DNA injection. Nat. Med. **2** (1996) 888–892.

There are 3 million deaths per annum worldwide due to tuberculosis, and AIDS is compounding the problem. A better vaccine than the live *Mycobacterium* currently in use, bacillus Calmette-Guerin (BCG), is needed. When mice were injected with plasmid DNA encoding a single mycobacterial antigen [65-kDa heat shock protein (hsp65)], they made specific cellular and humoral responses to the protein and became immune to subsequent challenge with *Mycobacterium tuberculosis*. Protection was equivalent to that obtained by vaccinating with live BCG, whereas immunizing with the protein was ineffective. Protection was also obtained with DNA encoding another mycobacterial antigen (36-kDa proline-rich antigen). These results suggest that DNA vaccination might yield improved vaccines to replace BCG.—Authors' Abstract

Wu, Q., *et al.* [A new SACT method and its comparison with PGL-I-ELISA.] China Lepr. J. **12** (1996) 108–113. (in Chinese)

An indirect serum antibody competition test (SACT) has been established using B_8F_4IV, a monoclonal antibody to PGL-I of *Mycobacterium leprae*, and with it the antibody levels in 100 patients (including LL 20, BL 20, BB 20, BT 20, and TT 20) and 28 healthy controls from non-endemic areas of leprosy were examined. Then a comparison of SACT with a PGL-I-ELISA was done. The result indicated that the positive rates of SACT and PGL-I-ELISA were 99% and 89% in the patients and 3.5% and 0 in the controls, respectively, with supposed theoretically normal values of OD $\geq$ 0.07 in ELISA and inhibition (%) $\geq$ 44% in SACT; that from LL to TT the OD values of ELISA were gradually decreased in concordance with declines in BIs, but there was no change like it in SACT. According to comparison of SACT with ELISA there was no significant difference in MB ($p < 0.1$) but in PB the difference was significant ($p < 0.05$) and in MB+PB, very significant ($p < 0.01$). So, with the point of screening the disease clinically with SACT and ELISA the sensitivities are 100% and 89%, the specificities 95% and 100%, and Youden indexes 0.98 and 0.9, respectively. The authors consider that both the methods are of high sensitivity and specificity, but SACT is more useful for detecting doubtful patients clinically and PGL-I-ELISA is more helpful to evaluating chemotherapy. If both are combined, it might be very useful for clinical serodiagnosis, finding subclinical infection and calculated relapse in leprosy.—Authors' English Abstract

Zhou, G., *et al.* [Use of S-100 protein staining for diagnosing tuberculoid leprosy.] China Lepr. J. **12** (1996) 112–113. (in Chinese)

Thirty-three cases of TT and BT leprosy were examined by the ABC method of S-100 protein. The nerves in epithelioid cell granulomas were markedly destroyed in 28 cases, being swollen, deformed and broken, making up 85% and no nerve was seen in five cases. In contrast, the sections of four cases of sarcoidosis and two cases of cutaneous tuberculosis showed that the S-100 protein positive nerves were located outside the granulomas and kept their normal structures. The authors believe that the S-100 protein stain technique is valuable in diagnosing paucibacillary leprosy when the diagnosis cannot be confirmed with H&E stain histopathologically.—Authors' English Abstract

Zwingenberger, K. and Wnendt, S. Immunomodulation by thalidomide: systematic review of the literature and of unpublished observations. J. Inflamm. **46** (1996) 177–211.

Three decades of immunological investigations using thalidomide are reviewed.

Both *in vitro* and *in vivo* investigations are in accordance with the clinical finding that thalidomide does not impede T-cell competence in the control of infection by mycobacteriae. The term immunosuppressant does not apply. The immunomodulatory effects of thalidomide are evident in a myriad of phenomenological changes, and a molecularly defined common denominator of these activities is not known at present.

Critical assessment with the objective to account for the clinical activity of thalidomide in specific human diseases leads to a focus on effects of thalidomide on phagocytic leukocytes and endothelia. The former are responsive to thalidomide by modulation of cytokine synthesis *in vitro* and *in vivo*; this activity can be shown using monocyte-specific stimuli in peripheral blood mononuclear cells but also in other phagocytic cells like microglia. For technical reasons, endothelial cells have until now been tested primarily *in vitro*. However, there is solid evidence now from intravital microscopy that the induction of adhesivity in postcapillary venules by LPS is modulated by thalidomide.

Altered surface antigen expression has been described on leukocytes obtained from humans and experimental animals treated with thalidomide, but convincing evidence is lacking for *in vitro* modulation of surface antigen expression on leukocytes (as opposed to the modulation of adhesion antigens on endothelial cells stimulated by LPS or exogenous TNF-alpha (TNF-α) in the presence of thalidomide). Therefore, *in vivo* redistribution is likely to account for some, if not all changes in circulating leukocyte phenotypes.

The immunopathological conditions most clearly responsive to thalidomide are vasculitic alterations of post-capillary venules either in the context of mycobacterial infection (in the case of erythema nodosum leprosum) or mucocutaneous apthae. In both instances (as in the majority of focal inflammatory lesions), leukocyte infiltration and cytokine responses, in particular TNF-α, are present. Thalidomide acts clinically not only by palliation of existing lesions but also by prevention of recurrence. The mechanism operates in skin, mucosa and parts of the nervous system and is most readily explained by synergism of TNF-α modulation and a separate point of action on leukocyte migration patterns.—Authors' Abstract

Microbiology

Aung, H., Toossii, Z., Wisnieski, J. J., Wallis, R. S., Culp, L. A., Phillips, N. B., Phillips, M., Averill, L. E., Daniel, T. M. and Ellner, J. J. Induction of monocyte expression of tumor necrosis factor alpha by the 30-kd alpha antigen of *Mycobacterium tuberculosis* and synergism with fibronectin. J. Clin. Invest. **98** (1996) 1261–1268.

Native 30-kD antigen, also known as alpha antigen, is a fibronectin-binding protein that is secreted by live *Mycobacterium tuberculosis*. This antigen may play an important biological role in the host-parasite interaction since it elicits delayed-type hypersensitivity response and protective immunity *in vivo* and T lymphocyte blastogenesis and IFN-gamma production *in vitro*. In the present study, we show that TNF-alpha protein is produced in monocyte culture supernatants in response to 30-kD antigen and the level is as high as that to purified protein derivative of *M. tuberculosis*. This stimulatory effect was not due to contamination with either bacterial lipopolysaccharide or mycobacterial lipoarabinomannan. The preincubation of monocytes with plasma fibronectin significantly enhanced the release of TNF-alpha into the culture supernatants in response to 30-kD antigen. This effect was blocked by polyclonal antibody to plasma fibronectin. In contrast, the monocytic cell line U937 failed to release TNF-alpha protein in the culture supernatants in response to 30-kD antigen with or without preincubation with plasma fibronectin. To determine whether this observation was due to differential binding of the 30-kD to fibronectin on these cells, a cell-based ELISA was used. Pretreatment of

monocytes with fibronectin enhanced their binding of the 30-kD antigen. U937 cells bound the 30-kD antigen weakly with or without fibronectin pretreatment. These results indicate that 30-kD antigen which is a known secretory antigen of *M. tuberculosis* is a stimulus for human monocytes to express TNF-alpha and that stimulatory effect may be mediated through plasma fibronectin.—Authors' Abstract

Banerjee, S. K., Bhatt, K., Rana, S., Misra, P. and Chakraborti, P. K. Involvement of an efflux system in mediating high level of fluoroquinolone resistance in *Mycobacterium smegmatis.* Biochem. Biophys. Res. Com. **226** (1996) 362–368.

A wild-type strain of *Mycobacterium smegmatis* mc (2) 155 was serially adapted to 64-fold of minimal inhibitory concentration of an antimycobacterial agent, ciprofloxacin. This clone (CIPr) exhibited crossresistance to ofloxacin and ethidium bromide. The rate of drug efflux was accelerated in CIPr compared to the wild-type strain. Verapamil, a calcium channel blocker, enhanced the drug accumulation in CIPr by diminishing the efflux and, thus, reversed the resistant phenotype. Additionally, a missense mutation was detected in the quinolone resistance determining region of the DNA-gyrase A subunit of CIPr. Taken together, these results suggest that drug efflux plays a major role in conferring such a high level of resistance in CIPr, in addition to the mutation in the DNA-gyrase locus.—Authors' Abstract

Bashyam, M. D., Kaushal, D., Dasgupta, S. K. and Tyagi, A. K. A study of the mycobacterial transcriptional apparatus: identification of novel features in promoter elements. J. Bacteriol. **178** (1996) 4847–4853.

Our earlier studies on transcriptional signals of mycobacteria had revealed that (i) strong promoters occur less frequently in the slowly growing pathogen *Mycobacterium tuberculosis* H37Rv than in the fast-growing saprophyte *M. smegmatis* and (ii) mycobacterial promoters function poorly in *Escherichia coli.* We now present evidence that RNA polymerases of *M. smegmatis, M. tuberculosis,* and *M. bovis* BCG recognize promoter elements with comparable efficiencies. Analysis of these randomly isolated mycobacterial promoters by DNA sequencing, primer extension, and deletion experiments revealed that their –10 regions are highly similar to those of *E. coli* promoters, in contrast to their –35 regions which can tolerate a greater variety of sequences owing presumably to the presence of multiple sigma factors with different or overlapping specificities for –35 regions, as reported earlier for the *Streptomyces* promoters. A comparison of the –10 and –35 binding domains of MysA, HrdB, and RpoD (the principal sigma factors of *M. smegmatis, Streptomyces aureofaciens,* and *E. coli,* respectively) showed that all three sigma factors have nearly identical –10 binding domains. However, the –35 binding domains of the principal mycobacterial and streptomycete sigma factors, although nearly identical to each other, are vastly different from the corresponding region of the sigma factor of *E. coli.* Thus, the transcriptional signals of mycobacteria have features in common with *Streptomyces* promoters but differ from those of *E. coli* because of major differences in the –35 regions of the promoters and the corresponding binding domain in the sigma factor.—Authors' Abstract

El Zaatari, F. A. K., Naser, S. A., Markesich, D. C., Kalter, D. C., Engstand, L. and Graham, D. Y. Identification of *Mycobacterium avium* complex in sarcoidosis. J. Clin. Microbiol. **34** (1996) 2240–2245.

Cell-wall-defective bacteria which later reverted to acid-fast bacilli have been isolated from sarcoid tissue. These have not been conclusively shown to be mycobacteria. Specific PCR assays were applied to identify mycobacterial nucleic acids in these cultured isolates and in fresh specimens obtained from patients with sarcoidosis. Positive amplification and hybridization were observed with *Mycobacterium avium* complex and/or *M. paratuberculosis*-specific probes in 5 of 6 cultured isolates and 2

fresh skin-biopsy samples and 1 cerebrospinal fluid specimen. There was no amplification or hybridization with *Mycobacterium tuberculosis* or *M. avium* subsp. *silvaticum* probes, respectively. Patients' sera were also tested for antibody reactivities by immunoblotting with *M. paratuberculosis* recombinant clones expressing the 36,000-molecular-weight antigen (36K antigen) (p36) and the 65K heat-shock protein (PTB65K). All 7 sarcoidosis, 4 of 6 tuberculosis, and all 6 leprosy patient serum specimens showed strong reactivity with p36 antigen. In contrast, 13 of 38 controls showed only weak reactivity with p36 ($p = 0.002$ for controls versus sarcoidosis samples). Similarly, PTB65K reacted with high intensity with sera from 5 of 5 sarcoidosis, 5 of 6 tuberculosis, and 5 of 6 leprosy patients, compared with its low-intensity reaction with 5 of 22 controls ($p = 0.001$ for controls versus sarcoidosis samples). This study demonstrates the isolation and/or identification of *M. paratuberculosis* or a closely related *M. avium* complex strain from sarcoid skin lesions and cerebrospinal fluid. Furthermore, the reactivity of antibodies in sarcoid patient sera against p36 and PTB65K antigens was comparable to the reactivity of sera obtained from patients with known mycobacterial diseases. Collectively, these data provide further support for the theory of the mycobacterial etiology of sarcoidosis.—Authors' Abstract

Kox, L. F. F., Noordhoek, G. T., Kunakorn, M., Mulder, S., Sterrenburg, M. and Kolk, A. H. J. Microwell hybridization assay for detection of PCR products from *Mycobacterium tuberculosis* complex and the recombinant *Mycobacterium smegmatis* strain 1008 used as an internal control. J. Clin. Microbiol. **34** (1996) 2117–2120.

A microwell hybridization assay was developed for the detection of the PCR products from both *Mycobacterium tuberculosis* complex bacteria and the recombinant *M. smegmatis* strain 1008 that is used as an internal control to monitor inhibition in the PCR based on the *M. tuberculosis* complex-specific insertion sequence IS6110. The test is based on specific detection with digoxigenin-labeled oligonucleotide probes of biotinylated PCR products which are captured in a microtiter plate coated with streptavidin. The captured PCR products are hybridized separately with two probes, one specific for the PCR product from IS6110 from *M. tuberculosis* complex and the other specific for the PCR fragment from the modified IS6110 fragment from the recombinant *M. smegmatis* 1008. The microwell hybridization assay discriminates perfectly between the two types of amplicon. The amount of PCR product that can be detected by this assay is 10 times less than that which can be detected by agarose gel electrophoresis. The test can be performed in 2 hr. It is much faster and less laborious than Southern blot hybridization. Furthermore, the interpretation of results is objective. The assay was used with 172 clinical samples in a routine microbiology laboratory, and the results were in complete agreement with those of agarose gel electrophoresis and Southern blot hybridization.—Authors' Abstract

Li, Z. M., Bai, G. H., von Reyn, C. F., Marino, P., Brennan, M. J., Gine, N. and Morris, S. L. Rapid detection of *Mycobacterium avium* in stool samples from AIDS patients by immunomagnetic PCR. J. Clin. Microbiol. **34** (1996) 1903–1907.

Direct PCR detection of bacteria in clinical samples is often hindered by the presence of compounds that inhibit the PCR. To improve and accelerate the diagnosis of *Mycobacterium avium-M. intracellulare* complex infections, an immunomagnetic PER (IM-PER) assay was developed. This IM-PCR procedure combines the separation of mycobacteria by antimycobacterial monoclonal antibody coupled to magnetic beads with an *M. avium-M. intracellulare* complex-specific PCR protocol based on 16S rRNA gene sequences. As few as 10 *M. avium* bacilli were detected in spiked human stool samples, a clinical specimen usually refractory to conventional PCR analysis, by the IM-PCR method. Moreover, *M. avium* organisms were detected in about 24 hr in 18 of 22 culture-confirmed fecal samples from AIDS patients. This IM-PCR pro-

tocol should allow for the rapid and sensitive detection of *M. avium* isolates in clinical specimens.—Authors' Abstract

McFadden, J. Recombination in mycobacteria. Mol. Microbiol. **21** (1996) 205–211.

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is thought to infect a quarter of the world's population and accounts for 3 million deaths each year. Leprosy, caused by *M. leprae,* continues to afflict millions. In many countries, the incidence of TB is increasing due to its association with the acquired immune deficiency syndrome (AIDS) and the emergence of multidrug resistance strains of tubercle bacilli. Genes that encode major antigens, enzymes, potential virulence determinants and drug resistance in mycobacteria have been isolated and characterized; however, further genetic analysis of pathogenic mycobacteria has been severely hampered by the difficulty in precisely defining the phenotype of both wild-type and mutant genes by utilizing homologous recombination to perform allele exchange. Recombination mechanisms have been intensely studied in *Escherichia coli* but it is unclear how far mechanistic pathways elucidated in this species are applicable to other organisms, such as mycobacteria. The aim of this review is to examine what is currently known about homologous recombination in mycobacteria. A model is proposed to account for both low levels of homologous recombination and high levels of illegitimate recombination found in the tubercle bacillus.—Author's Abstract

Meier, A., Heifets, L., Wallace, R. J., Zhang, Y. S., Brown, B. A., Sander, P. and Bottger, E. C. Molecular mechanisms of clarithromycin resistance in *Mycobacterium avium*: observation of multiple 23S rDNA mutations in a clonal population. J. Infect. Dis. **174** (1996) 354–360.

The peptidyltransferase region of the 23S rRNA gene (the probable target site for the macrolides) was investigated in blood isolates of *Mycobacterium avium* recovered from 38 patients before and after the development of clarithromycin resistance. Point mutations were identified in 100% of the 74 resistant relapse blood isolates but in none of 69 susceptible pretreatment isolates. Multiple mutations were identified in isolates from 23 (61%) of 38 patients. Of the 63 identified mutations, 95% involved adenine at bp 2058. Single-colony clones from cultures that were mixtures of more than one mutation revealed a single mutation within each clone. Pulsed field gel electrophoresis of genomic DNA restriction fragments revealed that 13 (81%) of 16 multiple mutations identified in the same patient were derived from a single infecting strain. *In vitro* investigation revealed the same point mutations observed *in vivo.* This study defines the probable mechanism of clarithromycin resistance in *M. avium* and provides *in vivo* evidence that mutational resistance is random and selection-directed.—Authors' Abstract

Menozzi, F. D., Rouse, J. H., Alavi, M., Laude Sharp, M., Muller, J., Bischoff, R., Brennan, M. J. and Locht, C. Identification of a heparin-binding hemagglutinin present in mycobacteria. J. Exp. Med. **184** (1996) 993–1001.

Adherence to mammalian host tissues is an important virulence trait in microbial pathogenesis, yet little is known about the adherence mechanisms of mycobacteria. Here we show that binding of mycobacteria to epithelial cells but not to macrophages can be specifically inhibited by sulfated carbohydrates. Using heparin-Sepharose chromatography, a 28-kD heparin-binding protein was purified from culture supernatants and cell extracts of *Mycobacterium bovis* and *M. tuberculosis.* This protein, designated heparin-binding hemagglutinin (HBHA), promotes the agglutination of rabbit erythrocytes, which is specifically inhibited by sulfated carbohydrates. HBHA also induces mycobacterial aggregation, suggesting that it can mediate bacteria-bacteria interactions as well. Hemagglutination, mycobacterial aggregation, as well as attachment to epithelial cells are specifically inhibited in the presence of anti-HBHA antibodies. Immunoelectron microscopy using anti-HBHA

monoclonal antibodies revealed that the protein is surface exposed, consistent with a role in adherence. Immunoblot analyses using antigen-specific antibodies indicated that HBHA is different from the fibronectin-binding proteins of the antigen 85 complex and p55, and comparison of the NH2-terminal amino acid sequence of purified HBHA with the protein sequence data bases did not reveal any significant similarity with other known proteins. Sera from tuberculosis patients but not from healthy individuals were found to recognize HBHA, indicating its immunogenicity in humans during mycobacterial infections. Identification of putative mycobacterial adhesins, such as the one described in this report, may provide the basis for the development of new therapeutic and prophylactic strategies against mycobacterial diseases.—Authors' Abstract

Misra, N., Habib, S., Ranjan, A., Hasnain, S. E. and Nath, I. Expression and functional characterisation of the clpC gene of *Mycobacterium leprae*: clpC protein elicits human antibody response. Gene **172** (1996) 99–104.

This paper reports the expression of a previously described gene [Nath and Laal, Nucleic Acids Res. 18 (1990) 4935], currently identified as the clpC gene of *Mycobacterium leprae*, using an *in vitro* rabbit reticulocyte lysate-coupled transcription/translation system. The produced protein moved as a 95-kDa band on SDS-PAGE. An additional band of 79 kDa was seen which may have resulted from a GTG codon downstream to the initiating ATG in the clpC sequence. A threefold increase in synthesis of the 95-kDa protein was achieved by altering the translation codon context sequence of the ATG start codon. The ClpC (caseinolytic protease C) amino acid sequence, which contained two nucleotide-binding sites, exhibited *in vitro* ATP binding. Of functional significance was its immunoreactivity in human subjects with mycobacterial infection. Leprosy and tuberculosis patients with active disease had antibodies which recognized ClpC in dot ELISA.—Authors' Abstract

Porichha, D., Brahmne, H. G., Mahapatra, D. C. and Reddy, B. N. Cellularity of macrophage granuloma and morphological index. Indian J. Lepr. **68** (1996) 217–222.

In the present study, morphological index (MI) and average macrophage count per microscopic field in skin sections of 94 lepromatous (LL) patients is correlated. The subjects included 14 cases with some histoid features. The MI in the lepromatous cases varied from < 1 to 40 and the corresponding macrophage counts ranged from 40 to 156. In cases with histoid changes the MI varied from 30 to 60 and the cell count ranged from 215 to 360. The histoid cases showed a higher MI and cell count compared to the other lepromatous cases. There was a positive correlation between MI and macrophage count and the hypercellular state appears to depend on living and multiplying bacteria.—Authors' Abstract

Ratledge, C. and Ewing, M. The occurrence of carboxymycobactin, the siderophore of pathogenic mycobacteria, as a second extracellular siderophore in *Mycobacterium smegmatis.* Microbiology **142** (1996) 2207–2212.

Carboxymycobactin, in which the usual intracellular mycobactin siderophore is modified by possession of a carboxylic acid group, has been isolated as a second extracellular siderophore from culture filtrates of *Mycobacterium smegmatis* grown under iron-deficient conditions. (The primary siderophore is an exochelin which is a trihydroxamate, pentapeptide derivative.) There may be up to 12 similar molecules produced with differing chain lengths that can be recognized by HPLC or HPTLC. The amount of carboxymycobactin is about 20 times higher when cultures are grown with glycerol instead of glucose. Formation is maximal with an initial pH of the medium of about 8.4. The proportion of carboxymycobactin to the total siderophores produced—mainly exochelins—is maximally 10% (usually 10–25 μg ml^{-1}). Formation of both extracellular siderophores (exochelin and carboxymycobactin) and of the intracellular mycobactin is max-

imal at the same initial concentration of iron added to the medium, 0.05–0.1 μg Fe ml^{-1}, though exochelin is synthesized 24 hr in advance of both carboxymycobactin and mycobactin.—Authors' Abstract

Schorey, J. S., Holsti, M. A., Ratliff, T. L., Allen, P. M. and Brown, E. J. Characterization of the fibronectin-attachment protein of *Mycobacterium avium* reveals a fibronectin-binding motif conserved among mycobacteria. Mol. Microbiol. **21** (1996) 321–329.

Mycobacterium avium is an intracellular pathogen and a major opportunistic infectious agent observed in patients with acquired immune deficiency syndrome (AIDS). Evidence suggests that the initial portal of infection by *M. avium* is often the gastrointestinal tract. However, the mechanism by which *M. avium* crosses the epithelial barrier is unclear. A possible mechanism is suggested by the ability of *M. avium* to bind fibronectin, an extracellular matrix protein that is a virulence factor for several extracellular pathogenic bacteria which bind to mucosal surfaces. To further characterize fibronectin binding by *M. avium*, we have cloned the *M. avium* fibronectin-attachment protein (FAP). The *M. avium* FAP (FAP-A) has an unusually large number of Pro and Ala residues (40% overall) and is 50% identical to FAP of both *M. leprae* and *M. tuberculosis*. Using recombinant FAP-A and FAP-A peptides, we show that two noncontinuous regions in FAP-A bind fibronectin. Peptides from these regions and homologous sequences from *M. leprae* FAP inhibit fibronectin binding by both *M. avium* and *M. bovis* bacillus Calmette-Guerin (BCG). These regions have no homology to eukaryotic fibronectin-binding proteins and are only distantly related to fibronectin-binding peptides of gram-positive bacteria. Nevertheless, these fibronectin-binding regions are highly conserved among the mycobacterial FAPs, suggesting an essential function for this interaction in mycobacteria infection of their metazoan hosts.—Authors' Abstract

Shivannavar, C. T., Katoch, V. M., Sharma, V. D., Patil, M. A., Katoch, K., Bharadwaj, V. P., Sharma, R. K., Bhatia, A. S. and Agrawal, B. M. Determination of mycobacterial phylogeny on the basis of immunological relatedness of superoxide dismutases. Int. J. System. Bacteriol. **46** (1996) 1164–1169.

Sixteen strains of cultivable mycobacteria were grown in Sauton's medium, and *Mycobacterium leprae* was purified from armadillo liver. Cell extracts were prepared from log-phase growths of each of the cultivable mycobacterial strains. Superoxide dismutase (SOD) enzyme was purified from all cultivable mycobacterial strains included in the study, and antibodies against purified SOD enzyme were raised in rabbits. Immunological distances (ImDs) between these anti-SOD antibodies and SOD antigens were determined by a previously described immunoprecipitation method and by a recently developed enzyme-linked immunosorbent assay (ELISA) technique. The reciprocal ImDs among mycobacterial strains were constant, reproducible and consistent by these two methods. An evolutionary tree was constructed on the basis of estimated ImDs. Except for *M. duvalii* and *M. terrae*, slowly and rapidly growing mycobacterial species appeared to be separately grouped by this analysis. Rapid growers clustered into a group which is near that of some slow-growing mycobacteria. *M. avium* falls almost in the middle of the evolutionary tree and the position of *M. leprae* was found to be between those of *M. avium* and *M. bovis* (BCG). Measurement of immunological relatedness of SODs provides an alternative system with which to study the taxonomical relatedness among mycobacteria.—Authors' Abstract

Sugita, Y., Miyamoto, M., Koseki, M., Ishii, N. and Nakajima, H. Diagnosis of leprosy by the practical application of the polymerase chain reaction. Eur. J. Dermatol. **6** (1996) 423–426.

We have developed a practical method of detecting *Mycobacterium leprae* DNA in leprosy patients using the polymerase chain reaction (PCR). We have designed a new set of primer oligonucleotides based on the DNA sequence encoding the *M. leprae* heat-shock protein 70 (hsp70). In order to

avoid nonspecific amplification of human or other bacterial DNA, regions of hsp70 with low amino-acid sequence homologies were selected for PCR primers, which also showed low homologies at the nucleic acid level. Instead of skin biopsy specimens, tissue fluid, which may contain *M. leprae,* was obtained from a needle inserted in the skin. The specificity of the amplified DNA fragment was confirmed by a simplified detection method, which produced the same results as Southern blot analysis. This study demonstrates useful, practical improvements of the PCR method that enable leprosy patients to be detected in the field.—Authors' Abstract

Wiese, M. and Seydel, U. Drug effects on intracellular mycobacteria determined by mass spectrometric analysis of the Na+-to-K+ ratios of individual bacterial organisms. Antimicrob. Agents Chemother. **40** (1996) 2047–2053.

The successful establishment of a drug screening system for intracellular cultivable and noncultivable mycobacteria based on the mass spectrometric determination of bacterial viability is described. To compare drug efficacies on intra- and extracellular mycobacteria, the mycobacteria were subjected to drug treatment either after phagocytosis by the mouse macrophage cell line RAW 264.7 or in cell-free medium. After reisolation, their viability was monitored by analyzing the intrabacterial sodium-to-potassium ratios for a limited number of individual organisms. This approach offers a reliable and quick tool for monitoring the influence of intracellular growth and of additional permeation barriers on intracellular drug efficacy and will, thus, provide useful information for the rational development and testing of optimized antimycobacterial drugs. In particular, the methodology is applicable to the noncultivable species *Mycobacterium leprae,* because the mass spectrometric analysis of the intrabacterial sodium-to-potassium ratio allows the determination of bacterial viability independent from their ability to multiply *in vitro.* Because of the improved metabolic activity of intracellularly growing *M. leprae* compared with that of extracellularly growing *M. leprae,* the spectrum of antileprosy drugs that can be tested *in vitro* could even be extended to those interfering with DNA replication and cell division.—Authors' Abstract

Ying, Y. A., Crane, D. D. and Barry, C. E. Stationary phase-associated protein expression in *Mycobacterium tuberculosis*: function of the mycobacterial alpha-crystallin homolog. J. Bacteriol. **178** (1996) 4484–4492.

The majority of active tuberculosis cases arise as a result of reactivation of latent organisms which are quiescent within the host. The ability of mycobacteria to survive extended periods without active replication is a complex process whose details await elucidation. We used two-dimensional gel electrophoresis to examine both steady-state protein composition and time-dependent protein synthetic profiles in aging cultures of virulent *Mycobacterium tuberculosis.* At least seven proteins were maximally synthesized 1 to 2 weeks following the end of log-phase growth. One of these proteins accumulated to become a predominant stationary-phase protein. N-terminal amino-acid sequencing and immunoreactivity identified this protein as the 16-kDa alpha-crystallin-like small heat shock protein. The gene for this protein was shown to be limited to the slowly growing *M. tuberculosis* complex of organisms as assessed by Southern blotting. Overexpression of this protein in wild-type *M. tuberculosis* resulted in a slower decline in viability following the end of log-phase growth. Accumulation of this protein was observed in log-phase cultures following a shift to oxygen-limiting conditions but not by other external stimuli. The protein was purified to homogeneity from overexpressing *M. smegmatis* in two steps and shown to have a significant ability to suppress the thermal denaturation of alcohol dehydrogenase. Collectively, these results suggest that the mycobacterial alpha-crystallin protein may play a role in enhancing long-term protein stability and, therefore, long-term survival of *M. tuberculosis.*—Authors' Abstract

Experimental Infections

Xu, D. L., Goto, Y., Amoako, K. K., Nagatomo, T., Fujita, T. and Shinjo, T. Establishment of Bcg^r congenic mice and their susceptibility resistance to mycobacterial infection. Vet. Microbiol. **50** (1996) 73–79.

Bcg congenic mice were developed by using C57BL/6 and DBA/2 strains of mice as progenitors. They were obtained by introgressively backcrossing the Bcg^r marker of DBA/2 onto C57BL/6. After 20 successive backcrossings, the heterozygous resistant mice were mated with each other to obtain homozygous mice as the Bcg^r congenic mice. The results of immunogenic and genetic markers coupled with those of a mixed lymphocyte reaction, all confirmed that the newly developed mice were highly congenic. These congenic mice were found to be resistant to *in vivo* infections by *Mycobacterium avium, M. intracellulare, M. bovis* BCG.—Authors' Abstract

Epidemiology and Prevention

Fine, P. E. M., Ponnighaus, J. M., Warndorff, D. K., Gruer, P. J. K., Oxborrow, S., Pharoah, P. D. P., Lucas, S. B., McDougall, A. C., Jenkins, P. A., Chauvula, D., *et al.* Randomised controlled trial of single BCG, repeated BCG, or combined BCG and *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. Lancet **348** (1996) 17–24.

Background: Repeat BCG vaccination is standard practice in many countries for prevention of tuberculosis and leprosy, but its effectiveness has not been evaluated. The addition of *Mycobacterium leprae* antigens to BCG might improve its effectiveness against leprosy. A double-blind, randomized, controlled trial to evaluate both these procedures was carried out in Karonga District, northern Malawi, where a single BCG vaccine administered by routine health services had previously been found to afford greater than 50% protection against leprosy, but no protection against tuberculosis.

Methods: Between 1986 and 1989, individuals lacking a BCG scar were randomly assigned BCG alone (27,904) or BCG plus killed *M. leprae* (38,251). Individuals with a BCG scar were randomly allocated placebo (23,307), a second BCG (23,456), or BCG plus killed *M. leprae* (8102). Incident cases of leprosy and tuberculosis were ascertained over the subsequent 5–9 years.

Findings: 139 cases of leprosy were identified by May 1995; 93 of these were diagnostically certain, definitely postvaccination cases. Among scar-positive individuals, a second BCG vaccination gave further protection against leprosy (about 50%) over a first BCG vaccination. The rate ratio for all diagnostically certain, definitely postvaccination cases, all ages, was 0.51 (95% CI 0.25–1.03, p = 0.05) for BCG versus placebo. This benefit was apparent in all subgroups, although the greatest effect was among individuals vaccinated below 15 years of age (RR = 0.40 [95% CI 0.15–1.01], p = 0.05). The addition of killed *M. leprae* did not improve the protection afforded by a primary BCG vaccination. The rate ratio for BCG plus killed *M. leprae* versus BCG alone among scar-negative individuals was 1.06 (0.62–1.82, p = 0.82) for all ages, though 0.37 (0.11–1.24, p = 0.09) for individuals vaccinated below 15 years of age.

Three hundred seventy-six cases of postvaccination pulmonary tuberculosis and 31 of glandular tuberculosis were ascertained by May 1995. The rate of diagnostically certain tuberculosis was higher among scar-positive individuals who had received a second BCG (1.43 [0.88–2.35], p = 0.15) than among those who had received placebo, and there was no evidence that any of the trial vaccines contributed to protection against pulmonary tuberculosis.

Interpretation: In a population in which a single BCG vaccination affords 50% or more protection against leprosy, but none against tuberculosis, a second vaccination can add appreciably to the protection against leprosy, without providing any protection against tuberculosis.—Authors' Abstract

Jesudasan, K., Vijayakumaran, P., Manimozhi, N., Raja, S., Bushanam, J. R., Kanagarajan, S. and Sundar Rao, P. S. S. Origin of new leprosy cases during general surveys in relation to previous survey findings. Lepr. Rev. **67** (1996) 183–189.

As part of the leprosy control activities in the area of Gudiyatham Thaluk, general surveys are done once every 3 to 5 years. The percentage of examination is about 90%. An analysis of all new cases registered for treatment between 1990–1994 was done to study whether these cases had been examined in the previous general survey. Of the new cases detected and registered, 566 cases (32.6%) were not examined during the previous survey. The significance of these findings in relationship to cost-effectiveness of general surveys, case-detection methodology and possible continuing of transmission of leprosy are discussed.—Authors' Summary

Mekhlafi, G. A. and Al-Qubati, Y. Retrospective analysis of 194 leprosy cases in the Republic of Yemen. Indian J. Lepr. **68** (1996) 227–234.

A review of the case files of 194 leprosy patients registered at a representative skin and venereal diseases outpatient clinic was done to assess the epidemiological and clinical patterns of the disease in Yemen. Almost all patients came from the poorer social groups and there was clustering of patients around some families. About 55% of the patients were aged 20 to 39 years and about 35% were aged over 40 years at the time of detection. Males were affected about three times as females; in males MB cases occurred about twice as often as PB cases and 12% of the cases presented as pure neuritic leprosy. Reactions were noticed in 39 cases (20%), 6 having type 1 and 27 having type 2 reaction. Bacterial index (BI) among 123 positive cases ranged from 0.1 to 6, about 70% of these cases showing a mean BI of more than 2.0. Patients' responses to treatment (MDT) were very good and BI decreased by about 1.55 (± 0.05) logs per year. About 50% of the patients had some disability (14% grade 1), and the disability rate among PB cases was about 70%. Our findings indicate the need for earlier diagnosis and better disability preventive measures.—Authors' Abstract

Nogueira, W., Marzliak, M. L. C., Goncalves, O. S. J. and Brasil, M. T. L. R. F. [Prospects for the elimination of leprosy.] Hansenol. Int. **20** (1995) 19–28. (in Portuguese)

This review of the prospects for the elimination of leprosy contains information specific for Brazil and for the state of São Paulo in particular. Noticeable changes have occurred in the country since 1991 as a result of investment in service reorganization with emphasis on personnel upgrading and constant supplies of medication. Prevalence figures for leprosy from 1924 to 1994 for the state of São Paulo have shown a decrease since the 1970s, with an even more marked decrease from 1991, after the introduction of multidrug therapy.—Trop. Dis. Bull. **93** (1996) 560

Ramasoota, P. and Intartitaya, T. Progress and impact of multidrug therapy (MDT) implementation to leprosy control in Thailand. Jpn. J. Lepr. **64** (1995) 314–319.

This paper reports the progress and impact of multidrug therapy (MDT) implementation for leprosy control in Thailand between 1984 and 1994. By 10 years of MDT implementation, the number of registered leprosy cases dropped from 44,406 in 1984 to only 4878 cases in 1994. The prevalence rate (per 10,000 population) declined 90% from 8.8 to 0.83; the detection rate of new cases (per 100,000 population) declined from 6.2 to 1.97. A total of 39,372 cases have been completely covered by MDT and 22,821 cases are under post-

MDT surveillance with the low relapse rate of only 1.46%. Other indicators showing a natural decline of leprosy were the increasing proportions of multibacillary leprosy cases and increasing mean age at onset of new cases of leprosy together with the decreasing proportion of children among new cases. Another impact of MDT was the increasing proportion of new patients who voluntarily attend treatment centers. However, there were still no satisfactory impacts on decreasing the proportion of cases with deformity and on decreasing the duration between onset and the first detection of a new case of leprosy.—Trop. Dis. Bull. **93** (1996) 562

Vijayakumaran, P., Manimozhi, N., Ravikumar, R. N., Jesudasan, K. and Sundar Rao, P. S. S. Leprosy among inmates of a prison. Indian J. Lepr. **68** (1996) 247–250.

A leprosy survey carried out in a district prison revealed a gross prevalence of 20 cases per 1000, and active prevalence of 10 cases per 1000, whereas prevalence of leprosy in the state was 1.12 per 1000. Such prisons thus form hyper-endemic pockets. The inmates are a closed community and there is a risk of cases among inmates spreading infection to others inside the prison during their sojourn there and to the community when they are released from the prison. Special efforts are required to identify and eliminate all identifiable sources of infection, especially at this point of time when we are aiming at elimination of leprosy as a public health problem.—Authors' Abstract

Rehabilitation

Bahrmand, A. R., Madani, H., Samar, G., Khalilzadeh, L., Bakayev, V. V., Yaghli, M. and Babaei, M. H. Detection and identification of non-tuberculous mycobacterial infections in 6,472 tuberculosis suspected patients. Scand. J. Infect. Dis. **28** (1996) 275–278.

Between March 1993 and March 1994, 82 patients with infection by nontuberculous mycobacteria (NTM) and 443 patients with tuberculosis (TB) were registered among 6472 patients with suspected tuberculosis in Iran. Skin-test reactivity to purified protein derivative (PPD) in patients demonstrated indurations of 10–14 mm or more for the majority of patients in both groups. Most patients with NTM infection had abnormal chest roentgenograms showing sporadic infiltrations, nodular abscesses, and cavities resembling TB radiologically. The similarity in age range, PPD skin reaction, and radiological evidence in patients infected with NTM or *Mycobacterium tuberculosis* (MTB) can mislead the physician. Some NTM species were recovered more often than others: *M. fortuitum* from 22 clinical specimens (26.8%) *M. gastri* 19 (23.1%); and *M. terrae* complex 15 (18.3%). The antimicrobial drug susceptibility tests of the isolated organisms showed that 42 (9.5%) isolates of MTB were resistant to isoniazid and 31 (7.0%) to streptomycin. A few strains (1.3%) were identified as being resistant to a combination of three primary drugs. These findings suggest that drug-resistant mycobacterial infections are becoming an important problem in the region.—Authors' Abstract

Bari, M. M., Islam, A. K. M. S. and Haque, A. K. M. A. Surgical reconstruction of leprotic foot drop. Lepr. Rev. **67** (1996) 200–202.

We have operated on 25 patients for correction of footdrop due to leprosy from March 1992 to July 1994. The method used was circumtibial transfer of the tibialis posterior to the tendons of extensor hellucis longus and the extensor digitorum longus in the foot together with lengthening of the Achilles tendon. The results were satisfactory in 20 of these cases as judged by adequate restoration of heel-toe gait and of active dorsiflexion. The follow-up period ranged from 6 months to 2 years. Inadequate postoperative physiotherapy was the reason for unsatisfactory results in five cases.—Authors' Summary

Diallo, A. M., Grauwin, M. Y., Hirzel, C., Ji, B., Lienhardt, C. and Tiendrebeogo, A. [Organization of a program for the prevention of disabilities and physical rehabilitation (PIRP) in the national leprosy control program (PNL); practical advice. Acta Leprol. **10** (1996) 29–35. (in French)

Patients treated and cured on the bacteriological level by multidrug therapy may nevertheless present handicaps, such as deformities resulting from the disease, which have personal and social consequences. It is actually the handicap and disability from which most patients suffer and which concern populations. The number of persons suffering from such handicaps worldwide has been estimated at 4 million. Therefore, the main goal is to gradually integrate the activities of the prevention of disabilities and physical rehabilitation program (PIRP) into the national leprosy control program (PNL). The persons involved in the implementation of the program outline the activities planned under the PIRP, detailed objectives, priorities, the means by which they will be implemented, the content of training programs, assessment criteria and documents available.—Authors' English Summary

Grauwin, M.-Y., Mane, I. and Cartel, J.-L. Pseudoepitheliomatous hyperplasia in trophic ulcers in leprosy patients; a 28-case study. Lepr. Rev. **67** (1996) 203–207.

Between 1984 and 1993, pseudoepitheliomatous hyperplasia developing in chronic ulcers were observed in 28 former Senegalese leprosy patients, which amounts to an annual frequency of 1.9 per 1000 ulcers. Correct diagnosis could only be made by histopathological examination of specimens taken from the depth of the lesion. Amputation was carried out on 17 patients and local excision on the other 10. Recurrence of growth was observed in 8 of the 10 patients treated by excision; in all of these 8 cases below-knee amputation had to be subsequently performed. From our experience, it may be assumed that local excision should be carried out only in the case of small tumors. Since the aim of surgical procedure is to allow the patient to have physical autonomy, below-knee amputation, followed by adaptation of prosthesis, should be the procedure chosen in the other cases.—Authors' Summary

Gupta, R., Dogra, N., Raje, M. and Majumder, S. Attempts to characterize the mechanisms involved in the growth inhibition of *Mycobacterium microti* in interferon-gamma or tumor necrosis factor-alpha activated J774A.1 cells. FEMS Microbiol. Lett. **140** (1996) 171–178.

The growth of *Mycobacterium microti* wa inhibited within J774A.1 macrophage cells activated with either interferon-gamma (IFN-γ) or tumor necrosis factor-alpha (TNF-α). Activation with INF-γ or TNF-α alone did not stimulated the production of nitrite in J774A.1 cells. IFN-γ but not TNF-α increased the production of hydrogen peroxide in a concentration-dependent manner but scavengers of reactive oxygen species did not influence the growth-inhibiting effect of IFN-γ within J774A.1 cells. Both IFN-γ and TNF-α enhanced the fusion of *M. microti* containing phagosomes with lysosomes and the ultimate degradation of bacteria. Our results showed that growth inhibition of *M. microti* within IFN-γ or TNF-α stimulated J774A.1 cells was independent of reactive oxygen intermediate and reactive nitrogen intermediate production.—Authors' Abstract

He, L., *et al.* [Amputation and artificial limbs of the legs in leprosy.] China Lepr. J. **12** (1996) 114–115. (in Chinese)

Ten amputated people cured of leprosy have been fitted up with artificial limbs 1 or 2 years ago. Follow up showed that 2 persons feel very satisfactory but to 5 of them it is only relative good. The authors consider that an artificial limb is very important for enhancing the ability to labor and live for amputated people, and it is absolutely necessary to follow them up for mending possible unfitness every half year.—Authors' English Abstract

Huang, S., *et al.* [Health status and economic burden of leprosy patients.] China Lepr. J. **12** (1996) 76–78. (in Chinese)

The survey of relation of different methods of payment for medical care to their rehabilitation for leprosy patients showed that effectiveness of rehabilitation in the patients with free medical care was better than those at their own expense. Although most of the patients were willing to accept surgical operation, only 28% of expenditure could be afforded because of their low earnings. To do rehabilitation management of leprosy patients, funds from government and society are necessary now.—Authors' English Abstract

Rao, S., Garole, V., Walawalkar, S., Khot, S. and Karandikar, N. Gender differentials in the social impact of leprosy. Lepr. Rev. **67** (1996) 190–199.

Prevalence rates of leprosy have reduced considerably in many states where multidrug therapy is in operation. However, reduction in prevalence alone is not sufficient as the social consequences of the diseases on the life of the patient are often severe and persist even after its cure. The present paper, therefore, investigates social impact with special reference to gender differentials. Data obtained from structured questionnaires (N = 606) are analyzed for this purpose. It was observed that the initial delay in identifying the skin changes as the symptoms of the disease were higher for females (29 months) than males (24 months). Even after identifying the symptoms, women were observed to depend exclusively on nonmedical treatment for a longer period (10 months) than males (6 months). Upon starting the medical treatment, females were observed to be more compliant than males, but the benefits of regularity appeared to be outweighed by the initial delay in starting medical treatment. The social impact on daily life was more severe for females than males as revealed by the isolation from daily activities, such as restrictions on participation in familial functions, restrictions on touching children. The paper highlights implications of gender bias on detection and treatment, and suggests modifications for control programs.—Authors' Summary

Seboka, G. and Saunderson, P. Cost-effective footwear for leprosy control programmes: a study in rural Ethiopia. Lepr. Rev. **67** (1996) 208–216.

A randomized, controlled trial of commercially available canvas shoes was carried out in a rural area of Ethiopia. Subjects with deformed and anesthetic feet, most with ulceration, were given either canvas shoes or plastazote/molded shoes and followed up for 1 year. Seventy-five percent of subjects with ulcers who used canvas shoes had no ulcer at the end of the study, while no significant change was noted in the plastazote group. The durability and acceptability of the shoes were also examined. Clients in remote areas who have no access to an orthopedic workshop, but who have anesthetic feet, with or without deformity, should have access to canvas shoes with an MCR insole. Two pairs are needed per year at a cost of US$ 6.70 per pair.—Authors' Summary

Yu, H. [Leprosy control in Xishan City, Jiangsu.] China Lepr. J. **12** (1996) 115–117. (in Chinese)

In Xishan City with a population of 1.09 million, Jiangsu, 428 patients with leprosy have been accumulatively registered since 1949 (294 males and 134 females with age of 6 to 69 years, MB 128 and PB 300) and 393 peasants. Five out of 349 cures have relapsed. The highest incidence was 3.27/100,000 in 1958 and the highest prevalence was 0.3‰ in 1973. Now, the prevalence is 0.0073‰ and no new patient was found in the last five years. A survey in 263 cases showed that 115 (43.7%) have some disability, of which 24 do not have the ability to labor and 8 have no ability to take care of themselves in daily life.—Author's English Abstract

Other Mycobacterial Diseases and Related Entities

Behr, C., Poupot, R., Peyrat, M. A., Poquet, Y., Constant, P., Dubois, P., Bonneville, M. and Fournie, J. J. *Plasmodium falciparum* stimuli for human gamma delta T cells are related to phosphorylated antigens of mycobacteria. Infect. Immun. **64** (1996) 2892–2896.

The presence in *Plasmodium falciparum* of a mitogenic factor for the major human blood gamma delta T-cell subset has been known for years. These gamma delta T cells bearing T-cell receptor V gamma 9 and V delta 2 variable regions also respond to *Mycobacterium tuberculosis,* through recognition of several phosphorylated nonpeptidic antigens. In this study, we undertook a better characterization of the malarial stimulus and show that the polyclonal activation of V gamma 9/V delta 2 gamma delta T cells by *P. falciparum* schizonts is also and exclusively attributable to two phosphorylated malarial compounds. The finding of such stimuli in eukaryotic cells evidences an antigenic link between intracellular parasites as different as *Plasmodium* and *Mycobacterium* species. Hence, phosphorylated antigens could be involved in a common pattern of transdisease T-cell responses against various human pathogens.—Authors' Abstract

Bras, A. and Aguas, A. P. Diabetes-prone NOD mice are resistant to *Mycobacterium avium* and the infection prevents autoimmune disease. Immunology **89** (1996) 20–25.

It was recently proposed that the diabetes genes of nonobese diabetic (NOD) mice are linked to the Bcg gene that is associated with resistance to infection by mycobacteria; however, it has not been established whether NOD mice are resistant or susceptible to the infection, although there are previous investigations on response of NOD mice to other intracellular parasites (e.g., Kaye, et al., Eur. J. Immunol. 22: 357–364). We have investigated here this question, as well as the consequences of mycobacterial infection on the natural history of murine diabetes. Female NOD mice were intraperitoneally infected with 10^8 viable bacilli of *Mycobacterium avium* at 2 months of age, i.e., before the mice show diabetes; they were studied up to the sixth month of age (when more than half of the untreated female NOD mice show glycosuria). To determine whether NOD mice were susceptible or resistant to *M. avium* infection, we have compared the kinetics of bacterial growths in liver and spleen of the mice with those determined in *M. avium*-susceptible (BALB/c) and resistant (C3H) strains of mice. NOD mice were able to control the *M. avium* infection, following a pattern similar to that observed in infected C3H mice. The mycobacterial infection prevented the expression of diabetes in all of the infected NOD mice, and it also decreased the incidence of proteinuria in the treated mice. The infected NOD mice showed a marked enhancement in antibodies against the 65,000 mycobacterial antigen [heat-shock protein (hsp) 65] up to the second month of infection and these elevated titers slowly decreased in the following months; anti-hsp65 antibodies were not detected in age-matched controls. This is the first demonstration that NOD mice are naturally resistant to mycobacterial infection, and we reinforce evidence on the role of the immune response triggered by mycobacteria and its hsp65 antigen in prevention of diabetes in NOD mice.—Authors' Abstract

Cangelosi, G. A., Brabant, W. H., Britschgi, T. B. and Wallis, C. K. Detection of rifampin- and ciprofloxacin-resistant *Mycobacterium tuberculosis* by using species-specific assays for precursor rRNA. Antimicrob. Agents Chemother. **40** (1996) 1790–1795.

rRNA precursor (pre-rRNA) molecules carry terminal stems which are removed during rRNA synthesis to form the mature rRNA subunits. Their abundance in bacterial cells can be markedly affected by antibiotics which directly or indirectly inhibit RNA synthesis. We evaluated the feasibility of rapidly detecting antibiotic-resistant *My-*

cobacterium tuberculosis strains by measuring the effects of brief *in vitro* antibiotic exposure on mycobacterial pre-rRNA. By hybridizing extracted *M. tuberculosis* nucleic acid with radiolabeled nucleic acid probes specific for pre-16S rRNA stem sequences, we detected clear responses to rifampin and ciprofloxacin within 24 and 48 hr, respectively, of exposure of cultured cells to these drugs. Detectable pre-rRNA was depleted in susceptible cells but remained abundant in resistant cells. In contrast, no measurable responses to isoniazid or ethambutol were observed. Probes for pre-rRNA were specific for the *M. tuberculosis* complex when tested against a panel of eight *Mycobacterium* species and 48 other bacteria. After 24 hr of incubation with rifampin, resistant *M. tuberculosis* strains were detectable in a reverse transcriptase PCR assay for pre-rRNA with a calculated lower limit of sensitivity of approximately 10^2 cells. Susceptible cells were negative in this assay at over 500 times the calculated lower limit of sensitivity. This general approach may prove useful for rapidly testing the susceptibility of slowly growing *Mycobacterium* species to the rifamycin and fluoroquinolone drugs and, with possible modifications, to other drugs as well.—Authors' Abstract

Charue, D., Chauvin, E., Duguet, C., Revuz, J. and Bagot, M. Thalidomide decreases the production of GM-CSF and TNF-alpha in the mixed epidermal cell-lymphocyte reaction. Eur. J. Dermatol. **6** (1996) 373–376.

Thalidomide is an effective treatment for several dermatological diseases. Recently, it has been used to treat Langerhans' cell histiocytosis. The mechanism of this effect is poorly understood. In order to try to define the mechanism of action of thalidomide, we studied its effects (26 to 2600 ng/ml) on lymphocyte proliferation in mixed allogeneic reactions, on the induction of allogeneic cytotoxic activity, and on the production of several cytokines in the mixed epidermal cell-lymphocyte reaction, using an ELISA or a RIA test. Thalidomide and its solvent had no effect on either lymphocyte proliferation or the cytotoxic activity induced in mixed allogeneic reactions. In mixed epidermal cell-lymphocyte reactions, the production of GM-CSF was decreased when either lymphoid cells or epidermal cells (EC) were preincubated with thalidomide. The production of TNF-alpha and IL-6 was decreased only when lymphoid cells were preincubated with thalidomide. The production of IL-1 beta was not decreased when either EC or lymphoid cells were preincubated with thalidomide. In conclusion, thalidomide decreases the production of several cytokines in MECLR, especially GM-CSF and TNF-alpha, which play a major role in the viability and function of Langerhans' cells. This effect of thalidomide on the lymphocyte-epidermal cell interactions may, at least partly, explain the effect of thalidomide on Langerhans' cell histiocytosis.—Authors' Abstract

Chum, H. J., O'Brien, R. J., Chonde, T. M., Graf, P. and Rieder, H. L. An epidemiological study of tuberculosis and HIV infection in Tanzania, 1991–1993. AIDS **10** (1996) 299–309.

In Tanzania during the past 6 years reported tuberculosis (TB) cases have nearly doubled, with proportionately much greater increases in smear-negative and extrapulmonary cases compared with smear-positive cases. This extensive study involved HIV testing of a representative country-wide sample of about one sixth of all new and relapsed TB cases registered between January 1991 and December 1993. A total of 6928 TB cases were tested. Overall HIV seroprevalence was 32%. Both crude and adjusted odds ratios for HIV infection were higher in women, those aged 25–44 years, urban residents, cases of smear-negative and extrapulmonary disease, and persons with a bacillus Calmette-Guérin vaccination scar. HIV seroprevalence among smear-positive relapse cases was no different from that among the newly diagnosed smear-positive pulmonary cases. Rates of initial drug resistance were low in both HIV-positive (4%) and HIV-negative (5.8%) patients. Rates of acquired resistance were higher (19% overall) and did not vary significantly by HIV serostatus. It is estimated that about two thirds of the increase in the

rate of smear-positive TB in Tanzania can be directly attributed to HIV infection.—Authors' Abstract

Corral, L. G., Muller, G. W., Moreira, A. L., Chen, Y. X., Wu, M. D., Stirling, D. and Kaplan, G. Selection of novel analogs of thalidomide with enhanced tumor necrosis factor alpha inhibitory activity. Mol. Med. **2** (1996) 506–515.

Background: Tumor necrosis factor alpha (TNF-α) is thought to mediate both protective and detrimental manifestations of the inflammatory response. Recently, thalidomide (α-N-phthalimidoglutarimide) was shown to partially inhibit monocyte TNF-α production (by 50%–70%) both *in vivo* and *in vitro*. More efficient inhibition of TNF-α may, however, be necessary to rescue the host from more acute and extensive toxicities of TNF-α-mediated inflammation.

Materials and Methods: Three structural analogs of thalidomide were selected for study based on increased activity against TNF-α production. The parent drug and the analogs were tested *in vitro* in human peripheral blood mononuclear cell cultures for their effects on lipopolysaccharide (LPS)-induced cytokine protein and mRNA production using ELISAs and Northern blot hybridization. The *in vitro* effects of the drugs were then confirmed *in vivo* in a mouse model of LPS induced lethality.

Results: The new compounds (two esters and one amide) showed increased inhibition of TNF-α production by LPS-stimulated human monocytes, relative to the parent drug thalidomide. The analogs and the parent drug enhanced the production of interleukin-10 (IL-10), but had little effect on IL-6 and IL-1-beta protein and mRNA production. When tested *in vivo,* the amide analog protected 80% of LPS-treated mice against death from endotoxin induced shock.

Conclusions: Analogs of thalidomide designed to better inhibit TNF-α production *in vitro* have correspondingly greater efficacy *in vivo.* These findings may have therapeutic implication for the treatment of human diseases characterized by acute and extensive TNF-α production, such as tuberculosis meningitis or toxic shock.—Authors' Abstract

Curco, N., Pagerols, X., Gomez, L. and Vives, P. *Mycobacterium kansasii* infection limited to the skin in a patient with AIDS. Br. J. Dermatol. **135** (1996) 324–326.

We describe a case of cutaneous *Mycobacterium kansasii* infection in a 56-year-old man with acquired immunodeficiency syndrome, who received treatment with trimethoprim-sulphamethoxazole for *Pneumocystis carinii* pneumonia. Resolution of the cutaneous lesion was observed without specific treatment.—Authors' Abstract

Dover, L. G. and Ratledge, C. Identification of a 29 kDa protein in the envelope of *Mycobacterium smegmatis* as a putative ferri-exochelin receptor. Microbiology **142** (1996) 1521–1530.

Evidence of a direct association between ferri-exochelin, the major extracellular siderophore of *Mycobacterium smegmatis,* and a 29-kDa protein has been obtained by three separate methods. (1) Direct binding of ^{55}Fe (III)-exochelin by the 29-kDa protein in an envelope preparation from iron-deficient cells was demonstrated by the extraction of a complex with the nondenaturing detergent CHAPS, and subsequent CHAPS-PAGE and autoradiography. (2) Affinity chromatography on a chemically synthesized ferri-exochelin-Sepharose 4B matrix led to the retention of the 29-kDa protein and a 25-kDa protein. The smaller protein was partially eluted with 1 mM ferri-exochelin although it did not form a stable complex with ferri-exochelin. The 29-kDa protein could not be eluted from the affinity matrix with 1 mM ferri-exochelin either alone or with 1 M NaCl. Only 2% (w/v) SDS could do this, but resulted in protein denaturation. (3) Incubation of ^{55}Fe-exochelin with CHAPS-solubilized envelope proteins in free solution followed by ion-exchange chromatography resolved three radioactive peaks; subsequent analysis by SDS-PAGE showed that the peak with the highest ^{55}Fe-binding activity per unit protein contained both the 29- and 25-kDa proteins. A direct association was demonstrated between the 29-kDa protein and ^{55}Fe-exochelin by gel filtration. The ev-

idence suggests that the 29-kDa iron-regulated envelope protein of *M. smegmatis* is a ferri-exochelin-binding protein and that the 25-kDa protein, which corresponds in size to a previously reported iron-regulated envelope protein in this bacterium, may have a role in the formation or maintenance of this complex. Proteins extracted from the cell envelope of iron-deficient *M. smegmatis* with CHAPS were dialyzed to remove the detergent, incorporated into liposome suspensions and then incubated with ^{55}Fe (III)-exochelin. This increased the retention of ^{55}Fe by 133-fold compared to proteins not placed in liposomes. Retention of ^{55}Fe was dependent upon the protein loading of the liposomes. Gel filtration confirmed that the iron was retained by these vesicles and even after dialysis the majority of ^{55}Fe was still retained by the vesicles. Re-solubilization of the labeled proteo-liposomes in various detergents gave limited recovery of a ferri-exochelin-protein complex. Attempts to resolve this complex by Triton X-100 PAGE led to separation of the two entities. The complex was stable, however, in a CHAPS-PAGE system.—Authors' Abstract

Hachem, R., Raad, I., Rolston, K. V. I., Whimbey, E., Katz, R., Tarrand, J. and Libshitz, H. Cutaneous and pulmonary infections caused by *Mycobacterium vaccae*. Clin. Infect. Dis. **23** (1996) 173–175.

Mycobacterium vaccae is a rapidly growing mycobacterial species that was previously not considered a human pathogen. We report 4 cases of *M. vaccae* infection that occurred in the southern United States; 1 patient had cutaneous disease, and 3 patients had cavitary lung disease. Two of the three patients with pulmonary disease had a history of exposure to cattle. The conditions of all patients improved with therapy: the cutaneous infection responded to therapy with minocycline and trimethoprim-sulfamethoxazole, and the pulmonary infections responded to therapy with ciprofloxacin.—Authors' Abstract

Hardman, W. J., Benian, G. M., Howard, T., McGowan, T. E., Metchock, B. and Murtagh, J. J. Rapid detection of mycobacteria in inflammatory necrotizing granulomas from formalin-fixed, paraffin-embedded tissue by PCR in clinically high-risk patients with acid-fast stain and culture-negative tissue biopsies. Am. J. Clin. Pathol. **106** (1996) 384–389.

A collection of inflammatory necrotizing granulomas (INGs) negative by acid-fast stain and culture (AFSC) were analyzed by polymerase chain reaction (PCR) for the presence of mycobacteria. Forty-two paraffin-embedded specimens with INGs were collected from patients at high risk for contracting tuberculosis. Twenty biopsies were positive and 22 were negative for mycobacteria by AFSC. Two universal primers specific for all mycobacteria were used to detect a 414 base pair (bp) fragment of 16S rRNA gene. Twenty of 20 biopsies were positive for mycobacteria by both AFSC and PCR (100%); whereas 19 of 22 biopsies negative by AFSC were positive by PCR (86%). Follow up of patients who were PCR positive but AFSC negative identified nine patients who had subsequent biopsies. Specimens from 8 of these 9 patients eventually grew *Mycobacterium tuberculosis*. Our results demonstrate that the detection of mycobacterial DNA by this method should be used in conjunction with AFSC for the initial diagnosis of mycobacterial infection.—Authors' Abstract

Hayman, J. A., Smith, I. M. and Flood, P. Pseudoepitheliomatous hyperplasia in *Mycobacterium ulcerans* infection. Pathology **28** (1996) 131–134.

Pseudoepitheliomatous hyperplasia of the epidermis occurring with *Mycobacterium ulcerans* skin infection may result in localization of the infected area with discharge of necrotic material, followed by healing leaving a depressed scar. The process represents more than simple re-epithelization of an ulcerated skin surface; it is a mechanism which produces active extrusion of necrotic material containing viable mycobacteria and should be seen as part of a protective physiological response to the infection.—Authors' Abstract

Hernandez Pando, R., Orozcoe, H., Sampieri, A., Pavon, L., Velasquillo, C., Larriva Sahd, J., Alcocer, J. M. and

Madrid, M. V. Correlation between the kinetics of Th1/Th2 cells and pathology in a murine model of experimental pulmonary tuberculosis. Immunology **89** (1996) 26–33.

T-helper 1 (Th1)/Th2 kinetics were studied by immunohistochemistry and molecular biology techniques (reverse transcriptase-polymerase chain reaction: RT-PCR, Southern-blot) during the course of pulmonary tuberculosis induced in BALB/c mice by the intratracheal instillation of the live and virulent *Mycobacterium tuberculosis* strain H37Rv. The histopathological study clearly showed two phases of the disease. The first one was an acute phase which was characterized by inflammatory infiltrate in the alveolar-capillary interstitium, blood vessel and bronchial wall with formation of granulomas. In this acute phase, which lasted from 1 to 28 days, a clear predominance of Th1 cells was observed, manifested by a high percentage of interleukin-2 (IL-2) positive cells in the inflammatory infiltrate granulomas demonstrated by immunohistology, as well as a gradual increment of interferon-gamma (INF-γ) m-RNA. This was followed by a chronic or advanced phase characterized by pneumonia, focal necrosis and fibrosis, with a Th0 balance due to an equivalent proportion of IL-2, and IL-4 positive cells in the lung lesions, that coincided with the highest level of INF-gamma and IL-4 mRNA. The cytofluorometric analysis of bronchial lavage cells, showed a predominance of CD4 T cells during the acute phase and CD8 T lymphocytes in the chronic phase. Gamma-delta T lymphocytes showed two peaks, at the beginning (3 days) and at the end (4 months) of the infection. These results suggest that T-lymphocyte subset kinetics and the pattern of cytokines produced in the lung during tuberculosis infection changed over time and correlate with the type and magnitude of tissue injury.—Authors' Abstract

Jackson, M., Berthet, F.-X., Otal, I., Rauzier, J., Martin, C., Gicquel, B. and Guilhot, C. The *Mycobacterium tuberculosis* purine biosynthetic pathway: isolation and characterization of the *purC* and *purL* genes. Microbiology **142** (1996) 2439–2447.

Genes from the *Mycobacterium tuberculosis* purine biosynthetic pathway were identified using purine auxotrophic mutants of *M. smegmatis* obtained by Tn*611* transposon mutagenesis. Two approaches were followed in parallel. The first consisted of the complementation of the *M. smegmatis* purine auxotrophs using a *M. tuberculosis* H37Rv shuttle cosmid library. In the second approach, specific probes corresponding to the regions adjacent to the insertion sites of Tn*611* in the *M. smegmatis* genome were used to screen a *M. tuberculosis* plasmid library by colony hybridization for inserts carrying homologous DNA fragments. Nucleotide sequence analysis of two *M. tuberculosis* genes isolated by these methods revealed high similarities with *purC* and *purL* genes from other bacterial and fungal sources. Transcriptional start sites were mapped for both genes, which revealed similar –10 boxes but with a higher GC content than the *Escherichia coli* σ^{70} consensus.—Authors' Abstract

Josse, R., Guedenon, A., Darie, H., Anagonou, S., Portaels, F. and Meyers, W. M. [*Mycobacterium ulcerans* skin infection: Buruli ulcers.] Med. Trop. **55** (1995) 363–373. (in French)

This well-illustrated review covers the epidemiology of Buruli ulcer (caused by *Mycobacterium ulcerans*), clinical aspects of the disease and its diagnosis, treatment, and pathogenesis and physiopathology.—Trop. Dis. Bull. **93** (1996) 662

Kim, S. J., Hong, Y. P., Lew, W. J., Yang, S. C. and Lee, E. G. Incidence of pulmonary tuberculosis among diabetics. Tuber. Lung Dis. **76** (1995) 529–533.

The authors performed a longitudinal epidemiological study based on civil servants in the Korean Republic to determine a comparative incidence of pulmonary tuberculosis (FIB) between diabetic and nondiabetic subjects. Their investigation was of newly developed PIE among diabetics and nondiabetics between 1988 and 1990, on

the basis of biennial medical examination and the medical records of those who claimed health insurance for tuberculosis. The 1990 medical examination and investigation of medical records of the health insurance claimants revealed that PTB had developed in 170 patients (including 37 smear and 8 culture positives) among 8015 diabetics, and in 4935 patients (including 538 smear and 342 culture positives) among 806,698 control subjects. Estimated annual incidence rates of PTB of (1) all types, (2) smear- and/or culture-positive versus (3) smear-positive cases were 1061, 281 and 231 per 10^5, respectively, among diabetics and 306,055 and 33 per 10^5 among nondiabetic controls. PTB developed in 167 of 7695 male diabetics and in 3 of 320 female diabetics; The greater the age, the more diabetics were found. The authors conclude that the relative risks (RR) of developing PTB of all types and bacteriologically confirmed cases were 3.47 times and 5.15 times higher in the diabetics than in the matched controls. A greater RR was observed in those at the age of 30–49 than in those of 50 years or more.—Trop Dis. Bull. **93** (1996) 557

Kobayashi, K., Yamazaki, J., Kasama, T., Katsura, T., Kasahara, K., Wolf, S. F. and Shimamura, T. Interleukin (IL)-12 deficiency in susceptible mice infected with *Mycobacterium avium* and amelioration of established infection by IL-12 replacement therapy. J. Infect. Dis. **174** (1996) 564–573.

Mycobacterium avium is an intracellular microorganism that infects and multiplies within macrophages. Cell-mediated immunity plays an important role in host defense, and interleukin (IL)-12, which is produced mainly by macrophages, is critical for its development. In a mouse model of disseminated *M. avium* infection, genetically susceptible BALB/c mice had increased mycobacterial growth and decreased IL-12 expression and developed large and numerous granulomas. In contrast, resistant DBA/2 mice exhibited reduced mycobacterial burden with increased IL-12 expression and developed fewer and smaller granulomas. In susceptible mice with established *M. avium* infection, IL-12 replacement therapy resulted in persistent reduction of mycobacterial burdens IL-12 itself, however, could not inhibit mycobacterial growth *in vitro.* By enhancing host defenses, IL-12 exerts a potent mycobactericidal activity *in vivo* with low toxicity. This suggests that IL-12 replacement therapy is rational for *M. avium* infection in susceptible hosts.—Authors' Abstract

Kocagoz, T., Hackbarth, C. J., Unsal, I., Rosenberg, E. Y., Nikaido, H. and Chambers, H. F. Gyrase mutations in laboratory-selected, fluoroquinolone-resistant mutants of *Mycobacterium tuberculosis* H37Ra. Antimicrob. Agents Chemother. **40** (1996) 1768–1774.

To characterize mechanisms of resistance to fluoroquinolones by *Mycobacterium tuberculosis,* mutants of strain H37Ra were selected *in vitro* with ofloxacin. Their quinolone resistance-determining regions of *gyrA* and *gyrB* were amplified and sequenced to identify mutations in gyrase A or B. Three types of mutants were obtained: (i) one mutant (TKp1) had no mutations in *gyrA* or *gyrB*; (ii) mutants that had single missense mutations in gyrA, and (iii) mutants that had two missense mutations resulting in either two altered gyrase A residues or an altered residue in both gyrases A and B. The TKp1 mutant had slightly reduced levels of uptake of [^{14}C]norfloxacin, which was associated with two- to fourfold increases in the MICs of ofloxacin, ciprofloxacin, and sparfloxacin. Gyrase mutations caused a much greater increase in the MICs of fluoroquinolones. For mutants with single *gyrA* mutations, the increases in the MICs were 4- to 16-fold, and for mutants with double gyrase mutations, the MICs were increased 32-fold or more compared with those for the parent. A *gyrA* mutation in TKp1 secondary mutants was associated with 32- to 128-fold increases in the MICs of ofloxacin and ciprofloxacin compared with the MICs for H37Ra and an eightfold increase in the MIC of sparfloxacin. Sparfloxacin was the most active fluoroquinolone tested. No sparfloxacin-resistant single-step mutants were selected at concentrations of > 2.5 μg/ml, and high-

level resistance (i.e., MIC ≥ 5 μg/ml) was associated with two gyrase mutations. Mutations in *gyrB* and possibly altered levels of intracellular accumulation of drug are two additional mechanisms that may be used by *M. tuberculosis* in the development of fluoroquinolone resistance. Because sparfloxacin is more active *in vitro* and selection of resistance appears to be less likely to occur, it may have important advantages over ofloxacin or ciprofloxacin for the treatment of tuberculosis.—Authors' Abstract

Maeda, S. and Kashiwabara, Y. Purification and properties of a membrane-bound phospholipase B from *Mycobacterium lepraemurium.* Biochim. Biophys. Acta **1303** (1996) 31–38.

The phospholipid deacylating enzyme was solubilized from the particulate (membrane) fraction of *Mycobacterium lepraemurium* with Triton X-100 and sodium cholate, and purified 1100-fold to homogeneous state by five steps of column chromatography: DE-52, PL-Sepharose (phosphatidylserine-attached sepharose), Mono P, heparin-Agarose and Mono Q column chromatography. The purified enzyme was composed of a single polypeptide chain and a molecular mass of 37 kDa was estimated for the protein by SDS-PAGE. The isoelectric point was determined about pH 4.6, and the protein was highly resistant to various kinds of proteolytic enzymes. The purified enzyme hydrolyzed both diacyl and monoacyl phospholipids, showing that this enzyme was classified to phospholipase B (phospholipase A^1/lysophospholipase). This phospholipase B had acidic pH optima and hydrolyzed both neutral phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and acidic phospholipids, such as phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglycerol (PG). Various fatty acids, such as 12:0, 14:0, 16:0, 18:0 and 18:1 at sn-1 position, and 18:1, 18:2, 18:3 and 16:0 at sn-2 position, were liberated from PC, suggesting no strict specificity toward the fatty acyl groups of phospholipids. From the comparison of degradation patterns of phosphatidylcholine with sn-1-[1-C-14]- and sn-2-[1-C-14]fatty acids, this enzyme was suggested to hydrolyze sn-1 position of phospholipid first and then sn-2 position, as the phospholipase B of *M. phlei.* This enzyme also attacked 1-acyl- and 2-acyl-lyso-PC at about same rates. The Km values for 1-acyl-2-oleoyl-PC and 2-oleoyl-lyso-PC were estimated as 1.6 and 0.75 mM, respectively.—Authors' Abstract

Mehra, V., Gong, J. H., Iyer, D., Lin, Y. G., Boylen, C. T., Bloom, B. R. and Barnes, P. F. Immune response to recombinant mycobacterial proteins in patients with tuberculosis infection and disease. J. Infect. Dis. **174** (1996) 431–434.

The capacity of four *Mycobacterium tuberculosis* recombinant antigens to elicit proliferation and cytokine production by human T cells was evaluated. Proliferative responses of peripheral blood mononuclear cells (PBMC) to all antigens were greater in healthy tuberculin reactors than in pulmonary tuberculosis patients, and proliferative responses of pleural fluid cells were greater than those of PBMC from patients with tuberculous pleuritis. The proliferative responses to the four recombinant antigens were similar in all patient groups, and there was no selective unresponsiveness to any antigen in pulmonary tuberculosis patients. The 38-kDa antigen induced less interferon-gamma than did the 10-, 30-, and 65-kDa antigens, and all four antigens induced similar amounts of interleukin-10. These results suggest that none of the four recombinant antigens are immunodominant, and that the 10-, 30-, and 65-kDa antigens are similar in their capacity to induce a potentially protective Th1-like response.—Authors' Abstract

Mehta, R. T. Liposome encapsulation of clofazimine reduces toxicity *in vitro* and *in vivo* and improves therapeutic efficacy in the beige mouse model of disseminated *Mycobacterium avium-M. intracellulare* complex infection. Antimicrob. Agents Chemother. **40** (1996) 1893–1902.

Disseminated infections caused by the *Mycobacterium avium-M. intracellulare* complex (MAC) are the most frequent op-

portunistic bacterial infections in patients with AIDS. MAC isolates are resistant to many of the standard antituberculous drugs. Failure to obtain significant activities of certain drugs is due to difficulty in achieving high concentrations at the sites where the infections reside. New and improved agents for the treatment of mycobacterial infections are therefore required. Earlier, the anti-MAC activities of various agents in free or liposomal form were studied; liposomes were used as drug carriers to ultimately target the drugs to macrophages where mycobacterial infections reside. Clofazimine was chosen for further studies because it could be effectively encapsulated and its activity was well maintained in liposomal form. The present studies with both erythrocytes and macrophages as the model systems show that liposomal drug is far less toxic *in vitro* than the free drug. The *in vivo* toxicity of clofazimine was also significantly reduced after liposome encapsulation. The therapeutic efficacies of free and liposomal drugs were compared in a beige mouse model of disseminated MAC infection. An equivalent dose of liposomal drug (10 mg/kg of body weight) was more effective in eliminating the bacteria from the various organs studied, particularly from the liver. Moreover, because of the reduced toxicity of liposomal drug, higher doses could be administered, resulting in a significant reduction in the numbers of CFU in the liver, spleen, and kidneys. The data demonstrate that liposomal clofazimine is highly effective in the treatment of MAC infections, even if the treatment is initiated after a disseminated infection has been established. The present studies thus suggest the potential usefulness of liposomal clofazimine for the treatment of disseminated MAC infections.—Author's Abstract

Menevse, A., Ulkuer, M. and Sultan, N. Rapid detection of *Mycobacterium tuberculosis* in clinical samples by polymerase chain reaction. Tohoku J. Exp. Med. **179** (1996) 1–9.

A polymerase chain reaction (PCR) for the rapid and specific detection of *Mycobacterium tuberculosis* has been used and evaluated for clinical applicability. Two oligonucleotide primers derived from the nucleotide sequence of an immunogenic protein MPB 64 amplified DNA from *M. tuberculosis* and *M. bovis*. No amplification was observed from any of 10 different mycobacterial strains. A total of 126 clinical samples were amplified and tested by both dot blot hybridization and restriction enzyme analysis. *M. tuberculosis* was detected by PCR in 38 smear and 42 culture-positive cases. An additional 16 culture-negative specimens were PCR positive, yielding an overall M. *tuberculosis* positivity rate of 46.0% (58/126) compared to 33.3% (42/126) by culture. The superior sensitivity of PCR to culture was more evident in nonpulmonary cases where PCR picked up 10 cases in addition to 6 culture positives out of 46 specimens. On the other hand, out of 80 pulmonary specimens only 6 cases in addition to 36 culture positives were picked up by PCR. The specificity of PCR was confirmed with dot blot hybridization and restriction enzyme analysis. This study corroborates that PCR offers a more sensitive and rapid alternative for the detection of *M. tuberculosis* to culture, and that it can be used in noncultured clinical specimens.—Authors' Abstract

Miyachi, H., Azuma, A., Hioki, E., Iwasaki, S., Kobayashi, Y. and Hashimoto, Y. Inducer-specific bidirectional regulation of tumor necrosis factor-alpha production. Biochem. Biophys. Res. Com. **226** (1996) 426–430.

Regulation by thalidomide [N (α)-phthalimidoglutarimide] of tumor necrosis factor alpha (TNF-α) production was found to be inducer-specific. Thalidomide enhances TNF-α production by human leukemia HL-60 cells induced with production by human leukemia HL-60 cells induced with 12-O-tetradecanolyphorbol 13-acetate (TPA), while it inhibits TNF-α production induced with okadaic acid (OA) in the same cell line. Some phthalimide analogs, including PP-33 [2-(2,6-diisopropylphenyl)-1H-isoindole-1,3-dione] and its 4,5.6,7-tetrafluoro derivative (FPP-33), also showed such an inducer-specific bidirectional TNF-α production-regulating activity. The structure-activity relationships of the compounds

tested are similar, but not identical, in the TPA-stimulated HL-60 and OA-stimulated HL-60 assay systems.—Authors' Abstract

Picardeau, M., Varnerot, A., Rauzier, J., Gicquel, B. and Vincent, V. *Mycobacterium xenopi* IS*1395,* a novel insertion sequence expanding the IS*256* family. Microbiology **142** (1996) 2453–2461.

An insertion sequence (IS) of *Mycobacterium xenopi* has been isolated and sequenced. This 1323-bp element, designated IS*1395,* is present in up to 18 copies in the *M. xenopi* genome and may be harbored in an *M. xenopi* extra-chromosomal element. It encodes a putative transposase of 415 amino acids which displays sequence homology to the *Staphylococcus aureus* IS*256* family. Members of this class of elements have been described in the genus *Mycobacterium*—for example, IS*1081* is present in the *M. tuberculosis* complex, IS*1245*-IS*1311* in *M. avium,* and IS*6120* in *M. smegmatis*; these elements exhibit an 89%, 45% and 16% amino acid identity with IS*1395,* respectively. Investigation of the host range of IS*1395* by Southern blot analysis revealed additional IS*1395*-related repeated sequences in *M. gordonae* and *M. celatum.* Moreover, IS*1395* represents a useful epidemiological tool for *M. xenopi* strain typing as it provides a diversity of restriction fragment length polymorphism patterns.—Authors' Abstract

Ravn, P. and Pedersen, B. K. *Mycobacterium avium* and purified protein derivative-specific cytotoxicity mediated by $CD4^+$ lymphocytes from healthy HIV-seropositive and -seronegative individuals. J. AIDS Hum. Retrovirol. **12** (1996) 433–441.

HIV is the greatest single risk factor for the development of tuberculosis. Diseases caused by *Mycobacterium tuberculosis* and mycobacteria are the most common opportunistic infections in HIV-infected persons, which may stem from a functional defect of the $CD4^+$ T-cell-mediated killing of macrophages harboring mycobacteria. Our objective was to investigate the *M. tuberculosis* and *M. avium*-specific cytotoxic capacity of T cells from healthy, bacille Calmette-Guerin-vaccinated, HIV-seropositive individuals. Blood mononuclear cells were obtained from 10 healthy HIV-seropositive and 10 healthy seronegative persons with no history of previous or active mycobacterial infection. Antigen-specific killing of macrophages presenting mycobacterial antigens (purified protein derivative or *M. avium* culture filtrate) was conducted. The phenotype of the killer cells was determined by a fluorescence-activated cell sorter after antigen stimulation and by using purified $CD4^+$ and $CD8^+$ cell subsets. Substantial, but reduced t antigen-specific cytotoxicity was observed in patients with asymptomatic HIV infection. The immunological dysfunction leading to reduced cytotoxic activity in healthy HIV-seropositive subjects could not be explained by a defect in the cytotoxic capacity of the individual $CD4^+$ lymphocyte after antigen stimulation, and it could not be explained by a reduction in the total number of $CD4^+$ cells before antigen stimulation. The antigen-specific cytotoxic activity was, however, closely related to the ability of the $CD4^+$ T cells to respond to mycobacterial antigens. The immunological dysfunction leading to reduced mycobacterial-specific cytotoxic activity in healthy HIV-seropositive subjects is caused either by a reduction in the number of antigen-responsive $CD4^+$ T cells (memory) or by an impairment of their ability to respond to antigenic stimuli.—Authors' Abstract

Salvetti, M., Ristori, G., Buttinelli, C., Fiori, P., Falcone, M., Britton, W., Adams, E., Paone, G., Grasso, M. G. and Pozzilli, C. The immune response to mycobacterial 70-kDa heat shock proteins frequently involves autoreactive T cells and is quantitatively disregulated in multiple sclerosis. J. Neuroimmunol. **65** (1996) 143–153.

Heat-shock proteins (hsp) are the most conserved molecules known to date that may also function as immune targets during infection. Hence, theoretically there is a high chance of crossreactive responses to epitopes shared by host and microbe hsp. If not properly regulated, these responses may contribute to the pathogenesis of autoim-

mune diseases. To determine if immune responses to hsp could contribute to the pathogenesis of multiple sclerosis, we raised T-lymphocyte lines specific for the purified protein derivative of *Mycobacterium tuberculosis* (PPD) from patients with multiple sclerosis, patients with tuberculosis, and from healthy individuals. These lines were then screened for their proliferative response to a *M. tuberculosis* 70-kDa hsp (M.tb.hsp70). The relative frequency of the recognition of highly conserved sequences of M.tb.hsp70 compared to variable ones was also assessed by mapping experiments on those PPD-specific T-lymphocyte lines which also recognized the mycobacterial 70-kDa hsp. In patients with multiple sclerosis, we observed a significantly higher estimated frequency of PPD-specific T lines responding to M.tb.hsp70 compared to healthy individuals and patients with tuberculosis. Furthermore, mapping experiments using recombinant proteins representing mycobacterial and human hsp70 sequences and a panel of synthetic peptides encompassing the whole sequence of *M. leprae* hsp70 showed that the response to conserved epitopes of hsp70 is a frequent event in each of the three conditions studied, often leading to the crossrecognition of microbial and human sequences. These findings implicate the 70-kDa hsp as potential autoantigens in multiple sclerosis.—Authors' Summary

Schmidt, H., Rush, B., Simonian, G., Murphy, T., Hsieh, J. and Condon, M. Thalidomide inhibits TNF response and increases survival following endotoxin injection in rats. J. Surg. Res. **63** (1996) 143–146.

Sepsis is a leading cause of death following major trauma and complicated abdominal surgery. Tumor necrosis factor (TNF) is believed to be a central mediator in the inflammatory response syndrome. Numerous methods of blunting the TNF response in sepsis have been attempted with suggestions of increased survival and decreased organ injury. Thalidomide, shown *in vitro* to selectively inhibit TNF production, has been used clinically in states of chronic TNF elevation with encouraging results. In this study, we examined the effect of thalidomide administration in a rat model of acute septic shock. Femoral artery cannulation was performed and baseline TNF measured. Dose response was determined by giving varying doses of thalidomide by gavage. Rats were injected intra-arterially with endotoxin and serial samples drawn. TNF was measured by ELISA. For survival, thalidomide was given by gavage and endotoxin injected intraperitoneally. Serum TNF elevation occurred after endotoxin injection with peak levels at 90 min. Thalidomide-treated rats had lower TNF levels at all time points ($p = < 0.01$ at 90 and 120 min), with the inhibition being dose dependent. Survival in treated rats exceeded that of untreated rats (53% vs 19%, $p = < 0.05$) at 48 and 72 hr. In conclusion, we found that thalidomide administration leads to increased survival following acute endotoxemia, which may be due to the observed TNF inhibition.—Authors' Summary

Silva, C. L., Silva, M. F., Pietro, R. C. L. R. and Lowrie, D. B. Characterization of T cells that confer a high degree of protective immunity against tuberculosis in mice after vaccination with tumor cells expressing mycobacterial hsp65. Infect. Immun. **64** (1996) 2400–2407.

Mice vaccinated by injection with tumor cells expressing the *Mycobacterium leprae* gene for hsp65 acquire a remarkably high degree of protection against challenge with *M. tuberculosis*. We used limiting-dilution analysis to assess the frequency of $CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$ splenocytes responding to mycobacterial hsp65 in such vaccinated mice. Cells of both phenotypes were present at very high and equal frequencies (approximately 1:100). Vaccination with live *M. bovis* BCG also increased the frequencies of both phenotypes of hsp65-reactive cells equally (to approximately 1:2500); whereas vaccination procedures that were not protective, with either dead BCG, hsp65 protein in incomplete Freund's adjuvant, or hsp65 mixed with tumor cells, resulted in preferential increase in $CD4^+$ $CD8^-$ cells. Twelve $CD4^+$ $CD8^-$ and 12 $CD4^-$ $CD8^+$ hsp65-responsive T-cell clones were obtained and characterized. All

showed conventional antigen recognition via major histocompatibility complex class II and class I pathways but differed in secretion of gamma-interferon and interleukin-4 and cytotoxicity. In tests of antimycobacterial activity against *M. tuberculosis*, both in infected macrophages *in vitro* and by adoptive transfer of protection with T-cell clones injected into irradiated mice, the most effective clones were the most cytotoxic and secretion of gamma-interferon made only a secondary contribution.—Authors' Abstract

Soler, R. A., Howard, M., Brink, N. S., Gibb, D., Tedder, R. S. and Nadal, D. Regression of AIDS-related Kaposi's sarcoma during therapy with thalidomide. Clin. Infect. Dis. **23** (1996) 501–503.

A 14-year-old girl with HIV infection and subcutaneous Kaposi's sarcoma (KS) received thalidomide therapy for oral ulcers, resulting in regression of KS lesions, disappearance of KS-associated herpes virus (KSHV) DNA from blood, and reduced viral load in tumor tissue. Administration of granulocyte colony-stimulating factor resulted in clinical exacerbation of KS and reappearance of KSHV DNA in blood.—Authors' Abstract

van Griethuysen, A. J., Janez, A. R. and Buiting, A. G. M. Comparison of fluorescent BACTEC 9000 MB system, Septi-Chek AFB system, and Lowenstein-Jensen medium for detection of mycobacteria. J. Clin. Microbiol. **34** (1996) 2391–2394.

The newly developed fluorescent BACTEC 9000 MB system for automated culture of mycobacteria was compared with the Septi-Chek AFB system and Lowenstein-Jensen medium (LJ). A total of 2005 clinical specimens were included in the study. Mycobacteria were isolated from 202 (10.1%) specimens, including 155 *Mycobacterium tuberculosis* complex isolates and 47 mycobacteria other than *M. tuberculosis* isolates. Of 135 isolates detected by the BACTEC system, the Septi-Chek AFB system, or both, 120 (91.6% were detected by the BACTEC system and 105 (80.2%) were detected by the Septi-Chek AFB system ($p < 0.02$). The recovery rate in the BACTEC system compared with that in the Septi-Chek AFB system was significantly higher for *M. tuberculosis* complex isolates ($p < 0.005$) and for isolates from acid-fast, smear-negative specimens ($p < 0.01$). Of 148 isolates detected by the BACTEC system, LJ, or both, 142 (95.9%) were detected by the BACTEC system and 118 (79.9%) were detected by LJ ($p < 0.001$). The recovery rate in the BACTEC system compared with that on LJ was significantly higher for *M. tuberculosis* complex isolates ($p < 0.001$). The BACTEC system detected more mycobacteria from both smear-positive and smear-negative specimens than LJ. The mean times to detection of mycobacteria were 17.6 days for the BACTEC system, 26.0 days for the Septi-Chek AFB system, and 29.4 days for LJ. The BACTEC fluorescent 9000 MB system is a rapid, sensitive, and efficient method for the isolation of mycobacteria.—Authors' Abstract

Walker, G. T. and Linn, C. P. Detection of *Mycobacterium tuberculosis* DNA with thermophilic strand displacement amplification and fluorescence polarization. Clin. Chem. **42** (1996) 1604–1608.

Strand displacement amplification (SDA) is an isothermal, *in vitro* method for diagnostics that amplifies a target DNA sequence by using a restriction enzyme and DNA polymerase. We have combined a new thermophilic form of SDA that involves restriction enzyme BsoBI and polymerase exo$^-$ Bca with fluorescence polarization for detection of *Mycobacterium tuberculosis* DNA by using the IS6110 insertion element as the target sequence. A 5′-fluorescein-labeled oligodeoxynucleotide detector probe hybridizes to the amplified product as it rises in concentration during SDA, and the single- to double-stranded conversion is monitored through an increase in fluorescence polarization. The associated change in polarization upon amplification of the target sequence is enhanced by specific polymerase biding to the double-stranded detector probe. Fewer than 10 *M. tuberculosis* genomes can be amplified and detected with an extremely simple pro-

tocol that takes only 20 min and uses relatively simple instrumentation and reagents, all of which can be purchased off the shelf.—Authors' Abstract

Wheeler, P. R. and Anderson, P. M. Determination of the primary target for isoniazid in mycobacterial mycolic acid biosynthesis with *Mycobacterium aurum* A^+. Biochem. J. **318** (1996) 451–457.

The target of the potent antituberculosis drug isoniazid was investigated in *Mycobacterium aurum* A^+, against which isoniazid has an MIC (minimum concentration required to give growth inhibition) of 0.3 μg/ml. Mycolic acid biosynthesis, measured by the incorporation of label from [1-C-14]acetate into mycolic acids, was inhibited differentially by isoniazid in cell-wall preparations of *M. aurum* A^+. Thus at an isoniazid concentration of 1 μg/ml, mycolic acid biosynthesis was inhibited by 80% while concomitant biosynthesis of non-hydroxylated fatty acids was inhibited by only 15%. Three lines of evidence identified 24:1 cis-5 elongase as the primary isoniazid target. First, 24:1 cis-5 did not restore isoniazid-inhibited mycolic acid biosynthetic activity in a crude cell-wall preparation, suggesting that the drug acts after the formation of the delta-5 double bond. Secondly, a 24:1 cis-5 elongase assay in which the product is mycolic acid is completely inhibited by isoniazid. Finally, the only intermediates that accumulate as a result of the addition of isoniazid are acids of 24 carbons. Both 24:0 and 24:1 are observed in a similar ratio whether or not isoniazid is present, even though concomitant mycolic acid biosynthesis is inhibited by isoniazid. These results are consistent with studies of the *M. tuberculosis* InhA protein by Dessen, Quemard, Blanchard, Jacobs and Sacchettini [(1995) Science 267, 1638–1641].—Authors' Abstract