CURRENT LITERATURE

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

General and Historical

de Feliciano, K. V. and Kovacs, M. H. [Opinions about Hansen's disease among the members of patients' social network in Recife.] Rev. Panam. Salud Pub. **1** (1997) 112–118.

This article describes a study done in Recife, Brazil, between November 1993 and July 1994 to explore the opinions office members of the social network (for example, family members, friends, and neighbors) of carriers of Hansen's disease regarding their estimation, interpretation, and management of physical manifestations of the disease in the time leading up to diagnosis. The sample consisted of 93 members of the social network, ranging in age between 20 and 70 years, who supported the course of action of 83 patients diagnosed in the study period. The analysis sought to detect differing capacities among the members of the patients' social network to discriminate between persons classified as cases (presence of disabilities or precursor lesions) or controls. The study found a lack of information about transmission of Hansen's disease and revealed a transitional phase in which there was expectation of cure along with a stigmatizing view of the consequences of the disease. Only one quarter of the study subjects suspected prior to diagnosis that the patient had Hansen's disease, which suggests low perception of the risk represented by the disease and reinforces the idea that its physical manifestations can be invisible. The results reveal a profile of perception and management of Hansen's disease that favors its propagation and the development or worsening of its physical and social consequences.—Authors' Abstract

Leprosy beyond the year 2000. Lancet **350** (1997) 1717.

The editorial reviews the World Health Organization's (WHO) goal of the "elimination of leprosy as a public health problem by the year 2000," and points out that this is defined by WHO as a prevalence of 1 case per 10,000 population (which case is defined by WHO as a person recommended by WHO to be currently receiving WHO multidrug therapy which is recommended by WHO to be of fixed duration and the durations of which have been recommended by WHO to be shorter and shorter). The wisdom of a timed objective for leprosy may be questioned, but it does no harm unless success is claimed misleadingly. In the past year, a growing doubt has developed about the wisdom of focusing on prevalence as an end point and neglecting those with long-term disability due to leprosy. There is no doubt that the emphasis on case finding and multidrug therapy has led to an impressively decreased overall toll of leprosy in the last decade; however, the incidence of leprosy has not decreased. The presumption that the year 2000 target will be reached is resulting now in a variety of cut backs in leprosy activities, including scientific research support, long-term care of patients, rural programs, etc. It is expected that these cut backs will be even more severe after the "elimination" goal has been declared, including the loss of some $10 million a year donated by the Japanese Sasakawa Foundation for leprosy control. The editorial concludes that, sadly, leprosy will be a "public health problem" beyond the year 2000.—RCH

Rao, P. S., Parkash, I. and Subramanian, M. Data utilization and analytical skills among NLEP managers. Indian J. Lepr. **69** (1997) 395–398.

Midlevel managers of the National Leprosy Elimination Programme, India, were assessed regarding their ability to turn routine data received from primary units into tools for management, analysis and decision making. Six-two managers (25 district leprosy officers, 37 medical officers) were administered a questionnaire containing 20 questions on calculations of rates, ratios, proportions, and percentages, as well as construction and interpretation of graphs. It was found that 11% of the study subjects scored very poor, 48% scored poor, 35% scored good, and only 5% of the managers scored very good. Among the district leprosy officers, 8%, 40%, 48% and 4% were rated as performing very poor, poor, good and very good, respectively. Among the medical officers, the corresponding figures were 14%, 54%, 27% and 5%.—Authors' Abstract

Wen, Y., *et al.* [The action of health education on popularizing knowledge of leprosy control.] China Lepr. J. **13** (1997) 191–194. (in Chinese)

Early detecting the patients is the key measure to eliminating leprosy completely from China. The study accomplished in Wenshan County, Yunnan, indicates that through systematic health education the knowledge level of leprosy in trained village doctors is obviously higher than those in the control and before the education ($p < 0.01$). Among the population, knowledge of leprosy is significantly raised also after health education ($p < 0.01$).—Authors' English Abstract

Yuasa, Y. [Synthesis of promin in Japan and global elimination of Hansen's disease.] Yakugaku Zasshi **117** (1997) 957–962. (in Japanese)

Professor Morizo Ishidate synthesized "Promin" for the treatment of leprosy/Hansen's disease which had been considered "incurable" until the discovery of the antileprosy effect of that drug by Dr. Faget of the U.S.A. in 1941. Professor Ishidate was the first to synthesize the drug in Japan in 1946 based on a brief news item in a Swiss journal smuggled in during the war. For this achievement, he is known as "father of chemotherapy for leprosy in Japan."

Professor Ishidate also contributed to the global fight against leprosy as Chairman of the Board of Directors of the Sasakawa Memorial Health Foundation (SMHF), which he helped to establish in May 1974 with the full financial backing of Mr. Ryoichi Sasakawa, President of the Japan Shipbuilding Industry Foundation (JSIF). Professor Ishidate, with his scientific knowledge as well as christianity-based humanitarian concern, advised Mr. Sasakawa how to spend JSIF money wisely for eliminating leprosy and nearly US$200 million was channeled through WHO and SMHF.

The successful outcome of the global multidrug therapy (MDT) program in the 1980s resulted in the adoption of a resolution by the World Health Assembly, "Elimination of Leprosy, as a public health problem" by the year 2000.

The synthesis of "Promin" in Japan and promoting of the global implementation of MDT are both achievements which can be attributed to Professor Ishidate.—Author's English Abstract

Chemotherapy

Alangaden, G. J. and Lerner, S. A. The clinical use of fluoroquinolones for the treatment of mycobacterial diseases. Clin. Infect. Dis. **25** (1997) 1213–1221.

Mycobacterial diseases often require prolonged therapy with multidrug regimens. Fluoroquinolones have excellent bactericidal activity against many mycobacteria; achieve

effective serum, tissue, and intracellular levels following oral administration; and produce few adverse effects. These properties have led to the increasing use of fluoroquinolones for the treatment of mycobacterial infections. We reviewed clinical studies and reports involving the use of fluoroquinolones for mycobacterial diseases. Ofloxacin, ciprofloxacin, sparfloxacin, and pefloxacin exhibit clinical efficacy against mycobacterial diseases, especially tuberculosis and leprosy. Fluoroquinolones have generally been administered in regimens that include other agents. However, when a fluoroquinolone has been found to be the sole active agent in a multidrug regimen, the ready emergence of resistance to fluoroquinolones has been recognized, just as when they have been used as monotherapy. Therefore, to forestall the emergence of resistance to fluoroquinolones during the treatment of mycobacterial diseases, these drugs should always be used in combination with at least one other active agent, and they should be used only when effective alternative drugs are not available.—Authors' Abstract

Ayers, J. D., Lowary, T. L., Morehouse, C. B. and Besra, G. S. Synthetic arabinofuranosyl oligosaccharides as mycobacterial arabinosyltransferase substrates. Bioorg. Med. Chem. Lett. **8** (1998) 437–442.

A series of arabinofuranosyl oligosaccharides found as constituent parts of the polysaccharide portion of the cell wall of *Mycobacterium tuberculosis* have been chemically synthesized. Screening of these oligosaccharides as substrates for arabinosyltransferases present in mycobacterial membrane preparations suggests that modified oligosaccharide analogs as small as disaccharides may be inhibitors of glycan biosynthesis. Such inhibitors would be of potential utility as lead compounds in the identification of new drugs for the treatment of mycobacterial infections.—Authors' Abstract

Calleja, C., Pascussi, J. M., Mani, J. C., Maurel, P. and Vilarem, M. J. The antibiotic rifampicin is a nonsteroidal ligand and activator of the human glucocorticoid receptor. Nature Med. **4** (1998) 92–96.

The glucocorticoid receptor (GR) belongs to a superfamily of ligand-regulated nuclear steroid hormone receptors. The steps in the signal transduction pathway leading to the biological effects of glucocorticoids (GCs) include sequentially binding of the steroid to the GR ligand-binding domain (LBD), receptor transformation, nuclear translocation, and either positive or negative gene transactivation. Rifampin (RIF) is a macrocyclic antibiotic used as an antituberculosis agent. As the incidence of tuberculosis has been increasing, in part because of the AIDS epidemic, a growing number of patients are being exposed to the adverse effects of this antibiotic. Indeed, this compound, as are the GCs, is often implicated in noxious drug interactions, because of its strong ability to induce drug-metabolizing enzymes. Moreover, in humans, RIF, as are the GCs, has been described as a potential immunodepressor, associated notably with the reduction of mitogenic responsiveness of human peripheral blood lymphocytes. Here, we report that RIF activates the human glucocorticoid receptor (hGR). Transient expression of wild-type, deleted or mutated GRs; sucrose density gradient sedimentation, and the BIAcore technique strongly suggest that RIF binds to the receptor with the physiological consequence that this antibiotic acts as an immunodepressor. Given the wide use of RIF in the treatment of coinfection of tuberculosis and HIV, this report is highly relevant to current medical practice.—Authors' Abstract

Cambau, E., Perani, E., Guillemin, I., Jamet, P. and Ji, B. Multidrug-resistance to dapsone, rifampicin, and ofloxacin in *Mycobacterium leprae*. Lancet **349** (1997) 103–104.

A 35-year-old man with lepromatous leprosy who had been treated with dapsone monotherapy was admitted to the Institut Marchoux in Bamako, Mali, in 1991. He was treated with a fully supervised regimen of 600 mg of rifampin and 400 mg of

ofloxacin daily for 28 days. A year later the patient relapsed with new lepromatous lesions. Bacilli from a skin biopsy were inoculated into mouse foot pads and were shown to be resistant to dapsone, rifampin and ofloxacin.—From the article.

Chen, J., *et al.* [Effects of a modified MDT regimen on 122 cases of MB leprosy.] China Lepr. J. **13** (1997) 185–187. (in Chinese)

Because of skin coloration, self-taking 50 mg B663 a day is very difficult to be regularly taken for some the patients and therefore the authors, on the basis of WHO-MDT, have drawn up a modified regimen consisting of supervised RMP 1200 mg and B663 1200 mg a month and self-taking DDS 100 mg a day. In 1983 to 1996, 122 cases of MB leprosy had been treated with the modified MDT: 6 died, 2 lost and 114 cases completed their course of the treatment including 32 new, 36 relapsed and 46 patients who used DDS monotherapy but were still active clinically with age of 22 to 70 years; disease duration of 1 to 54 years; BI 0.7 to 6.0. During treatment, the BI decreased by 0.52 for those with a BI of 3.0 or less and by 0.7 for those with a BI of over 3.0; 101 cases became negative by skin smear within 1 to 6 years, and the skin lesions began resolving 3 months after treatment and lost basically within 12 to 18 months. There was no relapse during follow up of 1 to 10 years for the 101 cured persons.—Authors' English Abstract

Cynamon, M. H., Speirs, R. J. and Welch, J. T. *In vitro* antimycobacterial activity of 5-chloropyrazinamide. Antimicrob. Agents Chemother. **42** (1998) 462–463.

5-Chloropyrazinamide and 5-chloropyrazinoic acid were evaluated for *in vitro* activity against *Mycobacterium tuberculosis, M. bovis,* and several nontuberculous mycobacteria by a broth dilution method. 5-Chloropyrazinamide was more active than pyrazinamide against all organisms tested. It is likely that this agent has a different mechanism of action than pyrazinamide.—Authors' Abstract

Gallo, M. E. N., Alvim, M. F. S., Nery, J. A. C. and Albuquerque, E. C. A. [A comparative study of two multidrug regimens of fixed duration in multibacillary hanseniasis—50.32 ± 19.62 and 39.70 ± 19.47 months of follow up.] Hansen. Int. **22** (1997) 5–14. (in Portuguese)

This study compares the bacilloscopic and clinical evolution of 140 multibacillary (MB) leprosy cases, divided in two groups and submitted to two treatment regimens with chemotherapic association with fixed dosage. Group I: 70 cases received rifampin (RMP) 600 mg and dapsone (DDS) 100 mg daily for 3 consecutive months, followed by DDS 100 mg daily self-administered for 21 months. Group II: RMP 600 mg and clofazimine (CLO) 300 mg once a month under supervision, plus self-administered doses of DDS 100 mg and CLO 50 mg daily, with a 24 supervised dose duration. No statistically meaningful differences were found ($p > 0.05$) on neuromotor and bacilloscopic evolution between the groups, neither on treatment nor on follow up after discharge. A significant statistical difference ($p < 0.05$) was found on reactional manifestations occurrence, where Group I showed a greater number of reactional cases during treatment and after discharge. This statistical difference was attributed to CLO presence in the Group II therapeutic regimen. Group I total follow up was 2110 patient-years with a mean of 50.32 ± 16.62 months, wherein 2 relapse cases were diagnosed. In Group II, submitted to the WHO-recommended multidrug treatment (WHO/MDT) regimen for MB patients, which is the regimen followed in our country, total follow up was 1897 patient-years, or mean 39.70 ± 19.47 months, and no relapse was diagnosed. In one of the relapse cases, a skin biopsy was inoculated into foot pads of mice (Shepard's technique) to verify bacillary viability and drug sensibility to RFM and DDS. The results suggest that in this case, the relapse was due to the resurgence of bacilli persisters sensitive to the drugs used. After the initiation of the standard WHO/MDT both cases have a satisfactory evolution.—Authors' English Summary

Horgen, L., Legrand, E. and Rastogi, N. Postantibiotic effect of amikacin, rifampin,

sparfloxacin, clofazimine and clarithromycin against *Mycobacterium avium*. Res. Microbiol. **148** (1997) 673–681.

Antimycobacterial drugs acting efficiently against *Mycobacterium avium* complex have in common low MICs and MBC/MIC ratios. The recently reported clinical efficacy of some of the newer drugs is also clearly linked to their pharmacokinetic properties, such as higher serum level and/or intracellular concentrations and half-life. In the present investigation, comparative postantibiotic effects (PAEs) of amikacin, rifampin, sparfloxacin, clofazimine and clarithromycin were investigated. Bacteria were exposed to MIC, MICx4 and MICx8 concentrations of each drug for 2 hr, the drug was removed by centrifugation and cells were thoroughly washed and resuspended in drug-free medium. Growth was compared to control organisms which underwent a similar treatment (but without drugs) and PAEs were assessed using the equation "T – C," where T equals the time required for colony counts to increase by 1 $\log^{10}$ in test samples after antibiotic exposure and C equals the time for 1 $\log^{10}$ growth in controls. Our results underlined two distinct patterns concerning PAE: pattern I included drugs for which PAE (in hours) was dose-dependent and varied (for MIC, MICx4 and MICx8 concentrations) for amikacin (10.3 ± 1.7, 14.7 ± 1.9 and 17.7 ± 4.1), rifampin (28.0 ± 7.6, 62.0 ± 18.5 and 71.0 ± 3.2) and clarithromycin (2.6 ± 1.0, 15.0 ± 4.0 and 22.0 ± 4.0); whereas pattern II included drugs with a stable PAE, relatively independent of the drug concentrations: sparfloxacin (11.0 ± 2.5, 12.3 ± 6.4 and 13.0 ± 2.1) and clofazimine (26.0 ± 2.8, 28.8 ± 2.5 and 27.3 ± 1.3). These results may be useful for guidance in scheduling of drug administration in *M. avium*-infected AIDS patients overburdened with too many drugs given for various opportunistic infections.—Authors' Abstract

Kar, P. K. and Dhaka, R. S. Borderline tuberculoid leprosy: clinicopathological evaluation of multidrug therapy. Med. J. Armed Forces India **53** (1997) 91–94.

The efficacy of multidrug therapy (MDT) as per WHO recommendations in 125 new cases of borderline tuberculoid (BT) leprosy in 116 men and 9 women from India were evaluated in 1991–1994. Age of the patients ranged from 18–50 years but the majority (80.8%) were aged 21–35 years. The site of lesion was the upper extremity in 65 (52%) cases. A skin smear for acid-fast *Mycobacterium leprae* was positive in 11 (8.8%) patients. All patients were given MDT consisting of rifampin 600 mg once a month and dapsone 100 mg daily for 6 months. At the end of 6 months, 42 (33.6%) patients had shown marked improvement, 14 (11.2%) had increase in activity, 51 (45.6%) had shown regression and 12 (9.6%) cases became clinically inactive. Histologically complete clearance of the infiltration was not observed in any patient. Compact granulomas persisted in 30 (24%) cases. In 1 (0.8%) patient, *M. leprae* were found in the skin smear at the end of 6 months. It is concluded that treatment with MDT for 6 months is inadequate to treat all types of BT leprosy cases.—Authors' Abstract

LeGuellec, C., Gaudet, M. L., Lamanetre, S. and Breteau, M. Stability of rifampin in plasma: consequences for therapeutic monitoring and pharmacokinetic studies. Ther. Drug Monit. **19** (1997) 669–674.

Interest in determining plasma levels of rifampin for adjustment of dosage regimens has increased, but conflicting results exist concerning rifampin stability. The authors developed a high-performance liquid chromatography assay to monitor rifampin plasma concentrations that was used to study the possible degradation of rifampin in plasma samples. This report describes the stability of rifampin in plasma kept at an ambient temperature for 24 hr or stored at –20°C for 2 weeks. The possible protective effect of adding ascorbic acid was also studied. The results indicate that rifampin degrades rapidly in plasma at an ambient temperature, and a 54% loss was observed within 8 hr. This degradation can be effectively prevented by adding ascorbic acid, thus prolonging stability for up to 12 hr. The same results were observed with samples obtained as part of routine drug monitoring. Degradation was found to be greater

at low rifampin concentrations. The authors subsequently demonstrated that decomposition of rifampin occurs after storage for 1 week at −20°C. However, in samples supplemented with ascorbic acid before freezing, no degradation was observed within 14 days at the two concentrations tested. Rifampin was more stable in specimens drawn from treated patients, suggesting possible *in vivo* stabilization of the molecule. Further studies are needed to determine stability of rifampin for longer storage periods. On the basis of these results, plasma samples obtained from patients receiving rifampin should be immediately supplemented width ascorbic acid and analyzed as soon as possible.—Authors' Abstract

Ogata, H., Kubo, M., Tamaki, K., Hirakata, H., Okuda, S. and Fujishima, M. Crescentic glomerulonephritis due to rifampin treatment in a patient with pulmonary atypical mycobacteriosis. Nephron **78** (1998) 319–322.

A 64-year-old male was treated continuously with rifampin, isoniazid and streptomycin for pulmonary atypical mycobacteriosis, *Mycobacterium kansasii.* Five weeks after beginning the treatment, the patient suddenly developed acute renal failure. A renal biopsy showed crescentic lesions characteristic of rapidly progressive glomerulonephritis with moderate interstitial changes. Serum antirifampin antibody was detected, and the cessation of rifampin treatment was followed by a rapid spontaneous recovery of the patient's renal function. This is, to our knowledge, the first case of rapidly progressive, crescentic glomerulonephritis associated with rifampin treatment where circulating antirifampin antibody is demonstrated and the renal function spontaneously improved after discontinuing rifampin treatment.—Authors' Abstract

Prabhakaran, K., Harris, E. B. and Randhawa, B. Suppression of the growth of six potentially pathogenic mycobacteria by beta-lactam/beta-lactamase inhibitors. Microbios **91** (1997) 7–14.

Drug-resistant tuberculosis and opportunistic infections by mycobacteria in immunocompromised subjects are not readily controlled with the antimycobacterial drugs now available. Beta-lactam antibiotics, the most widely used antibacterial agents, are ineffective against mycobacteria since they synthesize beta-lactamases. The beta-lactam/beta-lactamase-inhibitor combinations are used at present to treat infections caused by other beta-lactamase-positive organisms. Six potentially-pathogenic mycobacteria: *Mycobacterium avium, M. cheloneii, M. haemophilum, M. Microti, M. scrofulaceum* and *M. simiae,* were cultured in 7H9 medium (containing Tween 80 and albumin, dextrose, catalase) at 37°C for 10–14 days, with or without various concentrations (2–100 μg/ml) of ampicillin/sulbactam, amoxicillin/clavulanate and piperacillin/tazobactam. More than 50%–80% inhibition of the mycobacterial growth was observed at drug levels of 40–100 μg/ml in the medium; the drugs were active even when the detergent (Tween 80) was omitted. Against four of the mycobacteria, ampicillin/sulbactam proved to be the most active. The beta-lactam/beta-lactamas-inhibitor combinations may be of use as rational therapeutic agents against mycobacterial infections.—Authors' Abstract

Reilly, T. P., Bellevue, F. H., Woster, P. M. and Svensson, C. K. Comparison of the *in vitro* cytotoxicity of hydroxylamine metabolites of sulfamethoxazole and dapsone. Biochem. Pharmacol. **55** (1998) 803–810.

The differential incidence of adverse drug reactions (ADR) between trimethoprim-sulfamethoxazole and dapsone might be explained, in part, by differences in the inherent toxicity of the hydroxylamine metabolites of sulfamethoxazole and dapsone. To test this hypothesis, the *in vitro* cytotoxicities of sulfamethoxazole hydroxylamine, dapsone hydroxylamine, and monoacetyldapsone hydroxylamine were compared using peripheral blood mononuclear cells (PBMC) from healthy volunteers. After 3 hr of exposure to hydroxylamine metabolites, PBMC were washed thoroughly to remove residual hydroxylamine, and viability

was assessed 16 hr later by determination of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) conversion. A concentration-dependent toxicity was observed with each hydroxylamine metabolite. While dapsone hydroxylamine and monoacetyldapsone hydroxylamine were not significantly different, both showed significantly greater cytotoxic potency than sulfamethoxazole hydroxylamine ($p < 0.05$). This differential potency was not a function of differential stability in aqueous medium and was maintained over time. The effects of red blood cells (RBC), impermeable RBC "ghosts," and RBC lysate on hydroxylamine-induced cytotoxicity were determined using a two-compartment dialysis system. Amelioration of hydroxylamine-dependent cytotoxicity occurred when RBC were included in PBMC incubations. This apparent detoxifying effect was markedly greater using RBC lysate in comparison with impermeable "ghosts" ($p < 0.05$). No difference in detoxification was observed between sulfamethoxazole hydroxylamine and monoacetyldapsone hydroxylamine. Differences in the inherent cytotoxicity of their hydroxylamine metabolites do not appear to explain the differential incidence of ADR between trimethoprim-sulfamethoxazole and dapsone.—Authors' Abstract

Strayhorn, V. A., Baciewicz, A. M. and Self, T. H. Update on rifampin drug interactions. Arch. Intern. Med. **157** (1997) 2453—2458.

Rifampin is a potent inducer of cytochrome P-450 oxidative enzymes. Examples of well-documented, clinically significant interactions include warfarin, oral contraceptives, cyclosporine, glucocorticoids, ketoconazole, theophylline, quinidine, digitoxin, and verapamil. Recent reports have demonstrated clinically relevant interactions with protease inhibitors, zidovudine, delavirdine, itraconazole, nifedipine, midazolam or triazolam, nortriptyline, and doxycycline. To avoid reduced therapeutic response, therapeutic failure, or toxic reactions when rifampin is added to or discontinued from medication regimens, clinicians need to be cognizant of these interactions. Enhanced knowledge of known interactions will continue to develop, including research on induction of specific cytochrome P-450 isoenzymes. New rifampin interactions will be discovered with further investigations.—Authors' Abstract

Tingle, M. D., Mahmud, R., Maggs, J. L., Primohamed, M. and Park, B. K. Comparison of the metabolism and toxicity of dapsone in rat, mouse and man. J. Pharmacol. Exp. Therapeut. **283** (1997) 817–823.

The metabolism and toxicity of dapsone was compared *in vitro* and *in vivo* in rat, mouse and man. Metabolism was assessed by high-pressure liquid chromatography-mass spectrometry and methemoglobin formation has been used as a toxic endpoint. The greatest toxicity *in vitro* was seen in microsomes prepared from male Wistar rats (36.6 ± 1.5% methemoglobin), although toxicity was also seen in microsomes from the female rat (8.2 ± 1.3%), male CD1 (4.2 ± 1.6%) and human (10.9 ± 1.1%). The rank order of toxicity agreed with the formation of the hydroxylamine metabolite *in vitro*. All microsomes were also capable of catalyzing the reverse reaction, i.e., reduction of the hydroxylamine to dapsone. However, *in vivo* administration of dapsone resulted in significant ($p < 0.05$) methemoglobinemia only in male rats and humans. This species difference in the susceptibility to dapsone toxicity could not be attributed solely to the sensitivity of the target erythrocytes, because the order of sensitivity to dapsone hydroxylamine was human > mouse > rat. Analysis of bile and urine revealed the formation of dapsone hydroxylamine and its glucuronide in male rats and humans, but not in female rats or mice. This species difference in the metabolism and toxicity of dapsone has important implications in the safety evaluation of related compounds for man.—Authors' Abstract

Wang, H., *et al.* [Changes of disability in 240 persons cured of leprosy.] China Lepr. J. **13** (1997) 188–191. (in Chinese)

In 1990 and 1995, two surveys of disability among persons cured of leprosy with

DDS monotherapy in Chenggu County, Shaanxi Province, China, showed that the number, site and degree (as marks) of the disability in the latter survey increased by 46.8%, 53.1% and 48.8% respectively, as compared with those in the former one. But among those cured with MDT, there was no significant exacerbation of the disability in the same time. The authors pointed out that the follow up for persons cured of leprosy should be continued for a longer time.—Authors' English Abstract

Wang, J., *et al.* [Effects of retreatment with MDT on 472 persons cured of leprosy with DDS monotherapy.] China Lepr. J. **13** (1997) 198–199. (in Chinese)

For prevention of relapse, MDT has been given to 472 persons cured of leprosy with regular DDS monotherapy, including three regimens, i.e., I: monthly supervised RMP 600 mg plus daily self-taking DDS 100 mg for 6 months; II and III: monthly supervised RMP 1200 mg and B663 1200 mg plus daily self-taking DDS 100 mg for 6 months and for 1 year, respectively. Those who took regimens I, II and III were 329, 48 and 95 persons, including MB 62, 47 and 93, respectively. Since stopping the drugs, the three groups were followed up for 6, 8 and 7 years, respectively, and two relapses occurred only in original LL cases of group I.—Authors' English Abstract

Willcox, M. L. The impact of multiple drug therapy on leprosy disabilities. Lepr. Rev. **68** (1997) 350–366.

In an overview of controlled trials, it is shown that bactericidal drugs increase the short-term risk of type 1 reactions but prevent the long-term development of new impairments caused by bacterial proliferation. Clinical experience suggests that the clofazimine component of multiple drug therapy (MDT) has reduced the incidence of type 2 reactions or erythema nodosum leprosum (ENL). The principal impact of MDT, compared with monotherapy, has been to reduce the duration of active disease, thus preventing the deterioration of disability scores. Reduction of population disability rates is mainly achieved by earlier detection and treatment. MDT has a number of indirect benefits, such as improved compliance, decreased cost, and increased motivation and availability of leprosy workers. However, MDT must be supplemented by other measures to prevent and treat disabilities.—Author's Summary

Xue, W., *et al.* [Shortening treatment duration of leprosy with MDT plus ofloxacin.] China Lepr. J. **13** (1997) 197–198. (in Chinese)

For shortening the course of MDT, a four-drug regimen has been used to treat 18 leprosy patients, including 11 men and 7 women with age of 50.1 years and disease duration of 14.3 months, on an average, of whom 17 MB cases had a BI of 0.17 to 3.67, averaging 2.59, and being newly found in 16 and relapsed in 2. The regimen consists of supervised RMP 600 mg two times a week plus self-taking DDS 100 mg, B663 50 mg and ofloxacin 400 mg a day. After completion of a 4-month course of treatment, the skin lesions were reduced by over 50% in 11 cases, had disappeared in 3, and the mean BI of 2.59 was reduced to 1.2, (53.7%), including 2BT-negative cases. Twelve months after stopping the drugs, all skin lesions had disappeared in 8 cases, 12 became negative bacteriologically, and the BI of the remaining 5 was also below 1.0.—Authors' English Abstract

Clinical Sciences

Abraham, S., Ebenezer, G. J. and Jesudasan, K. Diffuse alopecia of the scalp in borderline lepromatous leprosy in an Indian patient. Lepr. Rev. **68** (1997) 336–340.

A case of borderline-lepromatous leprosy exhibiting alopecia of the scalp along with lepromatous lymphadenitis of the suboccipital lymph node is reported. To our knowledge generalized leprous alopecia of the

scalp with lepromatous lymphadenitis of the suboccipital node is a rare occurrence in female Indian patients.—Authors' Summary

Abraham, S., Vijayakumaran, P. and Jesudasan, K. Ulnar nerve abscess in a multibacillary patient during post-multidrug therapy surveillance. Lepr. Rev. **68** (1997) 333–335.

An old borderline-lepromatous leprosy patient, treated initially with dapsone monotherapy followed by MDT, 11 years later during surveillance presented with a 3-month-old asymptomatic cystic swelling arising from the right ulnar nerve, without exhibiting any evidence to document relapse of the disease. It responded promptly to corticosteroid therapy. This unusual clinical presentation of ulnar nerve abscess has not been reported elsewhere.—Authors' Summary

Benzekri, L. and Hassam, B. [Atypical cases of leprosy: a two-case study.] Acta Leprol. **10** (1997) 195–198. (in French)

This report describes two atypical cases of leprosy. A 48-year-old male patient presented with laryngeal dyspnea with adhesions of the oropharynx of which the biopises were inconclusive. The patient was cachectic with hyperesthesia of the extremities and two subcutaneous nodules. The biopsy of one nodule evoked thesaurismosis or dyslipoidosis while the bacilloscopy was positive in nasal smears. A 14-year-old female patient suffered from bullae which appeared spontaneously on erythematous skin on the legs and upper arms. Upon examination those areas were found to be hypoesthetic, as was a very large hamartoma on the left half of the body. A biopsy of healthy skin evoked the diagnosis of leprosy. The patient then developed BT leprosy and episodes of hysteria. The first observation led to several diagnoses: while laryngeal dyspnea is unusual in LL and while cutaneous histology of regressive LL contrasted with the abundance of the bacilloscopy. The diagnosis of the second case is that of indeterminate leprosy with premonitory neurological signs associated with pathomania and evolution to a multibacillary form.—Authors' English Summary

Bharath, S., Shamasundar, C., Raghuram, R. and Subbakrishna, D. K. Psychiatric morbidity in leprosy and psoriasis—a comparative study. Indian J. Lepr. **69** (1997) 341–346.

The psychiatric morbidity of 30 leprosy patients was compared with that of psoriasis in a clinic set up. The prevalence of psychiatric morbidity, was significantly less among leprosy patients (122/1000) than among those with psoriasis (476/1000); but the severity of the problem, as measured by General Health Questionnaire (GHQ), was significantly greater among leprosy patients ($p < 0.05$). There was no difference in the pattern of psychopathology diagnosis between the two groups. Depressive neurosis was the most common diagnosis in both the groups. The relevance of these findings in relation to leprosy is discussed.—Authors' Abstract

Cossermelli Messina, W., Neto, C. F. and Cossermelli, W. Articular inflammatory manifestations in patients with different forms of leprosy. J. Rheumatol. **25** (1998) 111–119.

Objective. To study the articular inflammatory manifestations of leprosy.

Methods. Sixty patients with leprosy from a public clinic in Sao Paulo, Brazil, participated in a study regarding their articular manifestations. The diagnosis and classification of leprosy were established by the clinical picture, skin smears, skin biopsy, and delayed hypersensitivity test to *Mycobacterium leprae* antigens (Mitsuda test). According to the Madrid and Ridley-Jopling classifications, 46 patients had lepromatous leprosy, 7 had borderline leprosy, 4 had tuberculoid leprosy, and 3 had indeterminate leprosy. History, general and articular examinations, and X-rays were employed and complemented in several cases by scintigraphic examinations with technetium methylene diphosphonate and computed tomographic studies.

Results. Three patients were excluded from study due to an association with a rheumatic disease. Among the 57 remaining patients, 44 had peripheral arthritis characterized by involvement of small joints (23/44), large joints (4/44), or both (17/44). The mean duration of arthritis was 11 years (range 1 mo. to 51 yrs.). Arthritis was detected in all subtypes of patients with leprosy. Supplementary radiological evaluation established the extent of inflammation and diagnosis of sacroiliitis. The diagnosis of sacroiliitis, based on the presence of sclerosis, erosions, and narrowing of the cartilage space in the sacroiliac joints, was established in 35 of 55 radiographs. Sacroiliitis varied from grade I to III, according to the Bennet and Wood classification, and was bilateral in most cases (30/35). There was no significant correlation between low back pain and the finding of sacroiliitis.

Conclusion. Articular inflammatory manifestations may exist in patients with different forms of leprosy, and can follow a chronic course. In addition, sacroiliitis is a common, previously unrecognized manifestation in patients with leprosy.—Authors' Abstract

Croft, R. P., Richardus, J. H. and Smith, W. C. S. Field treatment of acute nerve function impairment in leprosy using a standardized corticosteroid regimen—first year's experience with 100 patients. Lepr. Rev. **68** (1997) 316–325.

In this study, a fixed regimen of prednisolone for the treatment of acute nerve function impairment (NFI) in leprosy patients was developed and introduced at field level in one area (Thakurgaon) of the Danish-Bangladesh Leprosy Mission's field in northwestern Bangladesh. The assessment, management and follow up of patients were undertaken by leprosy control supervisors and physiotechnicians.

One-hundred patients were treated and followed up 6–8 months after completion of a 4-month course of prednisolone. At a level of change of 2 points (where a change of at least 2 points in the motor/sensory score was taken to indicate a change of status, i.e., full or partial recovery, or deterioration), 42/65 (64.6%) patients with sensory loss experienced some sensory recovery at completion of prednisolone treatment, and 40/65 (61.5%) at 6–8 months' follow up; 41/85 (48.3%) of patients with motor loss experienced improvement, and 42/85 (49.4%) at follow up. Analysis of the mean scores at the start of prednisolone treatment, completion and at follow up using the Student's *t* test showed highly significant ($p < 0.001$) differences between scores before and after treatment. The benefit is maintained as seen after a period of 6–8 months follow up.

It was concluded that treatment of acute NFI at field level by paramedical workers using a standardized regimen of prednisolone is feasible, practical and effective. —Authors' Summary

Courtright, P., Kim, S.-H., Lee, H.-S. and Lewallen, S. Excess mortality associated with blindness in leprosy patients in Korea. Lepr. Rev. **68** (1997) 326–330.

Vision loss and blindness are potential complications of leprosy. There is little data available to indicate the impact of eye complications on life expectancy and quality of life. We sought to determine the relative risk of death in blind leprosy patients compared to nonblind leprosy patients.

A population-based ocular survey of 510 mycobacteriologically negative leprosy patients in rural South Korea, conducted in 1988, formed the study population. After a 7-year period patients were traced to determine their status (alive, dead, lost to follow up).

Blind patients showed a 4.8-fold risk of death, even after adjusting for other factors, compared to nonblind patients. Young blind leprosy patients had the highest relative risk of death. Excess mortality was not associated with any specific cause of blindness, ocular pathology, or type of disease.

Findings from our study suggest that all leprosy patients with ocular disabilities (including those released from antileprosy treatment) should be targeted to receive eye care to prevent vision loss. Particular emphasis should be placed on young patients.—Authors' Summary

Darius, E. J., Selvasekar, A., Mani, M. N. and Jesudasan, K. Ulnar abscess: 4 months after release from control with paucibacillary-multidrug therapy. Lepr. Rev. **68** (1997) 173–174.

A 14-year-old girl in India, diagnosed as having borderline-tuberculoid leprosy, was given paucibacillary multidrug therapy (PB-MDT) for 6 months. During the period after release from treatment there were no complaints. Two years after release from treatment the patient was released from control, and 4 months after this she presented with swelling in the left arm above the elbow of 15 days' duration. A diagnosis of ulnar nerve abscess was made and steroids were started, with prednisolone (30 mg) given once a day. Improvement was shown within a week, with a reduction in fever and pain; 30 mg of steroids were given for 2 weeks, 20 mg for 2 weeks, 10 mg for 2 weeks, 5 mg for 4 weeks, and 5 mg on alternate days for 4 weeks. The nerve abscess responded well and the swelling was <3 mm in size at the end of the fourteenth week. The need for surgical intervention was therefore prevented.—Authors' Summary

Endoh, M., Ueki, A., Takahashi, K., Yamanaka, H., Izumi, S. and Tabira, T. Alpha-1-antichymotrypsin is not associated with the increased frequency of apolipoprotein-E-epsilon-4 allele in elderly non-demented leprosy patients. Dement. Geriatr. Cognit. Disorders **9** (1998) 26–28.

In our previous study, elderly leprosy patients showed a low prevalence of senile dementia of the Alzheimer type, but the frequency of apolipoprotein E (APO-E) epsilon 4 was elevated in nondemented elderly leprosy patients. Recent study has shown that Alzheimer's disease risk associated with APO-E epsilon 4 is significantly increased by the alpha^1-antichymotrypsin (ACT) genotype AA. Therefore we examined an association between ACT polymorphism and the APO-E epsilon 4 allele in 350 leprosy patients. None of our data showed an association of ACT genotype and APO-E epsilon 4 allele in leprosy patients. The allelic frequencies of the ACT gene did not differ even between demented patients with leprosy and age-matched controls. Our present data suggest that ACT polymorphism is not associated with the increased frequency of APO-E epsilon 4 in leprosy patients.—Authors' Abstract

Lyde, C. B. Pregnancy in patients with Hansen disease. Arch. Dermatol. **133** (1997) 626–627.

Background: Three pregnancies occurred in a cohort of 40 patients with Hansen's disease during a 6-year period. There are few recent reports in the English literature that deal with pregnancy in patients with Hansen's disease. These three cases are presented in the context of previously reported cohorts.

Observations: In patient 1, symptoms of the disease appeared during pregnancy. In patient 2, reactivation VS reaction occurred during pregnancy in a previously treated patient. In patient 3, the fetus was exposed to three antimicrobial drugs during the first trimester.

Conclusions: The pregnant state causes a relative decrease in cellular immunity. This decrease allows *Mycobacterium leprae* to proliferate, which may precipitate or worsen disease, leading to permanent nerve damage. Careful management of reactional states and treatment of patients with dapsone monotherapy can prevent this nerve damage. Infants are usually much less affected than mothers; however, selection of the mother's antimicrobial regimen must ensure adequate control of the bacteria while avoiding teratogenicity and *in utero* adverse effects.—Author's Abstract

Manzur, S., Qureshi, I. A., Farooq, M. A., Qureshi, P. S., Hakim, T. and Tarin, B. A. Radiological evaluation of bone changes in leprosy. J. Coll. Phys. Surg.-Pakistan **7** (1997) 199–202.

A study involving 44 patients with leprosy at the Radiology Department, Combined Military Hospital, Rawalpindi, in as-

sociation with the Rawalpindi Leprosy Hospital, Pakistan, during September 1995–August 1996 was conducted. All patients had bone changes of either specific or nonspecific type. Plain radiography was carried out with a view to evaluate its feasibility as a worthwhile source of supplementary data in planning strategy for treatment. The study group predominantly consisted of male patients between 51 and 60 years of age. Lepromatous leprosy accounted for 52% and borderline tuberculoid leprosy for 29% of the cases. The remaining belonged to either borderline lepromatous or indeterminate subtypes. Both specific and nonspecific lesions were most commonly seen in the lepromatous type, followed by borderline, and were more frequent and marked in feet than in hands. Cortical thinning/irregularity was the most common specific bone change in 79% of hands and 81% of feet. Generalized osteoporosis was seen in 77% of hands and 81% of feet, making it the most commonly seen nonspecific bone change.—Authors' Abstract

Muneishi, H., Taguchi, H., Sawada, T., Ikezoe, T., Matsui, S., Tanaka, S., Taniguchi, T., Onoue, O. and Miyoshi, I. Prevalence of HTLV-I in leprosy patients in two sanatoriums in Japan. J. Acquir. Immune Defec. Syndr. Hum. Retrovirol. **17** (1998) 380–383.

To determine the association between leprosy and HTLV-I, 450 and 394 leprosy patients in two sanatoriums in Japan (Sanatorium-A in Okayama Prefecture and Sanatorium-B in Gunma Prefecture) were investigated serologically for antibodies to HTLV-I. Serology was positive for HTLV-I in 38 (8.4%) of 450 leprosy patients in Sanatorium-A and in 34 (8.6%) of 394 patients in Sanatorium-B. Prevalence was much higher than that in the general population of these areas in Japan. A large proportion of HTLV-I-positive patients in both sanatoriums came from HTLV-I nonendemic areas in Japan, suggesting that HTLV-I infection occurred after the patients arrived at the sanatoriums. Infection through sexual contact or reuse of needles for frequent vaccination are possible routes of infection for HTLV-I in these cases.—Authors' Abstract

Ramesh, A., Sampath, V., Kumar, K. V. S., Janaki, V. R. and Boopalraj, J. M. Cauda equina syndrome masquerading as leprosy. Indian J. Lepr. **69** (1997) 275–279.

Cases are reported in 25- and 55-year-old men from India who presented with anesthesia over the saddle area, which was initially diagnosed as leprosy. Both patients were unresponsive to leprosy treatment and further examination revealed cauda equina syndrome. It is recommended that patients with trophic ulcer but without any other evidence of leprosy and with anesthesia in the saddle region should be investigated for cauda equina syndrome.—Authors' Abstract

Sampaio, R. N. R., Ribeiro, A. M. Q., Milfont, M. D. A. and Leite, E. [Onychromycosis and tinea pedis caused by *Microsporum nanum* in a patient with lepromatous leprosy.] An. Bras. Dermatol. **72** (1997) 571–574. (in Portuguese)

Microsporum nanum is a dermatophyte commonly found in swines (presenting or not cutaneous lesions) that seldom infects humans. In this report we describe a case of *M. nanum*, tinea pedis and tinea unguinum presented by it hog raiser affected by lepromatous leprosy. This patient was found to have plantar hyperkeratosis and onycholysis in all toenails and some fingernails of the right hand, and cultures of the skin and nail lesions yielded *M. nanum* colonies. Itraconazole pulse therapy resulted in improvement of ungueal lesions and resolution of plantar hyperkeratosis, although the plantar mycological smears remained positive after 15 months. Despite being known to affect the skin and the scalp, this seems to be the first report of ungueal involvement by *M. nanum*.—Authors' English Summary

Sayal, S. K., Das, A. L. and Gupta, C. M. Concurrent leprosy and HIV infection: a report of three cases. Indian J. Lepr. **69** (1997) 261–265.

Three cases of concurrent infection with HIV and leprosy are reported. One had developed borderline lepromatous leprosy 1

year after identifying HIV infection, while the other two had indeterminate leprosy and both conditions were identified at the same time in these two patients. All three cases showed satisfactory response to standard antileprosy multidrug therapy.—Authors' Abstract

Sethi, N. C., Madadi, A. J. and Bhandari, S. Serum zinc, copper, magnesium, proteins and superoxide dismutase in leprosy patients on multidrug therapy—a follow-up study. Indian J. Lepr. **68** (1996) 325–333.

Serum zinc, copper, magnesium, total proteins and albumin-globulin fractions and superoxide dismutase (SOD) were estimated in 80 untreated patients from India with TT/BT/BL/LL types of leprosy and in 40 control subjects. The investigations were repeated on days 30, 60 and 120 after the patients started multidrug therapy (WHO/MDT). Serum zinc was significantly lowered in all types of leprosy on days 0 and 30. Serum copper was significantly raised in all types of leprosy. This was not significant in BT/TT cases on days 60 and 120. There was a correlation between serum zinc and copper levels and the severity and type of leprosy. The lowering of serum magnesium values were not significant. With therapy, there was a shift of all three elements toward normal values. Serum total proteins reduction was not significant. Serum albumin was significantly lowered in all types of leprosy, while serum globulin was significantly raised. This rise in TT/BT was not significant on day 60 and 120 after starting treatment. Serum SOD was significantly reduced in all untreated cases and gradually increased with the clinical improvement under WHO/MDT.—Authors' Abstract

Sharma, V. K., Kaur, I., Vatve, M. and Kumar, B. Rifampicin-induced urticaria in leprosy. Lepr. Rev. **68** (1997) 331–332.

A 28-year-old housewife from Uttar Pradesh, India, had suffered from lepromatous leprosy with necrotic erythema nodosum leprosum (ENL) for the last 2 years. Her bacteriological and morphological indices were 4+ and 1%, respectively, and a skin biopsy confirmed the diagnosis of lepromatous leprosy with ENL. Her renal, hepatic and hematologic parameters were within normal limits except for hemoglobin of 8 g%. She was started on WHO-MBA (rifampin, dapsone and clofazimine) and prednisolone 40 mg daily. Within a half hour of the first loading dose, the patient developed severe itching over the trunk and extremities followed by urticaria. There was no rhinorrhea, fever, bronchospasm or hypotension. Urticaria subsided within 4–6 hr after administration of an antihistaminic, and there was no recurrence of symptoms during daily intake of dapsone and clofazimine. Urticaria recurred within 30 min of the 2nd and 3rd loading doses and increased in severity. Urticaria did not recur when rifampin was omitted from the 4th loading dose onward. The patient was treated with oflaxacin 400 mg daily for 8 weeks and continued on dapsone and clofazimine. There was no recurrence of urticaria during 2 years of follow up. An open patch test and prick test with rifampin dissolved in acetone were negative but administration of 300 mg rifampin under observation led to the development of itching and urticaria within 30 min.—Authors' Summary

Sirmour, S. K., Verma, P. K., Singh, J. N. and Okhandiar, R. P. Semen biochemistry of leprosy patients. Indian J. Lepr. **69** (1997) 251–254.

Studies have been made on the semen of three categories (borderline, borderline tuberculoid and lepromatous) of leprosy patients to evaluate the seminal biochemical constituents (fructose, glycerylphosphorylcholine and acid phosphatase) besides the physical properties (volume, pH, liquefaction time, sperm density and sperm motility). In all categories of leprosy patients, seminal pH, liquefaction time and sperm density underwent significant declines. The declines in the seminal volume and sperm motility were significant only in borderline leprosy. It was observed that seminal glycerylphosphorylcholine (GPC) concentration and acid phosphatase activity declined in all categories of leprosy patients but GPC showed a significant decline only in border-

line tuberculoid and acid phosphatase declined significantly only in borderline and lepromatous leprosy.—Authors' Abstract

Soni, N. K. Intranasal anaesthesia in lepromatous leprosy. Trop. Doc. **27** (1997) 89–90.

The definition and measurement of disabilities related to leprosy, the magnitude of the problem and current situation worldwide, and estimated global prevalence are discussed.—Authors' Abstract

Sujai, S., Vilvanathan, K., Nisha, K. and Arunthathi, S. Skin smears in leprosy: is reduction in number of sites justified? Acta Leprol. **10** (1997) 191–194.

An analysis of 377 sets of positive skin smears from leprosy patients was done to determine the minimum sites needed to detect all smear-positive leprosy patients. A combination of earlobe and a selective site could pick up 95.5% of the patients. An additional smear from the forehead increased the sensitivity to 97.7%. The results suggest that the sites for skin smears may be reduced to a combination of the earlobe and one selective smear site from a skin lesion to be able to detect most smear-positive leprosy patients.—Authors' Summary

Tendolkar, U. M., Varaiya, A., Ahuja, A. S., Motwane, S. A. and Gogate, A. S. Corneal ulcer caused by *Nocardia asteroides* in a patient with leprosy. J. Clin. Microbiol. **36** (1998) 1154–1156.

Nocardia asteroides is a rare cause of keratitis usually associated with trauma. We report a case of corneal ulceration caused by *N. asteroides* in a patient with leprosy. This is the first case report of nocardial keratitis from Southeast Asia. The diminished corneal sensation in a patient with leprosy could be a predisposing factor for development or exacerbation of corneal ulceration.—Authors' Abstract

Wilder-Smith, E., Wilder-Smith, A. and Egger, M. Peripheral autonomic nerve dysfunction in asymptomatic leprosy contacts. J. Neurol. Sci. **150** (1997) 33–38.

In endemic areas, subclinical autonomic nerve dysfunction may be a manifestation of infection with *M. leprae* and possibly allow detection before progression to clinical disease. Vasomotor reflex (VMR) testing was performed in 36 asymptomatic leprosy contacts (24 household contacts, 12 hospital contacts) and 47 age- and sex-matched controls in Pokhara, Nepal. Mean age was 30 years; two thirds were male. A Moor Instruments DRT4 laser doppler monitor was used for velocimetry of microvascular blood flow. The flow reduction following an inspiratory gasp was recorded from finger and toe tips. Mean percent reduction was 57.8 (standard deviation 14.6) among household contacts, 61.9 (17.5) among hospital contacts and 66.8 (7.8) among controls ($p = 0.1$ by analysis of variance). The prevalence of abnormal test results was 54% among household contacts, 42% among hospital contacts and 15% among controls ($p = 0.0005$ by chi-squared test for trend). Subclinical autonomic neuropathy is common among healthy contacts of leprosy patients. Prospective studies are now needed to clarify to what extent abnormal VMR tests predict the risk of progression to clinical disease.—Authors' Abstract

Immuno-Pathology

Agrewala, J. N., Kumar, B. and Vohra, H. Potential role of B7-1 and CD28 molecules in immunosuppression in leprosy. Clin. Exp. Immunol. **111** (1998) 56–63.

In order to understand the mechanism of unresponsiveness towards *Mycobacterium leprae* antigens in leprosy, we evaluated the role of *M. leprae* sonicate antigens in regu-

lating the expression of the costimulatory molecules B7-1, CD28, intercellular adhesion molecule-1 (ICAM-1), LFA-1 alpha, LFA-1 beta and Mac-1 on the lymphocytes of both leprosy patients and healthy subjects. It was observed that the expression of B7-1 and CD28 was significantly decreased but the levels of ICAM-1 and LFA-lcr were increased in patients with untreated borderline leprosy (BL)/lepromatous (LL) leprosy disease. No remarkable change was noticed in the case of borderline tuberculoid (BT) leprosy or treated BL/LL patients. Further, a striking finding was that lymphocytes from healthy subjects cultured with a particularly high dose of *M. leprae* sonicate antigens downregulated the expression of B7-1 and CD28 molecules, but upregulated the display of ICAM-1 and LFA-1 alpha. Furthermore, proliferation induced by *M. leprae* sonicate was inhibited only by anti-B7-1 antibody. *M. leprae* antigen-induced suppression of the proliferation of lymphocytes of healthy volunteers and LL patients was reversed by culturing the lymphocytes with purified protein derivative (PPD). It may be concluded from the findings in this study that downregulation of B7-1 and CD28 in BL/LL leprosy patients may be responsible for a defective T-cell signalling by the B7-1/CD28 pathway caused by *M. leprae* antigens. This may lead to clonal inactivation of *M. leprae*-reactive T cells, consequently the bacilli grow without restriction in macrophages.—Authors' Abstract

Arias, M., Rojas, M., Zabaleta, J., Rodriguez, J. I., Paris, S. C., Barrera, L. F. and Garcia, L. F. Inhibition of virulent *Mycobacterium tuberculosis* by Bcg^r and Bcg^s macrophages correlates with nitric oxide production. J. Infect. Dis. **176** (1997) 1552–1558.

The Nramp1 gene controls macrophage resistance or susceptibility to several intracellular microorganisms; however, there is conflicting evidence regarding its role during infection with virulent *Mycobacterium tuberculosis*. Nitric oxide (NO) is a potent antimycobacterial agent produced by macrophages, which is also regulated by Nramp1. The *in vitro* ability of B10R (resistant) and B10S (susceptible) murine macrophages to inhibit *M. tuberculosis* H37Rv and to produce NO in response to infection and interferon-gamma (IFN-γ) was compared. Infected B10R macrophages inhibited [H-3]uracil incorporation by *M. tuberculosis* and produced higher amounts of NO than did BIOS macrophages. IFN-γ increased the inhibitory activity of both cells. Inhibition of *M. tuberculosis* by IFN-γ-activated B10R macrophages was reversed by N-G-monomethyl-L-arginine (N(G)MMA). L-arginine restored NO production and increased the antimycobacterial activity by IFN-γ-stimulated N(G)MMA-treated macrophages. The Bcg/Nramp1 gene may regulate macrophages resistance or susceptibility to virulent *M. tuberculosis* by a differential capability of these cells to produce NO.—Authors' Abstract

Balaji, K. N. and Boom W. H. Processing of *Mycobacterium tuberculosis* bacilli by human monocytes for CD4+ alpha beta and gamma delta T cells: role of particulate antigen. Infect. Immun. **66** (1998) 98–106.

Mycobacterium tuberculosis readily activates both CD4+ and V delta 2+ gamma delta T cells. Despite similarity in function, these T-cell subsets differ in the antigens they recognize and the manners in which these antigens are presented by *M. tuberculosis*-infected monocytes. We investigated mechanisms of antigen processing of *M. tuberculosis* antigens to human CD4 and gamma delta T cells by monocytes. Initial uptake of *M. tuberculosis* bacilli and subsequent processing were required for efficient presentation not only to CD4 T cells but also to V delta 2+ gamma delta T cells. For gamma delta T cells, recognition of *M. tuberculosis*-infected monocytes was dependent on V delta 2+ T-cell-receptor expression.

Recognition of *M. tuberculosis* antigens by CD4+ T cells was restricted by the class II major histocompatibility complex molecule HLA-DR. Processing of *M. tuberculosis* bacilli for V delta 2+ gamma delta T cells was inhibitable by Brefeldin A; whereas processing of soluble mycobacterial antigens for gamma delta T cells was not sensitive to

Brefeldin A. Processing of *M. tuberculosis* bacilli for CD4+ T cells was unaffected by Brefeldin A. Lysosomotropic agents such as chloroquine and ammonium chloride did not affect the processing of *M. tuberculosis* bacilli for CD4+ and gamma delta T cells. In contrast, both inhibitors blocked processing of soluble mycobacterial antigens for CD4+ T cells. Chloroquine and ammonium chloride insensitivity of processing of *M. tuberculosis* bacilli was not dependent on the viability of the bacteria, since processing of both formaldehyde-fixed dead bacteria and mycobacterial antigens covalently coupled to latex beads was chloroquine insensitive. Thus, the manner in which mycobacterial antigens were taken up by monocytes (particulate versus soluble) influenced the antigen processing pathway for CD4+ and gamma delta T cells.—Authors' Abstract

Beuria, M. K., Parkash, O., Joshi, B., Mohanty, K. K., Katoch, K. and Sengupta, U. Levels of IgG subclasses in active and inactive cases in the disease spectrum of leprosy. Int. Arch. Allergy Immunol. **115** (1998) 61–66.

The present study was carried out to establish the role of IgG subclasses in leprosy. IgG subclasses to *Mycobacterium leprae* sonicated antigens (MLSA) and phenolic glycolipid-I (PGL-I) were determined in 124 patients with active leprosy across the disease spectrum and in 76 cases with inactive disease after completion of chemotherapy. IgG2 antibodies were found to be the predominant subclass across the disease spectrum. Lepromatous patients showed elevated levels of IgG1. IgG3 antibody levels were higher in lepromatous than that in tuberculoid patients. Patients with erythema nodosum leprosum showed a significant fall in IgG3 antibody to MLSA. While chemotherapy induced a reduction in IgG1, IgG2 and IgG3 to PGL-I in almost all types of leprous patients, for MLSA the reduction was noticed for these subclasses only in lepromatous patients. IgG4 responses to these antigens were low throughout the disease spectrum and did not alter with chemotherapy.—Authors' Abstract

Blackwell, J. M. Genetics of host resistance and susceptibility to intramacrophage pathogens: a study of multicase families of tuberculosis, leprosy and leishmaniasis in northeastern Brazil. Int. J. Parasitol. **28** (1998) 21–28.

Genetic analysis of disease phenotypes segregating in recombinant inbred, congenic and recombinant haplotype mouse strains permitted us to effectively "scan" the murine genome for genes controlling resistance and susceptibility to leishmanial infections. Five major regions were implicated which, because they show conserved synteny with regions of the human genome, immediately provide candidate gene regions for human disease susceptibility genes. A common intramacrophage niche for leishmanial and mycobacterial pathogens, and a similar spectrum of immune response and disease phenotypes, also led to the prediction that the same genes/candidate gene regions might be responsible for genetic susceptibility to mycobacterial infections such as leprosy and tuberculosis. Indeed, one of the murine genes (Nramp1) was identified for its role in controlling a range of intramacrophage pathogens, including leishmanial, salmonella and mycobacterial infections. In recent studies, multicase families of visceral leishmaniasis, tuberculosis and leprosy from northeastern Brazil have been analyzed to determine the role of these candidate genes/regions in humans. Complex segregation analysis provides evidence for one or two major genes controlling susceptibility to these diseases in this population. Family-based linkage analyses (e.g., combined segregation and linkage analysis; sib-pair analyses) and transmission disequilibrium testing have been used to examine the role of four regions in disease susceptibility and/or immune response phenotypes. Results to date demonstrate: 1) the major histocompatibility complex (MHC: H-2 in mouse, HLA in humans: mouse chromosome 17/human 6p; candidates class II and class III including tumor necrosis factor alpha/beta genes) shows both linkage to, and allelic association with, leprosy *per se,* but is only weakly associated with visceral leishmaniasis and shows neither linkage to, nor allelic association with, tuberculosis; 2) no evidence for

linkage between NRAMP1, the positionally cloned candidate for the murine macrophage resistance gene Ity/Lsh/Bcg (mouse chromosome 1/human 2q35), and susceptibility to tuberculosis or visceral leishmaniasis; 3) the region of human chromosome 17q (candidates NOS2A, SCYA2-5) homologous with distal mouse chromosome 11 is linked to tuberculosis susceptibility; and 4) the "T helper 2" cytokine gene cluster (proximal murine chromosome 11/human 5p; candidates IL4, IL5, IL9, IRF1, CD14) is not linked to human disease susceptibility for any of the three infections, but shows linkage to and highly significant allelic association with the ability to mount an immune response to mycobacterial antigens. The demonstration of an allelic association between IL-4 and immune response to mycobacterial antigen may provide a genetic explanation for the inverse association recently demonstrated between delayed hypersensitivity T helper 1 responses to mycobacterial antigen and atopic disorder in Japanese children. These studies demonstrate that the "mouse-to-human" strategy, refined by our knowledge of the human immune response to infection, can lead to the identification of important candidate gene regions in humans.—Author's Abstract

Bonato, V. L. D., Lima, V. M. F., Tascon, R. E., Lowrie, D. B. and Silva, C. L. Identification and characterization of protective T cells in hsp65 DNA-vaccinated and *Mycobacterium tuberculosis*-infected mice. Infect. Immun. **66** (1998) 169–175.

Immunization by intramuscular injection of plasmid DNA expressing mycobacterial 65-kDa heat shock protein (hsp65) protects mice against challenge with virulent *Mycobacterium tuberculosis* H37Rv. During infection or after immunization, CD4+/CD8– and CD8+/CD4-hsp65-reactive T cells increased equally in spleens. During infection, the majority of these cells were weakly CD44 positive ($CD44^{lo}$) and produced interleukin 4 (IL-4); whereas after immunization the majority were highly CD44 positive ($CD44^{hi}$) and produced gamma interferon (IFN-γ). In adoptive transfer of protection to naive mice, the total CD8+/CD4– cell population purified from spleens of immunized mice was more protective than that from infected mice. When the cells were separated into CD4+/CD8– and CD8+/CD4– types and then into $CD44^{hi}$ and $CD44^{lo}$ types, $CD44^{lo}$ cells were essentially unable to transfer protection, the most protective $CD44^{hi}$ cells were CD8+/CD4–, and those from immunized mice were much more protective than those from infected mice. Thus, whereas the $CD44^{lo}$ IL-4-producing phenotype prevailed during infection, protection was associated with the CD8+/$CD44^{hi}$ IFN-γ-producing phenotype that predominated after immunization. This conclusion was confirmed and extended by analysis of 16 hsp65-reactive T-cell clones from infected mice and 16 from immunized mice; the most protective clones, in addition, displayed antigen-specific cytotoxicity.—Authors' Abstract

Buhrer, S. S., Smits, H. L., Gussenhoven, G. C., Van Ingen, C. W. and Klatser, P. R. A simple dipstick assay for the detection of antibodies to phenolic glycolipid-I of *Mycobacterium leprae*. Am. J. Trop. Med. Hyg. **58** (1998) 133–136.

Among the many reported applications of the detection of antibodies to phenolic glycolipid-I (PGL-I) of *Mycobacterium leprae*, in particular, the use of seroprevalence as an indicator of the magnitude of the leprosy problem may turn out to be very useful in leprosy control programs. An operational function of serology within the leprosy control services requires a simple test system. We have developed a simple dipstick assay for the detection of antibodies to PGL-I and compared its performance with that of an ELISA. A high degree of agreement (97.2%) was observed between the ELISA and the dipstick assay when tested on 435 sera; the agreement beyond chance (kappa value) was 0.92. No significant difference was found between the dipstick assay and the ELISA when seropositivity rates obtained in groups of leprosy patients, household contacts, and controls were compared. The interpretation of the dipstick results as positive or negative was unequivocal, as illustrated by the high agreement between dif-

ferent persons reading the test (kappa values >0.88). Storage of the only reagents required, the dipsticks and the stabilized detection reagent, up to 3 weeks under tropical conditions of high temperatures, high humidity, and exposure to light, did not influence the results of the assay. The dipstick assay described here is an easy-to-perform method for the detection of IgM antibodies to PGL-I of *M. leprae;* it does not require any special equipment and the highly stable reagents make the test robust and suitable for use in tropical countries. An internal control validates the performance of the assay. This dipstick assay may be the method of choice for epidemiologic mapping of leprosy.—Authors' Abstract

Chiplunkar, S. V., Deshmukh, M. A., Kode, J. A., Gangal, S. G. and Deo, M. G. Ability of lymphokine-activated killer cells to lyse mycobacteria-infected cells. Acta Leprol. **10** (1997) 203–208.

Lymphokine-activated killer (LAK) cells were generated by interleukin-2 activation of peripheral blood lymphocytes obtained from lepromatous (LL) leprosy patients and healthy individuals. The ability of LAK cells to lyse targets (macrophages and T-24, a bladder carcinoma cell line) infected with mycobacteria (*Mycobacterium leprae* and mycobacterial strain ICRC) was assessed in a 51 chromium-release assay. It was observed that LAK cells generated from LL patients and healthy individuals could preferentially lyse *M. leprae* or ICRC-pulsed macrophages and T-24 cells, compared to nonpulsed targets. The ability of LAK cells to kill intracellular mycobacteria was demonstrated in colony forming assays. These results indicate a promising role for LAK cells in immunotherapy of leprosy.—Authors' Summary

De Hass, C. J. C., De Vos, N. M., Visser, M. R., Snippe, H. and Verhoef, J. Monocytes modulate enhancement of HIV-1 replication by *Mycobacterium tuberculosis.* Clin. Exp. Immunol. **111** (1998) 286–292.

To investigate the effects of *Mycobacterium tuberculosis* on HIV-1 replication, peripheral blood mononuclear cells (PBMC) of bacille Calmette-Guerin (BCG)-vaccinated donors and non-BCG-vaccinated donors were infected *in vitro* with a lymphotropic isolate of HIV-I and cultured in the presence of purified protein derivative (PPD). The addition of PPD resulted in enhanced HIV-I replication and lymphoproliferation in BCG-vaccinated donor PBMC, while PPD had no such effects in control PBMC. HIV-1 replication increased even more when monocytes were removed from PBMC, while lymphoproliferation was decreased. High percentages of monocytes were associated with a decreased HIV-1 replication and proliferation that could not be reversed by the addition of antibodies against the cytokines IL-1, transforming growth factor-beta or indomethacin. PPD stimulates PBMC to release IL-10, a cytokine known to downregulate proliferation and HIV-1 replication. PPD-induced effects on proliferation as well as HIV-1 replication could be partially blocked by adding a monoclonal antibody against MHC class II molecules, suggesting that part of the mechanism of PPD-induced enhancement is T-memory cell activation.—Authors' Abstract

Deretic, V., Via, L. E., Fratti, R. A. and Deretic, D. Mycobacterial phagosome maturation, rab proteins, and intracellular trafficking. Electrophoresis **18** (1997) 2542–2547.

One of the most prominent features of pathogenic mycobacteria, which include the potent human pathogens *Mycobacterium tuberculosis* and *M. leprae* and their opportunistic relatives *M. avium* and *M. marinum,* is their ability to survive and multiply in phagosomes of mononuclear phagocytic cells. The phagocytosed mycobacteria reside in a vacuolar compartment which is exempted from maturation into the phagolysosome. Recently, the arrest of the maturation of phagosomes containing *M. tuberculosis* complex organisms (*M. bovis* BCG) has been linked to the accumulation on the phagosomal membrane of the small GTP binding protein rab5, specific for the control of fusion within the early endosomal compartment. Furthermore, the *M. bovis* BCG phagosome is devoid of rab7, a rab

protein associated with the late endosome. The selective accumulation of rab5 and exclusion of rab7 defines the check point that has been compromised in mycobacterial phagosome maturation. Here we summarize these observations and relate them to other phenomena in the area of membrane and protein trafficking with the emphasis on phagosomes containing intracellular pathogens.—Authors' Abstract

Dipiro, J. T. Cytokine networks with infection: mycobacterial infections, leishmaniasis, human immunodeficiency virus infection, and sepsis. Pharmacotherapy **17** (1997) 205–223 (175 refs.).

Distinct cytokine profiles are clearly associated with and relate to the severity of several types of infections. Cytokine networks are apparent with selected human infectious diseases, such as mycobacterial infections (leprosy, tuberculosis), the parasitic infection leishmaniasis, human immunodeficiency virus (HIV) infection, and gram-negative sepsis. Cytokine profiles are determined to some extent by two functional subsets of T lymphocytes, Th1 and Th2. The Th1 cytokines (interferon-gamma interleukin-2 (IL-2), IL-12) enhance cell-mediated immunity, inhibit humoral immunity, and result in protective effect for pathogens that are removed primarily through cell-mediated immunity (*Mycobacterium tuberculosis, M. leprae,* leishmania). The Th2 cytokines (IL-4, IL-5, IL-10, IL-13) enhance humoral immunity and inhibit cell-mediated immunity, and result in a protective effect for pathogens removed primarily through humoral mechanisms. Progression of HIV infection is associated with a switch from a Th1 to a Th2 profile. For sepsis, uncontrolled activation of pro-inflammatory cytokines (IL-1, tumor necrosis factor-alpha, interferon-gamma) may be a fundamental defect that promotes the detrimental aspects of inflammation; whereas Th2 cytokines may be beneficial in controlling inflammation. Knowledge of basic cytokine immunopharmacology, networks, and relationships with infectious processes will aid clinicians in determining treatment approaches that are likely to be effective.—Author's Abstract

dos Santos, I. B. and Pereira, A. C., Jr. [Reactivity of cutaneous leprosy lesions to epicutaneous and intradermal immunologic agents.] An. Bras. Dermatol. **72** (1997) 539–545. (in Portuguese)

Background. We found two cases of skin immunological diseases whose extension was limited by leprous cutaneous lesions, thus suggesting different immunological reactions on the lesions and on the healthy skin of the same patient.

Objectives. To check experimentally, through epicutaneous testing with DNCB and intradermic testing with candida antigen, in the cutaneous lesion of TT and BT leprosy, if there are changes to the cellular immunity, in relation to an apparently normal skin in the same patient.

Methods. Thirty-nine volunteers with TT and BT leprosy were selected. In 26 patients epicutaneous tests with DNCB and 13 intradermic tests with candida antigen were applied. The results of such tests were evaluated by clinical responses, histopathological study and the immunohistochemical examination of the cellular components of the reactions.

Results. The clinical response to the epicutaneous application of DNCB on TT and BT leprous lesions was more intense than on the healthy skin of the same patient. There was no difference in the intradermic application of candida antigen on these areas. The Langerhans' cells showed reduction in number on the areas where DNCB was applied; the number remaining unaltered on the areas tested with candida antigen.

Conclusions. This study proved, experimentally, that a leprous cutaneous lesion (TT and BT) responds in a more intense way to the immunogen applied epicutaneously than the healthy skin of the same patient. It suggests that TT and BT leprosy interferes in the reactivity of the skin in the cutaneous lesion and that the Langerhans' cells do not participate in this reactivity.—Authors' English Summary

Hetland, G., Wiker, H. G., Hogasen, K., Hamasur, B., Svenson, S. B. and Harboe, M. Involvement of antilipoarabinomannan antibodies in classical comple-

ment activation in tuberculosis. Clin. Diagn. Lab. Immunol. **5** (1998) 211–218.

We examined alternative and classical complement activation induced by whole bacilli of *Mycobacterium bovis* BCG and *M. tuberculosis* products. After exposure to BCG, there were higher levels of the terminal complement complex in sera from Indian tuberculosis patients than in sera from healthy controls.

The addition of BCG with or without EGTA to these sera indicated that approximately 70% to 85% of the total levels of the terminal complement complex was formed by classical activation. Sera from Indian tuberculosis patients contained more antibody to lipoarabinomannan (LAM) than sera from healthy Indians. Levels of anti-LAM immunoglobulin G2 (IgG2), but not anti-LAM IgM, correlated positively with classical activation induced by BCG in the sera. By flow cytometry, deposition of C3 and terminal complement complex on bacilli incubated with normal human serum was demonstrated. The anticomplement staining was significantly reduced in the presence of EGTA and EDTA. Flow cytometry also revealed the binding of complement to BCG incubated with rabbit anti-LAM and then with factor B-depleted serum. This indicates that classical activation plays a major role in complement activation induced by mycobacteria and that anti-LAM IgG on the bacilli can mediate this response. Classical complement activation may be important for the extent of phagocytosis of *M. tuberculosis* by mononuclear phagocytes, which may influence the course after infection.—Authors' Abstract

Kampirapap, K. and Singtham, N. Anti-PGL-I antibody levels in Thai leprosy patients. Southeast Asian J. Trop. Med. Public Health **27** (1996) 728–733.

IgM antibody levels against phenolic glycolipid-I (PGL-I) were measured in 258 Thai leprosy patients between October 1992 and April 1994 by a commercially available *Mycobacterium leprae* particle agglutination test (MLPA). The percentage of seropositivity was much higher in newly untreated multibacillary (MB) patients (47 of 56, 83.9%) than in paucibacillary (PB) patients (8 of 45, 17.8%). Antibody titers in the MB group (56 patients) varied in the range 32–8192; whereas they varied in the range 32–256 in the PB group (45 patients). Patients being treated with multidrug therapy (MDT) were 68.3% (86 of 126) and 19.4% (6 of 31) seropositive in the MB and PB groups, respectively. Seropositivities in control serum specimens were 11.3% (53 of 468) in active pulmonary tuberculosis patients, 2.6% (2 of 77) in dermatological patients and 4.4% (8 of 179) in a healthy population. In conclusion, the anti-PGL-I assay using MLPA appears to be a sensitive and specific diagnostic tool for the diagnosis of MB patients. Additionally, it may provide an alternative to the BI determination in monitoring MB patients under MDT, and also in the surveillance of such patients after MDT.—Authors' Abstract

Klingler, K., Tchou Wong, K. M., Brandli, O., Aston, C., Kim, R., Chi, C. X. and Rom, W. N. Effects of mycobacteria on regulation of apoptosis in mononuclear phagocytes. Infect. Immun. **65** (1997) 5272–5278.

Since apoptosis is observed in tuberculous granulomas, we investigated the molecular mechanisms underlying the apoptotic pathway in an *in vitro* model of mycobacterial infection of mononuclear phagocytes. We postulated that *Mycobacterium tuberculosis* could trigger the apoptotic pathway in macrophages, resulting in death of the microorganism by modulating the expression of bc1-2, bax, bcl-x ^{L}, and bcl-x ^{S}. We found that the mRNA of bcl-2, an inhibitor of apoptosis, was downregulated in peripheral blood monocytes (PBM) between 2 and 6 hr following infection with *M. bovis* BCG or induction with heat-killed *M. tuberculosis* H37Ra. Western analysis showed a downregulation of the Bcl-2 protein, with a half-life of 24 hr. At the same time points, there was no change in the expression of Bax or Bcl-x^{S}, inducers of apoptosis, but Bcl-x, another inhibitor of apoptosis, was minimally upregulated by BCG. To determine if apoptosis could be a mechanism for growth inhibition *in vivo,* we obtained alveolar macrophages by bron-

choalveolar lavage from involved sites in patients with active pulmonary tuberculosis. Using the TUNEL (terminal deoxynucleotidyltransferase mediated nick end labeling) technique, we observed significantly more apoptosis in involved segments of five tuberculosis patients (14.8 ± 1.9%) than in those of normal controls (<1%, p = 0.02) or in uninvolved segments (4.3 ± 0.9%, p <0.05). We conclude that apoptosis of mononuclear phagocytes induced by *M. tuberculosis* occurs *in vivo* and that in an *in vitro* model of mycobacterial infection, apoptosis may be mediated by downregulation of Bcl-2.—Authors' Abstract

Lowrie, D. B., Silva, C. L. and Tascon, R. E. DNA vaccines against tuberculosis. Immunol. Cell Biol. **75** (1997) 591–594.

This edited transcript of a presentation at the "Vaccines Beyond 2000" conference describes a series of investigations by the authors throwing light on the mechanisms of protective immunity against tuberculosis in mice and raising hope for a new kind of vaccine to replace bacille Calmette-Guerin (BCG). DNA encoding only one or a few protein antigens was found capable of conferring persistent protection equal to the effect of BCG. The essential features seem to be an endogenous origin of the antigen within transfected mouse cells which favors the development of CD8+/CD44^{hi}/IFN-gamma-producing T cells with antigen-specific cytotoxicity. Such cells were the most efficient in adoptive transfer of protection from infected or DNA-vaccinated mice to naive mice.—Authors' Abstract

Moura, A. C. N. and Mariano, M. Lipids from *Mycobacterium leprae* cell wall suppress T-cell activation *in vivo* and *in vitro*. Immunology **92** (1997) 429–436.

The influence of *Mycobacterium leprae* cell-wall lipids on lymphocyte functions has been investigated *in vivo* (delayed-type hypersensitivity) and *in vitro*. The inflammatory response has been earlier evaluated by the mouse foot pad edema model and the delipidated mycobacteria evoked a mild but significant inflammatory response. Herein a higher level of hypersensitivity reaction was observed with delipidated bacilli than with the intact mycobacteria. The lipids obtained from the extract of *M. leprae* external cell wall were used to prepare liposomes, which have not been shown to be toxic to lymphocytes. The method of lipidic extraction and the sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the lipid fraction did not reveal any trace of proteins. Thin-layer chromatography of this extract detected four different bands with an apolar character, suggestive of mycolic and fatty acids. These same *M. leprae* liposomes potently suppressed lymph node cells, as well CD4+ and CD8+ T-cell lines, and an antigen-specific T-cell clone (T 4–9) proliferation, even under potent stimulus. Cholesterol-choline liposomes, unrelated to *M. leprae* liposomes, used as a control in the biological assays showed no significant effect on lymphoblastic activity, which points to the specificity of *M. leprae* lipids. These data demonstrated that *M. leprae* cell-wall lipids induce immune suppression in mice without causing any membrane alteration in T cells as assessed throughout kinetic studies *in vitro*. This fact is closely related to the downregulating effect induced by *M. leprae* lipids which we have previously observed in macrophage functions *in vivo* and *in vitro*. Although this lipidic fraction showed a suppressive action on T lymphocytes *in vitro* (proliferation) and *in vivo* (delayed-type hypersensitivity), its possible significance in the establishment of a specific immune response to *M. leprae* must be further investigated.—Authors' Abstract

Moura, A. C. N., Modolell, M. and Mariano, M. Down-regulatory effect of *Mycobacterium leprae* cell wall lipids on phagocytosis, oxidative respiratory burst and tumour cell killing by mouse bone marrow derived macrophages. Scand. J. Immunol. **46** (1997) 500–505.

The authors have previously demonstrated that lipids from *Mycobacterium leprae* cell walls inhibit macrophage functions and are endowed with antiinflammatory properties *in vivo*. To investigate these observations further, the authors describe here

the influence of dead *M. leprae* or of the lipids extracted from the cell wall of the mycobacterium, enclosed in liposomes, on the phagocytic, oxidative respiratory burst and tumoricidal ability of bone-marrow-derived macrophages *in vitro*. Dead *M. leprae* or its cell-wall lipids abrogated the oxidative respiratory burst and phagocytic ability of mouse bone-marrow-derived macrophages. A dose-dependent inhibitory effect of the bacterial lipid extract on tumor cell killing by lipopolysaccharide (LPS)-activated, bone-marrow-derived macrophages was demonstrated. However, when delipidated *M. leprae* were added to cultures of bone-marrow-derived macrophages, immune phagocytosis and superoxide production was upregulated. *M. leprae* or its lipids did not appear to be toxic to those cells assayed by the MTT (methyl thiazol tetrazolium) test. These data, added to our preceding observations, support the hypothesis that the downregulatory activity of *M. leprae* wall lipids on macrophage function might be one of the evasive mechanisms of the bacterium to enable it to perpetuate itself in the host tissues.—Authors' Abstract

Narayan, R., Maheshwari, P. K., Desikan, K. V. and Harinath, B. C. Detection of S-100 antigen and anticeramide antibody in sera of leprosy patients with and without reaction. Indian J. Lepr. **69** (1997) 347–352.

Levels of anticeramide antibodies and S-100 antigen in leprosy patients with and without reaction are compared in this study. The increase in levels of IgM anticeramide antibody in the tuberculoid group of patients with reaction, when compared to those without reaction, is significant ($p < 0.05$). Similarly, a significant increase ($p < 0.01$) was observed in the borderline group with reaction. No significant change in anticeramide antibody levels was observed in the lepromatous group of patients with and without reaction. Mean levels of S-100 were slightly lower in all three groups of patients with reaction, but differences were not statistically significant.—Authors' Abstract

Rambukkana, A., Salzer, J. L., Yurchenco, P. D. and Tuomanen, E. I. Neural targeting of *Mycobacterium leprae* mediated by the G domain of the Laminin-α2 chain. Cell (Cambridge) **88** (1997) 811–821.

The molecular basis of the neural tropism of *M. leprae* was investigated *in vitro* primary Schwann cells and Schwann cell-neuron co-cultures and in cell lines (human mammary cell line HBL 100, erythroleukemic cell line K562 and COS-7 cells) and found to be attributable to the specific binding of *M. leprae* to the laminin-2 (LN-α2) chain on Schwann cell-axon units. Using recombinant fragments of LN-2 (rLN-α2), the *M. leprae*-binding site was localized to the G domain. rLN-α2G mediated *M. leprae* binding to cell lines and to sciatic nerves of dystrophic *dy/dy* mice lacking LN-α2, but expressing laminin receptors. Anti-β_4 integrin antibody attenuated rLN-α2G-mediated *M. leprae* adherence, suggesting that *M. leprae* interacts with cells by binding to β_4 integrin via an LN-α2G bridge. In conclusion, the results indicate a novel role for the G domain of LN-2 in infection and reveal a model in which a host-derived bridging molecule determines nerve tropism of a pathogen.—Authors' Abstract

Rojas, R. E., Demichelis, S. O., Gimenez, M. F., Molinari, M. L. and Segal Eiras, A. Cross-reactivity of anti-10 kD heat shock protein antibodies in leprosy and tuberculosis patients. Medicina (B. Aires) **57** (1997) 581–586.

The response to recombinant 10-kD heat-shock protein (hsp) of *Mycobacterium leprae* (rML10) was evaluated by indirect ELISA in sera from leprosy patients, household contacts, tuberculosis patients and healthy controls in a leprosy-endemic area in the northeast of Argentina. Some technical parameters were analyzed: within-assay and between-assay variability, dose-response curves and detectability indexes (specificity and sensitivity) of ELISA applied to measure anti-10-kDa antibodies. High levels of these antibodies have already

been reported in positive bacilloscopy patients; herein we have also demonstrated that tuberculosis patients sera crossreact with this *M. leprae* antigen. This test seems to have a low sensitivity and specificity for leprosy detection; it confirms that antibodies against highly conserved hsp antigens are important in the polyclonal response against mycobacterial epitopes in leprosy as well as in tuberculosis.—Authors' Abstract

Sampaio, E. P., Moraes, M. O., Nery, J. A. C., Santos, A. R., Matos, H. C. and Sarno, E. N. Pentoxifylline decreases *in vivo* and *in vitro* tumour necrosis factor-alpha (TNF-α) production in lepromatous leprosy patients with erythema nodosum leprosum (ENL). Clin. Exp. Immunol. **111** (1998) 300–308.

Increasing evidence has implicated TNF-alpha as a pivotal molecule involved in the systemic inflammatory manifestations of erythema nodosum leprosum (ENL), an acute inflammatory complication that may occur in the chronic course of leprosy. In the present study, the mechanism of action of pentoxifylline (PTX) as an alternative therapy for the management of leprosy reactions has been evaluated. The effect of PTX on TNF-alpha production was examined in leprosy patients at the protein level and at the transcriptional level as well. Treatment of ENL patients with PTX (1200 mg daily) ameliorated the systemic symptoms and favored the evolution of reactional leprosy lesions. Serum TNF-alpha was assayed before and during treatment with PTX in 15 patients. The increased TNF-alpha levels seen in the circulation during the reaction were dramatically reduced within 3–7 days of therapy. No significant effect on serum IL-6 was noted. *In vitro* TNF-alpha production was assayed upon culture stimulation with *Mycobacterium leprae.* A reduction of inducible TNF-alpha in peripheral blood mononuclear cells (PBMC) was seen after 1–2 weeks of *in vivo* administration of PTX. Furthermore, no effect of the drug on IL-10 secretion was detected in these cultures. A kinetic analysis of the expression of TNF-alpha and IL-6 mRNA at the site of the leprosy lesion was performed in six reactional patients by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). The amount of TNF-alpha mRNA was increased in the tissue during ENL compared with before the reaction, and decreased thereafter following treatment for reaction (either PTX or thalidomide). These data suggest that PTX inhibits TNF-alpha production in ENL patients both *in vivo* and *in vitro,* and it may be useful in the treatment of leprosy patients undergoing ENL. —Authors' Abstract

Sampaio, E. P. and Sarno, E. N. Expression and cytokine secretion in the states of immune reactivation in leprosy. Bras. J. Med. Biol. Res. **31** (1998) 69–76.

Leprosy is a chronic inflammatory disease caused by *Mycobacterium leprae.* The human response to this pathogen exhibits intriguing aspects which are up to now not well understood. The present study discusses the probable mechanisms involved in T-cell specific unresponsiveness observed in lepromatous patients. Analysis of the cytokine profile either in blood leukocytes or in skin specimens taken from leprosy lesions indicates that some parameters of Th1 immune response are present in lepromatous patients under reactional states.—Authors' Abstract

Sethna, B. K., Birdi, T. J. and Antia, N. H. Adherence of *Mycobacterium leprae* to the nasal mucosa is influenced by surface integrity and viability. J. Biosci. **22** (1997) 575–583.

The intranasal route is one of the main routes of *Mycobacterium leprae* infection, and there is a paucity of information regarding the mode of spread of the pattern. The adherence of *M. leprae* to the nasal mucosa, its trapping within the sinuses of the head, and its fate after entry into the host was studied using a mouse model. A comparison of the adherence profile of *M. leprae* and *M. tuberculosis* showed that while larger numbers of *M. tuberculosis* were demonstrated within lungs, greater numbers

of *M. leprae* were present within the sinuses of the head. Adherence of *M. leprae* to the nasal mucosa was dependent on surface integrity since opsonization and heat killing resulted in decreased numbers of *M. leprae* in the nasal sinuses and a greater amount entering the lungs. The adherence appeared to be independent of the viability of the bacilli since similar numbers of formalin-fixed, rifampin-treated and viable *M. leprae* entered the lungs in the initial stages. However, the numbers of rifampin-treated *M. leprae* in the nasal sinuses were 12-fold lower than the numbers of viable *M. leprae.* These results indicated that both viability and surface integrity were important in the entry of *M. leprae* and its consequent dissemination.—Authors' Abstract

Smith, D., Hansch, H., Bancroft, G. and Ehlers, S. T-cell-independent granuloma formation in response to *Mycobacterium avium*: role of tumour necrosis factor-alpha and interferon-gamma. Immunology **92** (1997) 413–421.

We used *Mycobacterium avium* infection in severe combined immunodeficiency (SCID) mice to examine T-cell-independent mechanisms of inflammatory cell recruitment. SCID mice infected with a virulent strain of *M. avium* (TMC724) were able to recruit macrophages to sites of mycobacterial replication and formed organized and coherent granulomas in the absence of functional T cells.

Phagocyte recruitment was almost totally ablated by neutralization of either tumor necrosis factor-alpha (TNF-α) or interferon-gamma (IFN-γ) *in vivo,* demonstrating that granuloma formation was dependent on the presence of these cytokines. This was concomitant with a reduction in the *in situ* cytokine mRNA levels otherwise induced in infected mice, for chemokines, pro-inflammatory and regulatory cytokines, including TNF-α, IFN-γ, macrophage inflammatory protein-1 alpha, interleukin-1 beta and IL-10. Furthermore, *in vivo* treatment of infected mice with anti-asialo GM-I antisera, which depletes natural killer (NK) cells, prevented recruitment of inflammatory cells. *In vitro* studies confirmed that *M. avium* was able to elicit IFN-γ from SCID spleen in a dose-dependent manner. These data show for the first time that secretion of IFN-γ from NK cells can mediate a T-cell-independent pathway of granuloma formation and cellular infiltration in response to mycobacteria.—Authors' Abstract

Sousa, A. O., Henry, S., Maroja, F. M., Lee, F. K., Brum, L., Singh, M., Lagrange, P. H. and Aucouturier, P. IgG subclass distribution of antibody responses to protein and polysaccharide mycobacterial antigens in leprosy and tuberculosis patients. Clin. Exp. Immunol. **111** (1998) 48–55.

Immunoenzymatic assays were developed for the measurement of antibodies against mycobacterial lipoarabinomannan (LAM), a cell-free protein extract (CFX) of *Mycobacterium leprae,* and the 38-kD protein antigen of *M. tuberculosis.* Sera from 108 leprosy patients, belonging to all clinical-immunological forms of the spectrum, and 81 patients with localized or disseminated tuberculosis (TB) were tested for antibodies of the four IgG subclasses. Standard calibration curves were used to allow comparisons between results of different isotypes and specificities. Mean concentrations of total IgG antibodies were higher in the overall leprosy population than in TB patients. In leprosy, levels of anti-CFX increased from tuberculoid toward lepromatous forms, with a clear switch from IgG1 to IgG2 subclass predominance. A similar IgG1 to IgG2 conversion was observed in anti-LAM antibodies, although total levels of anti-LAM were similar in patients with tuberculoid and lepromatous forms. In TB antibodies against polysaccharide and protein antigens were both predominantly of IgG1 subclass, whatever the patient's clinical status, although lower in disseminated forms, probably due to concomitant HIV infection. A hypergammaglobulinemia was also found in most leprosy and TB patients. In TB this was due to increased IgG1 and IgG3, especially in HIV co-infected patients. Based on the current knowledge of the influence of T-cell-secreted cytokines on human immunoglobulin isotype expression, these results do not fit with a putative role of Th1 [such as found in TB and tuber-

culoid leprosy (TT)] and Th2 [such as found in lepromatous (LL) leprosy] environment in the isotypy of antibody responses in mycobacterial infections. Nor do variations of isotypy according to pathological conditions seem to be related to the biochemical nature of antigens, since antibodies to LAM and protein antigens had comparable evolutions of their subclass distribution. Other factors are to be investigated in order to understand better the significance and possible roles of antibodies in mycobacterial diseases.—Authors' Abstract

Tentori, L., Graziani, G., Porcelli, S. A., Sugita, M., Brenner, M. B., Madaio, R., Bonmassar, E., Giulani, A. and Aquino, A. Rifampin increases cytokine-induced expression of the CD1b molecule in human peripheral blood monocytes. Antimicrob. Agents Chemother. **41** (1998) 550–554.

In recent years, it has been shown that a nonclassical, major histocompatibility complex-independent system (i.e., CD1-restricted T-cell responses) is involved in T-cell immunity against nonpeptide antigens. The CD1 system appears to function by presenting microbial lipid antigens to specific T cells, and the antigens so far identified include several known constituents of mycobacterial cell walls. Among the four known human CD1 isoforms, the CD1b protein is the best characterized with regard to its antigen-presenting function. Expression of CD1b is upregulated on human blood monocytes upon exposure to granulocyte/macrophage-colony stimulating factor, alone or in combination with interleukin-4 (IL-4) (S. A. Porcelli, Adv. Immunol. 59:1–98, 1995). Rifampin (RFP) and its derivatives are widely used for chemoprophylaxis chemotherapy against *Mycobacterium tuberculosis*. However, this agent was found to reduce the mitogen responsiveness of human B and T lymphocytes, chemotaxis, and delayed-type hypersensitivity. The present study extends the immunopharmacological profile of RFP by examining its effects on CD1b expression by human peripheral blood monocytes exposed to GM-CSF plus IL-4. The results showed that clinically attainable concentrations (i.e., 2 or 10 μg/ml for 24 hr) of the agent produced a marked increase in CD1b expression on the plasma membrane, as evaluated by fluorescence-activated cell sorter analysis; whereas it had no effect on cytosolic fractions, as indicated by Western blot analysis. This was found to be the result of increased CD1b gene expression, as shown by Northern blot analysis of CD1b mRNA. These results suggest that RFP could be of potential value in augmenting the CD1b-restricted antigen recognition system, thereby enhancing protective cellular immunity to *M. tuberculosis*.—Authors' Abstract

Verhagen, C. E., Kraan, T. C. T. M. V., Buffing, A. A. M., Chand, M. A., Faber, W. R., Aarden, L. A. and Das, P. K. Type 1- and type 2-like skin-derived *Mycobacterium leprae*-responsive T-cell clones are characterized by coexpression of IFN-gamma/TNF-alpha and IL-4/IL-5/IL-13, respectively. J. Immunol. **160** (1998) 2380–2387.

In an earlier study, we generated a large number of *Mycobacterium leprae*-responsive and *M. leprae*-nonresponsive T-cell clones (TCC) from the lesional skin of immunologic unstable borderline leprosy patients. In that study, we divided TCC into type 1- and type 2-like on the basis of their IFN-gamma and IL-4 expressions. To explore whether other cytokines are co-produced along with IFN-gamma and IL-4, we investigated the secretion of a panel of other cytokines (TNF-alpha, IL-5, IL-6, IL-10, and IL-13) by a large number of these TCC. Upon analysis of 139 *M. leprae*-responsive TCC, we observed a positive correlation in the coproduction of IFN-gamma/TNF-alpha ($r = 0.81$), and in that of IL-4/IL-5 ($r = 0.83$), IL-4/IL-13 ($r = 0.80$), and IL-5/IL-13 ($r = 0.82$). Polarized type 1-like TCC produced dominantly IFN-gamma/TNF-alpha, and polarized type 2-like TCC predominantly IL-4/IL-5/IL-13. Most type 0-like TCC produced both sets of cytokines. In contrast, type 1- and type 2-like subsets of *M. leprae*-nonresponsive TCC ($N = 58$) did not show the same co-expression of these cytokines. Furthermore, when the differential expression of a broad panel of cytokines by individual *M. leprae*-

responsive TCC is considered, it appeared that additional phenotypes could be recognized. These results suggested that distinct isotypes of type 1- and type 2-like T cells, based on the secretion of a panel of cytokines, may reflect *M. leprae*-specific characteristics.—Authors' Abstract

Verhagen, C. E., Wierenga, E. A., Buffing, A. A. M., Chand, M. A., Faber, W. R. and Das, P. K. Reversal reaction in borderline leprosy is associated with a polarized shift to type 1-like *Mycobacterium leprae* T-cell reactivity in lesional skin—a follow-up study. J. Immunol. **159** (1997) 4474–4483.

Borderline leprosy patients often undergo acute changes in immune reactivity that manifest as reversal reaction (RR) in the course of the disease. RR is associated with an exacerbated, local delayed-type cellular immune response to *Mycobacterium leprae* and is responsible for severe tissue damage. We investigated whether RR episodes are associated with a change in T-cell subsets in the lesional skin with regard to their cytokine secretion profiles. *M. leprae*-responsive T-cell lines and thereafter T-cell clones (TCC) were generated from the lesional skin of seven untreated borderlineline leprosy patients (with or without RR) and again from three of these patients experiencing RR during treatment.

The phenotypes of the *M. leprae*-responsive TCC were either CD4+, CD8+, CD4–/CD8+/TCR gamma delta+, or CD4–/CD8–/TCR gamma delta+, although most of them were CD4+. Regardless of the clinical status of the untreated patients, a major subset of the *M. leprae*-responsive TCC was type 0-like and produced both IFN-gamma and IL-4. Interestingly, in all three patients who experienced a (re)occurrence of RR during treatment after the first analysis, a clear shift to polarized IFN-gamma production by the *M. leprae*-responsive TCC (type 1-like) was observed. This shift in T-cell subsets was also reflected in the observed decrease in serum IgG and IgM levels of the same patients during RR. These findings indicate that CD4+ *M. leprae*-responsive T cells with a polarized type 1-like phenotype might be responsible for the immune-mediated tissue damage occurring during RR.—Authors' Abstract

Vouret Craviari, V., Cenzuales, S., Poli, G. and Mantovani, A. Expression of monocyte chemotactic protein-3 in human monocytes exposed to the mycobacterial cell wall component lipoarabinomannan. Cytokine **9** (1997) 992–998.

Monocyte chemotactic protein-3 (MCP-3) is a C-C chemokine which interacts with the CCR1, CCR2 (MCP-1) and CCR3 receptors and has a distinct spectrum of action.

The present study was designed to assess whether mycobacterial components were able to induce expression and production of MCP-3 in human monocytes. Mycobacterial lipoarabinomannan (LAM) induced expression of MCP-3 mRNA in human peripheral blood mononuclear cells. The non-mannose-capped version of lipoarabinomannan (AraLAM) was considerably more potent than the mannose-capped version (ManLAM) or the simpler version phosphatidylinositol mannoside (PIM). Among mononuclear cells, monocytes were responsible for LAM-induced MCP-3 mRNA expression. Whole mycobacteria (*Mycobacterium bovis* BCG) strongly induced MCP-3 expression. Pretreatment with actinomycin D abolished LAM-induced MCP-3 expression; whereas cycloheximide only partially reduced the expression. LAM-induced MCP-3 expression was associated with the production of immunoreactive PTX3. Interleukin 10 (IL-10) and IL-13 inhibited the induction of MCP-3 by LAM. Thus mycobacterial cell-wall components induce expression of MCP-3 in human monocytes. MCP-3, a chemokine active on mononuclear phagocytes, NK cells, T cells and dendritic cells, may be relevant to the induction and expression of immunity against mycobacteria.—Authors' Abstract

Zea, A. H., Ochoa, M. T., Ghosh, P., Longo, D. L., Alvord, W. G., Valderrama, L., Falabella, R., Harvey, L. K., Saravia, N., Moreno, L. H. and Ochoa, A. C. Changes in expression of signal

transduction proteins in T lymphocytes of patients with leprosy. Infect. Immun. **66** (1998) 499–504.

Advanced stages of mycobacterial diseases such as leprosy and tuberculosis are characterized by a loss of T-cell function. The basis of this T-cell dysfunction is not well understood. The present report demonstrates major alterations in the expression of signal transduction molecules in T cells of leprosy patients. These alterations were most frequently observed in lepromatous (LL) leprosy patients. Of 29 LL patients, 69% had decreased T-cell receptor zeta-chain expression, 48% had decreased p56(lck) tyrosine kinase, and 63% had a loss of nuclear transcription factor NF-kappa B p65. An electrophoretic mobility shift assay with the gamma-interferon core promoter region revealed a loss of the Th1 DNA-binding pattern in LL patients. In contrast, tuberculoid leprosy patients had only minor signal transduction alterations. These novel findings might improve our understanding of the T-cell dysfunction observed in leprosy and other infectious diseases and, consequently, might lead to better immunologic evaluation of patients.—Authors' Abstract

Zhao, G., *et al.* [Examination of the sera of 278 leprosy (patients) with MLPA.] China Lepr. J. **13** (1997) 195–196. (in Chinese)

Serological examination by MLPA for 278 leprosy patients in several leprosaria, Hube Province, China, showed that the positivity in LL, BL, BB, BT and TT was 62.4%, 56.2%, 66.7%, 31.2% and 22.2%, respectively; in active cases, relapsed ones following DDS monotherapy, persons cured of leprosy, smear-positive patients and those with lepra reaction was 65.1%, 63.2%, 39.8%, 36.5% and 27.6%, respectively. The authors thought that the method is simple, has certain sensitivity and specificity, and could be used for monitoring of leprosy relapse.—Authors' English Abstract

Microbiology

Bunting, K., Cooper, J. B., Badasso, M. O., Tickle, I. J., Newton, M., Wood, S. P., Zhang, Y. and Young, D. Engineering a change in metal-ion specificity of the iron-dependent superoxide dismutase from *Mycobacterium tuberculosis* X-ray structure analysis of site-directed mutants. Eur. J. Biochem. **251** (1998) 795–803.

We have refined the X-ray structures of two site-directed mutants of the iron-dependent superoxide dismutase (SOD) from *mycobacterium tuberculosis.* These mutations which affect residue 145 in the enzyme (H145Q and H145E) were designed to alter its metal-ion specificity. This residue is either Gin or His in homologous SOD enzymes and has previously been shown to play a role in active-site interactions since its side chain helps to coordinate the metal ion via a solvent molecule which is thought to be a hydroxide ion. The mutations were based on the observation that in the closely homologous, manganese-dependent SOD from *M. leprae,* the only significant difference from the *M. tuberculosis* SOD within 10 angstroms of the metal-binding site is the substitution of Gin for His at position 145. Hence, an H145Q mutant of the *M. tuberculosis* (TB) SOD was engineered to investigate this residue's role in metal-ion dependence and an isosteric H145E mutant was also expressed. The X-ray structures of the H145Q and H145E mutants have been solved at resolutions of 4.0 angstroms and 2.5 angstroms, respectively, confirming that neither mutation has any gross effect on the conformation of the enzyme or the structure of the active site. The residue substitutions are accommodated in the enzyme's three-dimensional structure by small local conformational changes. Peroxide inhibition experiments and atomic absorption spectroscopy establish surprisingly that the H145E mutant SOD has manganese bound to it whereas the H145Q mutant SOD re-

tains iron as the active-site metal. This alteration in metal specificity may reflect on the preference of manganese ions for anionic ligands.—Authors' Abstract

Carriere, C., Riska, P. F., Zimhony, O., Kriakov, J., Bardarov, S., Burns, J., Chan, J. and Jacobs, W. R. Conditionally replicating luciferase reporter phages: improved sensitivity for rapid detection and assessment of drug susceptibility of *Mycobacterium tuberculosis.* J. Clin. Microbiol. **35** (1997) 3232–3239.

TM4 is a lytic mycobacteriophage which infects mycobacteria of clinical importance. A luciferase reporter phage, ph4E40, has been constructed from TM4 and was previously shown to be useful for the rapid detection and drug-susceptibility testing of *Mycobacterium tuberculosis.* However, the lytic nature of the phage results in a loss of detectable light output and limits the sensitivity of detection. We describe several strategies aimed at improving the luciferase activity generated by TM4 luciferase phages, including a) varying the position of the luciferase gene in the phage genome, b) isolating host-range mutants of the phage, and c) introducing temperature-sensitive mutations in the phage such that it will not replicate at the infecting temperature. Several new phages generated by these methods show increased intensity of luciferase production compared to the first-generation reporter phage phAE40, and one phage, phAE88, also demonstrates an enhanced duration of luciferase activity.

This has allowed the detection of as few as 120 BCG cells and the determination of drug susceptibilities of *M. tuberculosis* in as little as 1 day.—Authors' Abstract

Chatterjee, D. and Khoo, K. H. Mycobacterial lipoarabinomannan: an extraordinary lipoheteroglycan with profound physiological effects. Glycobiology **8** (1998) 113–120.

Detailed structural and functional studies over the last decade have led to current recognition of the mycobacterial lipoarabinomannan (LAM) as a phosphatidylinositol-anchored lipoglycan with diverse biological activities. Fatty acylation has been demonstrated to be essential for LAM to maintain its functional integrity although the focus has largely been on the arabinan motifs and the terminal capping function.

It has recently been shown that the mannose caps may be involved not only in attenuating host-immune response, but also in mediating the binding of mycobacteria to and subsequent entry into macrophages. This may further be linked to an intracellular trafficking pathway through which LAM is thought to be presented by CD1 to subsets of T cells. The implication of LAM as a major histocompatibility complex (MHC)-independent T-cell epitope and the ensuing immune response is an area of intensive studies. Another recent focus of research is the biosynthesis of arabinan which has been shown to be inhibitable by the antituberculosis drug ethambutol. The phenomenon of truncated LAM as synthesized by ethambutol-resistant strains provides an invaluable handle for dissecting the array of arabinosyltransferases involved, as well as generating much needed structural variants for further structural and functional studies. It is hoped that with more systematic investigations based on clinical isolates and human cell lines, the true significance of LAM in the immunopathogenesis of tuberculosis and leprosy can eventually be explained.—Authors' Abstract

Chua Intra, B., Ivanyi, J., Hills, A., Thole, J., Moreno, C. and Vordermeier, H. M. Predominant recognition of species-specific determinants of the GroES homologues from *Mycobacterium leprae* and *M. tuberculosis.* Immunology **93** (1998) 64–72.

The *Mycobacterium leprae* and *M. tuberculosis* 10000 MW heat-shock protein homologues of GroES have previously been identified as major immunogens for human T cells. We used synthetic peptides to characterize the determinants recognized by murine T cells. The findings suggest that, despite 90% sequence identity between these two proteins, T cells recognize promi-

nently the species-specific determinants localized within amino-acid residues 21–40 and 49–72. Analysis of the molecular determinants of species specificity for the *M. leprae* GroES sequence 25–40, using T-cell hybridomas and major histocompatibility complex (MHC)-binding assays, led to the identification of epitope cores and critical residues. Interestingly, closely overlapping epitope cores were found to be restricted by either H-2A^d (24–34) or H-2E^d (28–34). Furthermore, the site recognized by the *M. leprae*-specific monoclonal antibodies ML06 and ML10 was also localized in the overlapping sequences 25–31 and 25–29. In conclusion, we demonstrated that immunodominant species-specific T- and B-cell epitopes can be found in a mycobacterial heat-shock protein despite its highly conserved amino-acid sequence. This finding suggests the feasibility of identifying a sufficient number of *M. leprae*-specific determinants for a composite T-cell immunodiagnostic reagent for tuberculoid leprosy.—Authors' Abstract

Cunningham, A. F. and Spreadbury, C. L. Mycobacterial stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alpha-crystallin homology. J. Bacteriol. **180** (1998) 801–808.

Most cases of tuberculosis are due to reactivation of endogenous infection which may have lain quiescent or dormant for decades. How *Mycobacterium tuberculosis* survives for this length of time is unknown, but it is hypothesized that reduced oxygen tension may trigger the tubercle bacillus to enter a state of dormancy. *M. bovis* BCG and *M. tuberculosis* H37Rv were cultured under aerobic, microaerobic, and anaerobic conditions. Their ultrastructural morphology was analyzed by transmission electron microscopy (TEM), and protein expression profiles were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). TEM revealed that the microaerobically and anaerobically cultured bacilli but not the aerobically cultured bacilli developed a strikingly thickened cell wall outer layer. The thickening was not observed in aerobically cultured stationary-phase bacilli or in anaerobically cultured *M. smegmatis*. A highly expressed protein was detected by SDS-PAGE in microaerobic and anaerobic cultures and was identified as the 16-kDa small heat-shock protein or alpha-crystallin homolog. Immunolocalization by colloidal gold immunoelectron microscopy identified three patterns of protein distribution in *M. bovis* BCG cultured under low oxygen tension. The 16-kDa protein was strongly associated with the cell envelope, fibrous peptidoglycan-like structures, and intracellular and peripheral clusters. These results suggest that tubercle bacilli may adapt to low-oxygen conditions by developing a thickened cell wall and that the 16-kDa protein may play a role in stabilizing cell structures during long-term survival, thus helping the bacilli survive the low-oxygen tension in granulomas. As such, the cell-wall thickening and the 16-kDa protein may be markers for the dormant state of *M. tuberculosis*.—Authors' Abstract

Dhople, A. M. Comparative *in vitro* activities of rifamycin analogues against *Mycobacterium leprae*. Indian J. Lepr. **69** (1997) 377–384.

Comparative activities of various rifamycin analogs against leprosy were studied by evaluating their effects on *in vitro* growth of *Mycobacterium leprae* in DH medium as described earlier. Among the seven analogs studied, KRM-1648 was found to be the most potent in inhibiting the growth of rifampin-sensitive strains of *M. leprae*, MIC being 0.05 µg/ml. This was followed by KRM-2312 and T_9 (MIC of each being 0.1 µg/ml) and rifabutin (MIC 0.2 µg/ml). Rifampin along with KRM-1657 and KKM-1668 were least effective, MIC for each being 0.4 µg/ml. The effects of each at their respective MICs were bactericidal. The results were similar for rifampin-resistant strains of *M. leprae*, but the MICs were higher than those obtained with rifampin-sensitive strains of *M. leprae*. Thus, even though rifampin has been the first-line drug in the treatment of leprosy, the results in present studies suggest that other ri-

famycin analogs are available that are more potent than rifampin against both rifampin-sensitive as well as rifampin-resistant strains of *M. leprae.*—Author's Abstract

Ehrt, S., Shiloh, M. U., Ruan, J., Choi, M., Gunzburg, S., Nathan, C., Xie, Q. W. and Riley, L. W. A novel antioxidant gene from *Mycobacterium tuberculosis.* J. Exp. Med. **186** (1997) 1885–1896.

Among the major antimicrobial products of macrophages are reactive intermediates of the oxidation of nitrogen (RNI) and the reduction of oxygen (ROI). Selection of recombinants in acidified nitrite led to the cloning of a novel gene, MoxR1, from a pathogenic clinical isolate of *Mycobacterium tuberculosis.* Expression of MoxR1 conferred upon *Escherichia coli* and *M. smegmatis* enhanced ability to resist RNI and ROI, whether the bacteria were exposed to exogenous compounds in medium or to endogenous products in macrophages. These studies provide the first identification of an RNI resistance mechanism in mycobacteria, point to a new mechanism for resistance to ROI, and raise the possibility that inhibition of the MoxR1 pathway might enhance the ability of macrophages to control tuberculosis.—Authors' Abstract

Ginkel, S. Z., Dooley, T. P., Suling, W. J. and Barrow, W. W. Identification and cloning of the *Mycobacterium avium* folA gene, required for dihydrofolate reductase activity. FEMS Microbiol. Lett. **156** (1997) 69–78.

Dihydrofolate reductase is an essential bacterial enzyme necessary for the maintenance of intracellular folate pools in a biochemically active reduced state. In this report, the *Mycobacterium avium* folA gene was identified by functional genetic complementation, sequenced, and expressed for the first time. It has an open reading frame of 543 bp with a G+C content of 73%. The translated polypeptide sequence shows 58% identity to the consensus sequence of the conserved regions from eight other bacterial dihydrofolate reductases. Recombinant *M. avium* dihydrofolate reductase was expressed actively in *Escherichia coli,* and SDS-PAGE analysis revealed a 20-kDa species, agreeable with that predicted from the polypeptide sequence.—Authors' Abstract

Gonzalez y Merchand, J. A., Garcia, M. J., Gonzalez Rico, S., Colston, M. J. and Cox, R. A. Strategies used by pathogenic and nonpathogenic mycobacteria to synthesize rRNA. J. Bacteriol. **179** (1997) 6949–6958.

One rRNA operon of all mycobacteria studied so far is located downstream from a gene thought to code for the enzyme UDP-N-acetylglucosamine carboxyvinyl transferase (UNAcGCT), which is important to cell-wall synthesis. This operon has been designated rrnA^{f} for fast-growing mycobacteria and rrnA^{s} for slow growers. We have investigated the upstream sequences and promoter activities of rrnA^{f} operons of typical fast growers which also possess a second rrn (rrnB^{f}) operon and of the rrnA operons of the fast growers *Mycobacterium abscessus* and *M. chelonae,* which each have a single rrn operon per genome. These fast growers have a common strategy for increasing the efficiency of transcription of their rrnA operons, thereby increasing the cells' potential for ribosome synthesis. This strategy involves the use of multiple (three to five) promoters which may have arisen through successive duplication events. Thus, we have identified a hypervariable multiple promoter region (HMPR) located between the UNAcGCT gene and the 16S rRNA coding region. Two promoters, P1 and PCL1, appear to play pivotal roles in mycobacterial rRNA synthesis; they are present in all of the species examined and are the only promoters used for rRNA synthesis by the pathogenic slow growers. P1 is located within the coding region of the UNAcGCT gene, and PCL1 has a characteristic sequence that is related to but distinct from that of the additional promoters. In fast-growing species, P1 and PCL1 produce less than 10% of rRNA transcripts, so the additional promoters found in the HMPR are important in increasing the potential for rRNA synthesis during rapid growth. In contrast, rrnB operons appear to

be regulated by a single promoter; because less divergence has taken place, rrnB appears to be younger than rrnA.—Authors' Abstract

Gupta, U. D. and Katoch, V. M. Understanding the phenomenon of persistence in mycobacterial infections. Indian J. Lepr. **69** (1997) 385–393.

Persistence of live organisms despite chemotherapy for long periods is a significant problem in both leprosy and tuberculosis. The consequence of this persistence is varying rates of relapses which undermine the success of treatment. The mechanisms of the dormancy are ill-understood, and as explanation a switch to alternate modes of metabolism, such as glyoxylate bypass and other shunts, has been suggested. This presentation reviews the information available on this aspect. In-depth studies by designing and investigating model system(s) using molecular genetic approaches may help in gaining better understanding of the mechanisms of dormancy and persistence in mycobacterial infections and devising appropriate strategies and tools for the better management of these complications.—Authors' Abstract

Gupta, U. D., Katoch, K., Natarajan, M., Sharma, V. D., Sharma, R. K., Shivannavar, C. T. and Katoch, V. M. Viability determination of *M. leprae*: comparison of normal mouse foot pad and bacillary ATP bioluminescence assay. Acta Leprol. **10** (1997) 209–212.

Correlation between viability assessment by mouse foot pad and ATP bioluminescence was studied in biopsy specimens from multibacillary leprosy cases. Biopsies were processed for inoculation into mouse foot pad and estimation of bacillary ATP levels by bioluminescent assay by earlier established procedures. ATP content as pg/million bacilli was estimated and correlation was assessed with growth in the mouse foot pad. It was observed that when the ATP content was >36 pg/million bacterial cells, (>1% probable viables) there was growth in the mouse foot pad from all the specimens. Similar results were observed when the ATP content was in the range of 3.6 to 35.99 pg/million cells (0.1% to 1% probable viables). The positivity rates in the mouse foot pad decreased when the ATP content decreased further. No positive growth in the specimens below 0.04 pg/million bacilli (<0.001% viable organisms) was observed. These findings show an overall correlation between viability assessed by mouse foot pad and ATP bioluminescence. These observations validate the concept of ATP content of viable unit of *M. leprae* being in the order of 10^{-15} g/live cell which is in the same order of magnitude as a colony forming unit of cultivable mycobacteria.—Authors' Summary

Hu, Y. M., Butcher, P. D., Sole, K., Mitchison, D. A. and Coates, A. R. M. Protein synthesis is shut down in dormant *Mycobacterium tuberculosis* and is reversed by oxygen or heat shock. FEMS Microbiol. Lett. **158** (1998) 139–145.

Oxygen-limiting conditions are critical to the survival of the bacteria in tuberculosis. *Mycobacterium tuberculosis* can survive anaerobiosis *in vitro* for long periods of time only after a gradual transition to a microaerophilic stationary phase. The underlying mechanism behind stationary phase adaption needs to be elucidated. The protein profiles of *M. tuberculosis* during long-term stationary phase growth and under strict anaerobic incubation were monitored by [S-35] methionine labelling, SDS-PAGE and fluorography. These experiments have established that protein synthesis gradually decreased over 50 days in the long-term stationary phase cultures which were considered to be microaerophilic. There was an 80% linear decrease in the level of total protein synthesis during the first 40 days of microaerophilic growth and then the rate of protein synthesis faded quickly. For the first time we have shown that total protein synthesis shutdown occurred when bacilli were incubated under further anaerobic conditions. Viability, estimated by cfu counts, remained constant during stationary phase growth and under anaerobic incubation. Furthermore, when oxygen was introduced into the anaerobic culture, protein synthesis

restarted. Also heat shock at 45°C, 48°C and 50°C rapidly induced protein synthesis in stationary and anaerobic cultures. These data indicate that dormant bacteria shut down protein synthesis but remain responsive to specific stimuli which restore protein synthesis. In addition the dormant bacilli induced by anaerobiosis developed more heat resistance than nondormant organisms.—Authors' Abstract

Hughes, M. S., Beck, L. A., Skuce, R. A. and Neill, S. D. Development of mycobacterial species-specific DNA probes by subtraction hybridization. FEMS Microbiol. Lett. **156** (1998) 31–36.

Subtraction hybridization was used to identify sequences of *Mycobacterium bovis* DNA which might be of diagnostic value. Genomic DNA from *M. avium,* isolated commonly from cattle and whose tuberculin is used in the comparative intradermal tuberculin test, was subtracted from *M. bovis* genomic DNA. A novel sequence, of 131 bp, which appears to be *M. tuberculosis* complex-specific was identified. The specificity of this sequence was stringently tested by a probe and polymerase chain reaction (PCR) assay. Nucleotide identity determination and sequence comparisons revealed that the 131-bp sequence is located directly upstream of a potential isocitrate dehydrogenase (IDH) coding gene and may be of diagnostic value, enabling differentiation of *M. tuberculosis* complex mycobacteria from other mycobacterial species.—Authors' Abstract

Katoch, V. M., Saxena, N., Shivannavar, C. T., Sharma, V. D., Katoch, K., Sharma, R. K. and Murthy, P. Effect of trifluoperazine on *in vitro* ATP synthesis by *Mycobacterium leprae.* FEMS Immunol. Med. Microbiol. **20** (1998) 99–102.

The effect of trifluoperazine (TFP), a calmodulin antagonist, was investigated on *in vitro* ATP levels of human-derived *Mycobacterium leprae. M. leprae* were obtained from biopsies from multibacillary forms of leprosy and were incubated in a modified Dubos medium system which supports limited *in vitro* synthesis of *M. leprae.* This incubation was carried out in the absence and presence of different concentrations of trifluoperazine. Samples for estimation of bacillary ATP levels were taken at day 0 and at 14 days of incubation. TFP inhibited ATP levels in *M. leprae* and this inhibitory effect was marginal at 2.5 μg ml^{-1} (35% inhibition), highly significant at 5 μg ml^{-1} (87% inhibition) and almost total at 10 μg ml^{-1} (98.5% inhibition). This compound appears to have potential as an antileprotic drug and also as a broad spectrum antimycobacterial agent in view of its antitubercular activity reported earlier.—Authors' Abstract

Klann, A. G., Belanger, A. E., Abanes De Mello, A., Lee, J. Y. and Hatfull, G. F. Characterization of the dnaG locus in *Mycobacterium smegmatis* reveals linkage of DNA replication and cell division. J. Bacteriol. **180** (1998) 65–72.

We have isolated a W-induced temperature-sensitive mutant of *Mycobacterium smegmatis* that fails to grow at 42°C and exhibits a filamentous phenotype following incubation at the nonpermissive temperature, reminiscent of a defect in cell division. Complementation of this mutant with an *M. smegmatis* genomic library and subsequent subcloning reveal that the defect lies within the *M. smegmatis* dnaG gene encoding DNA primase. Sequence analysis of the mutant dnaG allele reveals a substitution of proline for alanine at position 496. Thus, dnaG is an essential gene in *M. smegmatis,* and DNA replication and cell division are coupled processes in this species. Characterization of the sequences flanking the *M. smegmatis* dnaG gene shows that it is not part of the highly conserved macromolecular synthesis operon present in other eubacterial species but is part of an operon with a dgt gene encoding dGTPase. The organization of this operon is conserved in *M. tuberculosis* and *M. leprae,* suggesting that regulation of DNA replication, transcription, and translation may be coordinated differ-

ently in the mycobacteria than in other bacteria.—Authors' Abstract

Lee, R. E., Brennan, P. J. and Besra, G. S. Mycobacterial arabinan biosynthesis: the use of synthetic arabinoside acceptors in the development of an arabinosyl transfer assay. Glycobiology **7** (1997) 1121–1128.

Information on the biosynthesis of the D-arabinans of the cell wall of *Mycobacterium tuberculosis* is rapidly emerging with the promise of new targets for drug development against tuberculosis. Accordingly, arabinosyl transferase assays were developed utilizing synthesized [1-C-14]-beta-D-arabinofuranosyl-1-monophosphoryldecaprenol as donor and a variety of O- and S-alkyl arabinosides as acceptors. These were: alpha-D-Araf-(1—>5)-alpha-D-Araf-O- and -S-alkyl diarabinosides and alpha-D-Araf-(1—>5)-alpha-D-Araf-(1—>5)-alpha-D-Araf-O- and -S-alkyl triarabinosides. Whereas the O- and S-alkyl monosaccharide acceptors were inactive, the O- and S-alkyl disaccharide and the O- and S-alkyl trisaccharide acceptors (<C12) possessed considerable acceptor activity, and the trisaccharide acceptors were more potent than the corresponding disaccharides. The O-alkyl disaccharide acceptors with a C8 alkyl chain were more active than those containing the C6 or C10 analogs. Chemical analysis of the enzymatically synthesized products of the reactions demonstrated that beta-D-arabinofuranosyl-1-monophosphoryldecaprenol was an effective donor for two of the three potential arabinosyl transferases: beta-D-arabinofuranosyl-1-monophosphoryldecaprenol : arabinan alpha (1—>5) arabinosyl transferase and beta-D-arabinofuranosyl-1-monophosphoryldecaprenol : arabinan beta (1—>2) arabinosyl transferase. The beta (1—>2) arabinosyl transferase activity was more in evidence in the presence of the O-alkyl disaccharide acceptor; whereas both transferases were about equivalent in the presence of the S-alkyl trisaccharide acceptor. The tuberculosis drug ethambutol, a known mycobacterial arabinosyl transferase inhibitor, was inactive within these arabinosyl transferase/acceptor based assay systems, supporting other evidence that a third activity, responsible for the formation of alpha 1—>3 linkage, is the drug target. —Authors' Abstract

Lety, M. A., Nair, S., Berche, P. and Escuyer, V. A single point mutation in the embB gene is responsible for resistance to ethambutol in *Mycobacterium smegmatis.* Antimicrob. Agents Chemother. **41** (1997) 2629–2633.

Ethambutol [EMB; dextro-2,2′-(ethylenediimino)-di-1-butanol] is an effective drug when used in combination with isoniazid for the treatment of tuberculosis. It inhibits the polymerization of arabinan in the arabinogalactan and lipoarabinomannan of the mycobacterial cell wall. Recent studies have shown that arabinosyltransferases could be targets of EMB. These enzymes are encoded by the *emb* locus that was identified in *Mycobacterium smegmatis, M. leprae, M. avium,* and *M. tuberculosis.* We demonstrate that a missense mutation in the *M. smegmatis embB* gene, one of the genes of the *emb* locus, confers resistance to EMB. The level of resistance is not dependent on the number of copies of the mutated *embB* gene, indicating that this is a true mechanism of resistance. The mutation is located in a region of the EmbB protein that is highly conserved among the different mycobacterial species. We also identified in this region two other independent mutations that confer EMB resistance. Furthermore, mutations have recently been described in the same region of the EmbB protein from clinical EMB-resistant *M. tuberculosis* isolates. Together, these data strongly suggest that one of the mechanisms of resistance to EMB consists of missense mutations in a particular region of the EmbB protein that could be directly involved in the interaction with the EMB molecule.—Authors' Abstract

Manca, C., Lyashchenko, K., Colangeli, R. and Gennaro, M. L. MTC28, a novel 28-kilodalton proline-rich secreted antigen specific for the *Mycobacterium tu-*

berculosis complex. Infect. Immun. **65** (1997) 4951–4957.

Proteins that are actively secreted by *Mycobacterium tuberculosis* serve as major targets of immune responses in the infected host. To identify and purify novel proteins in the filtrates of *M. tuberculosis* cultures, a bacteriophage lambda library of *M. tuberculosis* H37Rv DNA was immunoscreened by using an anticulture filtrate rabbit antiserum. Of 20 positive clones isolated, six were analyzed and found to express the genes for two known components of the early culture filtrate, the secreted 45/47-kDa antigen complex and the KatG protein, and two novel genes. Here we report the molecular cloning and nucleotide sequence of one of the new genes encoding a culture filtrate protein of 310 amino-acid (aa) residues.

We called this gene mtc28. The deduced polypeptide sequence contained an NH2-terminal, highly hydrophobic 32-aa region having properties of a secretion signal peptide. The putative 278-aa mature MTC28 protein was characterized at its NH2 and COOH termini by a high content of proline and alanine residues organized in an (AP)(n) motif. Thus, MTC28 is a new member of a group of proline-rich antigens found in *M. tuberculosis* and *M. leprae.* As shown by DNA hybridization experiments, the mtc28 gene was present only in species of the *M. tuberculosis* complex. Purified recombinant MTC28 antigen evoked strong, delayed-type hypersensitivity and antibody responses in guinea pigs immunized with *M. bovis* BCG, but not in guinea pigs immunized with *M. avium.* The strong immunological activity of MTC28 and the absence of B- and T-cell epitopes crossreactive with a common environmental mycobacterial species, such as *M. avium,* make this novel antigen an attractive reagent for immunodiagnosis of tuberculosis.—Authors' Abstract

Naito, M., Ohara, N., Matsumoto, S. and Yamada, T. The novel fibronectin-binding motif and key residues of mycobacteria. J. Biol. Chem. **273** (1998) 2905–2909.

The binding motifs of the immunodominant antigen (Ag) alpha-Ag (Ag85 complex B) of *Mycobacterium kansasii* for human fibronectin were examined using digested fragments.

We defined two fibronectin-binding epitopes on 27 amino acids from 84 to 110 and on 20 amino acids from 211 to 230. The epitopes were almost conserved in the closely related Ag85 complex of other mycobacteria species. Inhibition of fibronectin binding to intact alpha-Ag molecules was observed with peptide-(84–110), but not with peptide-(211–230). Peptide-(84–110) could also inhibit fibronectin binding to all components of the Ag85 complex of bacillus Calmette-Guerin (Ag85A, Ag85B, and Ag85C). Further study with synthetic peptides defined 11 residues from 98 to 108 as the minimum motif. Six residues [(98) FEWYYQ (103)] were critical for interacting with fibronectin. The motif revealed no homology to other known prokaryotic and eukaryotic fibronectin-binding proteins. The defined motif of alpha-Ag is novel and unique for mycobacteria.—Authors' Abstract

Oftung, F. and Lundin, K. E. A. Identification of mycobacterial HSP70 reactive human T cell clones discriminating between *M. tuberculosis* and *M. leprae.* FEMS Immunol. Med. Microbiol. **20** (1998) 145–151.

M. tuberculosis-reactive CD4+ cell clones were established from a BCG-vaccinated donor and tested for proliferative responses against complex mycobacterial antigens like *M. tuberculosis, M. leprae,* and PPD, as well as the recombinant *M. tuberculosis* hsp70 and hsp65 antigens from both *M. tuberculosis* and *M. leprae.* This screening permitted the identification of T-cell clones specifically recognizing the mycobacterial hsp70 or hsp65 antigen. All hsp65-reactive T-cell clones were crossreactive for *M. tuberculosis* and *M. leprae;* whereas three hsp70-reactive T-cell clones only recognized *M. tuberculosis.* In addition, HLA typing and blocking experiments with anti-HLA antibodies revealed that antigen presentation to all *M. tuberculosis*-reactive T-cell clones was restricted by HLA-DR3 molecules. We have thereby demonstrated the presence of human T-cell specificities

directed against the mycobacterial hsp70 antigen that are able to discriminate between *M. tuberculosis* and *M. leprae.*—Authors' Abstract

Oftung, F., Wiker, H. G., Deggerdal, A. and Mustafa, A. S. A novel mycobacterial antigen relevant to cellular immunity belongs to a family of secreted lipoproteins. Scand. J. Immunol. **46** (1997) 445–451.

The gene sequence of a novel 24.1-kDa *Mycobacterium tuberculosis* protein was identified within the Sanger Centre (U.K.) *M. tuberculosis* genome database (cosmid MTCY24G1) by searching with a 126-bp DNA sequence isolated from a genomic *M. leprae* lambda g11 library with *M. leprae*-reactive human T-cell clones as probes. The 24.1-kDa antigen is common to the vaccine strain *M. bovis* BCG, as well as *M. leprae.*

The 699-bp open reading frame encodes a 233 amino-acid long precursor protein with a signal peptide sequence for secretion and a consensus motif for lipid conjugation, which suggests that the mature protein is an exported lipoprotein antigen. The molecular mass of the mature protein antigen from *M. leprae* sonicate was shown to correspond to the deduced size of the *M. tuberculosis* protein by T-cell Western analysis. Homology searches revealed two other similarly sized, hypothetical, secreted mycobacterial lipoproteins within the *M. tuberculosis* genome database.—Authors' Abstract

Ramakrishnan, S., Sukhaswami, M. B., Patil, K. M. and Eswaran, C. Sequence data analysis reveals a relationship between LSR2, the recombinant fusion protein mimicking *M. leprae* and VIF of bovine immunodeficiency virus (BIV). J. Biomol. Struct. Dyn. **15** (1997) 605–609.

In the course of a computer simulation study looking for active sites for the interaction between MHC II and T-7—a 12 residue long peptide of LSR2—a recombinant fusion protein mimicking the native bacillus *M. leprae,* an interesting relationship between the antigenicity of LSR2 and VIF of BIF has come to light. Computer analysis study has revealed that this stretch of residue from 36 to 48 of LSR2 is highly antigenic. The experimental observation seems to confirm the role of this 12 residue peptide in antibody response. In an effort to determine whether a significant sequence level relationship exists between this and any other known protein, the sequence homology of both protein and nucleic acid was studied. It is found that this 12 residue long peptide (T-7) of LSR2 is homologous with viral infectivity factor (VIF) of the bovine immunodefiency virus (BIV). Homology with translated nucleic acid sequence also indicates the same fact. The VIF gene which codes for this protein is known to be essential for ability of cell-free virus preparation to infect cells. These results lead to the question of whether this 12 residue long peptide, which is common to both proteins, plays a role in their infectivity.

Whether mutations in the peptide or elimination of this peptide from the protein and studying the effect of this on the diseases themselves may help in controlling them, is another important question relevant to medical researchers.—Authors' Abstract

Raynaud, C., Etienne, C., Peyron, P., Laneelle, M. A. and Daffe, M. Extracellular enzyme activities potentially involved in the pathogenicity of *Mycobacterium tuberculosis.* Microbiology-U.K. **144** (1998) 577–587.

To evaluate the potential contribution of extracellular enzymes to the pathogenicity of mycobacteria, the presence of selected enzyme activities was investigated in the culture filtrates of the obligate human pathogen *Mycobacterium tuberculosis, M. bovis* BCG, the opportunistic pathogens *M. kansasii* and *M. fortuitum,* the nonpathogenic species *M. phlei* and *M. smegmatis.*

For *M. tuberculosis* and *M. bovis,* 22 enzyme activities were detected in the culture filtrates and/or cell surfaces, of which eight were absent from the culture fluids of nonpathogens: alanine dehydrogenase, glutamine synthetase, nicotinamidase, isonicotinamidase, superoxide dismutase, catalase, peroxidase and alcohol dehydrogenase. These activities, which correspond to secreted enzymes, formed a significant part

(up to 92%) of the total enzyme activities of the bacteria and were absent from the culture fluids and the cell surfaces of the nonpathogenic species *M. smegmatis* and *M. phlei.* The extracellular location of superoxide dismutase and glutamine synthetase seemed to be restricted to the obligate pathogens examined. The difference in the enzyme profiles was not attributable to the growth rates of the two groups of bacteria. The presence of the eight enzyme activities in the outermost compartments of obligate pathogens and their absence in those of nonpathogens provides further evidence that these enzymes may be involved in the pathogenicity of mycobacteria. In addition, the eight enzyme activities were demonstrated in the cell extract of *M. smegmatis.* Stepwise erosion of the cell surface of *M. smegmatis* to expose internal capsular constituents showed that the various enzyme activities, with the possible exception of superoxide dismutase, were located more deeply in the cell envelope of this bacterium. This suggests that the molecular architecture of the mycobacterial envelopes may play an important role in the pathogenicity of these organisms.—Authors' Abstract

Shankar, S., Kapatral, V. and Chakrabarty, A. M. Mammalian heterotrimeric G-protein-like proteins in mycobacteria: implications for cell signalling and survival in eukaryotic host cells. Mol. Microbiol. **26** (1997) 607–618.

Mammalian heterotrimeric GTP-binding proteins (G proteins) are involved in transmembrane signalling that couples a number of receptors to effectors mediating various physiological processes in mammalian cells. We demonstrate that bacterial proteins such as a Ras-like protein from *Pseudomonas aeruginosa* or a 65-kDa protein from *Mycobacterium smegmatis* can form complexes with human or yeast nucleoside diphosphate kinase (Ndk) to modulate their nucleoside triphosphate synthesizing specificity to GTP or UTP. In addition, we demonstrate that bacteria such as *M. smegmatis* or *M. tuberculosis* harbor proteins that cross-react with antibodies against the alpha-, beta- or the gamma subunits of heterotrimeric G proteins. Such antibodies also alter the GTP synthesizing ability of specific membrane fractions isolated from glycerol gradients of such cells, suggesting that a membrane-associated Ndk-G-protein homolog complex is responsible for part of GTP synthesis in these bacteria. Indeed, purified Ndk from human erythrocytes and *M. tuberculosis* showed extensive complex formation with the purified mammalian alpha- and beta-G-protein subunits and allowed specific GTP synthesis, suggesting that such complexes may participate in transmembrane signalling in the eukaryotic host. We have purified the alpha-, beta- and gamma-subunit homolog from *M. tuberculosis,* and we present their internal amino-acid sequences as well as their putative homologies with mammalian subunits and the localization of their genes on the *M. tuberculosis* genome. Using oligonucleotide probes from the conserved regions of the alpha- and gamma-subunit of *M. tuberculosis* G-protein homolog, we demonstrate hybridization of these probes with the genomic digest of *M. tuberculosis* H37Rv but not with that of *M. smegmatis,* suggesting that *M. smegmatis* might lack the genes present in *M. tuberculosis* H37Rv. Interestingly, the avirulent strain H37Ra showed weak hybridization with these two probes, suggesting that these genes might have been deleted in the avirulent strain or are present in limited copy numbers as opposed to those in the virulent strain H37Rv.—Authors' Abstract

Sharma, R. K., Shivannavar, C. T., Katoch, K., Sharma, V. D., Natrajan, M., Saxena, N. and Katoch, V. M. Microdensitometric scanning procedure for quantitative assessment of hybridization or rRNA targeting probes in leprosy. Acta Leprol. **10** (1997) 213–217.

In order to develop an objective criteria of grading of positivity of hybridization signals of gene probes targeting rRNA, a microdensitometric scanning procedure was standardized. Ribosomal RNA was extracted from the bacilli harvested from biopsies of leprosy cases across the spectrum and blotted on nitrocellulose membranes. *M. leprae*-specific rRNA targeting

oligonucleotide probes were end-labelled and hybridization was done by the technique standardized and published earlier. The autoradiographs were developed and microdesitometric scanning was done by altering different parameters. Positivity was graded in five grades and compared with visual positivity. Microdensitometric scanning procedure and the 5-grade system appear to be useful and reproducible. Signals in paucibacillary specimens were in 2+ to 3+ grading range; whereas those in multibacillary specimens varied in grade from 2+ to 5+. This approach appears to have potential usefulness for assessing the bacillary load (possibly viable) in the clinical specimens from leprosy cases.—Authors' Abstract

Sreevatsa and Katoch, K. Viability of *M. leprae* while undergoing laboratory procedures. Indian J. Lepr. **69** (1997) 353–359.

Studies were carried out to assess whether various methodological procedures adopted while conducting experiments or maintaining *M. leprae* under different conditions affected the number of organisms made available or their viability. Results of mouse foot pad experiments showed that bacilli survived for 1 day at 37°C, 7 days at 20°C to 30°C and for 90 days in lyophilized conditions. Repeated daily exposure of the material preserved in a refrigerator at +4°C to room temperature showed that bacilli survived for only up to 5 days; whereas with single exposure they survived up to 14 days. *M. leprae* were found to lose infectivity after 30 minutes of exposure to various disinfectants and ultraviolet light. Centrifugation at high speed did not affect the viability of *M. leprae.*—Authors' Abstract

Supply, P., Magdalena, J., Himpens, S. and Locht, C. Identification of novel intergenic repetitive units in a mycobacterial two-component system operon. Mol. Microbiol. **26** (1997) 991–1003.

Mycobacterial interspersed repetitive units (MIRUs), a novel class of repeated sequences, were identified within the intercistronic region of an operon coding for a mycobacterial two-component system, named *senX3-regX3.* Southern blot analysis and homology searches revealed the presence of several homologous sequences in intergenic regions dispersed throughout the genomes of *Mycobacterium bovis* BCG, *M. tuberculosis* and *M. leprae.* These could be grouped into three major families containing elements of 77–101 bp, 46–53 bp and 58–101 bp. Based on the available mycobacterial sequences, the total number of MIRUs is estimated to be about 40–50 per genome. Similar to previously identified small repetitive sequences, the MIRUs of the two-component operon are transcribed on a polycistronic mRNA. Unlike previously identified small repetitive sequences, however, MIRUs do not contain dyad symmetries, comprise small open reading frames (ORFs) whose extremities overlap those of the contiguous ORFs, and are oriented in the same translational direction as those of the adjacent genes. Analyses of the sequences at the insertion sites suggest that MIRUs disseminate by transposition into DTGA sites involved in translational coupling in polycistronic operons.—Authors' Abstract

Williams, K. J., Chung, G. A. C. and Piddock, L. J. V. Accumulation of norfloxacin by *Mycobacterium aurum* and *Mycobacterium smegmatis.* Antimicrob. Agents Chemother. **42** (1998) 795–800.

The modified fluorescence method was used to determine the accumulation of norfloxacin by *Mycobacterium aurum* A+ and *M. smegmatis* mc^2155. By using an exogenous norfloxacin concentration of 10 μg/ml, a steady-state concentration (SSC) of 160 to 180 ng of norfloxacin/mg of cells was obtained for *M. aurum,* and an SSC of 120 to 140 ng of norfloxacin/mg of cells obtained for *M. smegmatis.* For both species of mycobacteria, the SSC was achieved within 5 min. The silicon oil method was investigated and gave higher SSCs than the modified fluorescence method. Further studies on the mechanism of norfloxacin accumulation by *M. aurum* were performed. An increase in the pH of the wash buffer from 7.0 to 9.0 did not significantly affect the final SSC obtained. Accumulation was nonsaturated over a norfloxacin concentration range

of 0 to 100 μg/ml, and the proton motive force inhibitor 2,4-dinitrophenol (1 and 2 mM), whether it was added before or after norfloxacin was added, had no effect on the final SSC obtained. 2,4-Dinitrophenol also had no effect on norfloxacin accumulation by *M. smegmatis*. Furthermore, norfloxacin accumulation by *M. aurum* was unaffected by the presence of either Tween 80 or subinhibitory concentrations of ethambutol in the growth medium. Therefore, it is proposed that norfloxacin accumulation by mycobacteria occurs by simple, energy-independent diffusion.—Authors' Abstract

Experimental Infections

Dhople, A. M. and Williams, S. L. The activity of rifabutin against *Mycobacterium leprae* in armadillos. Int. J. Antimicrob. Agents **9** (1998) 169–173.

The activity of rifabutin (LM 427) against *Mycobacterium leprae* was evaluated in armadillos inoculated earlier with human-derived *M. leprae*. Rifabutin was administered daily at a dose of 6 mg/kg body weight/day. The effect of rifabutin on *M. leprae* harvested from armadillos was determined by measuring the intracellular levels of ATP (an indicator of metabolic activity) of *M. leprae* and also their ability to multiply in the mouse foot pads and *in vitro* in DH medium. Within 2 weeks of initiating the treatment, ATP levels declined to 21% of the original (pretreatment level) and these *M. leprae* failed to multiply in the footpads of mice as well as in the *in vitro* culture system. This suggests that rifabutin was able to kill all *M. leprae* within 2 weeks. After 8 weeks the treatment was terminated and results showed that *M. leprae* from the treated armadillos remained nonviable in the mouse foot pad system as well as in the *in vitro* system, indicating bactericidal action of rifabutin. The results suggest that rifabutin can be a substitute for rifampin in the leprosy multidrug therapy regimen.—Authors' Abstract

Epidemiology and Prevention

Abel, L., Sanchez, F. O., Oberti, J., Thuc, N. V., Van Hoa, L., Lap, V. D., Skamene, E., Lagrange, P. H. and Schurr, E. Susceptibility to leprosy is linked to the human NRAMP1 gene. J. Infect. Dis. **177** (1998) 133–145.

Leprosy is a debilitating infectious disease of human skin and nerves. Genetic factors of the host play an important role in the manifestation of disease susceptibility. The human NRAMP1 gene is a leprosy susceptibility candidate locus since its murine homolog Nramp1 (formerly Lsh/Ity/Bcg) controls innate resistance to *Mycobacterium lepraemurium*. In this study, 168 members of 20 multiplex leprosy families were genotyped for NRAMP1 alleles and four closely linked polymorphic markers. Highly informative haplotypes overlapping the NRAMP1 gene were constructed, and the haplotype segregation into leprosy-affected offspring was analyzed. It was observed that the segregation of NRAMP1 haplotypes into affected siblings was significantly nonrandom. This finding is consistent with the hypothesis that NRAMP1 itself is a leprosy susceptibility locus.—Authors' Abstract

Castellazi, Z., Alcantara, F., Diaz, H. B., Canario, S. and Ledesma, R. [Epidemiological evaluation of a group of leprosy patients diagnosed in 1996.] Rev. Dom. Dermatol. **24** (1997) 35–41. (in Spanish)

Brief information about the behavior of leprosy in Dominican Republic and an epidemiologic analysis of 283 new patients diagnosed in 1996 are presented.—Authors' English Summary

Cui, L. [A case of leprosy hypersensitive to both DDS and RMP.] China Lepr. J. **13** (1997) 204. (in Chinese)

A 38-year-old man had been diagnosed to have leprosy when he visited a skin hospital because of pesticide poisoning in 1986 and given WHO-MDT. Three days later drug eruption occurred and was diagnosed as hypersensitivity to DDS. So, only RMP and B663 were taken but the drug eruption occurred again. Last, dual hypersensitivity to DDS and RMP has been proved.—Author's English Abstract

Ebenezer, L., Arunthathi, S. and Kurian, N. Profile of leprosy in children: past and present. Indian J. Lepr. **69** (1997) 255–259.

The profile of leprosy in children currently seen in a referral hospital is compared with that of children with leprosy admitted in the 1970s. Children with leprosy under the age of 15 years in 1974 and 1979 comprised one group (Group I) while those during 1989 and 1994 constituted the second group (Group II). The variables studied included age, sex, type of leprosy, deformity and contact status. Multidrug therapy (MDT) was introduced in the treatment of leprosy in 1982. The probable change it has made in the presentation of leprosy in children is discussed.—Authors' Abstract

Levee, G., Schurr, E. and Pandey, J. P. Tumor necrosis factor alpha, interleukin-1-beta and immunoglobulin (GM and KM) polymorphisms in leprosy—a linkage study. Exp. Clin. Immunogenet. **14** (1997) 160–165.

In order to determine the genetic components of susceptibility to leprosy in six multiplex French Polynesian families, linkage analysis was carried out between a putative disease gene and six polymorphic loci: G1M, G2M, KM, IL-1 beta, TNF-alpha (1, 2) and TNF-alpha (A, G) using the lod score method. The three modes of inheritance, assuming a full penetrance value or reduced penetrance values (80% and 40%) for the susceptible allele, as well as with affected ones only, were tested. The results of this study provide no evidence for linkage between leprosy and the markers tested.—Authors' Abstract

Lietman, T., Porco, T. and Blower, S. Leprosy and tuberculosis: the epidemiological consequences of cross-immunity. Am. J. Public Health **87** (1997) 1923–1927.

Objectives. This study tested the hypothesis, first proposed by Chaussinand, that individual-level immunity acquired from exposure to tuberculosis may have contributed to the disappearance of leprosy from western Europe.

Methods. The epidemiological consequences of crossimmunity were assessed by the formulation of a mathematical model of the transmission dynamics of tuberculosis and leprosy.

Results. The conditions under which *Mycobacterium tuberculosis* could have eradicated *M. leprae* were derived in terms of the basic reproductive fates of the two infections and the degree of crossimmunity.

Conclusions. If the degree of crossimmunity between two diseases within an individual is known, then the epidemiological consequences of this crossimmunity can be assessed with transmission modeling. The results of this analysis, in combination with previous estimates of the basic reproductive rate of tuberculosis and degree of crossimmunity, imply that tuberculosis could have contributed to the decline of leprosy if the basic reproductive rate of leprosy was low.—Authors' Abstract

Ramaprasad, P., Fernando, A., Madhale, S., Rao, J. R., Edward, V. K., Samson, P. D., Klatser, P. R., de Wit, M. Y. L., Smith, W. C. S. and Cree, I. A. Transmission and protection in leprosy: indications of the role of mucosal immunity. Lepr. Rev. **68** (1997) 301–315.

Recent advances in treatment have achieved a large drop in the prevalence of active leprosy cases, but the incidence is at best decreasing slowly. Most people within leprosy-endemic populations have been exposed to *Mycobacterium leprae* but few develop disease, and it seems likely that the

majority of the population develops protective immunity. If the site of initial infection is in the nose, dissemination of bacilli around the body to skin and nerve implies that the initial infection is bacilliferous, and it has been shown that nasal *M. leprae* are detectable by polymerase chain reaction (PCR) of nasal swabs. Since salivary anti-*M. leprae* IgA (sMLIgA) levels are correlated with protection, we have surveyed groups of leprosy patients, contacts and the general population for both their sMLIgA and nasal PCR positivity. A total of 304 subjects were enrolled in the study: PCR and mucosal challenge tests were performed in 204 of these individuals. sMLIgA was present in 66% of treated patients, 76% of leprosy workers and 72% of healthy contacts. However, only 33% of indigenous subjects were sMLIgA+ in contrast to the earlier studies showing 74% positivity. PCR for *M. leprae* was present in both household contacts (2%) and indigenous controls (5%). In a subsequent follow-up study, nasal swabs were taken from 97 of those studied in the first series: three PCR+ individuals followed up after 1 year became negative, while of the remaining 94 PCR– individuals retested, 2 became positive. Of 112 subjects retested with the mucosal challenge test for sMLIgA, 22 converted from positive to negative and 12 from negative to positive. These results suggest that there is widespread subclinical transmission of *M. leprae* with transient infection of the nose resulting in the development of a mucosal immune response, despite the fact that few individuals will develop clinical disease. This may explain the current lack of effect of multidrug therapy (MDT) control programs on incidence, although the reduction in the general population immunity is consistent with some effect of MDT on transmission.—Authors' Abstract

Vijayakumaran, P., Mahipathy, P. V., Misra, R. K., Petro, T. S., Ramanujan, R., Karunakaran, S. and Abraham, O. Hidden cases of leprosy (in prison). Indian J. Lepr. **69** (1997) 271–274.

Leprosy survey conducted in eight prisons in seven districts of Bihar State in India revealed a prevalence of 13.3 per 1000 which was 12 times more than the recorded prevalence of leprosy in the state. Thus, this finding supports the view that prisons could form a hyperendemic pocket for leprosy. Regular NLEP services need to be extended to the inmates of the prisons.—Authors' Abstract

Rehabilitation

Bourrel, P. The metacarpophalangeal stabilization test: its surgical interest. Indian J. Lepr. **69** (1997) 5–11.

The metacarpophalangeal stabilization test to assess proximal interphalangeal stiffness is described and a simple therapeutic plan is proposed for the choice of appropriate techniques for palliative claw hand surgery in leprosy patients.—Author's Abstract

Bourrel, P. Two objective "archivable" tests for voluntary muscle testing in ulnar and median nerve paralysis. Indian J. Lepr. **69** (1997) 13–23.

Two tests are described for the assessment of ulnar and median nerve function for use under field conditions ("flap flexion" of the fingers for ulnar nerve, and tip-to-tip thumb opposition to the fourth finger for median nerve). It is suggested that these tests could be used to assess the results of treatment of leprosy neuritis by medical treatment, or by medical treatment completed by surgical decompression of nerves and also of corrective surgery of claw hand, or loss of opposition of the thumb.—Author's Abstract

Husain, S., Mishra, B., Prakash, V. and Malaviya, G. N. Evaluation of results of

surgical decompression of median nerve in leprosy in relation to sensory-motor functions. Acta Leprol. **10** (1997) 199–201.

Median nerve decompression was performed in 29 leprosy patients of whom 20 were followed up for varying periods. It has been observed that the decompression was beneficial, sensory recovery was seen in 90% cases and in 45% cases the muscle strength improved and the process of deterioration was arrested in another 25% of cases.—Authors' Abstract

Kaur, G., Sachdeva, G., Bhutani, L. K. and Bamezai, R. Association of polymorphism at COL3A and CTLA4 loci on chromosome 2q31-33 with the clinical phenotype and *in-vitro* CMI status in healthy and leprosy subjects: a preliminary study. Hum. Genet. **100** (1997) 43–50.

Two genetic loci, COL3A and CTLA4, located within the chromosome 2q31-33 region in the vicinity of the proposed syntenic site of the mouse "Bcg" locus were genotyped by the polymerase chain reaction in leprosy patients and healthy individuals. All of the subjects studied were assessed as *in-vitro* responders/nonresponders to mycobacterial antigens. Simple sequence length polymorphism analysis revealed five (236 to 312 bp) and eight (84 to 120 bp) allelomorphs for COL3A and CTLA4, respectively. Our preliminary analysis showed a significant association between the 250-bp COL3A allelomorph in the homozygous condition and the multibacillary form of leprosy ($p < 0.05$; relative risk = 5.5). Another allelic (312 bp) variant of COL3A was significantly correlated with nonresponsiveness to *M. leprae* antigens *in vitro* ($p < 0.01$). The 104-bp allelomorph of CTLA4 was not observed in any of the 25 cases of leprosy. This absence was statistically significant ($p < 0.05$) when compared with normal healthy controls and depicted a high relative risk (RR = 25.83). An additional observation of the predominance of a unique 84-bp CTLA4/CTLA4-like allelomorph was observed in the Indian subjects studied.—Authors' Abstract

Ma, D., *et al.* [Follow up of 25 years for prevention of leprosy with BCG vaccination.] China Lepr. J. **13** (1997) 203–204. (in Chinese)

BCG had been vaccinated for 429 children (229 boys and 200 girls) with age less than 15 years in 1986 in a village of leprosy–high endemic area, Sichuan Province. Since then a 25-year follow up was done. Before the vaccination an OT test showed that 458 of 492 children in the village were negative, of which 29 were not vaccinated because of diseases or other causes. By 1993 among the vaccinated there was one person to have suffered from leprosy and that occurred 11 years after the vaccination.—Authors' English Abstract

Malaviya, G. N. Unfavourable results after surgical correction of claw fingers in leprosy. Indian J. Lepr. **69** (1997) 43–52.

General complications and problems occurring after surgical correction of claw fingers in leprosy patients are discussed. Specific unfavorable outcomes include recurrence of clawing, over-correction, superficialis minus deformity, arch reversal, median nerve damage, deviation of fingers, adhesion formation and reduced grip strength.—Author's Abstract

Sachdeva, G., Kaur, G., Bhutani, L. K. and Bamezai, R. Genetic variations at the T cell receptor γ locus in circulating peripheral blood mononuclear cells of clinically categorized leprosy patients. Hum. Genet. **100** (1997) 30–34.

The allelic polymorphisms at exon 3 and exon 2 of the T-cell receptor (TCR) Cγ2 (TRGC2) gene, generating 18-kb and 5.4-kb *Hind*III fragments, respectively, were found to be more frequent in multibacillary (MB) leprosy patients than in the controls ($p < 0.005$ and $p < 0.001$, respectively) when screened with the IDP2.11 probe. The frequencies of heterozygotes for the 18-kb allele and homozygotes for the 5.4-kb allele were found to be significantly higher in the MB patients than in the controls ($p < 0.001$). Interestingly, the 8.0-kb allele, originating

from the triplication of exon 2 of Cγ2, was observed exclusively in the paucibacillary (PB) leprosy patients. Further, when DNA samples were screened with the pH60 probe for the *Hind*III RFLP at the TCR Jγ2 (TRGJ2) gene segment, the 2.1-kb allele was again more prevalent in leprosy patients with the MB form of the disease than in the PB patients and the controls ($p < 0.025$). The frequency of homozygotes for the 2.1-kb allele was also significantly higher in the MB patients than in the PB patients ($p < 0.010$) and the controls ($p < 0.025$). A significant difference was observed in the frequencies of detectable rearrangements involving the Vγ7/8 and Vγ9 gene segments at the γ locus between circulating peripheral blood mononuclear cells of the MB leprosy patients and the controls. These rearrangements were detected less frequently in the MB patients ($p < 0.001$ for Vγ7/8 and $p < 0.005$ for Vγ9).—Authors' Abstract

Salafia, A. and Chauhan, G. Joshi external stablising system (JESS) in proximal interphalangeal joint (PIP) contractures in leprosy. Indian J. Lepr. **69** (1997) 331–339.

The authors present their experience in the use of the JESS (Joshi External Stabilizing System) for correction of proximal interphalangeal joint contracture deformity in 68 fingers. The use of the JESS has made this surgery easier and faster in releasing contractures, and it has given better correction than the methods so far used by the same authors, like capsulotomy, local flaps and free skin grafting. The procedure is simple and has no serious side effects; it can be repeated if need be. The JESS is easy to apply, economical, reliable, reusable, well accepted by the patient. Compared to the other distractors made in the U.S.A. and Europe, the JESS has an added advantage in that it costs so very much less (US$5–10) that our leprosy hospitals can afford it. In our patients, we have achieved full extension in 75% and good extension in 10.3% of the cases. These figures are much better than what was possible in the past with local flaps and free skin grafting. With those procedures we had excellent results in only 53% of the cases and poor result in 28%.—Authors' Abstract

Sane, S. B., Kulkarni, V. N. and Mehta, J. M. Restoration of abduction-opposition in paralysed thumb in leprosy. Indian J. Lepr. **69** (1997) 83–92.

The results from 121 patients in India who underwent various different surgical procedures for the restoration of paralytic thumb in leprosy are reviewed (143 operations on 136 hands). Results from 111 hands were available for analysis and were graded as excellent in 37 cases, good in 46, fair in 26 and poor in 2.—Authors' Abstract

Sane, S. B., Mehta, J. M. and Kulkarni, V. N. Application of "measured tension" technique in correction of claw fingers by tendon transfer in leprosy. Indian J. Lepr. **69** (1997) 63–70.

A method of pre-operative measurement of the tension required to correct clawing in a particular finger of patients with leprosy is described. The procedure involves using a spring balance and applying the same tension, using the same spring balance intraoperatively, to the slip to be sutured; 396 hands in India were operated upon with this technique and 45.5% had an excellent result and 31.8% a good result.—Authors' Abstract

Sarma, G. R., Subrahmanyam, S., Deenabandhu, A., Babu, C. R. N., Madhivathanan, S. and Kesavaraj, N. Exposure to pulsed magnetic fields in the treatment of plantar ulcers in leprosy patients—a pilot, randomized, double-blind, controlled clinical trial. Indian J. Lepr. **69** (1997) 241–250.

A pilot, randomized, double-blind, controlled clinical trial to study the effect of exposure to pulsed magnet fields (PMF) on the rate of healing of plantar ulcers in leprosy patients was undertaken. Twenty patients were randomly allocated to receive standard wound-care treatment (controls) and 20 others received standard treatment

plus exposure to PMF (sinusoidal form, 0.95 to 1.05 Hz, amplitude ± 2400 nano Teslas) (study group) for 4 weeks. Assessment of the outcome of treatment was based on the volume of ulcers, calculated from the maximal length, breadth and depth of the ulcer recorded on the day of admission, at 1 and 2 weeks and at the end of treatment. The analysis of the results was based on 15 control patients and 18 PMF patients after deletion of 4 patients due to irregularity in attendance and 3 others on account of suspected malignancy of the ulcers. In the control group, the geometric mean volumes of the ulcers were 2843 and 1478 mm^3 on the day of admission and at the end of the treatment ($p = 0.03$); the corresponding values in the PMF group were 2428 and 337 mm^3, respectively ($p < 0.001$). A decrease in the volume of 40% or more was observed in 53% of control patients and 89% of PMF patients ($p = 0.02$); a decrease of 80% or more was observed in none of the controls and in 33% of PMF patients. These findings strongly suggest that exposure to PMF causes a significantly more rapid healing of plantar ulcers in leprosy patients.—Authors' Abstract

van Brakel, W. H. and Anderson, A. M. Impairment and disability in leprosy: in search of the missing link. Indian J. Lepr. **69** (1997) 361–376.

This paper describes the results of a survey aimed at studying the relationship between impairment and disability in leprosy. Persons affected by leprosy attending the Green Pastures Hospital, Pokhara, or one of the field clinics in the Western Region of Nepal visited during the study period were interviewed using a standardized questionnaire. Two-hundred-sixty-nine subjects were included in the study. For the analysis, "disability" was defined as activities being done with "much difficulty," "only with help" or being "impossible." The most commonly affected indoor activities were cutting nails (22%), washing clothes (16%), using scissors (17%) and tying a knot (18%). Among the outdoor activities, cutting grass, digging, harvesting, threshing and milking a cow or buffalo were the most commonly affected (22%–26%). Sensory impairment of the thumb and/or index finger at the 2 g level was a very significant risk factor for disability activities involving the hand(s). Muscle weakness of the thumb and mobile clawing of the fingers had a strong association with disability in several activities. Sensory impairment of the sole was the strongest determinant of disability in activities involving the lower limb. We recommend that efforts should be made to include disability as a standard activity for monitoring and evaluation of rehabilitation, both for individuals and on the program level.—Authors' Abstract

Vlassoff, C., Khot, S. and Rao, S. Double jeopardy: women and leprosy in India. World Health Stat. Q. **49** (1996) 120–126.

Studies are reported from Bihar and Maharashtra states in India, describing the process of "dehabilitation" among male and female leprosy patients, and gender-sensitive interventions to address existing problems in leprosy control are suggested. The study examined the impact of leprosy on marriage and family relations. While both men and women were negatively affected in terms of their family and marital lives, women suffered more isolation and rejection. Psychologically, women appeared more vulnerable because they were deprived of personal contact with others in the domestic environment. The importance of providing information to both leprosy patients and their families about the disease and its treatment, including the possibility of cure with multidrug therapy and of counselling family members about their role in helping patients cope and recover, is discussed.—Authors' Abstract

Zhang, L., *et al.* [Anaesthesia of hands and feet in 152 disabled leprosy patients.] China Lepr. J. **13** (1997) 199–202. (in Chinese)

Analysis of sensation loss in disabled hands and feet of 152 leprosy patients in Jiangsu Province China, showed that the sensation loss was symmetric and the loss rate in MB was significantly higher than in PB, which in tibial, ulnar and median

nerves was 81.3%, 56.2% and 44.1%, respectively. The authors emphasized that the monitoring of the sensory function in the hands and feet is most important in leprosy control.—Authors' English Abstract

Zhang, L., *et al.* [Damage of tibial nerve in 734 leprosy patients.] China Lepr. J. **13** (1997) 187–188. (in Chinese)

Damaged tibial nerves were examined in 734 cases of leprosy in the north of Jiangsu Province, China. The results showed that the injury rate (76.3%) of sensory fibers is much higher than those of vegetative and motor fibers. The authors speculate that *M. leprae* can damage all these fibers without selectivity, and emphasize that special protection must be given to the patient's insensible feet, especially for those with MB leprosy.—Authors' English Abstract

Zhu, Z., *et al.* [Effects of protective footwear on preventing plantar ulcer.] China Lepr. J. **13** (1997) 202–203. (in Chinese)

The effects of protective shoes in the prevention of plantar ulcer were observed among 49 leprosy patients with average age of 51 years, including 39 men and 10 women. The Huili brand of gym shoes made in Shanghai, China, was used for 2 years. By the end of 2 years, 12 of 19 plantar ulcers, had healed and no new ulcer had occurred in patients with dryness and rhagades of the skin on their soles. While the shoes were used, they did also self-care, including soaking the feet in lukewarm water and then applying oil.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Aguiar, J. and Stenou, C. [Buruli ulcers in rural areas of Benin: management of 635 cases.] Med. Trop. **57** (1997) 83–90.

During 1991–1996, 635 cases of Buruli ulcer (caused by *Mycobacterium ulcerans*) were treated at the Health and Nutrition Centre, Zagnanado, in a rural area of southern Benin. 66% of the cases were children or adolescents from damp, swampland and areas. Surgery was the only effective treatment and is recommended to be performed as early as possible. In these patients a wide excision of the ulcer and surrounding tissue was performed 797 times followed by skin grafting, using thin skin grafts in 574 cases. Antibiotic treatment was started immediately in order to avoid or treat secondary infection. All patients recovered except five who died of intercurrent infection.—Authors' English Abstract

Bermudez, L. E., Petrofsky, M., Kolonoski, P. and Young, L. S. Emergence of *Mycobacterium avium* populations resistant to macrolides during experimental chemotherapy. Antimicrob. Agents Chemother. **42** (1998) 180–183.

Macrolide resistance is an emerging problem in AIDS patients who receive these agents for treatment or prophylaxis against *Mycobacterium avium* (MAC) infection. We compared the emergence of resistant MAC strains during therapy with clarithromycin (clarithromycin resistance was defined as MIC $\geq$32 μg/ml) and azithromycin (azithromycin resistance was defined as MIC $\geq$128 μg/ml) in C57BL/6 beige mice. Treatment with clarithromycin and azithromycin resulted in a decrease of 98.5% in the number of viable bacteria in spleens at week 8 and 99% at week 12 compared with the number of bacteria present in spleen before the initiation of therapy ($p < 0.001$). Splenic homogenates were also plated onto 7H11 agar plus clarithromycin at 32 μg/ml or azithromycin at 128 μg/ml. Resistance emerged significantly more of-

ten in mice treated with clarithromycin (100% of treated mice at both 8 and 12 weeks) than in those receiving azithromycin (0% at week 8 and 14% at week 12). The frequencies of resistance of the MAC population in the spleen to clarithromycin were 2.1×10^{-3} at week 8 and 1.1×10^{-2} at week 12; whereas resistance to azithromycin was absent at week 8 (all mice) and was $\sim 3.5 \times 10^{-5}$ (mean for the three positive animals) at week 12. Clarithromycin was more effective in initial reduction of MAC burden in tissue after 8 and 12 weeks of treatment, but resistant strains emerged significantly more frequently after treatment with clarithromycin than after treatment with azithromycin.—Authors' Abstract

Carlson, L. D. C., Wallis, C. K. and Coyle, M. B. Standardized BACTEC method to measure clarithromycin susceptibility of *Mycobacterium genavense.* J. Clin. Microbial. **36** (1998) 748–751.

A standardized clarithromycin susceptibility test for *Mycobacterium genavense* is reported. The BACTEC radiometric broth dilution test method recommended for the *M. avium* complex was modified to develop a reliable and reproducible procedure. Test development involved optimization of medium pH and inoculum densities for antibiotic vials as well as growth control vials. MIC control organisms include *M. simiae, M. avium*, and *M. xenopi.* Growth control vials required two to three inoculum dilutions, which varied for each species. Clarithromycin MICs and MBCs for 12 isolates and one colonial variant of *M. genavense* ranged from ≤0.06 to 0.25 μg/ml.—Authors' Abstract

Collins L. A., Torrero, M. N. and Franzblau, S. G. Green fluorescent protein reporter microplate assay for high-throughput screening of compounds against *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **42** (1998) 344–347.

An optimal assay for high-throughput screening for new antituberculosis agents would combine the microplate format and low cost of firefly luciferase reporter assays and redox dyes with the ease of kinetic monitoring inherent in the BACTEC system. The green fluorescent protein (GFP) of the jellyfish *Aequorea victoria* is a useful reporter molecule which requires neither substrates nor cofactors due to the intrinsically fluorescent nature of the protein. The gene encoding a red-shifted, higher-intensity GFP variant was introduced by electroporation into *Mycobacterium tuberculosis* $H_{37}Ra$ and *M. tuberculosis* $H_{37}Rv$ on expression vector pFPV2. A microplate-based fluorescence assay (GFP microplate assay [GFPMA]) was developed and evaluated by determining the MICs of existing antimycobacterial agents. The MICs of isoniazid. rifampin, ethambutol, streptomycin, amikacin, ofloxacin, ethionamide, thiacetazone, and capreomycin, but not cycloserine, determined by GFPMA were within 1 $\log_2$ dilution of those determined with the BACTEC 460 system and were available in 7 days. Equivalent MICs of antituberculosis agents in the BACTEC 460 system for both the reporter and parent strains suggested that introduction of pFPV2 did not influence drug susceptibility, in general. GFPMA provides a unique tool with which the dynamic response of *M. tuberculosis* to the existing and potential antituberculosis agents can easily, rapidly, and inexpensively be monitored.—Authors' Abstract

Debol, S. M., Herron, M. J. and Nelson, R. D. Anti-inflammatory action of dapsone: inhibition of chemoattractant-induced signal transduction. J. Leukoc. Biol. **62** (1997) 827–836.

Dapsone has clinical utility as an antiinflammatory agent but the mechanism of this action remains unknown. We have previously reported that dapsone inhibits beta^2 integrin (CD11b/CD18)-mediated adherence of human neutrophils *in vitro* and now describe studies designed to discover how dapsone-mediated inhibition of this neutrophil function occurs. Results indicate that dapsone interferes with the activation or function of the G-protein (G^i type) that initiates the signal transduction cascade common to chemotactic stimuli. They also show that dapsone-mediated suppression of this pathway inhibits the generation of sec-

ond messengers essential to the activation of beta^2 integrin molecules, as well as respiratory and secretory functions of neutrophils exposed to chemoattractants. We propose that the inhibition of chemoattractant-induced signal transduction by dapsone suppresses neutrophil recruitment and local production of toxic respiratory and secretory products in the affected skin of dermatitis herpetiformis and other neutrophilic dermatoses.—Authors' Abstract

Doucet Populaire, F., Capobianco, J. O., Zakula, D., Jarlier, V. and Goldman, R. C. Molecular basis of clarithromycin activity against *Mycobacterium avium* and *Mycobacterium smegmatis*. J. Antimicrob. Chemother. **41** (1998) 179–187.

Clarithromycin, the 6-O-methyl derivative of erythromycin, is approved for treatment of *Mycobacterium avium* infections and for prophylaxis in patients at risk.

Since clarithromycin is more active against mycobacteria than the parent compound erythromycin, we evaluated the interaction of erythromycin and clarithromycin with cells and ribosomes isolated from *M. avium* and *M. smegmatis*. The MIC of clarithromycin was 32 and 64 times lower than that of erythromycin for *M. smegmatis* and *M. avium*, respectively.

The cellular uptake rate for clarithromycin was two- to fivefold faster than for erythromycin, and cell-associated clarithromycin reached a plateau twofold higher than that of erythromycin after 3 hr. Energy was not required for uptake. Fractionation of cell-associated clarithromycin yielded 12% in the walls, 21% bound to ribosomes, with the remainder being lost during work-up. In addition, three- to sixfold more clarithromycin was associated with the isolated cell integument compared with erythromycin. The k_d for clarithromycin binding to ribosomes was 2.9- and 3.5-fold tighter for *M. smegmatis* and *M. avium*, respectively, than for erythromycin, due mainly to a slower off-rate. The log partition coefficients of the non-ionized form (log P = u) for clarithromycin and erythromycin were 3.24 and 2.92, respectively. Thus, clarithromycin is more hydrophobic than erythromycin. This would favor more rapid diffusion within and across hydrophobic regions of the cell integument, since once a solute saturates a membrane the net flux across the membrane must equal the net flux within the membrane as dictated by diffusion. We conclude that the lower MIC of clarithromycin for *M. avium* and *M. smegmatis* is due to a combination of increased cellular uptake, the major factor, possibly through a peripheral hydrophobic layer, and increased binding affinity to ribosomes.—Authors' Abstract

Dunzendorfer, S., Schratzberger, P., Reinisch, N., Kahler, C. M. and Wieder, C. J. Effect of thalidomide on neutrophil respiratory burst, chemotaxis, and transmigration of cytokine- and endotoxin-activated endothelium. Naunyn-Schmiedebergs Arch. Pharmacol. **356** (1997) 529–535.

Vascular endothelium activated by endotoxin and cytokines plays an important role in organ inflammation and blood leukocyte recruitment. Neutrophils, which are a homogeneous population of effector cells, are rapidly attracted in large numbers to sites of inflammation where they form an early response to infection or injury.

Excessive production of various interleukins, TNF, arachidonic acid metabolites, and other substances by neutrophils and macrophages results in systemic endothelial cell injury, a fundamental problem. In the present study, we investigated *in vitro* the effects of thalidomide (THD) on activation of endothelial cells for enhanced transmigration of neutrophils by lipopolysaccharide (LPS), tumor necrosis factor-alpha (TNF), and interleukin-1 (IL-1). Modulation of endotoxin- and cytokine-induced neutrophil chemotaxis and respiratory burst by THD were also studied. Treatment of HUVEC with THD in combination with LPS, TNF, and IL-1, respectively, antagonized LPS-activated transmigration of neutrophils but stimulated the effects of TNF and IL-1. All of the agents used—THD, LPS, TNF, and IL-1—inhibited neutrophil chemotaxis. Addition of THD to the neutrophils had no effect on LPS-inhibited chemotaxis; whereas the TNF- and IL-1-induced chemotaxis was modulated in a bimodal manner. However, THD failed to in-

fluence neutrophil respiratory burst activity. Results demonstrate that THD differentially affects mediator-induced activation of HUVEC and neutrophils.—Authors' Abstract

Ellner, J. J. Regulation of the human immune response during tuberculosis. J. Lab. Clin. Med. **130** (1997) 469–475.

Pulmonary tuberculosis is characterized by depression of purified protein derivative-stimulated (PPD-stimulated) blastogenesis in peripheral blood mononuclear cells (PBMCs) as well as decreased production of interleukin-2 (IL-2) and interferon-gamma (IFN-γ). Circulating T cells and monocytes (MNs) are nonspecifically activated *in situ.* PPD directly stimulates the primed MNs from patients with tuberculosis (TB) to overproduce a panoply of cytokines including transforming growth factor-beta (TGF-beta) and IL-10, which serve to depress PPD-stimulated blastogenesis and cytokine expression. Cross-modulation by these immunosuppressive MN products is superimposed on a primary T-cell abnormality that persists for at least 12 months after the diagnosis of TB and involves apoptotic mechanisms.—Author's Abstract

Erb, K. J., Holloway, J. W., Sobeck, A., Moll, H. and LeGros, G. Infection of mice with *Mycobacterium bovis*-Bacillus Calmette-Guerin (BCG) suppresses allergen-induced airway eosinophilia. J. Exp. Med. **187** (1998) 561–569.

It has been proposed that the increase in prevalence and severity of atopic disorders inversely correlates with exposure to infectious diseases such as tuberculosis. We have investigated this issue by combining an intranasal *Mycobacterium bovis*-bacillus Calmette-Guerin (BCG) infection with a murine model of allergen (ovalbumin [OVA]) induced airway eosinophilia. BCG infection either 4 or 12 wk before allergen airway challenge resulted in a 90%–95% and 60%–70% reduction in eosinophilia within the lungs, respectively, compared to uninfected controls. The inhibition of airway eosinophilia correlated with a reduced level of IL-5 production by T cells from the lymph node draining the site of OVA challenge. Interestingly, BCG infection of the lung had no effect on IgG1 and IgE OVA-specific serum immunoglobulin or blood eosinophil levels. Furthermore, BCG-induced inhibition of airway eosinophilia was strongly reduced in interferon (IFN)-gamma receptor-deficient mice and could be partially reversed by intranasal IL-5 application. Intranasal BCG infections could also reduce the degree of lung eosinophilia and IL-5 produced by T cells after *Nippostrongylus brasiliensis* infection. Taken together, our data suggest that IFN-γ produced during the T-helper cell (Th1) immune response against BCG suppresses the development of local inflammatory Th2 responses in the lung. Most importantly, this inhibition did not extend to the systemic immunoglobulin response against OVA. Our data support the view that mycobacterial infections have the potential to suppress the development of atopic disorders in humans.—Authors' Abstract

Eriksson, T., Bjorkman, S., Roth, B., Fyge, A. and Hoglund, P. Enantiomers of thalidomide: blood distribution and the influence of serum albumin on chiral inversion and hydrolysis. Chirality **10** (1998) 223–228.

The aim of this investigation was to elucidate the distribution and reactions of the enantiomers of thalidomide at their main site of biotransformation *in vivo,* i.e., in human blood. Plasma protein binding, erythrocyte : plasma distribution, and the kinetics of chiral inversion and degradation in buffer, plasma, and solutions of human serum albumin (HSA) were studied by means of a stereospecific HPLC assay. The enantiomers of thalidomide were not extensively bound to blood or plasma components. The geometric mean plasma protein binding was 55% and 66%, respectively, for (+) - (R) and () - (S)-thalidomide. The corresponding geometric mean blood : plasma concentration ratios were 0.86 and 0.95 (at a hematocrit of 0.37) and erythrocyte : plasma distributions were 0.58 and 0.87. The rates of inversion and hydrolysis of the enantiomers increased with pH over the range of 7.0–7.5. HSA, and to a lesser ex-

tent human plasma, catalyzed the chiral inversion, but not the degradation, of (+) - (R) - and (-) - (S) thalidomide. The addition of capric acid or preincubation of HSA with acetylsalicylic acid or physostigmine impaired the catalysis to varying extents. Correction for distribution in blood enhances previously observed differences between the pharmacokinetics of the enantiomers *in vivo*. The findings also support the notion that chiral inversion *in vivo* takes place mainly in the circulation and in albumin-rich extravascular spaces while hydrolysis occurs more uniformly in the body. In addition, the chiral inversion and hydrolysis of thalidomide apparently occur by several different mechanisms.—Authors' Abstract

Fenton, M. J., Vermeulen, M. W., Kim, S., Burdick, M., Strieter, R. M. and Kornfeld, H. Induction of gamma interferon production in human alveolar macrophages by *Mycobacterium tuberculosis*. Infect. Immun. **65** (1997) 5149–5156.

Gamma interferon (IFN-γ) is a cytokine which plays a critical role in resistance to *Mycobacterium tuberculosis* infection. While T lymphocytes and natural killer cells are a major source of IFN-γ, previous demonstrations that it can be produced by murine macrophages prompted us to examine the capacity of human alveolar macrophages to express IFN-γ. Here we report that *in vitro* infection of alveolar macrophages with *M. tuberculosis* induces both the release of IFN-γ protein and a transient increase in IFN-γ mRNA levels. The IFN-producing cells were shown to be macrophages by reverse transcription-*in situ* PCR. We also observed that *M. tuberculosis* stimulation resulted in IFN-γ-dependent expression of the chemokines IFN-γ, gamma-inducible protein 10 and monokine induced by IFN-γ suggesting that macrophage-derived IFN-γ can function in an autocrine and/or paracrine manner. The existence of a positive regulatory loop was suggested by the observation that exogenous IFN-γ protein could induce IFN-γ mRNA expression in uninfected alveolar macrophages. Interleukin-12 was also found to be a potent inducer of IFN-γ production, and *M. tuberculosis*-induced IFN-γ production appears to be mediated, at least in part, by IL-12. In contrast, *M. tuberculosis*-induced IFN-γ production by alveolar macrophages could be blocked by exogenous IL-10.

These studies are the first to demonstrate an autoregulatory role for IFN-γ produced by alveolar macrophages infected *in vitro* with *M. tuberculosis*.—Authors' Abstract

Goldfeld, A. E., Delgado, J. C., Thim, S., Bozon, M. V., Uglialoro, A. M., Turbay, D., Cohen, C. and Yunis, E. J. Association of an HLA-DQ allele with clinical tuberculosis. JAMA **279** (1998) 226–228.

Context. Although tuberculosis (TB) is the leading worldwide cause of death due to an infectious disease, the extent to which progressive clinical disease is associated with genetic host factors remains undefined.

Objective. To determine the distribution of HLA antigens and the frequency of two alleles of the tumor necrosis factor alpha (TNF-γ) gene in unrelated individuals with clinical TB (cases) compared with individuals with no history of clinical TB (controls) in a population with a high prevalence of TB exposure.

Design. A two-stage, case-control molecular typing study conducted in 1995–1996.

Setting. Three district hospitals in Svay Rieng Province in rural Cambodia.

Patients. A total of 78 patients with clinical TB and 49 controls were included in the first stage and 48 patients with TB and 39 controls from the same area and socioeconomic status were included in the second stage.

Main outcome measures. Presence of HLA class I and class II alleles determined by sequence-specific oligonucleotide probe hybridization and presence of two TNF-α alleles determined by restriction fragment length polymorphism analysis.

Results. In the first stage, 7 DQB1*0503 alleles were detected among 156 alleles derived from patients with TB; whereas no DQB1*0503 alleles were found among the 98 alleles derived from controls ($p = 0.04$). There was no detectable difference in the distribution of the 2 TNF-α alleles in patients with TB compared with controls. In

the second stage, we tested for the presence of a single variable, the DQB1*0503 allele, and found 9 DQB1*0503 alleles among 96 alleles derived from patients with TB and no DQB1*0503 alleles among 78 alleles in controls ($p = 0.005$).

Conclusion. The HLA-DQB1*0503 allele is significantly associated with susceptibility to TB in Cambodian patients and, to our knowledge, is the first identified gene associated with development of clinical TB.—Authors' Abstract

Hamuryudan, V., Mat, C., Saip, S., Ozyazgan, Y., Siva, A., Yurdakul, S., Zwingenberger, K. and Yazici, H. Thalidomide in the treatment of the mucocutaneous lesions of the Behcet syndrome—a randomized, double-blind, placebo-controlled trial. Ann. Intern. Med. **128** (1998) 443.

Background: Recurrent oral and genital ulcers are the most frequent problem in the management of the Behcet syndrome. Uncontrolled experience suggests that thalidomide may help prevent recurrences of these ulcers.

Objective: To determine the efficacy of two thalidomide dosages in the treatment of mucocutaneous lesions of the Behcet syndrome.

Design: Randomized, double-blind, placebo-controlled trial.

Setting: Specialist outpatient clinic for the Behcet syndrome in Turkey.

Patients: 96 male patients with the Behcet syndrome who primarily had mucocutaneous lesions without major organ involvement.

Intervention: Thalidomide, 100 mg/d or 300 mg/d, or placebo for 24 weeks.

Measurements: Sustained absence of any oral and genital ulceration during treatment (complete response) and changes in the number of mucocutaneous lesions. An additional evaluation was done 4 weeks after treatment ended.

Results: A complete response occurred in 2 of the 32 patients (6% [95% CI, 0.8% to 20.8%]) receiving thalidomide, 100 mg/d; in 5 of the 31 patients (16% [CI, 5.5% to 33.7%]) receiving thalidomide, 300 mg/d; and in none of the 32 patients (0% [CI, 0% to 10.9%]) receiving placebo ($p = 0.031$). The suppressive effect of thalidomide with either dosage was evident at 4 weeks for oral ulcers ($p < 0.001$) and at 8 weeks for genital ulcers ($p < 0.001$) and follicular lesions ($p = 0.008$). This effect persisted during treatment but diminished rapidly after treatment was discontinued. Both thalidomide dosages led to significant increases in the number of erythema nodosum lesions during the first 8 weeks of treatment ($p = 0.03$). Polyneuropathy developed in 4 patients (1 in the 100-mg/d group and 3 in the 300-mg/d group); in 3 of these patients, the condition was diagnosed after the trial had ended.

Conclusions: Thalidomide is effective for treating the oral and genital ulcers and follicular lesions of the Behcet syndrome. A dosage of 100 mg/d is as effective as a dosage of 300 mg/day.—Authors' Abstract

Hayashi, T., Catanzaro, A. and Rao, S. P. Apoptosis of human monocytes and macrophages by *Mycobacterium avium* sonicate. Infect. Immun. **65** (1997) 5262–5271.

Mycobacterium avium is an intracellular organism which multiplies predominantly within human macrophages. This organism has previously been shown to induce apoptosis to human macrophages. With a view to identifying *M. avium* components that induce cell death in infected host cells, sonicated extracts of *M. avium* as well as individual components isolated from the *M. avium* sonicate were tested in various assays with a human monocytic cell line (THP-1). THP-1 cells incubated with *M. avium* sonicate showed significantly reduced viability after a 2-day exposure compared to control cells incubated with media alone. This effect was dose dependent, with only 6.6% ± 5.2% and 48.8% ± 10.3% of the cells being viable by trypan blue exclusion at 600 and 300 μg/ml, respectively. Control cells, on the other hand, exhibited a viability of 98.8% ± 1.0%. In addition, an 80% ammonium sulfate fraction of the *M. avium* sonicate and the previously characterized 68-kDa protein were found to have similar effects on THP-1 cells. In both cases, the reduction in viability was due to

apoptosis characterized by chromatin condensation, DNA fragmentation by agarose gel electrophoresis, or terminal deoxynucleotidyl transferase-mediated d-UTP nick end labeling (TUNEL) and release of nuclear matrix protein (NMP) into the culture medium. *M. avium* sonicate-induced apoptosis of THP-1 cells was completely inhibited by the commonly used antioxidants pyrrolidinedithiocarbamate (PDTC) and butylated hydroxyanisole (BHA), indicating that the generation of free oxygen radicals may be responsible for inducing cell death. *M. avium* sonicate was found to induce apoptosis of monocyte-derived macrophages (MDMs) as well.

This effect was not reversed in the presence of PDTC and was not accompanied with DNA fragmentation when determined by agarose gel electrophoresis, as seen in the case of THP-1 cells. However, these MDMs were found to contain fragmented DNA by TUNEL. These findings suggest that the mechanism of cell death in MDMs may be different from that observed with THP-1 cells. Furthermore, these results provide new insight into the effect of *M. avium* components on host cell responses during *M. avium* infection.—Authors' Abstract

Hoffner, S. E., Gezelius, L. and Olsson Liljequist, B. *In-vitro* activity of fluorinated quinolones and macrolides against drug-resistant *Mycobacterium tuberculosis*. J. Antimicrob. Chemother. **40** (1997) 885–888.

The increasing incidence of drug-resistant *Mycobacterium tuberculosis* necessitates therapeutic alternatives. The *in-vitro* activities of seven fluoroquinolone and macrolide compounds were tested against 23 clinical isolates of *M. tuberculosis,* including 17 multidrug-resistant strains. Sparfloxacin was the most active fluoroquinolone, with MICs of 1mg/L for all tested strains, followed by levofloxacin and ciprofloxacin. Trovafloxacin had no inhibitory activity at the concentrations tested. The MICs of the macrolides were generally higher, clarithromycin being the most active with MICs of ≤8 mg/L for 8 of the 23 strains.—Authors' Abstract

Kim, J. H., Cho, E. H., Kim, K. S., Kim, H. Y. and Kim, Y. M. Cloning and nucleotide sequence of the DNA gyrase *gyrA* gene from *Serratia marcescens* and characterization of mutations in *gyrA* of quinolone-resistant clinical isolates. Antimicrob. Agents Chemother. **42** (1998) 190–193.

The sequence of the DNA gyrase *gyrA* gene of *Serratia marcescens* ATCC 14756 was determined. An open reading frame of 2640 nucleotides coding for a polypeptide with a calculated molecular mass of 97,460 was found, and its sequence complemented the sequence of an *Escherichia coli gyrA* temperature-sensitive mutation. Analysis of the PCR products of the quinolone resistance-determining regions of *gyrA* genes from six quinolone-resistant clinical isolates revealed a single amino-acid substitution, Ser-83 to Arg or Asp-87 to Tyr, in all six mutants, suggesting that a mutational alteration in *gyrA* is a common mechanism of quinolone resistance in *S. marcescens.*—Authors' Abstract

Labo, M., Gusberti, L., De Rossi, E., Speziale, P. and Riccardi, G. Determination of a 15437 bp nucleotide sequence around the *inhA* gene of *Mycobacterium avium* and similarity analysis of the products of putative ORFs. Microbiology **144** (1998) 807–814.

A 15437 bp region encompassing the *inhA* locus from the *Mycobacterium avium* chromosome was cloned and sequenced. From the sequencing data generated and the results of homology searches, the primary structure of this region was determined. This region contains four known genes (*acnA, fabG, inhA* and *hemH*) and two genes, *invA* and *invB,* whose products display homology with p60 invasion protein of *Listeria monocytogenes.* Six proteins encoded by putative ORFs contained an RGD motif (often involved in binding to macrophage integrins), while ORF1 and MoxR are probably transcriptional regulators. The rest of the putative products encoded by ORFs in the sequenced region showed little homology with the proteins contained in the

databases and were considered to be unknown proteins.—Authors' Abstract

Lalvani, T., Brookes, R., Wilkinson, R. J., Malin, A. S., Pathan, A. A., Andersen, P., Dockrell, H., Pasvol, G. and Hill, A. V. S. Human cytolytic and interferon gamma-secreting CD8+ T lymphocytes specific for *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 270–275.

Protective immunity to *Mycobacterium tuberculosis* is poorly understood but mounting evidence, at least in animal models, implicates major histocompatibility complex class I-restricted CD8+ T cells as an essential component. By using a highly sensitive assay for single-cell interferon gamma release, we screened an array of *M. tuberculosis* antigen-derived peptides congruent with HLA class I allele-specific motifs. We identified CD8+ T cells specific for epitopes in the early secretory antigenic target 6 during active tuberculosis, after clinical recovery and in healthy contacts. Unrestimulated cells exhibited peptide-specific interferon-gamma secretion; whereas lines or clones recognized endogenously processed antigen and showed cytolytic activity. These results provide direct evidence for the involvement of CD8+ cytotoxic T lymphocytes in host defense against *M. tuberculosis* in humans and support current attempts to generate protective cytotoxic T lymphocyte responses against *M. tuberculosis* by vaccination.—Authors' Abstract

Lowrie, D. B., Silva, C. L. and Tascon, R. E. Genetic vaccination against tuberculosis. Springer Semin. Immunopathol. **19** (1997) 161–173.

New weapons are needed in the fight against tuberculosis. Recent research indicates that a vaccine better than BCG may be within reach. A diverse range of protein antigens can give encouragingly high levels of protective immunity in animal models when administered with adjuvants or as DNA vaccines. Accelerated arrest of bacterial multiplication followed by sustained decline in bacterial numbers are key parameters of protection, and so the vaccine must target antigens produced by both actively multiplying and growth-inhibited bacteria. Consistent with this, the protective antigens have been found among secreted and stress proteins (e.g., Ag85, ESAT-6, hsp65, hsp70). Species-specific antigens are not needed; hence these remain available for diagnostic tests. Adoptive transfer of protection from vaccinated or infected mice into naive mice by transfer of purified T cells and clones shows that protection is expressed by antigen-specific cytotoxic T cells that produce interferon-gamma and lyse infected macrophages. These cells are produced in response to endogenous antigen. DNA vaccination appears to be an excellent way of generating these cells and may be able to give long-lasting protection.—Authors' Abstract

Mainali, E. S. and McMurray, D. N. Protein deficiency induces alterations in the distribution of T-cell subsets in experimental pulmonary tuberculosis. Infect. Immun. **66** (1998) 927–931.

Previous research has suggested that dietary protein deficiency alters resistance to experimental pulmonary tuberculosis, in part by affecting the distribution and trafficking of antigen-reactive T cells. In this study, guinea pigs were maintained on either a protein-deficient (10% ovalbumin) or control (30% ovalbumin) diet and infected 4 to 6 weeks later with a low dose of virulent *Mycobacterium tuberculosis* H37Rv by the respiratory route. Monoclonal antibodies directed against the CD4 or CD8 markers on guinea pig lymphocytes were used in a flow cytofluorometric assay to determine the proportion of each subset in the peripheral circulation, spleen, and bronchotracheal lymph nodes at 4 weeks after infection. In uninfected guinea pigs, only the spleen exhibited an effect of diet on T-cell distribution, with small but consistent reductions in the proportions of both CD4 and CD8 T lymphocytes. However, following infection, protein deficiency exerted a profound effect on T-cell distribution. Malnourished, tuberculous guinea pigs harbored only 20% and 60% of the T cells (as

a proportion of total lymphoid cells) found in the spleen and blood, respectively, of their well-nourished counterparts. Normal relative proportions of CD4 and CD8 cells were observed, however. In striking contrast, the bronchotracheal lymph nodes of protein-deprived guinea pigs with tuberculosis contained more than twice the numbers of T cells of control guinea pigs, and the normal CD4-to-CD8 ratio was reversed. Peripheral T-cell function, as measured by the delayed hypersensitivity skin test to tuberculin, and antigen-induced lymphoproliferation *in vitro* were markedly suppressed in protein-malnourished animals. Conversely, purified protein derivative-induced (but not concanavalin A-induced) proliferation was significantly enhanced in cultures of lymph node cells from protein-deprived tuberculous animals. Taken together, these results suggest that immunological abnormalities and loss of antimycobacterial resistance in the lungs of protein-deficient guinea pigs may be explained, in part, by sequestration of antigen-reactive T cells in the lymph nodes draining the site of infection.—Authors' Abstract

Marriott, J. B., Cookson, S., Carlin, E., Youle, M., Hawkins, D. A., Nelson, M., Pearson, P., Vaughan, A. N., Gazzard, B. and Dalgleish, A. G. A double-blind placebo-controlled phase II trial of thalidomide in asymptomatic HIV-positive patients: clinical tolerance and effect on activation markers and cytokines. AIDS Res. Hum. Retrovir. **13** (1997) 1625–1631.

A randomized double-blind, placebo-controlled study was performed to determine the safety, efficacy, and effect of thalidomide on a variety of immunological and biochemical parameters in asymptomatic human immunodeficiency virus (HIV)-positive patients. Nineteen male patients with elevated markers of immune activation and CD4 cell counts above 400/mm^3 were randomized to either placebo or thalidomide at 100 mg/day for 24 weeks.

However, only 3 (of 10) patients receiving thalidomide completed all 24 weeks compared to 6 (of 9) patients receiving placebo. This was mainly due to fatigue (somnolence is a recognized side effect), although this was also seen to a lesser extent in the placebo group and so may not be drug attributable. No significant changes in CD4/CD8 count, activation markers, TNF-alpha, or TNFR1 were observed. However, a nonsignificant trend toward inhibition of mitogen-induced TNF-alpha production was observed in the thalidomide arm. The lack of systemic effect and the lower tolerance of thalidomide (at this dose) in asymptomatic patients highlights the need for pharmacokinetic analysis to address possible absorption problems and the need for more potent and less toxic TNF-alpha inhibitors to be developed for use in this type of study.—Authors' Abstract

Matsiota Bernard, P., Vrioni, G. and Marinis, E. Characterization of rpoB mutations in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates from Greece. J. Clin. Microbiol. **36** (1998) 20–23.

There is a geographic distribution of *Mycobacterium tuberculosis* strains with various rpoB gene mutations that account for rifampin resistance. We studied 17 rifampin-resistant clinical isolates from patients in Greece to identify rpoB mutations. The aim of our study was the evaluation of a commercially available line probe assay kit (INNO-LiPA Rif, TB) to detect rpoB mutations and rifampin resistance. The results obtained with the commercially available assay were compared to those obtained by automated DNA sequence analysis of amplified PCR products. Randomly amplified polymorphic DNA (RAPD) analyses of the isolates were also performed. The overall concordance of the line probe assay with a phenotypic rifampin susceptibility test was 94%. Three distinct rpoB mutations in codons Ser (531), His (526), and Asp(516) were correctly identified with the kit, but mutations in external regions and insertions were detected only by automated DNA sequence analysis. The changes in codons Ser (531) and His (526) accounted for the majority of rifampin resistance, as previously described for isolates from other geographic

areas. The results obtained by RAPD analyses of the isolates suggested that clonally related *M. tuberculosis* strains can have subclones bearing distal mutant rpoB alleles. We conclude that this line probe assay kit, which is fast and with which tests are easy to perform, can be used for the rapid detection of rifampin resistance in *M. tuberculosis* before the availability of results by conventional methods and for epidemiological studies, but that negative results obtained by this method do not rule out rifampin resistance.—Authors' Abstract

Medina, E. and North, R. J. Resistance ranking of some common inbred mouse strains to *Mycobacterium tuberculosis* and relationship to major histocompatibility complex haplotype and Nramp1 genetype. Immunology **93** (1998) 270–274.

Six common inbred strains of mice and their F-1 hybrids were examined for resistance to infection with the H37Rv strain of *Mycobacterium tuberculosis.* According to survival times after inoculation of 10^5 CFU intravenously (i.v.), the mice could be classified as being either highly susceptible (CBA, DBA/2. C3H, 129/SvJ) or highly resistant (BALB/c and C57BL/6). F-1 hybrids of susceptible and resistant strains were resistant. Although an examination of a limited number of H-2 congenic strains showed that the H-2(k) haplotype could confer susceptibility on a resistant strain, it was evident that nonmajor histocompatibility complex genes were much more important. Resistant strains all possessed the susceptibility allele of the antimicrobial resistance gene Nramp1. Results obtained with selected strains infected with 10^2 CFU of *M. tuberculosis* by aerosol agreed with the results obtained with mice infected i.v. The size of the bacterial inoculum was important in distinguishing between resistant and susceptible strains, in that a 10^7 inoculum overcame the resistance advantage of one strain over another.—Authors' Abstract

Moller, D. R., Wysocka, M., Greenlee, B. M., Ma, X. J., Wahl, L., Flockhart, D. A., Trinchieri, G. and Karp, C. L. Inhibition of IL-12 production by thalidomide. J. Immunol. **159** (1997) 5157–5161.

The immunomodulatory properties of thalidomide are currently being exploited therapeutically in conditions as diverse as erythema nodosum leprosum, chronic graft-vs-host disease, rheumatoid arthritis, and sarcoidosis. The relevant mechanism of action of thalidomide in these diseases remains unclear. The important role recently ascribed to IL-12, a cytokine critical to the development of cellular immune responses, in the pathogenesis of several of these conditions led us to examine whether thalidomide affects the production of IL-12. Thalidomide potently suppressed the production of IL-12 from human PBMC and primary human monocytes in a concentration-dependent manner. Thalidomide-induced inhibition of IL-12 production was additive to that induced by suboptimal inhibiting doses of dexamethasone, and occurred by a mechanism independent of known endogenous inhibitors of IL-12 production. These results suggest that thalidomide may have therapeutic utility in a wide range of immunologic disorders that are characterized by inappropriate cellular immune responses.—Authors' Abstract

Or, R., Feferman, R. and Shoshan, S. Thalidomide reduces vascular density in granulation tissue of subcutaneously implanted polyvinyl alcohol sponges in guinea pigs. Exp. Hematol. **26** (1998) 217–221.

The efficacy of thalidomide in the treatment or erythema nodosum leprosum is a well established fact; there is also accumulating evidence of its therapeutic value in a number of other inflammatory and immune-mediated conditions. In addition, thalidomide has been shown to be an inhibitor of angiogenesis induced by basic fibroblast growth factor (bFGF). Nevertheless, its mechanism of action remains speculative. Using guinea pigs, orally administered thalidomide significantly enhanced the response of multinucleated foreign body giant cells ($p < 0.05$) in subcutaneously implanted, polyvinyl alcohol sponges. Fur-

thermore, the drug exerted a dual effect in that it reduced vascular density ($p < 0.05$), which was not abolished by recombinant human bFGF, and at the same time amplified the granulomatous response with and without bFGF ($p < 0.05$ and $p < 0.01$, respectively). The results of our experiments represent a further step toward understanding the mechanism of action of thalidomide, with implications for its potential use in wound healing and scar formation as well as in the control of tumorigenesis.—Authors' Abstract

Pham Huy, C., Galons, H., Voisin, J., Zhu, J. R., Righenzi, S., Warnet, J. M., Claude, J. R. and Duc, H. T. *In vitro* and *in vivo* immunosuppressive potential of thalidomide and its derivative, N-hydroxythalidomide, alone and in combination with cyclosporin A. Int. J. Immunopharmacol. **19** (1997) 289–296.

Thalidomide (Thd) has been shown to have interesting immunosuppressive properties and strong action against TNF-alpha. It is used for treating a variety of immune-mediated pathology and inflammatory diseases. The purpose of this work was to evaluate the *in vitro* and *in vivo* immunosuppressive effects of Thd and its derivative, N-Hydroxythalidomide (H-Thd), alone and in combination with cyclosporin A (CsA), upon different *in vitro* lymphocyte activation pathways and *in vivo* local graft-versus-host-reaction (GVHR). At different concentrations, both Thd and H-Thd alone inhibited the lymphocyte proliferation induced by alloantigen (MLR), mitogens (ConA, PWM) and superantigen (SEB) with an activity of 50%–75% that of CsA; however, in some tests, immunosuppressive potency of H-Thd was shown to be higher than that of Thd. *In vivo* using GVHR, Thd and H-Thd alone proved as active as CsA. The association *in vitro* and *in vivo* of each compound with CsA at different low concentrations produced an additive effect as strong as CsA used alone at high therapeutic concentrations.

In summarizing, this study revealed that: 1) despite its weaker potency *in vitro* than that of CsA, H-Thd presents interesting immunosuppressive properties similar to, and in some cases, better than Thd, and 2) the combination of H-Thd or Thd with CsA at suboptimal concentrations leads to high activity.—Authors' Abstract

Pitchappan, R. M., Agrewala, J. N., Dheenadhayalan, V. and Ivanyi, J. Major histocompatibility complex restriction in tuberculosis susceptibility. J. Biosci. **22** (1997) 47–57.

The distribution of the human leukocyte antigen D-related allele 2 (HLA D2) and its relation to clinical severity in patients with pulmonary tuberculosis in India, and the humoral and cell-mediated immune responses of sputum-positive and -negative patients were investigated. The role of MHC restriction in disease pathogenesis was also investigated by observing lymphocyte transformation test responses of patients and controls to selected mycobacterial peptides and correlating them to their MHC class II haplotypes, assigned by PCR-SSP typing. T-cell clones were generated against peptide 16.3 in a normal HLA DR15/DR9 healthy donor and characterized. HLA DR2 predisposed for a more severe form of pulmonary tuberculosis encoding a high-responder status. The spectrum of immune reactivity to mycobacteria was "innate," and it was demonstrable in healthy individuals from an endemic area. There was no correlation between the purified protein derivative response and peptide responses. It was also found that once a person was a high responder to P16- and P38-derived peptides (6/22), he/she (whether a patient or control) was a high responder for a wide range of mycobacterial peptides. The majority of the T-cell clones generated *in vitro,* to peptide 16.3 (amino acids 21–40) of the 16-kDa mycobacterial antigen, is HLA DR restricted, permissive and of Th1 phenotype. The results suggested that MHC class II restriction plays a role in peptide recognition and the immune response. Nonetheless the outcome and specificity of the immune reactivity and the resultant disease pathogenesis may depend on the promiscuity of peptide recognition and cytokine profiles.—Authors' Abstract

Proenca, N. G., de Freitas, T. P., Guidotti, A. and Seguim, M. C. [Treatment of eruptive pustular psoriasis (von Zumbusch type) with thalidomide.] An. Bras. Dermatol. **72** (1997) 575–578. (in Portuguese)

The eruptive pustule psoriasis of von Zumbusch is a rare and severe variety of psoriasis. It is difficult to treat, even employing immunosupressive drugs. Two cases of von Zumbusch psoriasis were treated with thalidomide. The initial dose was 100 mg per day, but in the second patient the dosage had to be increased to 200 mg per day very soon. Excellent results were obtained in both patients, with remission in the second week. During the following months the patients remained in remission, with a maintenance dosage of 50 mg of thalidomide per day. This is a new indication for the use of thalidomide.—Authors' English Summary

Rovelli, A., Arrigo, C., Nesi, F., Balduzzi, A., Nicolini, B., Locasiulli, A., Vassallo, E., Miniero, R. and Uderzo, C. The role of thalidomide in the treatment of refractory chronic graft-versus-host disease following bone marrow transplantation in children. Bone Marrow Transplant. **21** (1998) 577–581.

Chronic graft-versus-host disease (cGVHD) is a frequent complication of allogeneic bone-marrow transplantation (BMT). Thalidomide was found to have immunosuppressive properties, and it has been used in a limited number of children with cGVHD. We report our experience with refractory and/or high-risk cGVHD in 14 children. Six children showed complete clinical response to thalidomide in a median time of 2 months. Four children had partial responses and four failed. Side effects were usually mild (somnolence, constipation) and only two patients developed sensory peripheral neuropathy. An increased incidence of infectious complications attributable to thalidomide was not observed. Nine out of 10 responding patients are alive 49–111 months post-BMT. Thalidomide can be effective particularly in children with prevailing mucocutaneous cGVHD. All patients should be carefully monitored to detect peripheral neuropathy early.—Authors' Abstract

Santamaria, A., Ordaz Moreno, J., Rubio Osornio, M., Solis Hernandez, F. and Rios, C. Neuroprotective effect of dapsone against quinolinate- and kainate-induced striatal neurotoxicities in rats. Pharmacol. Toxicol. **81** (1997) 271–275.

We tested the ability of dapsone, a well-known antibiotic and antiinflammatory drug, to attenuate both the quinolinic acid (an NMDA agonist of glutamate receptors)- and kainic acid (a non-NMDA agonist of glutamate receptors)-induced *in vivo* neurotoxicities in rats. Circling behavior and striatal gamma-aminobutyric acid (GABA) depletion were considered as behavioral and neurochemical end-points of brain toxicity. Rotation behavior, evaluated 6 days after the intrastriatal injection of quinolinic acid (130 ± 19 ipsilateral turns/hr), was attenuated by doses of 12.5 mg/kg and 25 mg/kg of dapsone (50 ± 9 and 63 ± 9 turns/hr, respectively). Striatal GABA levels (237.3 ± 15.1 μg/g in control rats), found depleted at day 7 after quinolinic acid (98.3 ± 8.6 μg/g), were also protected by dapsone at doses of 12.5 and 25 mg/kg (167.7 ± 19.5 and 236.4 ± 46.6 μg/g, respectively). No protective effects were observed on quinolinic acid-induced neurotoxicity, as evaluated by both parameters, at lower doses of dapsone (6.25 and 9.375 mg/kg). The action of dapsone, at the dose of 12.5 mg/kg, was also measured on kainic acid-induced depletion of the striatal GABA levels. Animals treated with dapsone + kainic acid (182.8 ± 27.1 μg/g) showed significant attenuation of GABA depletion, as compared to rats treated with kainic acid alone (122.2 ± 19.9 μg/g). These findings provide evidence to suggest that dapsone is acting as a neuroprotective agent against excitotoxicity induced by glutamate receptor agonists.—Authors' Abstract

Selvaraj, P., Uma, H., Reetha, A. M., Xavier, T., Venkatesan, P., Prabhakar, R. and Narayanan, P. R. Association of HLA-class I antigens and haplotypes

with relapse or pulmonary tuberculosis in patients treated with short course chemotherapy. Indian J. Tuberc. **44** (1997) 9–12.

The association between HLA antigen(s) and/or haplotypes and relapse in patients successfully treated for pulmonary tuberculosis was examined. Serological determination of HLA-A, -B, -DR and -DQ antigens was performed in patients from India with quiescent pulmonary tuberculosis and bacteriologically relapsed patients after treatment with short-course chemotherapy with rifampin, isoniazid, pyrazinamide and streptomycin or ethambutol in various combinations for 6–8 months. An increased antigen frequency of HLA Al ($p = 0.03$) and B17 was seen in patients with bacteriological relapse compared with those with quiescent disease. The relative risks (RR) were Al = 2.8 and B17 = 3.2, respectively. The haplotypes Al-B17 (RR = 3.3), B17-DR7 (RR = 3.0), Al-DR7 ($p = 0.04$; RR = 9.3) were very common in patients with bacteriological relapse. This increase of HLA-Al, B17 antigens or the haplotypes Al-B17, B17-DR7 or Al-DR7 ($p \doteq 0.004$) was present irrespective of the treatment regimen. Results suggested that HLA-Al (and -Bl7) antigen(s), as such, and/or haplotypes Al-DR7 or non-HLA genes linked closer to HLA-A, -B and -DR loci may be associated with relapse of pulmonary tuberculosis after chemotherapy.—Authors' Abstract

Sfondrini, L., Morelli, D., Menard, S., Maier, J. A. M., Singh, M., Melani, C., Terrazzini, N., Colombo, M. P., Colnaghi, M. I. and Balsari, A. Anti-tumor immunity induced by murine melanoma cells transduced with the *Mycobacterium tuberculosis* gene encoding the 38-kDa antigen. Gene Ther. **5** (1998) 247–252.

The *Mycobacterium tuberculosis* Ag38 gene, which encodes a highly immunogenic protein, was cloned into a retroviral vector in-frame with the leader and the transmembrane portion of the nerve growth factor receptor, and transduced into the murine melanoma cell line B16–B78. Significant protection was observed in mice immunized with the transduced melanoma cells and subcutaneously challenged with parental melanoma cells since only 20% of mice developed tumors. Necroscopy of mice immunized with the transduced melanoma cells revealed dramatic inhibition of experimental metastases induced by intravenous (i.v.) inoculation of parental melanoma cells. Moreover, vaccination with transduced cells significantly prolonged survival of mice challenged i.v. with parental melanoma cells. These data indicate that the presence of the mycobacterial 38-kDa protein greatly enhances immunological recognition of structures expressed by the parental melanoma cells. Comparision of Th1 and Th2 responses in mice immunized with parental melanoma cells versus mice receiving the transduced cells revealed a clear predominance of Th1 responses when the Ag38 protein was endogenously expressed. This transduction approach may represent a promising immunotherapeutic strategy for the treatment of cancer patients.—Authors' Abstract

Shafran, S. D., Singer, J., Zarowny, D. P., Deschense, J., Phillips, P., Turgeon, F., Aoki, F. Y., Toma, E., Miller, M., Duperval, R., Lemieux, C. and Schlech, W. F. Determinants of rifabutin-associated uveitis in patients treated with rifabutin, clarithromycin and ethambutol for *Mycobacterium avium* complex bacteremia: a multivariate analysis. J. Infect. Dis. **177** (1998) 252–255.

Uveitis occurred in a substantial proportion of AIDS patients receiving rifabutin, 600 mg daily, together with clarithromycin and ethambutol for treatment of *Mycobacterium avium* bacteremia. A case-control study was undertaken to examine potential risk factors for developing uveitis. Of eight parameters examined, only baseline body weight predicted the development of uveitis by both univariate and multivariate analyses ($p = 0.001$). The incidence of uveitis was 14% in patients weighing >65 kg, 45% in patients between 55 and 65 kg, and 64% in patients <55 kg. Concomitant therapy with fluconazole, a drug known to raise serum rifabutin concentrations, was not associated with an increased incidence of uveitis. The risk of uveitis was markedly re-

duced when rifabutin was given at 300 mg daily in combination with clarithromycin and ethambutol.—Authors' Abstract

Silver, R. F., Li, Q., Boom, W. H. and Ellner, J. J. Lymphocyte-dependent inhibition of growth of virulent *Mycobacterium tuberculosis* H37Rv within human monocytes: requirement for CD4+ T cells in purified protein derivative-positive, but not in purified protein derivative-negative subjects. J. Immunol. **160** (1998) 2408–2417.

Protective human immunity to *Mycobacterium tuberculosis* (M. Tb) has proven difficult to characterize, in part because of technical obstacles to *in vitro* infection of human cells with virulent M. Tb. We established a reproducible method of infecting human monocytes (MN) with the virulent M. Tb strain H37Rv that did not reduce MN viability. TNF-alpha had no effect on replication of H37Rv within BM, and IFN-gamma mediated only a 1.9-fold reduction in bacterial growth. In contrast, nonadherent cells (NAC) from purified protein derivative (PPD)-positive and PPD-negative subjects reduced intracellular growth of H37Rv by 6- and 10.6-fold, respectively ($p = 0.007$ and $p = 0.005$). CD4+ T cells were essential to growth inhibition mediated by NAC of PPD-positive subjects; whereas containment of M. tb by NAC of PPD-negative subjects did not require CD4+ cells. CD8+ T cells did not contribute to protection mediated by NAC of either group. Supernatants of cocultured H37Rv-infected MN and NAG only partially reduced intracellular growth of M. Tb despite containing nanogram concentrations of TNF-alpha and IFN-gamma. Neutralizing antibodies to TNF-alpha, IFN-gamma, and IL-12 failed to affect the NAC-mediated growth limitation. NAC treated with emetine retained approximately 40% of their capacity to contain intracellular H37Rv, however. These studies indicate that protective human recall responses to M. Tb are mediated primarily by CD4+ T cells; whereas CD4-CD8- lymphocytes may contribute to innate immunity to M. Tb. The ability of NAC to activate M. Tb-infected MN is only partly attributable to soluble mediators and may also involve contact-mediated mechanisms. —Authors' Abstract

Sommer, C., Marziniak, M. and Myers, R. R. The effect of thalidomide treatment on vascular pathology and hyperalgesia caused by chronic constriction injury of rat nerve. Pain **74** (1998) 83–91.

Tumor necrosis factor alpha (TNF) may be involved in the pathogenic mechanisms of neuropathic pain by affecting endothelial cells and by upregulation of receptor sensitivity in afferent nerve fibers. To test the hypothesis that TNF plays a role in the vascular changes and the pain-related behavior in an experimental painful neuropathy in rats produced by tying loosely constrictive ligatures around one sciatic nerve, we investigated the effect of thalidomide, a selective blocker of TNF-production in activated macrophages. In rats in which treatment with thalidomide was started preoperatively, there was diminished mechanical allodynia and thermal hyperalgesia during the early stage of the disease. TNF immunohistochemistry revealed reduced endoneurial immunoreactivity on day 5 post-surgery as compared to sham-treated animals. The pathologic vascular changes were also reduced in thalidomide-treated rats. Starting treatment with thalidomide at a time point when hyperalgesia was already present did not alter the course of tie pain-related behavior. We conclude that preemptive treatment with a substance that blocks production of TNF reduces pain-related symptoms and pathologic vascular changes in the chronic constriction injury model of neuropathic pain.—Authors' Abstract

Sousa, A. O., Salem, J. I., Lee, F. K., Vercosa, M. C., Cruaud, P., Bloom, B. R., Lagrange, P. H. and David, H. L. An epidemic of tuberculosis with a high rate of tuberculin energy among a population previously unexposed to tuberculosis, the Yanomami Indians of the Brazilian Amazon. Proc. Natl. Acad. Sci. U.S.A. **94** (1997) 13227–13232.

A survey of an emerging tuberculosis epidemic among the Yanomami Indians of

the Amazonian rain forest provided a unique opportunity to study the impact of tuberculosis on a population isolated from contact with the tubercle bacillus for millennia until the mid-1960s. Within the Yanomami population, an extraordinary high prevalence of active tuberculosis (6.4% of 625 individuals clinically examined) was observed, indicating a high susceptibility to disease, even among bacille Calmette-Guerin-vaccinated individuals. Observational studies on cell-mediated and humoral immune responses of the Yanomami Indians compared with contemporary residents of the region suggest profound differences in immunological responsiveness to *Mycobacterium tuberculosis* infection. Among the Yanomami, a very high prevalence of tuberculin skin test anergy was found. Of patients with active tuberculosis, 46% had purified protein derivative of tuberculosis reactions <10 mm; similarly 58% of recent bacillus Calmette-Guerin vaccines exhibited skin test reactions <5 mm. The Yanomami also had higher titers of antibodies against *M. tuberculosis* glycolipid antigens (>70%) than the control subjects composed of Brazilians of European descent (14%). The antibodies were mostly of the IgM isotype. Among the tuberculosis patients who also produced IgG antibodies, the titers of IgG4 were significantly higher among the Yanomami than in the control population. Although it was not possible to analyze T-cell responses or patterns of lymphokine production *in vitro* because of the remoteness of the villages from laboratory facilities, the results suggest that the first encounter of the Yanomami Indian population with tuberculosis engenders a diminished cell-mediated immune response and an increased production of antibody responses relative to other populations with extensive previous contact with the pathogen. These findings suggest that tuberculosis may represent a powerful selective pressure on human evolution that over centuries has shaped the nature of human immune responses to infection.—Authors' Abstract

Stevens, R. J., Andujar, C., Edwards, C. J., Ames, P. R. J., Barwick, A. R., Khamashta, M. A. and Hughes, G. R. V. Thalidomide in the treatment of the cutaneous manifestations of lupus erythematosus: experience in sixteen consecutive patients. Br. J. Rheumatol. **36** (1997) 353–359.

We review the efficacy, tolerability and safety of low-dose thalidomide in the treatment of refractory disfiguring rash in 16 patients with cutaneous manifestations of lupus. Rashes, which included discoid lupus erythematosus (DLE), subacute cutaneous lupus (SCLE), photosensitive malar rash and nonspecific chronic erythema, were diagnosed on clinical grounds supported by skin biopsy in 11/16 patients. Using starting doses of 50–100 mg/day, 7/16 (44%) patients gained complete or near-complete remission of skin disease and 6/16 (37%) partial remission. Three out of 16 patients failed to respond. Maximum benefit was achieved within 16 weeks in all patients. Doses of 25–50 mg/day were effective in maintaining response. Rapid relapse occurred in 6/8 (75%) patients following drug withdrawal, but the response to thalidomide in those requiring repeat courses appeared to be maintained. There was no detectable improvement in systemic disease. One patient developed symptoms of mild peripheral neuropathy which resolved on drug withdrawal. Our experience suggests that thalidomide is effective in the treatment of severe skin manifestations of lupus refractory to other treatment and can be used safely in specialist rheumatological practice.—Authors' Abstract

Veitch, M. G. K., Johnson, P. D. R., Flood, P. E., Leslie, D. E., Street, A. C. and Hayman, J. A. A large localized outbreak of *Mycobacterium ulcerans* infection on a temperate southern Australian island. Epidemiol. Infect. **119** (1997) 313–318.

Mycobacterium ulcerans, the organism which causes Buruli or Bairnsdale ulcer, has never been isolated in culture from an environmental sample. Most foci of infection are in tropical regions. The authors describe the first 29 cases of *M. ulcerans* infection from a new focus on an island in temperate southern Australia, 1992–1995. Cases were mostly elderly, had predomi-

nantly distal limb lesions and were clustered in a small region in the eastern half of the main town on the island. The authors suspected that an irrigation system which lay in the midst of the cluster was a source of infection. Limitation of irrigation was associated with a dramatic reduction in the number of new cases. These findings support the hypothesis that *M. ulcerans* has an aquatic reservoir and that persons may be infected directly or indirectly by mycobacteria disseminated locally by spray irrigation.— Authors' Abstract

Venkataprasad, N., Jacobs, M. R., Johnson, J. L., Klopman, G. and Ellner, J. J. Activity of new quinolones against intracellular *Mycobacterium avium* in human monocytes. J. Antimicrob. Chemother. **40** (1997) 841–845.

The ability to inhibit the *in vitro* growth of mycobacteria within human monocytes is a useful screening assay for novel chemotherapeutic agents. In this study the MICs of a panel of new quinolones were determined by the broth microdilution method for two strains of *Mycobacterium avium.* Sixteen such compounds with MIC-(90)s ranging from 2 to >32 mg/L were subsequently selected for the 7-day monocyte assay using ciprofloxacin for comparison. The degree of inhibition of intracellular growth correlated with the MICs. PD 139586, PD 143289, PD 135144, PD 119421 and PD 131575 were the most active new agents with activities superior to those of ciprofloxacin and sparfloxacin.— Authors' Abstract

Washko, R. M., Hoefer, H., Kiehn, T. E., Armstrong, D., Dorsinville, G. and Frieden, T. R. *Mycobacterium tuberculosis* infection in a green-winged macaw (*Ara chloroptera*): report with public health implications. J. Clin. Microbiol. **36** (1998) 1101–1102.

Mycobacterium tuberculosis was isolated from the eyelid, skin, tongue, and lungs of a green-winged macaw (*Ara chloroptera*). Two persons living in the same household were culture positive for pulmonary tuberculosis 3 to 4 years before tuberculosis was diagnosed in the bird. Although humans have not been shown to acquire tuberculosis from birds, an infected bird may be a sentinel for human infection.—Authors' Abstract

Yang, Q. H., Khoury, M. J., James, L. M., Olney, R. S., Paulozzi, L. J. and Erickson, J. D. The return of thalidomide: are birth defects surveillance systems ready? Am. J. Med. Genet. **73** (1997) 251–258.

In the 1960s, thalidomide caused limb deficiencies in thousands of infants worldwide. The limb deficiencies were frequently of the intercalary type. As a result, numerous countries started birth defect surveillance programs. In 1967, the Centers for Disease Control (CDC) started the Metropolitan Atlanta Congenital Defects Program (MACDP), a population-based surveillance system, to provide early warning against new teratogens. Recent studies have shown that thalidomide may be beneficial for a range of conditions, including cancer and AIDS, and it may once again become widely available. Here, we examine the ability of MACDP to detect an increase in the birth prevalence of limb deficiency as an early warning of fetal exposure to thalidomide. We calculated base rates for all limb deficiencies, for bilateral nonsyndromic intercalary or preaxial deficiencies, and for all nonsyndromic intercalary limb deficiencies among Atlanta infants born from 1968 through 1993. We used relative risk estimates from previous studies and a range of pregnancy exposure rates for thalidomide. We tested the statistical power of MACDP to detect subtle changes in the birth prevalence of these defects using Poisson and cumulative sum (CUSUM) techniques. The base rates for all limb deficiencies, for bilateral intercalary or preaxial deficiencies, and for all intercalary limb deficiencies were 0.53, 0.035, and 0.022/ 1000, and the estimated relative risks were 175, 4, 570, and 8180, respectively. We varied the assumed rate of exposure to thalidomide from 1/10,000 to 5/100. With a 1/1000 exposure rate, both Poisson and CUSUM techniques will detect a rate change in in-

tercalary limb deficiency in about 6 months of monitoring, and a rate change in bilateral intercalary or preaxial deficiencies in about 12 months of monitoring. When monitoring all limb deficiencies, a pregnancy exposure rate of 3.5% or less would go unnoticed by the Poisson method and would take more than 50 years for the CUSUM method to signal an alarm with a 1/1000 exposure rate. However, for rates of exposure less than 1/1000, a progressively longer period of time or larger sample are needed to detect a rate change by both methods. Our findings highlight the importance of enlarging the monitored population and correct case classification in birth defects surveillance.—Authors' Abstract

Zhanel, G. G., Saunders, M. H., Wolfe, J. N., Hoban, D. J., Karlowsky, J. A. and Kabani, A. M. Comparison of CO_2 generation (BACTEC) and viable-count methods to determine the postantibiotic effect of antimycobacterial agents against *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **42** (1998) 184–187.

The postantibiotic effects (PAEs) of antimycobacterial agents determined with a BACTEC TB-460 instrument (CO_2 production) and by a traditional viable-count method against *Mycobacterium avium* complex (MAC) were not significantly different ($p > 0.05$). The longest PAEs following a 2-hr exposure to 2× the MIC were induced by amikacin (10.3 hr), rifampin (9.7 hr), and rifabutin (9.5 hr), while the shortest PAEs resulted from clofazimine (1.7 hr) and ethambutol (1.1 hr) exposure. CO_2 generation is a valid and efficient means of determining *in vitro* PAEs against MAC.—Authors' Abstract