CURRENT LITERATURE

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

General and Historical

Chen, X., *et al.* [Socio-medical investigation on leprosy following MDT(I); knowledge, attitude and behaviour of leprosy patients to MDT.] China Lepr. J. **13** (1997) 133–136. (in Chinese)

In order to ensure the effectiveness of WHO's MDT and to timely detect and solve the social, psychological and behavioral problems in MDT implementation, an investigation was carried out in 370 leprosy patients under MDT in Jiangsu Province, China. The results showed that a majority of the patients were willing to receive MDT and their attitudes toward MDT were associated with their knowledge on MDT. The busy farming season and patients' frequent movement rather than the discoloration due to MDT did significantly influence their compliance. Most of the patients hoped the professional leprosy workers would provide MDT service for them.—Authors' English Abstract

Le Grand, A. Women and leprosy: a review. Lepr. Rev. **68** (1997) 203–211.

Gender inequalities in health have a significant impact on women's health. In leprosy gender inequalities could be even more serious, since it is a highly stigmatized disease. A review has been made of the most recent literature dealing with gender and leprosy. First some data are presented on gender inequalities in rates of case detection, deformities and reversal reactions among leprosy patients. Then the major factors contributing to those differences are discussed. The paper ends with some recommendations for further research on gender and leprosy.—Author's Summary

Prabhavalkar, A. B. and Ansari, K. Patterns of indoor admissions of leprosy patients in a pre-MDT and post-MDT era and a role for leprosy referral hospital in leprosy integration programme. Indian J. Lepr. **69** (1997) 159–167.

Analysis of the admission patterns in a leprosy referral hospital during the last 27 years (1966 through 1993) shows a decrease in the number of annual admissions since 1987. While there were 1550 admissions during 1981–1983, only 842 patients were admitted during 1991–1993. There was no great change in the reasons for admission, about 46% for reactions, 37% for ulcers, 5% for neuritis and about 12% for other problems. However, compared to the pre-MDT days, admissions for neuritis have increased. A case is made for the continuation of special leprosy referral hospitals even beyond the year 2000 A.D., i.e., even after elimination of leprosy as a public health problem.—Authors' Abstract

Terencio de las Aguas, J. [The centenary of the International Leprosy Congresses.] Rev. Leprol. Fontilles **21** (1997) 175–194. (in Spanish)

The centennial of the International Leprosy Congress has been reached. There have been fifteen, together with other important events on leprosy, number of participants, topics reviewed and conclusions. Finally, the importance of the Beijing (China) Congress of 1998 with its slogan is mentioned "Working toward a World without Leprosy."—Author's English Summary

Xu, Y., *et al.* [On optimal input of resources for leprosy control after its basic eradica-

tion.] China Lepr. J. **13** (1997) 130–132. (in Chinese)

Now in China, the number of leprosy patients is greatly reduced because, since the 1950s, especially after the 1980s, the leprosy control work has been crowned with great success due to adoption of modern knowledge and techniques. In such a condition, the input of resource for it should naturally be changed relevantly. With a survey in two counties, Guangdong, of which one had higher endemic of leprosy and the other lower, the authors suggest that after basic eradication of leprosy, i.e., the prevalence and the incidence were below 0.01‰ and 0.5/100,000 for a county where leprosy was endemic, the local government should directly dispose one to three workers, of which one must be a qualified doctor responsible for future leprosy control, including early detection and treatment of the new patients, physical and social rehabilitation for those with leprosy disability, health education for eradication of stigma of leprosy and guidance of local health care services in leprosy control, and supply them relevant funds and national subsidies, which totally might be about RMB 15 to 50 thousand yuan a year on the basis of the task. So, it will greatly save the resources and promote efficiency.—Authors' English Abstract

Chemotherapy

Bermudez. L. E. and Inderlied, C. B. Effect of *Mycobacterium avium* infection on the influx, accumulation, and efflux of KRM-1648 by human macrophages. Microb. Drug Resist. Mechanisms Epidemiol. Dis. **3** (1997) 277–282.

KRM-1648 is a new benzoxazinorifamycin with activity *in vitro* and *in vivo* against organisms of the *Mycobacterium avium* complex. We investigated the ability of C-14-KRM-1648 to concentrate within human monocyte-derived macrophages *in vitro*. KRM-1648 is rapidly taken up by uninfected macrophages, with 90% of the initial concentration added to the monolayer found within macrophages by 1 hr and approximately 80% at 2 hr. Comparable results were obtained in assays using macrophages that have been infected with an AIDS-related strain of *M. avium* for 24 hr. In contrast, macrophages infected with *M. avium* for 3 days showed an impaired ability to concentrate KRM-1648, primarily because of a significant efflux of the antibiotic (intracellular concentration of 86% of the available drug was present within macrophages at 1 hr vs. 47% at 2 hr). Daily administration of KRM-1648 to a macrophage monolayer for 3 consecutive days resulted in significant accumulation of the drug within phagocytic cells. Although the efflux was greater in *M. avium*-infected macrophages than in uninfected cells, consecutive administration of KRM-1648 led to a total intracellular accumulation of drug that exceeded the initial level and appeared to continue to accumulate. The ability of KRM-1648 to rapidly accumulate in human macrophages, including *M. avium*-infected cells, may explain, in part, the improved therapeutic effectiveness in animal models against *M. avium* and *M. tuberculosis*.—Authors' Abstract

Jamis Dow, C. A., Katki, A. G., Collins, J. M. and Klecker, R. W. Rifampin and rifabutin and their metabolism by human liver esterases. Xenobiotica **27** (1997) 1015–1024.

1. The main metabolites of rifampin and rifabutin in man are their respective 23 deacetylated derivatives, but the enzyme(s) responsible for these biotransformations are not known.

2. In experiments with human liver slices and human liver microsomes, the 25 deacetylated derivatives of these drugs were the main metabolites observed. Slices and microsomes metabolized rifabutin 3–6-fold

faster than rifampin, in agreement with their relative clearance in patients. Rifabutin partitioned into slices more avidly than rifampin.

3. In microsomal incubations, deacetylation did not require NADPH, but the amount of metabolite at the end of incubation was affected by NADPH. With NADPH the amount of 25 deacetyl rifabutin decreased; whereas the amount of 25 deacetyl rifampin increased slightly. A panel of liver microsomes from seven donors showed a 3–4-fold difference in the formation of 25 deacetyl rifabutin or 25 deacetyl rifampin, with strong correlation between the production of the two metabolites (r^2 –0.94).

4. The production of 25 deacetyl rifabutin and 25 deacetyl rifampin by human liver microsomes was not significantly affected by 1 μM 4 chloromercuricbenzoic acid or bis-(4-nitrophenyl) phosphate, but was completely inhibited by 1 μM paraoxon or 1 μM diisopropylfluorophosphate. These results indicate that in man rifampin and rifabutin are deacetylated to their main metabolites by B-esterases.—Authors' Abstract

Ji, B., Jamet, P., Sow, S., Perani, E. G., Traore, I. and Grosset, J. H. High relapse rate among lepromatous leprosy patients treated with rifampin plus ofloxacin daily for 4 weeks. Antimicrob. Agents Chemother. **41** (1997) 1953–1956.

Fifty-one lepromatous leprosy patients, all of whom had relapsed after previous dapsone (DDS) monotherapy, were treated between 1990 and 1991 with 600 mg of rifampin (RMP) plus 400 mg of ofloxacin (OFLO) daily for 4 weeks, and the great majority of the patients were followed up at least once a year after completion of the treatment. After only 173 patient-years of follow up, 5 relapses had been detected; the overall relapse rate was 10.0% (confidence limits, 1.7% and 18.3%), or 2.9 relapses (confidence limits, 0.4 and 5.4) per 100 patient-years. The unacceptably high relapse rate indicated that 4 weeks of treatment with daily RMP-OFLO was unable to reduce the number of viable *Mycobacterium leprae* organisms to a negligible level. In addition, the *M. leprae* from one of the relapses were proved to have multiple resistance to DDS, RMP, and OFLO. To avoid further relapses, the follow up was terminated and the great majority of the patients were retreated with the standard 2-year multidrug therapy from 1994. No further relapse has been diagnosed since the beginning of retreatment.—Authors' Abstract

Job, C. K., Jayakumar, J. and Aschhoff, M. Delayed resolution versus treatment failure in paucibacillary leprosy patients under six months fixed duration multidrug therapy. Indian J. Lepr. **69** (1997) 131–142.

Thirty paucibacillary (PB) patients were given multidrug therapy (MDT) PB regimen for 6 months and were examined clinically and histopathologically before therapy, at 6 months and 12 months after therapy; and in four patients, at 18 to 23 months after MDT. Histopathological activity was present in 50% and 25% of patients after 6 months and 12 months, respectively, after MDT. At 18 to 23 months, the four patients continued to have active lesions both clinically and histopathologically. On the basis of this study it is found that fixed duration of MDT is effective in a large majority of patients especially those with indeterminate leprosy. However, there is "delayed resolution" in a significant number of patients which in a few instances may turn out to be "treatment failures." Therefore, a regular follow up of high-risk patients for at least 2 years and, if possible, 5 years, with freedom to intervene with additional antiinflammatory or antileprosy therapy as desired, is recommended.—Authors' Abstract

Lai, Z., *et al.* [Five-year monitoring for 120 persons who had MB leprosy and used MDT.] China Lepr. J. **13** (1997) 145–146. (in Chinese)

Since 1986, 120 patients, including 88 men and 32 women (LL 83, BL: 33 and BB 4 with age of 35.5 years and BI 2.8 on the average) have adopted WHO's MDT for treatment of their MB leprosy in 17 counties (cities) of Liangshan Prefecture,

Sichuan, China, and by the end of 1995 completed follow up of 5 years after MDT. After stop of the treatment their clinical conditions and BI were continuously improving and decreasing. At follow up of the fourth year all of them have clinically been cured. By the fifth year, BIs of 114 persons (95%) were negative, histopathological sections of 96 cases (80%) showed nonspecific changes, leprosy reaction was no longer seen and there was no relapse.—Authors' English Abstract

Queiroz, R. H. C., Souza, A. M., Melchior, E., Gouveia, E. G. and Carvalho, D. Influence of acetylator phenotype on the haematological and biochemical effects associated with dapsone in leprosy patients. Lepr. Rev. **68** (1997) 212–217.

Methemoglobinaemia and hemolytic anemia were the principal side effects observed in 30 leprosy patients undergoing long-term treatment with dapsone as a single drug or as part of multidrug therapy. Hepatic, pancreatic and renal evaluations showed no relevant clinical changes. Since N-acetylation is a major metabolic pathway for dapsone, slow acetylation phenotype may be a risk factor for the development of these reactions. To confirm this hypothesis we correlated acetylator phenotype and the hematological and biochemical effects induced by dapsone. No excess proportion of slow acetylators was found. We conclude that slow acetylators are not at greater risk of developing hematological side effects of dapsone than fast acetylators.—Authors' Summary

Scior, T., Raddatz, G., Figueroa, R., Roth, H. J. and Bisswanger, H. A. Molecular modeling study on dapsone and sulfonamides comparing structures and properties with respect to antileprosy activity. J. Mol. Model. **3** (1997) 332–337.

Despite the very close structural relationship between dapsone (4,4′-diaminodiphenyl sulfone, 4,4′ sulphonyldianiline, diphenyl sulphone, DDS) and sulfanilamide (p-aminobenzene sulfonamide), being the prototype of all other sulfonamides, only dapsone shows remarkable efficient pharmacological activity against *Mycobacterium leprae.* Cells of certain microorganisms need para-aminobenzoic acid (PABA), the latter playing the role of natural substrate to the biosynthesis of folic acid. Sulfones and sulfonamides show competitive antagonism as chemical analogs of PABA. It is most surprising that, despite of sharing this molecular mechanism, only dapsone shows antileprosy activity *in vivo.* The study was accomplished upping molecular mechanics (SYBYL) and semiempirical methods (MOPAC). The calculations of aromaticity, charges, protonation by MOPAC, and of lipophilicity by our empirical program LIPOP(hilicity) give evidence that dapsone is more lipophilic (log p values 0.97) than sulfanilamide (–0.67). The extremely lipophilic cell wall of *M. leprae* contributes to the surprising difference in antileprosy activity. Sulfonamides are more or less deprotonated (45% to 99%) at physiological pH units; whereas dapsone is totally undissociated. This results in different permeability rates into the bacterial cells *in vivo* compared to other sulfones and sulfonamides. The unique combination of high lipophilicity and low ionic dissociation favors antileprotic potency in dapsone. On principle, amide groups do not hinder activity, but cause acidity and subsequently dissociation.—Authors' Abstract

Single-Lesion Multicentre Trial Group. Efficacy of single dose multidrug therapy for the treatment of single-lesion paucibacillary leprosy. Indian J. Lepr. **69** (1997) 121–129.

A multicenter double-blind controlled clinical trial was carried out to compare the efficacy of a combination of rifampin 600 mg plus ofloxacin 400 mg plus minocycline 100 mg (ROM) administered as single dose with that of the standard 6-month WHO/MDT/PB regimen. The subjects included 1483 cases with one skin lesion who were previously untreated, were smear-negative, and had no evidence of peripheral nerve trunk involvement, and they were randomly divided into study and control groups. The total duration of the study from the day of intake was 18 months, and 1381 patients completed study. Only 12 patients were categorized as treatment failure and no differ-

ence was observed between the two regimens. Occurrence of mild side effects and leprosy reactions were minimal (less than 1%) in both groups. This study showed that ROM is almost as effective as the standard WHO/MDT/PB in the treatment of single lesion PB leprosy.—Authors' Abstract

Venkatesan, K., Mathur, A., Girdhar, A. and Girdhar, B. K. Excretion of clofazimine in human milk in leprosy patients. Lepr. Rev. **68** (1997) 242–246.

Clofazimine is an important and effective constituent of multidrug therapy for leprosy. A study has been conducted to determine the distribution of clofazimine in maternal milk so that the safety of breast feeding during maternal ingestion of the drug can be ascertained. Eight female leprosy patients (LL/BL) on clofazimine, 50 mg daily or 100 mg on alternate days for 1–18 months, (mean 5.0 ± 1.81 months; median 3.25 months) and in the early lactating phase were studied. Blood samples and milk specimens were collected 4–6 hr after the last daily dose. Clofazimine was assayed in the milk and plasma samples by HPTLC. Mean plasma and milk clofazimine levels were 0.9 ± 0.03 μg/ml and 1.33 ± 0.09 μg/ml, respectively. The ratio of milk to plasma drug concentration ranged from 1.0 to 1.7 with a mean of 1.48 ± 0.08. The amount of drug ingested by the infants was 0.199 ± 0.013 mg/kg/day which represented 22.1 ± 1.9% of the maternal dose.—Authors' Summary

Wang, Y., *et al.* [Histopathological features five years after MDT in 572 cases of MB leprosy.] China Lepr. J. **13** (1997) 89–90. (in Chinese)

Since 1986, skin biopsies of 572 cases of MB leprosy taking MDT (BB 27, BL 88 and LL 457) have been done in succession. Most of them had taken DDS monotherapy for 1 to 2 years. Before MDT their clinical and pathological manifestations were coincident, with a BI of 2.0 to 6.0 in their sections. The results showed that when MDT was stopped the infiltration in all of them began to regress and by the end of the fifth year of the surveillance most of them showed nonspecific inflammations.—Authors' English Abstract

Wozel, G., Blasum, C., Winter, C. and Gerlach, B. Dapsone hydroxylamine inhibits the LTB4-induced chemotaxis of polymorphonuclear leukocytes into human skin: results of a pilot study. Inflamm. Res. **46** (1997) 420–422.

Objective: Dapsone (4,4′diaminodiphenylsulfone) is effective in treating leprosy, chronic inflammatory conditions and opportunistic infections in HIV patients. By the oral route, the sulfone is metabolized to monoacetyldapsone (MADDS) and dapsone hydroxylamine (DDS-NOH). We have addressed the question as to whether these dapsone metabolites have anti-inflammatory properties of their own *in vivo.*

Treatment and Methods: After 2 weeks topical pretreatment with MADDS (1%), DDS-NOH (1%) and clobetasol proprionate (CP; 0.05%) dissolved in acetone, as a reference, 10 ng leukotriene B-4 (LTB4) were applied on the upper arms of eight healthy volunteers. After 24 hr, biopsies were taken and the polymorphonuclear leukocytes (PMN) were quantified fluorometrically using elastase as a marker enzyme.

Results: MADDS did not show any inhibitory activity on trafficking of PMN compared to the corresponding control and nontreated area (untreated: 790 ± 450 PMN/10 μg skin; MADDS: 1099 ± 556 PMN/10 μg skin); whereas DDS-NOH caused a statistically significant inhibition of PMN accumulation as did the reference CP (DDS-NOH: 128 ± 143 PMN/10 μg skin; CP: 86 ± 131 PMN/10 μg skin, $p < 0.01$).

Conclusion: These results indicate that DDS-NOH has anti-inflammatory potential which might contribute to the effectiveness of dapsone therapy.—Authors' Abstract

Zhou, T. [Monitoring for seven years after MDT in 604 cases of MB leprosy.] China Lepr. J. **13** (1997) 87. (in Chinese)

Since 1986, 1145 cases of leprosy have used WHO's MDT in Qianxinan Prefecture, Guizhou Province; 681 among them have been monitored for 7 years after completion of the course of 2 years, of which 77

cases died or moved out. By the end of the fourth year of monitoring all of them have been cured and there were no relapses.—Author's English Abstract

Zu, Y., *et al.* [On the course of MDT for MB leprosy.] China Lepr. J. **13** (1997) 81–83. (in Chinese)

Since 1986, 89 leprosy patients (LL 36, BL 38 and BB 15) with the disease duration of 1 to 41 years have been treated with modified WHO-MDT. Among them, the time was 17.8 months for clearance of the skin lesions and 27.9 months for bacteriological negativity, on the average, and 47 cases (56%) have been cured within 4 years. The authors suggest that the treatment duration should be over 4 years.—Authors' English Abstract

Clinical Sciences

Arunthathi, S. and Satheesh, K. K. Does clofazimine have a prophylactic role against neuritis? Lepr. Rev. **68** (1997) 233–241.

A study was undertaken with the aim of testing the usefulness of clofazimine as a prophylactic agent against neuritis and nerve damage. A modified regimen, using initial high doses of clofazimine followed by regular multibacillary multidrug therapy (MB-MDT) WHO regimen, was given to a series of consecutive cases of high-risk borderline leprosy patients, fulfilling defined selection criteria (N = 65). These patients were studied for the incidence of neuritis/type 1 reaction, over a period of 2 years. Results were compared with a matched series of consecutive cases treated only with regular MB-MDT WHO regimen (N = 57). The difference in incidence rates of neuritis between the two groups was significant ($p < 0.01$), suggesting that clofazimine may have a useful prophylactic role against neuritis/type 1 reaction and nerve damage.—Authors' Summary

Bernink, E. H. M. and Voskens, J. E. J. Study on the detection of leprosy reactions and the effect of prednisone on various nerves, Indonesia. Lepr. Rev. **68** (1997) 225–232.

This paper presents a retrospective study on the detection of the treatment of leprosy reactions in a field situation, and the effect of prednisone on the various affected nerves. Two patient cohorts were analyzed. The leprosy control program in the testing area is not backed up by a specialized referral leprosy hospital, but patients are treated on an ambulatory basis at peripheral health centers by trained multipurpose health workers supervised by the health center doctors. For operational purposes the guidelines and procedures for reaction management in the field were adjusted and partially simplified. In both studies it appeared that the time of the occurrence of severe reactions was the same: 80% or more of the severe reactions occurred in the first year of treatment, the majority in the first few months after the start of the multidrug (MDT) treatment. One third of all reaction patients suffered from a silent neuritis. Well-instructed fieldworkers proved to be competent in detecting and treating leprosy reactions. Treatment of severe reactions with prednisone in the field situation can preserve or considerably improve the functions of the affected nerves. It is interesting that often the motor function of a nerve was found to be impaired without any loss in sensibility, which was tested using the ballpoint pen method.—Authors' Summary

Chakrabarti, A., Kumar, B., Das, A. and Mahajan, V. K. Atypical post-kala-azar dermal leishmaniasis resembling histoid leprosy. Lepr. Rev. **68** (1997) 247–251.

An adult male with atypical lesions of post-kala-azar dermal leishmaniasis (PKDL) is described. He had extensive ulcerated

noduloplaque lesions on his hands, feet and genitalia. He had been diagnosed and treated for leprosy in the past. He came from an area endemic for kala-azar and leprosy and had a previous history of kala-azar. There was an abundance of *Leishmania donovani* bodies in slit-skin smears and in histopathology sections. There was a good therapeutic response to sodium stibogluconate. An ulcerative variant of PKDL has been described but is extremely rare. Extensive lesions with ulceration have not been described before to the best of our knowledge. The epidemiological significance of the case is discussed.—Authors' Summary

Chan, J., *et al.* [Antibody to HIV-1 in the sera of leprosy patients.] China Lepr. J. **13** (1997) 89. (in Chinese)

The antibody to HIV-1 in the sera of 65 leprosy patients (LL 62 and BL 3) with a mean age of 42 years was examined by using ELISA. Two positive cases have been found and both are the LL form of leprosy with bacteriological positivity. The authors point out that this is a false-positive reaction and its cause is yet unknown.—Authors' English Abstract

Ishikawa, S., Tanaka, H., Mizushima, M., Hashizume, H., Ishida, Y. and Inoue, H. Osteoporosis due to testicular atrophy in male leprosy patients. Acta Med. Okayama **51** (1997) 279–283.

A study was conducted to examine the relationship of testicular atrophy to bone metabolism in male leprosy patients. The study consisted of 31 leprosy patients (mean age: 62.0 years) and 31 healthy control men (mean age: 60.0 years). Measurements were made of their serum levels of free testosterone (FT), estradiol (E-2), luteinizing hormone (LH) and 25-hydroxyvitamin D (25 OHD). Bone mineral density (BMD) was measured at radial sites and the lumbar vertebral bodies (L2–L4) by dual-energy X-ray absorptiometry using a Hologic QDR-2000 densitometer. FT and E-2 levels were significantly lower and LH levels higher in leprosy patients than in controls. This represents a primary hypogonadal pattern. A value of 7.20 pg/ml of FT (= mean – 1 S.D. of control) was used as a cut-off value, and the subjects were subdivided into a hypogonadal group (HG) and a non hypogonadal group (non-HG). When the subjects were compared for differences in age, age at onset of disease, duration of disease, body mass index and BMD, only the duration of disease and BMD were significantly different between the two groups, Furthermore, BMD of the forearm significantly correlated with FT levels ($r = 0.689$, $p < 0.0001$). Low BMD may be due to orchitis and testicular atrophy.—Authors' Abstract

Kumar, P., Saxena, R., Mohan, L., Thacker, A. K. and Mukhija, R. D. Peripheral nerve abscess in leprosy: report of twenty cases. Indian J. Lepr. **69** (1997) 143–147.

During the year 1994–1995, 20 of the 67 leprosy patients attending the dermatology department with any kind of nerve involvement were found to be having nerve abscess. These abscesses occurred in all types of leprosy (except indeterminate) and a variety of nerve trunks and cutaneous nerves. In none of the instances was the abscess associated with reaction. All of the patients were surgically treated, without any steroid therapy. All cases showed significant improvement whenever there was nerve function deficit. Similarly, pain was relieved in all cases, when it was present.—Authors' Abstract

Lockwood, D. N. J. The management of erythema nodosum leprosum: current and future options. Lepr. Rev. **67** (1996) 253–259 (57 refs.).

Treatments of erythema nodosum leprosum are reviewed with particular emphasis on data derived from trials that used a randomized controlled design. The drugs discussed include: acetylsalicylic acid (aspirin), colchicine, indomethacin, chloroquine, prednisolone, thalidomide and clofazimine. Possible future treatment options discussed include: methylprednisolone,

plasma exchange, intravenous immunoglobulin, cyclosporin A, tumor necrosis factor α antibodies, tenidap and zinc supplementation.—Author's Abstract

Malaviya, G. N., Husain, S., Mishra, B., Girdhar, A. and Girdhar, B. K. Protective sensibility—its monofilament nylon threshold equivalents in leprosy patients. Indian J. Lepr. **69** (1997) 149–158.

An attempt has been made to define the levels of "protective sensibility" in terms of perception thresholds to monofilament nylon-induced touch/pressure stimuli. Certain problems were observed while interpreting the observations. There appears to be a range of threshold values instead of a clear cut-off point. We suggest that a monofilament nylon stimulus two times the normal threshold value for that patient be taken as cut-off point. This will make the observations of Birke and Sims (1986) and Hammond and Klenerman (1987) reasonable without having any need to exclude the cases who defy the boundaries laid by them. Since the genesis of plantar ulcer is multifactorial, it appears logical to include all patients who have a certain degree of hypoesthesia for a special ulcer care program. The likely problems while using monofilament nylons in the field and their possible solutions have also been outlined.—Authors' Abstract

Shi, Z., *et al.* [Psychological and social factors making leprosy patients to suicide themselves.] China Lepr. J. **13** (1997) 148–149. (in Chinese)

Since 1967, in the municipal leprosary, Taixing City, Jiangsu, China, 130 suicide leprosy patients were given emergency treatment, including MB 45 and PB 85, 55 men and 75 women with the age of 14 to 59 years, of which 125 (96.25%) are peasants with lower cultural level. The causes of suicide mainly were pessimism and disappointment originating from social and family discrimination and that the need in living could not be met. The authors point out that health education and psychological therapy are essential to prevention of suicide among those who have or had leprosy.—Authors' English Abstract

Wang, D., *et al.* [Serological surveillance with PGL-I and LAM-β for leprosy relapse.] China Lepr. J. **13** (1997) 138–140. (in Chinese)

In Gansu province, China, 35 persons whose leprosy had been cured with DDS monotherapy 18.6 years ago on the average, including 24 men and 11 women (MB 24 and PB 11) were twice examined with ELISA in 1994 and 1995 with a lapse of one year. The blood specimens were taken from the earlobe. PGL-I and LAM-β were used as antigens for the ELISA, and OD values of positivity were defined as >0.18 for the IgG-LAM-β and >0.16 for IgM-PGL-I. Twenty-three of 48 MB samples and 5 of 22 PB samples showed positivity of IgG-LAM-β, being 48% and 23%, and the samples of 24 MB and 6 PB showed positivity of IgM-PGL-I, being 50% and 27.6%, respectively. The OD values of IgG-LAM-β were 0.18 to 1.70, of which one was 0.86 with IgM-PGL-I OD value of 0.41 who had been diagnosed as relapse of MB leprosy and the others had IgM-PGL-I OD value of 0.2 or less without relapse, showing that only IgM-PGL-I has relevance to BI.—Authors' English Abstract

Yan, L., *et al.* [Corneal lesions in 228 leprosy patients.] China Lepr. J. **13** (1997) 65–67. (in Chinese)

In Taixing City, Jiangsu Province, China, among 1045 leprosy patients 228 cases (21.82%) have corneal lesions which were uniocular in 130 and biocular in 98, including corneal anesthesia (65.35%), keratitis (59.21 %), hypoesthesia in the cornea (34.65%), nebula (25%) and leucoma (19.3%). The causes of the corneal lesions were mainly injury of fifth nerve, lagophthalmos and ectropion; 41.67% of low vision and 25.44% of blindness originated from corneal ulcers, leucoma and scars; 18% of the low vision are curable. The corneal lesions have concern with form and duration of leprosy, reaction and treatment.—Authors' English Abstract

Immuno-Pathology

Altare, F., Jouanguy, E., Newport, M., Lamhamedi, S., Fischer, A., Levin, M. and Casanova, J. L. IFNγR1, a human mycobacterial susceptibility candidate gene. Bull. Inst. Pasteur. **95** (1997) 143–146.

Human interferon gamma receptor ligand binding chain (IFNγR1) deficiency is an autosomal recessive inherited disorder. Affected children show severe, profound and apparently selective susceptibility to weakly pathogenic mycobacteria, such as bacillus Calmette-Guerin (BCG) and nontuberculous mycobacteria. This article reviews the evidence supporting a causative relationship between the genotype (IFNγR1 mutations) and the phenotype (mycobacterial infections) of these children.—Authors' Abstract

Aseffa, A., Dietrich, M. A. and Shannon, E. J. Effect of thalidomide on apoptosis of lymphocytes and neutrophils. Immunopharmacol. Immunotoxicol. **19** (1997) 313–326.

Thalidomide causes congenital anomalies and it is immunomodulatory. These properties could be explained by an ability to alter the orderly process of programmed cell death during embryogenesis and modulation of apoptosis of lymphoid and/or myeloid cells in the immune response. Apoptosis of lymphoid and myeloid cells was studied by measuring the percentage of cells capable of excluding propidium iodide and expressing phosphatidylserine on their outer membrane. In addition, expression of Fc gamma RIII (CD16) was used to assess neutrophil apoptosis. Thalidomide did not affect the rate of apoptosis of CTLL-2 cells deprived of, or supplemented with, IL-2; of T-cells (mitogen-stimulated or resting) or of neutrophils. However, neutrophils obtained from HIV-infected patients treated with thalidomide showed reduced expression of CD16, a surrogate marker for apoptosis of neutrophils. Thalidomide's effect on neutrophil apoptosis *in vivo* warrants further investigation.—Authors' Abstract

Bergeron, A., Bonay, M., Kambouchner, M., Lecossier, D., Riquet, M., Soler, P., Hance, A. and Tazi, A. Cytokine patterns in tuberculous and sarcoid granulomas—correlations with histolopathologic features of the granulomatous response. J. Immunol. **159** (1997) 3034–3043.

Cytokines play an important role in granuloma formation, but the extent that cytokine profiles are similar in different granulomatous diseases and whether differences in the histopathologic features of the granulomatous response results from differences in cytokine production have not been evaluated. To investigate these questions, we used RT-PCR to quantify the expression of mRNAs coding for 16 cytokines in granulomatous lymph nodes from patients with tuberculosis and sarcoidosis and from control tissues, and we sought correlations between the level of expression of these cytokines and the histopathologic features of the granulomas. Expression of mRNAs coding for a number of cytokines (IL-1 beta, IFN-gamma, TNF-alpha, granulocyte-macrophage (GM)-CSF, IL-12 (p40), and lymphotoxin-beta) was increased in tuberculous and sarcoid granulomas compared with that of control tissues. All sarcoid granulomas were shown to express a Th1 pattern of cytokine mRNAs, while tuberculous lymph nodes expressed either a Th1 or a Th0 profile. CM-CSF and lymphotoxin-beta mRNAs were more abundant in sarcoid than in tuberculous granulomas; whereas IL-8 mRNA was strongly expressed only in tuberculous lymph nodes. Strong expression of GM-CSF, TNF-alpha, and IL-8 by granulomas was shown to be correlated, respectively, with the presence of florid granulomatous lesions, the absence of central necrosis, and the presence of neutrophil infiltration. These results demonstrate that the formation of tuberculous and sarcoid granulomas in humans is associated with the expression of characteristic cytokine profiles and indicate that the expression of certain cytokines is associated with the development of specific pathologic features in the resulting granulomas.—Authors' Abstract

Blackwell, J. M., Black, G. F., Peacock, C. S., Miller, E. N., Sibthorpe, D., Gnananandha, D., Shaw, J. J., Silveira, F., Lins Lainson, Z., Ramos, F., Collins, A. and Shaw, M. A. Immunogenetics of leishmanial and mycobacterial infections: the Belem family study. Phil. Trans. R. Soc. London Ser. B—Biol. Sci. **352** (1997) 1331–1345.

In the 1970s and 1980s, analysis of 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 of the genome were implicated in the control of infections caused by different *Leishmania* species which, because they show conserved synteny with regions of the human genome, immediately provides 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 leishmania, salmonella and mycobacterium infections. In recent studies, multicase family data on visceral leishmaniasis and the mycobacterial diseases, tuberculosis and leprosy, have been collected from northeastern Brazil and analyzed to determine the role of these candidate genes/regions in determining disease susceptibility. Complex segregation analysis provides evidence for one or two major genes controlling susceptibility to tuberculosis in this population. Family-based linkage analyses (combined segregation and linkage analysis; sib-pair analysis), which have the power to detect linkage between marker loci in candidate gene regions and the putative disease susceptibility genes over 10–20 centimorgans, and transmission disequilibrium testing, which detects allelic associations over 1 centimorgan (ca. 1 megabase), have been used to examine the role of four regions in determining disease susceptibility and/or immune response phenotype. Our results demonstrate: (i) the major histocompatibility complex (MHC: H-2 in mouse, HLA in man: mouse chromosome 17/human 6p; candidates class II and class III including TNF 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; (ii) 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 could be demonstrated in this Brazilian population; (iii) the region of human chromosome 17q (candidates NOS2A, SCYA2–5) homologous with distal mouse chromosome 11, originally identified as carrying the Sell gene controlling healing versus nonhealing responses to *Leishmania major,* is linked to tuberculosis susceptibility; and (iv) the "T helper 2" cytokine gene cluster (proximal murine chromosome 11/human 5q; candidates IL4, IL5, IL9, IRF1, CD14) controlling later phases of murine *L. major* infection, is not linked to human disease susceptibility for any of the three infections, but shows linkage to and highly significant allelic association with ability to mount an immune response to mycobacterial antigens. These studies demonstrate that the "mouse-to-man" strategy, refined by our knowledge of the human immune response to infection, can lead to the identification of important candidate gene regions in man.—Authors' Abstract

Chensue, S. W., Warmington, K., Ruth, J. H., Lukacs, N. and Kunkel, S. L. Mycobacterial and schistosomal antigen-elicited granuloma formation in IFN-gamma and IL-4 knockout mice—analysis of local and regional cytokine and chemokine networks. J. Immunol. **159** (1997) 3565–3573.

Types 1 (IFN-gamma/TNF-dominant) and 2 (IL-4/IL-5-dominant) granulomatous inflammation were analyzed in mice with knockout of IFN-gamma or IL-4 genes. Lung granulomas were elicited by beads

coated with purified protein derivative (PPD) of *Mycobacteria bovis* or soluble *Schistosoma mansoni* egg Ags. Parameters included granuloma size, composition, and macrophage function; white blood cell differentials; lymph node cytokine profiles; and cytokine/chemokine mRNA expression by lungs. Type 1 (PPD) and 2 (soluble *Schistosoma mansoni* egg Ags) responses showed characteristic cytokine and chemokine profiles in control mice. IFN-gamma knockout converted the PAD response to a type 2-like pattern with eosinophil infiltration and decreased TNF and RANTES, but increased IL-4, IL-5, IL-10, IL-13, monocyte chemoattractant protein-3 (MCP-3), and eotaxin expression. IL-4 knockout exacerbated type 1 inflammation with increased IL-2/IFN-gamma production by lymph nodes and IL-1 production by granuloma macrophages but, unexpectedly, IFN-gamma transcripts were reduced in the lungs. Regarding the type 2 response, IL-4 was needed for maximal blood eosinophilia but, surprisingly, its absence had a minimal effect on type 2 granuloma size and composition despite regional reductions of IL-5 and IL-10 as well as local reductions of TNF-alpha, MCP-1, MCP-3, and eotaxin. Thus, the type 2 granuloma was not converted to a type 1 composition with IL-4 knockout, but showed persistent expression of IL-13 and some degree of IL-5 and MCP-3, suggesting that these cytokines could potentially support a compensatory type 2 response. IFN-gamma knockout did not augment type 2 granuloma size or Th2 cytokines in lymph nodes and unexpectedly reduced IL-4 transcripts in lungs. This study offers important implications regarding inflammation and its relationship to local and regional cytokine expression.—Authors' Abstract

Dlugovitzky, D., Torres Morales, A., Rateni, L., Farroni, M. A., Largacha, C., Molteni, O. and Bottasso, O. Circulating profile of Th1 and Th2 cytokines in tuberculosis patients with different degrees of pulmonary involvement. FEMS Immunol. Med. Microbiol. **18** (1997) 203–207.

To investigate whether differences in the degree of pulmonary tuberculosis lesions could be accompanied by changes in the pattern of circulating cytokines, 29 untreated tuberculosis patients showing mild (N = 10), moderate (N = 5) or advanced (N = 14) pulmonary disease, and 12 age-matched healthy controls (mean ± S.D., 36 ± 15 years) were studied. ELISA methods for the evaluation of interferon-gamma, interleukin-2 (IL-2), IL-4, and IL-10 indicated that all patients had increased serum levels of the four cytokines in relation to controls. Mean titers of interferon-gamma and IL-2 in mild and moderate patients appeared higher than in those with advanced disease, whereas moderate and advanced patients showed the higher levels of IL-4 in comparison to mild cases. Raised levels of IL-10 were more prevalent in advanced disease, and statistically different from those in mild patients. This cytokine pattern may help to explain findings wherein mild tuberculosis is characterized by preserved cellular immune responses while advanced disease is accompanied by an impairment of such parameters.—Authors' Abstract

Ebenezer, G. J., Suneetha, S. and Arunthathi, S. Clinical and histopathological activity in paucibacillary leprosy patients after fixed-duration multidrug therapy. Lepr. Rev. **68** (1997) 218–224.

In 37 clinically diagnosed borderline-tuberculoid (BT) leprosy patients skin biopsies were done prior to starting multidrug therapy (MDT) and at the end of 6 months of therapy. Clinical and histopathological activity, graded as active, resolving and inactive, were studied at the end of 6 months of MDT. Of the 37 clinically diagnosed BT patients 24 could be confirmed by histopathology as having BT leprosy, while the other 13 biopsies showed features of indeterminate (I) leprosy. After 6 months of MDT, out of the 24 histopathologically confirmed BT patients, 4 (17%) showed clinical activity and 8 (33%) showed histopathological activity. Of the 13 histopathologically diagnosed indeterminate cases all were clinically inactive but histological activity persisted in 3 cases (23%). Out of the 37 clinically diagnosed BT patients 3 showed both clinical and histopathological activity at the end of MDT. This study em-

phasizes the importance of performing histopathological examinations on leprosy patients undergoing research studies for the confirmation of diagnosis and for proper classification of the disease. The histopathological activity that outlasts the MDT may be due to the bacillary fragments that persist but clinical activity coupled with histopathological activity seen in 3 patients at the end of 6 months may foreshadow a relapse and these patients and others like them need to be followed up for longer durations.—Authors' Summary

Garcia, V. E., Sieling, P. A., Gong, J. H., Barnes, P. F., Uyemura, K., Tanaka, Y., Bloom, B. R., Morita, C. T. and Modlin, R. L. Single-cell cytokine analysis of gamma delta T cell responses to nonpeptide mycobacterial antigens. J. Immunol. **159** (1997) 1328–1335.

TCR gamma delta T cells are considered important in the rapid immune response to intracellular infection. We investigated the early response of peripheral blood gamma delta T cells to the nonpeptide Ag isopentenyl pyrophosphate and to its synthetic analog ethyl pyrophosphate. In healthy donors, an increase in the number of gamma delta T cells was detected as soon as 4 days after stimulation with the nonpeptide Ags. Single-cell analysis of cytokine production was performed by intracellular staining of IFN-gamma and IL-4. Gamma delta T cells were found to rapidly expand and produce IFN-gamma in response to nonpeptide Ags. Furthermore, IL-12 augmented the IFN-gamma response. In contrast, gamma delta T cells from the majority of HIV+ donors did not expand or express IFN-gamma in response to nonpeptide Ags, even in the presence of IL-12. These findings indicate a role for nonpeptide-reactive gamma delta T cells in effective cell-mediated immunity for intracellular pathogens. —Authors' Abstract

Hernandez Pando, R., Pavon, L., Arriaga, K., Orozco, H., Madrid Marina, V. and Rook, G. Pathogenesis of tuberculosis in mice exposed to low and high doses of an environmental mycobacterial saprophyte before infection. Infect. Immun. **65** (1997) 3317–3327.

Mycobacteria are ubiquitous in the environment, but they are not part of the normal human microbial flora. It has been suggested that variable contact with mycobacteria can influence susceptibility to mycobacterial pathogens and the efficacy of subsequent *Mycobacterium bovis* BCG vaccination. To test this, mice were immunized with high or low doses of an environmental saprophyte, *M. vaccae,* that is intensely immunogenic as an autoclaved preparation. Two months later, they received an intratracheal challenge with *M. tuberculosis* H37Rv. Recipients of a low Th1-inducing dose (10^7 organisms) were partially protected and maintained a high ratio of interleukin 2 (IL-2)-positive to IL-4-positive cells in the perivascular, peribronchial, and granulomatous areas of the lung; whereas in unimmunized controls the IL-4-positive cells increased markedly between days 21 and 28. In contrast, recipients of the high dose (10^9 organisms), which primes Th2 as well as Th1 cytokine production, died more rapidly than unimmunized controls and showed massive pneumonia from day 7. The ratio of IL-2-positive to IL-4-positive cells in all compartments of the lung rapidly fell to 1 by day 14 for these animals. These events correlated with cytokine mRNA profiles and with increases in the local toxicity of tumor necrosis factor alpha (TNF-alpha), demonstrable only when a major Th2 component was present. These data indicate that cross-reactive epitopes present in an environmental saprophyte can evoke either protective responses or responses that increase susceptibility to *M. tuberculosis.* The latter are associated with the presence of a Th2 component and increased sensitivity to TNF-alpha.—Authors' Abstract

Kremer, L., Estaquier, J., Brandt, E., Ameisen, J. C. and Locht, C. *Mycobacterium bovis* bacillus Calmette Guerin infection prevents apoptosis of resting human monocytes. Eur. J. Immunol. **27** (1997) 2450–2456.

Apoptosis plays an essential role in the development and homeostasis of multicel-

lular organisms. Some infectious agents interfere with this programmed cell death to their own benefit. Here, we show that infection of resting human monocytes with *Mycobacterium bovis* bacillus Calmette Guerin (BCG) increases monocyte viability by preventing them from undergoing apoptosis. Heat-killed BCG also prevented apoptosis, indicating that replication of BCG is not required to prevent cell death. Analysis of BCG-infected monocytes revealed an upregulation of the A1 mRNA; whereas the bc1–2 mRNA was not upregulated. Interestingly, preinfection with BCG renders the cells resistant to interleukin (IL)-10-induced apoptosis which may be one of the mechanisms mycobacteria use to modulate immune responses. BCG infection was also accompanied by an impairment of the capacity of monocytes to secrete IL-10 and by an induction of the capacity to secrete tumor necrosis factor-alpha, two cytokines known to induce and prevent human monocyte apoptosis, respectively. Since it has been reported that apoptosis is involved in killing of intracellular mycobacteria, the prevention of apoptosis may represent a strategy for mycobacterial survival in the infected host.—Authors' Abstract

Ladel, C. H., Blum, C., Dreher, A., Reifenberg, K., Kopf, M. and Kaufmann, S. H. E. Lethal tuberculosis in interleukin-6-deficient mutant mice. Infect. Immun. **65** (1997) 4843–4849.

Tuberculosis is a chronic infectious disease which causes major health problems globally. Acquired resistance is mediated by T lymphocytes and executed by activated macrophages. *In vitro* studies have emphasized the importance of macrophage activation for mycobacterial growth inhibition. *In vivo,* the protective host response is focused on granulomatous lesions in which *Mycobacterium tuberculosis* is contained. A cellular immune response of the T helper 1 (Th1) type is considered central for control of tuberculosis. Using interleukin-6 (IL-6)-deficient mice, we here demonstrate a crucial role of this pluripotent cytokine in protection against *M. tuberculosis* but not against *M. bovis* BCG. Infection with *M. tuberculosis* was lethal for the IL-6-deficient mice at inocula that were still controlled by IL-6-competent mice. Spleen cells from *M. tuberculosis*-infected IL-6(–/–) mouse mutants produced elevated levels of IL-4 and reduced levels of gamma interferon compared to the control levels. Cytofluorometric analyses of spleen cells from *M. tuberculosis*-infected mice revealed more-profound alterations in T-cell ratios in IL-6(–/–) mice than in control mice. We assume that IL-6 contributes to host resistance by its proinflammatory activity and by its influence on cytokine secretion.—Authors' Abstract

Ottenhoff, T. H. M., Spierings, E., Nibbering, P. H. and de Jong, R. Modulation of protective and pathological immunity in mycobacterial infections. Int. Arch. Allergy Immunol. **113** (1997) 400–408.

Mycobacterial infections represent major problems to global health care. Tuberculosis is feared particularly because of its high mortality rates; whereas in leprosy the occurrence of immunopathology, particularly nerve damage, is a major problem since the bacillus itself is relatively harmless. Thus, both effective vaccination strategies as well as novel immunomodulating regimens are warranted for the control of morbidity and mortality in mycobacterial diseases. Since CD4+ Th1 cells and type-1 cytokines play a key role both in protective immunity and immunopathology in mycobacterial infections, we here describe new pharmacological and cytokine-based strategies to regulate Th1 immunity.—Authors' Abstract

Riedel, D. D. and Kaufmann, S. H. E. Chemokine secretion by human polymorphonuclear granulocytes after stimulation with *Mycobacterium tuberculosis* and lipoarabinomannan. Infect. Immun. **65** (1997) 4620–4623.

Macrophages (MAC) and polymorphonuclear granulocytes (PNG) are professional phagocytes which perform essential functions in antibacterial defense. The intracellular bacterium *Mycobacterium tuberculosis* persists and replicates in resting

macrophages. Although it is generally assumed that activated MAC are central to protection against *M. tuberculosis,* PNG may also contribute to defense. We wondered whether PNG produce proinflammatory chemokines after stimulation by *M. tuberculosis* or its major cell wall component, lipoarabinomannan (LAM). In this study, we showed that *M. tuberculosis*- and LAM-activated human PNG secrete the leukocyte attractant interleukin-8 (IL-8) and the PNG-specific chemokine GRO-alpha in a dose-dependent manner. Treatment of PNG with the leukotriene-B4 inhibitor MK-886 prior to stimulation with *M. tuberculosis* or LAM partially blocked IL-8 and GRO-alpha induction, suggesting involvement of the 5-lipoxygenase pathway in the secretion of these chemokines. We conclude that PNG contribute to early resistance to *M. tuberculosis* via chemokine secretion.—Authors' Abstract

Rojas, M., Barrera, L. F., Puzo, G. and Garcia, L. F. Differential induction of apoptosis by virulent *Mycobacterium tuberculosis* in resistant and susceptible murine macrophages—role of nitric oxide and mycobacterial products. J. Immunol. **159** (1997) 1352–1361.

Resistance and susceptibility of macrophages to mycobacteria are under the control of the Bcg/Nramp1 gene, which also controls the NO– production in response to macrophage activators. There is recent evidence indicating that mycobacteria induce apoptosis in infected macrophages. Using murine macrophage lines, congenic at the Bcg/Nramp1 gene, this report shows that B10R are more prone than B10S macrophages to undergo apoptosis after exposure to live virulent *Mycobacterium tuberculosis* H37Rv or PPD. As determined by cell viability, DNA fragmentation, hypoploidy, and the terminal deoxynucleotide transferase dUTP-biotin nick-end labeling assay, induction of apoptosis correlated with NO– production. Aminoguanidine and anti-TNF-alpha inhibited NO– production and apoptosis. B10R and B10S macrophages were equally affected by sodium nitroprusside, a donor of NO–, but its effect, mainly in B10R cells, was enhanced by the presence of *M. tuberculosis.* Nonvirulent mycobacteria induced lower levels of NO– and did not cause cell death. Killed *M. tuberculosis* mannose-capped lipoarabinomannan (ManLAM), and LPS rescued macrophages from apoptosis albeit they induce NO–. These findings suggest the existence of opposite pathways: metabolically active mycobacteria promotes apoptosis; whereas their structural components inhibit it. Apoptosis may be a critical mechanism by which the Nramp1 gene controls the macrophage infection with virulent mycobacteria.—Authors' Abstract

Rojas, R. E., Demichelis, S. O., Sarno, E. N. and Segal Eiras, A. IgM anti-phenolic glycolipid I and IgG anti-10-kDa heat shock protein antibodies in sera and immune complexes isolated from leprosy patients with or without erythema nodosum leprosum and contacts. FEMS Immunol. Med. Microbiol. **19** (1997) 65–74.

The aim of the present work was to evaluate the levels of anti-PGL-I and anti-10-kDa heat-shock protein antibodies in serum and immune complexes isolated from leprosy patients, convivients and controls. Leprosy patients with erythema nodosum leprosum or without it were included and a comparative study was done to investigate intergroup differences. Immune complexes were precipitated from serum by polyethylene glycol 3.5%; antibody levels were measured in sera and in dissociated immune complexes by ELISA. Serum antibody levels were then correlated with immune complex-associated antibody levels. The results showed that the erythema nodosum leprosum group differed from controls, contacts and non-erythema nodosum leprosum patients in their immune complex levels. IgM anti-PGL-I and IgG anti-10-kDa heat-shock protein antibodies were constituents of the immune complexes in patients with erythema nodosum leprosum, who exhibited a significant difference in their immune complex composition compared with controls, contacts and non-erythema nodosum leprosum patients while free antibody levels (anti-PGL-I and anti-10-kDa) did not differentiate between erythema nodosum leprosum and non-erythema nodosum leprosum patients, the measurement of immune complex-associated antibodies demonstrated

a significant difference between the two clinical conditions. Furthermore, the measurement of immune complex-associated anti-PGL-I IgM made it possible to differentiate between contacts and controls. The significance of these results is discussed.—Authors' Abstract

Rook, G. A. W. Intractable mycobacterial infections associated with genetic defects in the receptor for interferon gamma: what does this tell us about immunity to mycobacteria? Thorax **52** Suppl. 3 (1997) S41–S46.

The attenuated strain of *Mycobacterium bovis* bacille Calmette-Guerin (BCG) is the most widely used vaccine in the world. In most children, inoculation of live BCG vaccine is harmless although it occasionally leads to a benign regional adenitis. In rare cases, however, vaccination causes disseminated BCG infection, which may be lethal. Impaired immunity of the host is generally thought to be the pathogenic mechanism. Disseminated BCG infection has been reported in children with inherited immune disorders. Most of these children had severe combined immunodeficiency, which is characterized by an absence of T cells, and some had chronic granulomatous disease, which is marked by an impairment of the phagocyte respiratory burst. Rare cases of BCG infection have also been reported in association with the acquired immunodeficiency syndrome. We examined the five genes coding for interferon-gamma, interferon-gamma R1, IRF1, TNF-alpha and TNF-alpha R1 in a child with fatal idiopathic disseminated BCG infection. We found a mutation of the gene for interferon-gamma R1. There was no detectable interferon-gamma R1 on the cells from the affected child. These findings provide further evidence of the importance of interferon-gamma in the response to mycobacterial infection.—Author's Abstract

Roy, S., McGuire, W., Mascie Taylor, C. G. N., Saha, B., Hazra, S. K., Hill, A. V. S. and Kwiatkowski, D. Tumor necrosis factor promoter polymorphism and susceptibility to lepromatous leprosy. J. Infect. Dis. **176** (1997) 530–532.

Genetically determined differences in immune responses to environmental agents may underlie susceptibility to many autoimmune and infectious diseases. Leprosy provides an example of a polarity in the type of immune response made to an infectious agent, and there is evidence that the major histocompatibility complex is genetically linked to leprosy type. It was found that HLA-DR2 is associated with both tuberculoid and lepromatous types of leprosy; however, a variant at position –308 of the promoter of the neighboring tumor necrosis factor (TNF) gene was increased in frequency in lepromatous (odds ratio = 3.0, p = 0.02) but not tuberculoid leprosy. Some studies have found higher serum levels of TNF in lepromatous than tuberculoid leprosy, and high TNF levels are found in malaria and leishmaniasis, which are also associated with this TNF allele. It is speculated that this association reflects genetic variability in cytokine production, which influences the immune response to and clinical outcome of leprosy.—Authors' Abstract

Russell, D. G., Sturgill Koszycki, S., Van Heyningen, T., Collins, H. and Schaible, U. E. Why intracellular parasitism need not be a degrading experience for *Mycobacterium*. Phil. Trans. R. Soc. London Ser. B—Biol. Sci. **352** (1997) 1303–1310.

The success of mycobacteria as pathogens hinges on their ability to infect and persist within the macrophages of their host. However, activation of host macrophages by cytokines from a productive cellular immune response can stimulate the cells to kill their resident pathogens. This suggests that the interaction between host cell and microbe is in delicate balance, which can be tipped in favor of either organism. Biochemical analysis of mycobacterial vacuoles has shown them to be integral to the host cell's recycling endosomal system. As such they show limited acidification and hydrolytic activity despite possession of known lysosomal constituents such as cathepsins D, B and L, and LAMP 1. Even in established infections, they remain dynamic compartments accessible to several plasmalemma-derived constituents.

Once the macrophage has been activated by IFN-gamma and TNF-alpha the vacuoles coalesce and acidify. This marks a distinct alteration in vacuole physiology and leads to stasis and death of the mycobacteria. Mycobacteria have developed several strategies to avoid this outcome. Most notably, live bacilli induce sustained release of IL-6 from infected macrophages. IL-6 blocks the ability of both polyclonal primary T cells and T-cell hybridomas to respond to appropriate stimuli. Such an activity could render the centers of infection foci, such as granulomas, anergic and thus avoid release of macrophage-activating cytokines. This paper discusses both the mechanisms by which mycobacteria try to ensure their success as intracellular pathogens and the relevance of these strategies to the overall understanding of mycobacterial diseases.—Authors' Abstract

Yin, Y., *et al.* [Serological activity of recombinant α-antigen of *M. leprae.*] China Lepr. J. **13** (1997) 68–70. (in Chinese)

Two techniques of ELISA for the detection of antibodies to *M. leprae* have been developed. They use the recombinant α1- and α2-antigen of *M. leprae,* and the sera from leprosy patients (L 38, B 19 and T 36) were analyzed with the techniques. PGL-I ELISA was used as a reference test. The results showed that the sensitivity of α1- and α2-ELISA and the PGL-I ELISA on L leprosy sera were 97%, 92% and 94%, respectively. The corresponding results for B and T leprosy sera were 84%, 58%, 68% and 47%, 38%, 30%, respectively. The α1 ELISA has the highest sensitivity.—Authors' English Abstract

Zugel, U. and Kaufmann, S. H. E. Activation of CD8 T cells with specificity for mycobacterial heat shock protein 60 in *Mycobacterium bovis* bacillus Calmette-Guerin-vaccinated mice. Infect. Immun. **65** (1997) 3947–3950.

Heat-shock protein 60 (hsp60)-specific CD8 T cells lysed *Mycobacterium bovis* BCG-infected macrophages *in vitro* and adoptively transferred protection against mycobacterial infection. Moreover, CD8 T cells with this hsp60 specificity were activated *in vivo* by BCG vaccinations. Our studies suggest there is participation of hsp60-specific CD8 T cells in BCG-induced immunity.—Authors' Abstract

Microbiology

Alcaide, F., Pfyffer, G. E. and Telenti, A. Role of embB in natural and acquired resistance to ethambutol in mycobacteria. Antimicrob. Agents Chemother. **31** (1997) 2270–2273.

The mycobacterial embCAB operon encodes arabinosyl transferases, putative targets of the antimycobacterial agent ethambutol (EMB). Mutations in embB lead to resistance to EMB in *Mycobacterium tuberculosis.* The basis for natural, intrinsic resistance to EMB in nontuberculous mycobacteria (NTM) is not known; neither is the practical implication of resistance to EMB in the absence of embB mutations in *M. tuberculosis* well understood. The conserved embB resistance-determining region (ERDR) of a collection of 13 strains of NTM and 12 EMB-resistant strains of *M. tuberculosis* was investigated. Genotypes were correlated with drug susceptibility phenotypes. High level natural resistance to EMB (MIC, greater than or equal to 64 μg/ml) was associated with a variant amino acid motif in the ERDR of *M. abscessus, M. chelonae,* and *M. leprae.* Transfer of the *M. abscessus* emb allele to *M. smegmatis* resulted in a 500-fold increase in the MICs. In *M. tuberculosis,* embB mutations were associated with MICs of ≥20 μg/ml while resistance not associated with an ERDR mutation generally resulted in MICs of ≥10 μg/ml. These data further support the notion that the emb region determines intrinsic and acquired resistance to EMB and might help in the re-

assessment of the current recommendations for the screening and treatment of infections with EMB-resistant *M. tuberculosis* and NTM.—Authors' Abstract

Besra, G. S., Morehouse, C. B., Rittner, C. M., Waechter, C. J. and Brennan, P. J. Biosynthesis of mycobacterial lipoarabinomannan. J. Biol. Chem. **272** (1997) 18460–18466.

The mycobacterial lipoglycans, lipomannan (LM) and lipoarabinomannan (LAM), are potent immunomodulators in tuberculosis and leprosy. Little is known of their biosynthesis, other than being based on phosphatidylinositol (PI), and they probably originate in the phosphatidylinositol mannosides (PIMs; PIMans). A novel form of cell-free incubation involving *in vitro* and *in situ* labeling with GDP-[(14)]Man of the polyprenyl-P-mannoses (C35C50-P-Man) and the simpler PIMs of mycobacterial membranes, reisolation of the [C-14]Man-labeled membranes, and *in situ* chase demonstrated the synthesis of a novel ru(1 → 6)-linked linear form of LM at the expense of the C-33/C-50-P-Man. There was little or no synthesis under these conditions of PIMan(5) with its terminal alpha(1 → 6)Man unit or the mature LM or LAM with copious alpha(1 → 2)Man branching. Synthesis of the linear LM, but not of the simpler PIMan(2), was susceptible to amphomycin, a lipopeptide antibiotic that specifically inhibits polyprenyl-P-requiring translocases. A mixture of P[H-3]I and P[H-3]IMan(2) was incorporated into the linear LM, supporting other evidence that, like the PIMs, LM and LAM, it is a lipid-linked mannooligosaccharide and a new member of the mycobacterial glycosylphosphatidylinositol lipoglycan/glycolipid class. Hence, the simpler PIMs originate in PI and GDP-Man, but further growth of the linear backbone emanates from C-35-/C-50-P-Man and is amphomycin-sensitive. The origin of the alpha(1 → 1)Man branches of mature PIMan(5), LM, and LAM is not known at this time but is probably GDP-IMan.—Authors' Abstract

Bibi, F., Espitia, C., Alito, A., Zumarraga, M., Romano, M. I., Cravero, S. and Cataldi, A. A novel 27 kDa lipoprotein antigen from *Mycobacterium bovis.* Microbiology **143** (1997) 3599–3605.

A novel *Mycobacterium bovis* antigen was identified from an expression library using sera from naturally infected cattle. The *Escherichia coli* recombinant clone expressed a 27-kDa protein, named P27. A rabbit serum against the recombinant antigen recognized a protein of 27 kDa in cellular extracts from *M. bovis* and *M. tuberculosis.* No protein was recognized in the culture supernatant. Sequence analysis indicated that P27 has a molecular mass of 24 kDa, showing a characteristic signal sequence for lipoprotein modification (a signal peptidase type II site). The gene is identical to a gene identified in the *M. tuberculosis* genome sequencing project. Cellular fractionation experiments suggested that P27 is an integral membrane protein. The antigen was recognized by individual sera and peripheral blood mononuclear cells (PBMC) from diseased cattle. PCR experiments with specific primers directed to the P27 structural gene indicated that it is only present in the *M. tuberculosis* species complex. In conclusion, a novel immunogenic lipoprotein in *M. bovis/M. tuberculosis* has been identified. The results presented here and elsewhere suggest that mycobacterial lipoproteins should be considered in the design of new recombinant vaccines and diagnostic methods.—Authors' Abstract

Durbach, S. I., Andersen, S. J. and Mizrahi, V. SOS induction in mycobacteria: analysis of the DNA-binding activity of a LexA-like repressor and its role in DNA damage induction of the *recA* gene from *Mycobacterium smegmatis.* Mol. Microbiol. **26** (1997) 643–653.

The protein encoded by the *lexA* gene from *Mycobacterium leprae* was overproduced in *Escherichia coli.* The recombinant protein bound to the promoter regions of the *M. leprae lexA, M. leprae recA* and *M. smegmatis recA* genes at sites with the sequences 5′-GAACACATGTTT AND 5′-GAACAGGTGTTC, which belong to the "Cheo box" family of binding sites recognized by the SOS repressor from *Bacillus subtilis.* Gel mobility shift assays were used to confirm that proteins with the same site

specificity of DNA binding are also present in *M. tuberculosis* and *M. smegmatis.* Complex formation was impaired by mutagenic disruption of the dyad symmetry of the *M. smegmatis recA* Cheo box. LexA binding was also inhibited by preincubation of the *M. smegmatis* and *M. tuberculosis* extracts with anti-*M. leprae* LexA antibodies, suggesting that the mycobacterial LxA proteins are functionally conserved at the level of DNA binding. Finally, exposure of *M. smegmatis* to DNA-damaging agents resulted in induction of the *M. smegmatis recA* promoter with concomitant loss of DNA binding of LexA to its Cheo box, confirming that this organism possesses the key regulatory elements of a functional SOS induction system.—Authors' Summary

Fullner, K. J. and Hatfull, G. F. Mycobacteriophage L5 infection of *Mycobacterium bovis* BCG: implications for phage genetics in the slow-growing mycobacteria. Mol. Microbiol. **26** (1997) 755–766.

Mycobacteriophage L5 is a well-characterized temperate phage that forms stable lysogens in *Mycobacterium smegmatis.* The host range of L5 is, however, unclear because previous reports suggested that it does not infect slow-growing mycobacteria such as *M. tuberculosis* and bacille Calmette-Guérin (BCG). Moreover, luciferase reporter phage derivatives of L5 failed to produce light from BCG, suggesting that infection is blocked at or before the stage of DNA injection. In this study, we demonstrate that L5 infection of slow-growing mycobacteria specifically requires a high concentration of Ca^{2+}, conditions that differs from those required for infection of *M. smegmatis* by L5 and for infection of BCG by the closely related phage D29. In addition, we show that there are specific genetic determinants of L5 that confer the ability to infect slow-growing mycobacteria, without altering infection of *M. smegmatis.* These observations extend the use of phage L5 for the diagnosis and analysis of tuberculosis and other mycobacterial diseases.—Authors' Summary

Hoppe, H. C., de Wet, B. J. M., Cywes, C., Daffe, M. and Ehlers, M. R. W. Identification of phosphatidylinositol mannoside as a mycobacterial adhesion mediating both direct and opsonic binding to nonphagocytic mammalian cells. Infect. Immun. **65** (1997) 3896–3905.

The molecular basis for the binding of *Mycobacterium tuberculosis* to nonphagocytic cells, which are readily infected *in vitro,* and the *in vivo* significance of this interaction are incompletely understood. Of six cell types tested, we found that only two, Chinese hamster ovary (CHO) fibroblasts and primary porcine aortic endothelial cells, were able to bind *M. tuberculosis* H37Rv efficiently *in vitro.* Binding to both CHO and endothelial cells was markedly (three- to fivefold) enhanced by 10% to 20% human or bovine serum, suggesting that the bacteria were coated by a serum opsonin. Preincubation with individual candidate opsonins revealed that recombinant human mannose-binding protein (rMBP), fibronectin, and transferrin were each able to enhance binding threefold. Preincubation of bacteria in serum depleted of mannan-binding lectins or in genetic MBP-deficient serum resulted in enhancements that were only similar to 60% and 58%, respectively, of that produced by preincubation in control serum. In contrast, serum depleted of fibronectin or transferrin retained its opsonizing capacity, suggesting that the latter two are not significant opsonins in whole serum. Binding of *M. tuberculosis* and *M. smegmatis* to both CHO and endothelial cells on the presence or absence of serum was blocked (60% to 70%) by a monoclonal antibody, MAb 1D1, selected for recognition of intact bacilli. The 1D1 antigen was purified from mycobacterial cell walls and chemically identified as a polar phosphatidylinositol mannoside (PIM). Latex beads coated with purified 1D1 antigen bound to CHO cells, which was enhanced threefold by serum and abolished by periodate treatment, suggesting a requirement for the PIM mannoses in opsonic adhesion. This was likely mediated, at least in part, by serum MBP, as rMBP bound strongly to 1D1 antigen in both thin-layer chromatography overlay and plate binding assays, the latter in a mannan-inhibitable manner. This is the first demonstration that mycobacterial PIMs can function as adhesins for binding to nonphagocytic cells, both directly and af-

ter opsonization with serum proteins, including MBP.—Authors' Abstract

Hou, W., *et al.* [A PCR for 16s rRNA of *M. leprae.*] China Lepr. J. **13** (1997) 70–72. (in Chinese)

A PCR test for the detection of *M. leprae* has been developed on the basis of the gene coding the species-specific fragment of 16s rRNA of *M. leprae.* The test is specific and repeatable. Its deduction low limit is 200 bacilli.—Authors' English Abstract

Lee, R. E., Smith, M. D., Nash, R. J., Griffiths, R. C., McNeil, M., Grewal, R. K., Yan, W. X., Besra, G. S., Brennan, P. J. and Fleet, G. W. J. Inhibition of UDP-Gal mutase and mycobacterial galactan biosynthesis by pyrrolidine analogues of galactofuranose. Tetrahedron Lett. **38** (1997) 6733–6736.

Some pyrrolidine analogs of galactofuranose-synthesized from carbohydrate lactones—are the first known inhibitors of *E. coli* K12 UDP-Gal mutase and mycobacterial galactan biosynthesis. This inhibition may form a new chemotherapeutic strategy for the treatment of human pathogens which contain integral galactofuranosyl structures such as tuberculosis and leprosy.—Authors' Abstract

Lepage, S., Dubois, P., Ghosh, T. K., Joris, B., Mahapatra, S., Kundu, M., Basu, J., Chakrabarti, P., Cole, S. T. Nguyen Disteche, M. and Ghuysen, J. M. Dual multimodular class A penicillin-binding proteins in *Mycobacterium leprae.* J. Bacteriol. **179** (1997) 4627–4630.

The ponA gene of cosmid L222 of the *Mycobacterium leprae* genome library encodes a multimodular class A penicillin-binding protein (PBP), PBP1*. The PBP, labelled with a polyhistidine sequence, has been produced in *Escherichia coli,* extracted from the membranes with 3-[(3-cholamidopropyl)-dimethylammonio]-1 propane-sulfonate (CHAPS) and purified by Ni2+-nitrilotriacetic acid-agarose chromatography. In contrast to the pon1-encoded class A PBP1, PBP1* undergoes denaturation at temperatures higher than 25°C, it catalyzes acyl transfer reactions on properly structured thiolesters, and it binds penicillin with high affinity.—Authors' Abstract

Mehrotra, J., Mittal, A., Dhindsa, M. S. and Sinha, S. Fractionation of mycobacterial integral membrane proteins by continuous elution SDS-PAGE reveals the immunodominance of low molecular weight subunits for human T cells. Clin. Exp. Immunol. **109** (1997) 446–450.

Integral membrane proteins (IMP) represent a serologically distinct class of mycobacterial antigens which are potent stimulators of human T cells (Mehrotra *et al.,* Clin Exp Immunol 1995; 102:626). The range of LMP from *Mycobacterium fortuitum* was resolved by continuous elution SDS-PAGE to recover 31 discrete fractions covering bands up to approximate to 58 kD. The fractions, after removal of SDS, were subjected to human T-cell proliferation assays for the identification of immunodominant molecule(s). A low molecular weight (<20 kD) fraction was able to stimulate T cells from 11 out of 12 donors comprising mainly tuberculoid leprosy patients. The described protocol is well suited to situations where large quantities of antigenic protein mixtures must be processed in order to get the purified molecules/fractions in amounts required for immunoepidemiological studies.—Authors' Abstract

Mukhopadhyay, S., Basu, D. and Chakrabarti, P. Characterization of a porin from *Mycobacterium smegmatis.* J. Bacteriol. **179** (1997) 6205–6207.

A pore-forming protein with an M-r of 40,000 has been extracted from the cell wall of *Mycobacterium smegmatis* with buffer containing the detergent Zwittergent 3–12 and 0.5 M NaCl and purified on an anion-exchange column. Although the pore diameter was large (2 nm), the specific activity was much lower than those of non-specific porin channels of enteric bacteria.

The channel allowed the permeation of small hydrophilic molecules such as sugars and amino acids. Its N-terminal sequence did not show any similarity to those of other porins sequenced so far.—Authors' Abstract

Osawa, N. Growth of *Mycobacterium leprae* in macrophages *in vitro.* Proc. Japan Acad. Ser. B.—Phys. Biol. Sci. **73** (1997) 138–143.

Macrophages were obtained from peritoneal cavities of mice and infected *in vitro* with *Mycobacterium leprae.* After 3 weeks, the multiplication of the bacteria was observed inside of the infected cells. Three weeks after the infection of the medium was replaced and antibiotic free medium was added to culture flask which was incubated at 32°C. Three months later, amorphous turbidity was observed under the surface layer of the medium. This turbidity slowly progressed with time. Next the sample was centrifuged and CBA macrophages added. After 3 weeks Ziehl-Neelsen positive rods were observed in 0.2%–0.6% of the cells. These observations not only indicated the release of *M. leprae* from infected macrophages, but also suggested the possibility of the microorganism growing in the medium itself. With these observations and experiences accumulated over 15 years of trial and error, I discuss various potential improvements for the medium and the environment to permit more efficient growth of *M. leprae in vitro.*—Author's Abstract

Osawa, N. Growth of *Mycobacterium leprae in vitro.* Proc. Japan Acad. Ser. B.—Phys. Biol. Sci. **73** (1997) 144–149.

Mycobacterium leprae is cultured *in vitro* with a newly devised ML medium. Two different samples were tested: one *M. leprae* Thai-53 strain which was passaged through nude mice in 1976, and another clinically isolated *M. leprae* K-1 strain; 10^8 cells from these samples were cultured with ML medium at 32°C in glass flasks and the medium gradually become turbid [as measured by optical density (OD) at 510 nm]. The addition of ethylene oxide treated Cutina LE (Henkel, Germany) to the ML medium significantly increased the turbidity. The turbidity increase without Cutina LE was from 0.339 to 0.687 in 65 days, while the addition of Cutina LE resulted in OD change from 0.05 to 0.67 in 1 week. The observation of the surface of Cutina LE by phase-contrast microscope showed the adherent bacteria with apparent active multiplication. The identity of the turbidity was confirmed to be *M. leprae* by the use of polymerase chain reaction.—Author's Abstract

Quan, S., Venter, H. and Dabbs, E. R. Ribosylative inactivation of rifampin by *Mycobacterium smegmatis* is a principal contributor to its low susceptibility to this antibiotic. Antimicrob. Agents Chemother. **41** (1997) 2456–2460.

Mycobacterium smegmatis inactivates rifampin by ribosylating this antibiotic. The gene responsible for this ability was cloned and was shown to confer low-level resistance to this antibiotic (MIC increase, about 12-fold) in related organisms. A 600-bp subclone responsible for ribosylating activity and resistance carried an open reading frame of 429 bp. Targeted disruption of the gene in *M. smegmatis* resulted in mutants with much increased susceptibility to rifampin (MICs of 1.5 instead of 20 μg/ml) as well as the loss of antibiotic-inactivating ability. Also, disruption of this gene led to a much lower frequency of occurrence of spontaneous high-level rifampin-resistant mutants.—Authors' Abstract

Ribeiro, G., Viveiros, M., David, H. L. and Costa, J. V. Mycobacteriophage D29 contains an integration system similar to that of the temperate mycobacteriophage L5. Microbiology **143** (1997) 2701–2708.

A mycobacteriophage D29 DNA fragment cloned in pRM64, a shuttle plasmid that transforms *Mycobacterium smegmatis,* was sequenced. The determined sequence was 2592 nucleotides long and had a mean G+C content of 63.7 mol%, similar to that of mycobacterial DNA. Four ORFs were

identified: one with strong homology to dCMP deaminase genes; one homologous to mycobacteriophage L5 gene 36, whose function is unknown; one encoding a possible excisase; and one encoding an integrase. The intergenic region between the putative excisase gene and the integrase gene had a lower than average G+C content and showed the presence of the same *attP* core sequence as mysobacteriophage LS. Transformation experiments using subclones of pRM64 indicated that the integrase gene and all the intergenic region were essential for stable transformation. A subclone containing the integrase gene and the core *attP* sequence was able to transform but recombinants were highly unstable. Southern analysis of total DNA from cells transformed with pRM64 and its derivatives showed that all the plasmids were integrated at one specific site of the bacterial chromosome. A recombinant exhibiting a high level of resistance to the selective drug kanamycin had two plasmids integrated at different sites. These results demonstrated that the D29 sequences contained in pRM64 were integrative, indicating that the generally held view of D29 as a virulent phage must be reviewed.—Authors' Abstract

Schorey, J. S., Carroll, M. C. and Brown, E. J. A macrophage invasion mechanism of pathogenic mycobacteria. Science **277** (1997) 1091–1093.

Tuberculosis is the leading cause of death due to an infectious organism, killing an estimated 3 million people annually. *Mycobacterium tuberculosis,* the causative agent of tuberculosis, and other pathogenic mycobacteria require entry into host macrophages to initiate infection. An invasion mechanism was defined that was shared among pathogenic mycobacteria including *M. tuberculosis, M. leprae,* and *M. avium* but not by nonpathogenic mycobacteria or nonmycobacterial intramacrophage pathogens. This pathway required the association of the complement cleavage product C2a with mycobacteria, resulting in the formation of a C3 convertase. The mycobacteria-associated C2a cleaved C3, resulting in C3b opsonization of the mycobacteria and recognition by macrophages.—Authors' Abstract

Smith, D. R., Richterich, P., Rubenfield, M., Rice, P. W., Butler, C., Lee, H. M., Kirst, S., Gundersen, K., Abendschan, K., Xu, Q. X., *et al.* Multiplex sequencing of 1.5 Mb of the *Mycobacterium leprae* genome. Genome Res. **7** (1997) 802–819.

The nucleotide sequence of 1.5 Mb of genomic DNA from *Mycobacterium leprae* was determined using computer-assisted multiplex sequencing technology. This brings the 2.8-Mb *M. leprae* genome sequence to similar to 66% completion. The sequences, derived from 43 recombinant cosmids, contain 1046 putative protein-coding genes, 44 repetitive regions, 3 rRNAs, and 15 tRNAs. The gene density of one per 1.4 kb is slightly lower than that of *Mycoplasma* (1.2 kb). Of the protein coding genes, 44% have significant matches to genes with well-defined functions. Comparison of 1157 *M. leprae* and 1564 *M. tuberculosis* proteins shows a complex mosaic of homologous genomic blocks with up to 22 adjacent proteins in conserved map order. Matches to known enzymatic, antigenic, membrane, cell wall, cell division, multidrug resistance, and virulence proteins suggest therapeutic and vaccine targets. Unusual features of the *M. leprae* genome include large polyketide synthase (pks) operons, inteins, and highly fragmented pseudogenes.—Authors' Abstract

Srikanth, N. C., Gangadhar, S., Narasimha Reddy, Y., Apte, S. S., Lakshmipathy, V., Apparao, A. V. N., Krishna, D. R. and Prabhakar, M. C. Characterization of leprosy based on the nasal lipid profile. Indian J. Lepr. **69** (1997) 179–181.

While extracting *M. leprae* from the nasal flushings of leprosy patients it was found that these organisms were trapped in the waxy layer, between the aqueous and the chloroform layers. Thin layer chromatography (TLC) analysis of this layer, using a chloroform-methanol-water system, revealed different spots when sprayed with acid alcohol and heated at 160°C. The TLC profile of lipids of lepromatous and border-

line (MB according to the WHO terminology) leprosy patients was distinctly different from that of tuberculoid leprosy patients and normal human volunteers. A simple, economical and fast procedure to characterize patients belonging to different spectra has been developed.—Authors' Abstract

Sun, Z., Scorpio, A. and Zhang, Y. The *pncA* gene from naturally pyrazinamide-resistant *Mycobacterium avium* encodes pyrazinamidase and confers pyrazinamide susceptibility to resistant *M. tuberculosis* complex organisms. Microbiology **143** (1997) 3367–3373.

The antituberculosis drug pyrazinamide (PZA) needs to be converted into pyrazinoic acid (POA) by the bacterial pyrazinamidase (PZase) in order to show bactericidal activity against *Mycobacterium tuberculosis. M. avium* is naturally resistant to PZA. To investigate whether this natural resistance to PZA is due to inability of the *M. avium* PZase to convert PZA to bactericidal POA, the *M. avium* PZase gene (*pncA*) was cloned by using the *M. tuberculosis pncA* gene as a probe. Sequence analysis showed that the *M. avium pncA* gene is 561 bp long, encoding a protein with a predicted size of about 19.8 kDa; but Western blotting showed that the *M. avium* PZase migrated as a 24-kDa band when expressed in *M. bovis* BCG and *Escherichia coli.* Sequence comparison revealed that *M. avium* PZase has 67.7% and 32.8% amino acid identity with the corresponding enzymes from *M. tuberculosis* and *E. coli,* respectively. Southern blot analysis with the *M. avium pncA* gene as a probe showed that *M. terrae, M. gastri, M. marinum, M. fortuitum, M. xenopi, M. gordonae, M. szulgai, M. celatum* and *M. kansasii* have close *pncA* homologues; whereas *M. chelonae* and *M. smegmatis* did not give significant hybridization signals. Transformation with the *M. avium pncA* gene conferred PZA susceptibility to PZA-resistant *M. tuberculosis* complex organisms, indicating that the nonsusceptibility of *M. avium* to PZA is not due to an ineffective PZase enzyme, but appears to be related to other factors such as transport of POA.—Authors' Abstract

Experimental Infections

Matsuoka, M., Nomaguchi, H., Yukitake, H., Ohara, N., Matsumoto, S., Mise, K. and Yamada, T. Inhibition of multiplication of *Mycobacterium leprae* in mouse foot pads by immunization with ribosomal fraction and culture filtrate from *Mycobacterium bovis* BCG. Vaccine **15** (1997) 1214–1217.

Immunization of mice with the ribosomal fraction from ruptured *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) and the culture filtrate reduced remarkably the multiplication of *M. leprae* in the foot pads of mice. This is the first reported case of the protective activity against *M. leprae* multiplication in mice of the BCG ribosomal fraction and culture filtrate. The inhibition was more evident with the culture filtrate than with the ribosomal fraction. When the ribosomal proteins separated from ribosomal RNA were injected into mice, only a slight inhibition was observed. Ribosomal RNA alone did not inhibit at all, in contrast to the conclusion reported by Youmans and Youmans.—Authors' Abstract

Singh, N., Birdi, T. J., Chandrashekar, S. and Antia, N. H. Schwann cell extracellular matrix protein production is modulated by *Mycobacterium leprae* and macrophage secretory products. J. Neurol. Sci. **151** (1997) 13–22.

Extracellular matrix (ECM) protein deposition is an important feature of leprous nerves, where Schwann cells (SCs) and macrophages are the main hosts for *Mycobacterium leprae.* Since, SCs are involved in the synthesis of ECM proteins and its production is regulated by macro-

phage secretory factors, the present study aimed to determine *in vitro* the effect of *M. leprae* infection and macrophage secretory products on secretion of ECM proteins by SCs in two strains of mice, Swiss White (SW) and C57BL/6, that are known to differ in their nerve pathology and macrophage functions in response to infection. Following 6 days of *M. leprae* infection, SCs from SW mice responded with increase-d secretion of C-14-leucine radiolabelled proteins and a concomitant increase in laminin and collagens type I, III and IV, as determined by enzyme-linked immunosorbent assay. In contrast, infected C57BL/6 SCs responded with decreased secretion of total proteins and fibronectin. Exposure of SCs to macrophage-conditioned medium resulted in decreased ECM protein secretion in both strains of mice. This decrease was a function of protein breakdown by macrophage-derived proteases and also active regulation by macrophage-secreted cytokines. A similar effect of *M. leprae* and macrophage secretory products on SC metabolism in leprous nerves would have major ramifications on damage and repair activities. In addition ECM proteins would also influence the composition of the infiltrating cell population in lepromatous and tuberculoid nerves.—Authors' Abstract

Epidemiology and Prevention

Bertolli, J., Pangi, C., Frerichs, R. and Halloran, M. E. A case-control study of the effectiveness of the BCG vaccine for preventing leprosy in Yangon, Myanmar. Int. J. Epidemiol. **26** (1997) 888–896.

Background: Five randomized trials, a follow-up study, and six case-control investigations of BCG vaccine's effectiveness (VE) for preventing leprosy have been conducted internationally, with widely varying estimates of VE. Because of the difficulty of generalizing from disparate results, local estimates of VE are needed for health planning purposes and are currently particularly relevant, given the World Health Organization's (WHO) goal to eliminate leprosy by the year 2000.

Methods: We conducted a case-control study in Yangon, Myanmar. Residents of Yangon between the ages of 6 years and 24 years who were listed in the National Leprosy Registry as being on active treatment for leprosy between December 1992 and April 1993 were eligible to participate in the study as cases. Control subjects were matched to the cases on age, sex, and neighborhood.

Results: One or more doses of BCG were associated with a VE of 66%. The results show a significant trend of increasing VE with increasing number of BCG doses (one dose, VE = 55%; two doses, VE = 68%; three doses, VE = 87%). One dose of BCG vaccine appeared to provide protection substantially higher than that found in an earlier vaccine trial in Myanmar, but consistent with results from case-control studies in other countries.

Conclusions: These data suggest that BCG reduces the risk of leprosy in Myanmar, and that BCG vaccination of infants, along with early case-finding and treatment, should be considered an important part of the leprosy intervention strategy.—Authors' Abstract

Chen, J. [A survey of residential hygiene among leprosy patients.] China Lepr. J. **13** (1997) 150–152. (in Chinese)

The residential environment of 70 newly detected cases of leprosy (PB 45 and MB 25) has been examined and compared with that of local healthy residents since 1975 in Liyang City, Jiangsu, China. There totally were 352 members in their families; the sources of infection for them were inside their families in 7 cases, within their villages in 54 and unknown in 9. Their houses all are thatch or tile and brick structures with earth-filled ground and without any windows, but those of local healthy resi-

dents are tile and brick or concrete with concrete ground and front and rear windows. In their houses, the average content of CO_2 was 0.35% and the total of bacteria was 23,253/m^3 in the air on the average, but those in healthy residents are 0.16% and 6325/m^3, respectively. The author points out that the residential environment of leprosy patients clearly was worse than that of local healthy residents, so improvement of this condition will be conducive to the eradication of leprosy.—Author's English Abstract

Ferra Torres, T. and Carrazana Hernandez, G. B. [Method of detection and source of infection in the incidence of leprosy.] Rev. Leprol. Fontilles **21** (1997) 161–165. (in Spanish)

It was determined the means of detection and sources of infection of the 81 incidences of leprosy reported in Camagüey town during 1989–1993.

Considered in the means of detection were three groups: spontaneous, household contacts and risk population. Within the sources of infection it was considered the following relations: father, mother, brother-sister, husband/wife, other family members, neighbors and work-mates. It was confirmed that 82.7% of the cases visited the doctor spontaneously, 13.6% was detected through house calls and 3.7% through examination of risk population. The principal source of infection was represented by neighbors (19.8%) followed by the families (father, mother, brother-sister and others) (14.8%). Spouses were not determined as a source of infection.—Authors' English Summary

Fine, P. E. M. and Smith, P. G. Vaccination against leprosy—the view from 1996. Lepr. Rev. **67** (1996) 249–252.

The implications of the "Karonga Prevention Trial" of leprosy vaccine in Malawi are discussed. The trial evaluated 2 vs. 1 BCG vaccination and BCG plus killed *Mycobacterium leprae* vaccination vs. BCG alone. The trial found that BCG booster reduced leprosy risk by about 50% and that the combined BCG plus *M. leprae* vaccine showed no convincing evidence of imparting more protection against leprosy than BCG alone. There was no evidence that either vaccine regimen imparted any protection against pulmonary tuberculosis although some protection was seen against glandular tuberculosis.—Authors' Abstract

Lechat, M. F., Shrager, D. I., Declercq, E., Bertrand, F., Blettner, W. A. and Blumberg, B. S. Decreased survival of HTLV-I carriers in leprosy patients from the Democratic Republic of the Congo: a historical prospective study. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **15** (1997) 387–390.

In this historical prospective study using sera stored for 22 years, we investigated the effect of HTLV-I infection on survival in a population of leprosy patients in the Democratic Republic of the Congo (formerly Zaire). We also determined the distribution of HTLV I by subpopulation, age, and gender. Stored sera taken from a population of leprosy patients and controls in 1969 were tested for HTLV-I. Follow-up survival data on these patients were obtained in 1991. The sera collected in 1969 from 520 individuals was used to determine the prevalence of HTLV-I. Included in this number were 328 patients resident in the sanatorium. Survival and other data were available for 327 of these. A multivariate survival analysis using a logistic regression model was performed to evaluate the influence of HTLV-I status, age, type of leprosy, gender, duration of hospitalization, and ethnic group on survival. The overall prevalence of HTLV-I among the 520 individuals in the prevalence study was 34%, with 37.4% in the leprosy group and 25.2% in the control group ($p < 0.01$). Multivariate analysis using logistic regression showed that females of the Mongo and Ngombe ethnic group taken together were significantly more likely to be infected than the other groups (OR = 3.67, 95% CI: 2.14 to 6.30). A comparison of the death rates directly standardized for age and sex showed that the rate was significantly higher for HTLV-I positive (5.5/100 person-years of observation) compared with HTLV-I nega-

tive (3.6/100 person-years of observation). A survival analysis using the Cox model showed a risk ratio of 1.4 (CI: 1.04 to 1.89) for those infected with HTLV-I. An increase in the death rate was associated with HTLV-I infection in leprosy inpatients. The decreased survival associated with HTLV-I infection may result from an increased susceptibility to a variety of diseases.—Authors' Abstract

Ma, J. [Effects of MDT for 13 years in Mengla County, Yunnan.] China Lepr. J. **13** (1997) 149–150. (in Chinese)

In Mengla County with a population of 185,260, Yunnan, since 1983 WHO's MDT has been used, and by the end of 1995 a total of 318 cases of leprosy was treated with it, of which 305 were cured, 13 died before completion of the course and 10 died after the cure. The 295 persons (MB 122 and PB 173) were under monitoring for 10 years for MB and for 12 years for PB, and among them there was no relapse. Since adoption of MDT the detection rate of leprosy has decreased from 10.24/100,000 (1983) to 5.93/100,000 (1995) and the prevalence from 1.32‰ to 0.076‰ for the same time, but in 1995 there still were 13 new cases of leprosy. The author pointed out that leprosy control work is to be continued, which the government must give its attention to.—Author's English Abstract

Ma, Q. [Leprosy in Guangzhou city before and after MDT.] China Lepr. J. **13** (1997) 84–85. (in Chinese)

By the end of 1995, 7417 leprosy patients have been accumulatively registered and 34 active cases remained in Guangzhou. WHO/MDT has been used since 1986. In the periods from 1976 to 1985, 507 new patients and 1986 to 1995, 127 have been found, of which 156 persons cured with DDS monotherapy relapsed. From 1976 to 1995, the prevalence reduced from 0.42‰ to 0.08‰ and the incidence from 163/100,000 to 0.27/100,000.—Author's English Abstract

Rajdekar, M. P., Thakar, U. H., Pharande, A. M., Naik, S. S. and Ganapati, R. Hidden sources of infection in unapproachable areas. Indian J. Lepr. **69** (1997) 169–171.

The population living in the hilly terrains of Panvel Taluka, District Raigad of Maharashtra State, India, was examined in a special campaign carried out during a time when the majority of the population will be stationed at the hills. Examination of 10,499 persons revealed 108 leprosy cases (PR 10.3/1000) of which 72 were paucibacillary (PB) and 29 were multibacillary (MB) cases. Among the PB cases, only two had single-lesion type and among the 29 MB cases, 14 were smear-positive, having a BI more than 4. These untreated advanced leprosy cases, in view of their frequent migrations in order to earn their livelihood, may be responsible for transmitting the infection in the plains areas where multidrug therapy has been practiced since 1990. In order to achieve early leprosy elimination, it is necessary to cover populations in difficult areas like the one mentioned by special action programs.—Authors' Abstract

Shen, J., *et al.* [Differences of subclinical infection rates and onset of leprosy between various villages in a high-endemic area.] China Lepr. J. **13** (1997) 142–145. (in Chinese)

A serological survey was done with ELISA for IgM against ND-O-BSA among 1833 healthy residents in six villages of Wenshan and Guangnan counties, Yunnan, China, where prevalence of leprosy was high, and then all of the positive persons and some negative ones were followed up with the same method yearly for 3 successive years. At first, the result showed that the rate of subclinical infection of leprosy in the six villages was different, that is, the highest (25.86%) was in the village MIZ next to a colony, the others were 21.38% in TBN with good transport, 7.82% in inaccessible MZG, 13.6% in XSK and 18.86% in NZ. In the last two villages there are active cases of leprosy. The results during the follow up were also different in the villages, but it has nothing to do with prevalence of leprosy. For example, in NZ with continuous positivity of 12.5% three new patients

were found but in MZG with more continuous positivity no new patient. The authors believe that sustained ascent of the antibody titer is a danger index to clinical leprosy.—Authors' English Abstract

Shen, J., Li, W., Yan, L., *et al.* [Study on the recent trends of leprosy epidemiology in Yangzhou Prefecture and Dongtai City, Jiangsu Province.] Chin. J. Clin. Dermatol. **26** (1997) 160–163. (in Chinese)

The paper reported the results of an epidemiological study on leprosy in Yangzhou Prefecture and Dongtai City during 1982–1993. A total of 757 active cases were diagnosed during 1982–1993. Among them, 565 were newly diagnosed and 192 were relapsed cases after dapsone (DDS) monotherapy. The results showed that during 1982–1993, the number of annually diagnosed cases gradually decreased, the case-detection rate declined from 1.6/100,000 to 0.49/100,000, the ratio of newly diagnosed lepromatous cases increased from 0.39 to 0.63, the average age of the cases at detection increased from 40.3 to 45.7 years, and the mean time from onset of leprosy to diagnosis shortened. But there were no significant changes in the disability rate (≥WHO grade 2) and the ratio of child-case in new patients. The authors consider that the leprosy situation in these areas has improved markedly, but the leprosy control and early case finding need to be strengthened in some areas so as to decrease the disability rate and the ratio of child-cases. Because the relapsed cases after DDS monotherapy accounted for a noticeable proportion in the total number of cases, monitoring relapse in patients cured with DDS monotherapy and also MDT need to be strengthened.—Authors' English Abstract

Xu, K., *et al.* [Leprosy endemic and its control in Yunnan Province.] China Lepr. J. **13** (1997) 80–81. (in Chinese)

In Yunnan Province with a population of 38,024,475 and 128 counties, 48,603 leprosy patients have been accumulatively detected for the period of 1954 to 1994, including MB 26,235 and PB 22,368, and 35,239 men and 13,368 women, 35,151 cases (MB 18,037 and PB 17,114) have been cured (693 relapsed; MB 601 and PB 92), 10,295 died (MB 5846 and PB 4448) and 1017 moved out. Since 1980 WHO's MDT has been adopted and by the end of 1994 there remained 2141 active cases, the prevalence reduced from 0.82% to 0.06%, the incidence from 19.37/100,000 to 0.31/100,000 and the patients with the disease duration of <2 years in newly detected ones increased from 43.4% to 62.9%. In recent years, disability rate in new cases was 23.6%.—Authors' English Abstract

Yang, J., *et al.* [Integration of vertical system and general medical services for case-finding in leprosy.] China Lepr. J. **13** (1997) 75–80. (in Chinese)

After training medical workers in towns and villages and developing health education on leprosy for residents, the number of cases detected through primary health service has increased by 25% in 10 towns of Wenshan County, Yunnan, China, as compared with previous one, and the cases whose infection source was unknown increased from 36.1% to 68.9% and the rate disability of Grades II and III decreased from 33.3% to 13.3% in newly detected patients. The cost of detecting a patient was reduced significantly and spots as yet untouched by case finding activity lessened, too. The authors believe that the combination of leprosy control service with primary health system is effective and feasible.—Authors' English Abstract

Yang, X., *et al.* [Effect of oral DDS for prevention of leprosy.] China Lepr. J. **13** (1997) 85. (in Chinese)

To prevent leprosy, 62 children with a mean age of 8.7 years, being born and living in leprosaria with their parents suffering from leprosy, have been given oral DDS for 2 years and no case of leprosy was found among them during a follow up of 15 years.—Authors' English Abstract

Zheng, D., *et al.* [Endemic situation of leprosy in 1996, Guangdong.] China Lepr. J. **13** (1997) 147–148. (in Chinese)

In Guangdong, 94,252 leprosy patients have accumulatively been found by the end of 1996. It was reported by 21 prefectures and 78 counties (cities) in 1996 that the numbers of new leprosy patients have been continuously reducing and there only were 148 new ones, including 14 relapsed and 7 children with age less than 14 years. Now in the province there are 472 active patients, the prevalence is 0.007‰ and less than 0.01% in 80 of 99 cities (counties). Up to now, there is no reliable evidence of the incidence decreased by MDT. The authors think that after decrease of leprosy patients health education for eliminating stigma of leprosy and rehabilitation for the patients and cured persons should be emphasized.—Authors' English Abstract

Rehabilitation

Carpintero, P., Garcia-Frasquet, A., Pradilla, P., Garcia, J. and Mesa, M. Wrist involvement in Hansen's disease. J. Bone Joint Surg. [B.] **79B** (1997) 753–757.

We performed a neurological and radiological study of the wrists of 58 patients with Hansen's disease and 60 age-matched healthy control subjects. Significant differences ($p < 0.01$) were found between the groups in the carpal glenoid sector, the radial physeal widening index, the carpal ulnar distance, the carpal index and in distal radio-ulnar discrepancy. Comparison of the results in three subgroups of leprosy patients with sensory impairment (group A-1), motor deficit (A-2) and no neurological impairment (A-3), showed significant differences ($p < 0.01$) between group A-1 and the other two. This suggests that in these patients the changes in the carpus and radiocarpal joint may be caused by neuropathic arthropathy of the wrist. Our findings are of particular interest since there are few reports of neuropathic arthropathy in non-weightbearing joints.—Authors' Abstract

Chen, J. [A survey of the social estate of leprosy patients.] China Lepr. J. **13** (1997) 140–142. (in Chinese)

The situations of 166 persons cured of leprosy at their homes have been surveyed in Liyang City, Jiangsu, China, including 131 men and 35 women, of whom 30.1% were over 60 years old. Among them 151 persons are working at various posts according to their own abilities, and 15 persons without the ability depend on social relief or their families. The mean annual income of each person in the families was over RMB 3000 yuan for 46 persons, 1000 to 3000 yuan for 57, 500 to 1000 for 30 and <500 for 15, being equal to the living standard of local population. Among them, eight were single and 23 got married after their leprosy was cured, of whom 19 have healthy persons as their spouses; 101 persons have built new houses. Now they are already treated the same as other residents on visiting village medical station or town hospital. Among their sons and daughters, 314 of 367 adults are already married, all of school age are at school, those who can work all have jobs. All of the people around them are willing to contact them and know that leprosy is unfearful and curable, but in about one third of them the contact has a bit reduced. The author believes that discrimination against leprosy decreased greatly in this region.—Author's English Abstract

Dan, Z., *et al.* [Disability among 1522 cases of leprosy using MDT.] China Lepr. J. **13** (1997) 136–138. (in Chinese)

Among 1522 cases of leprosy under MDT the disability rate is 57.9% in Hunan Province, China, of which 63.3% was Grade I and 36.7% Grades II and III, including claw hand in 39.9%, plantar ulcer

in 28.5%, lagophthalmos in 13.6% and facial paralysis in 3.7%. The visible disability mainly took place before using WHO's MDT, but there also were new disability after MDT among those who had some disability. The authors suggest that for patients who have had disability prednisone should be given for 6 successive months when MDT is given.—Authors' English Abstract

Liu, L., *et al.* [Feasibility of plastic surgery in rehabilitation for leprosy patients.] China Lepr. J. **13** (1997) 86–87. (in Chinese)

The needs for recovery surgery among 209 persons disabled of leprosy have been surveyed in Qingdao City. Of them 86 have disability of grades II and III with a mean age of 48 years, but of which only 33 persons (38.4 %) are willing to receive the operation. Out of 53 persons who are not willing to take it, with a mean age of 52 years, 30 were because they can not afford the operation for income shortage and the rest are in fear of affecting their families and jobs or consider it useless for the aged like they are.—Authors' English Abstract

Ma, J. [Effect of protective footwear on plantar ulcers.] China Lepr. J. **13** (1997) 88. (in Chinese)

Since 1992, on the basis of TLMI's device, 33 leprosy patients with 44 plantar ulcers and 163 with numb soles have been given and were wearing protective footwear for 1 year. By the end of the year, of 44 ulcers 24 (54.5%) healed and 12 significantly improved, and no ulcer developed in the people with numb soles except three at the beginning of the test.—Author's English Abstract

Quintana Ginestar, M. V., Ruiz-Hidalgo, G. D. and Terencio de las Aguas, J. [Tepezcohuite: an alternative treatment for neurotrophic ulcers in leprosy; a case report.] Rev. Leprol. Fontilles **21** (1997) 167–173. (in Spanish)

Tepezcohuité oil was used in the treatment of trophic ulcers of the foot of long evolution in a lady diagnosed as cured lepromatous leprosy. No infections appeared during the cicatrization of the ulcers. It can be used as an alternative therapeutics in countries where it is common and easy to obtain.—Authors' English Summary

Terencio de las Aguas, J. [Osteoarticular lesions in leprosy.] Rev. Leprol. Fontilles **21** (1997) 195–219. (in Spanish)

A total of 800 patients are studied for osteoarticular lesions that are classified as specific, produced by *M. leprae,* and neurotrophic and in this group the ones complicated by local infections.

The most affected bones are the short ones of the hands and feet, especially the distal phalanges. Specific lesions made up 6% of the total. Neurotrophic lesions were most common (82%), especially in the feet with ostrolysis and osteoarthritis of the phalanges and metatarsals, less in the metacarpals. These occur on some patients with long-standing peripheral neuropathy.

Other lesions observed are cranial, tarsal disintegration, "Panaris of Marvan" and tibio-fibular periostitis.

The importance of these lesions for the social and vocational reinsertion of these patients and their prevention is discussed. —Author's English Summary

Xu, C., *et al.* [Situations of persons cured of leprosy after returning home and community's response.] China Lepr. J. **13** (1997) 83–84. (in Chinese)

The situation of 687 persons, having returned home from leprosaria after leprosy had been cured, was surveyed in 1994. The result showed that 195 died, including 11 suicides and 78 have been refused to be received by their families, of them 34 could not do self-service in daily life. On the attitude of 913 members of their families to them, 436 (47.8%) are willing to live together with them and the others are not willing to do so. Of their 1767 neighbors 719 (40.7%) are not willing to get along with them because of fear of being infected and dislike of their appearance. The authors pointed out that for improvement of the situation, sustained health education is essential.—Authors' English Abstract

Xu, Y. [The manufacture and improvement of simple artificial limb for leprosy patients—analysis of 132 cases.] China Lepr. J. **13** (1997) 72–75. (in Chinese)

Among 132 persons with one leg amputated for leprosy complication and using simply constructed artificial limb in Guangdong, the use of the artificial limbs has been examined and a comparison between them and more modern polyester ones was completed. The author thinks that the simple artificial limb is cheap and practical, and suggests several proposals to improve it.— Author's English Abstract

Other Mycobacterial Diseases and Related Entities

Al Shamali, M., Khan, I., Al Nakib, B., Al Hassan, F. and Mustafa, A. S. A multiplex polymerase chain reaction assay for the detection of *Mycobacterium paratuberculosis* DNA in Chrohn's disease tissue. Scand. J. Gastroenterol. **32** (1997) 819–823.

Background: *Mycobacterium paratuberculosis* is implicated as a possible cause of Crohn's disease. However, due to lack of an appropriate diagnostic method, this has been a subject of significant controversy. Our aim was therefore to develop a multiplex polymerase chain reaction (MPCR) for the detection of *M. paratuberculosis* DNA in Crohn's disease tissue.

Method: Biopsy samples were collected by endoscopic forceps from terminal ileum, and genomic DNA was isolated. *M. paratuberculosis*-specific marker genes were amplified by using the present MPCR method.

Results: Here we report a new MPCR for detection of *M. paratuberculosis* DNA in Crohn's disease tissue. In this technique two genetic markers, IS900 and a newly described specific marker of MP2, were amplified in a single tube simultaneously. The method was evaluated using biopsy specimens from 10 Crohn's disease patients, 6 ulcerative colitis patients, and 21 irritable bowel syndrome patients. The patients were characterized by using standard clinical and histologic observations. The present MPCR method could not detect *M. paratuberculosis* DNA in the biopsy specimens. However, the marker genes were amplified from the samples that were spiked with *M. paratuberculosis* before DNA extraction. The marker genes were also not detected in 10 closely related mycobacterial strains and human genomic DNA.

Conclusions: The present MPCR method is highly specific and can detect *M. paratuberculosis* DNA more reliably. These findings do not support an etiologic role of *M. paratuberculosis* in Crohn's disease.—Authors' Abstract

Amara, R. R. and Satchidanandam, V. Differential immunogenicity of novel *Mycobacterium tuberculosis* antigens derived from live and dead bacilli. Infect. Immun. **65** (1997) 4880–4882.

Mouse serum raised against killed antigen preparations of *Mycobacterium tuberculosis* failed to recognize most of the recombinant antigens of *M. tuberculosis* that were originally identified by reactivity to tuberculosis (TB) patient sera. Similar results were obtained with serum from guinea pigs immunized with live and killed mycobacteria. Antibodies raised against seven random TB patient serum-reactive antigens detected each of these antigens in the sonicate preparation. The nucleotide sequences of the genes for these seven antigens revealed that all represented hitherto unreported genes of *M. tuberculosis.* Our results suggest differential presentation to the host immune system of the same antigens derived from live and killed mycobacteria.— Authors' Abstract

Bardarov, S., Kriakov, J., Carriere, C., Yu, S. W., Vaamonde, C., McAdam, R. A., Bloom, B. R., Hatfull, G. F. and

Jacobs, W. R. Conditionally replicating mycobacteriophages: a system for transposon delivery to *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U.S.A. **94** (1997) 10961–10966.

Transposon mutagenesis provides a direct selection for mutants and is an extremely powerful technique to analyze genetic functions in a variety of prokaryotes. Transposon mutagenesis of *Mycobacterium tuberculosis* has been limited in part because of the inefficiency of the delivery systems. This report describes the development of conditionally replicating shuttle phasmids from the mycobacteriophages D29 and TM4 that enable efficient delivery of transposons into both fast- and slow-growing mycobacteria. These shuttle phasmids consist of an *Escherichia coli* cosmid vector containing either a mini-Tn10(kan) or Tn5367 inserted into a nonessential region of the phage genome. Thermosensitive mutations were created in the mycobacteriophage genome that allow replication at 30°C but not at 37°C (TM4) or 38.5°C (D29). Infection of mycobacteria at the nonpermissive temperature results in highly efficient transposon delivery to the entire population of mycobacterial cells. Transposition of mini-Tn10(kan) occurred in a site-specific fashion in *M. smegmatis*; whereas Tn5367 transposed apparently randomly in *M. phlei,* bacille Calmette-Guerin (BCG), and *M. tuberculosis.* Sequence analysis of the *M. tuberculosis* and BCG chromosomal regions adjacent to Tn5367 insertions, in combination with *M. tuberculosis* genomic sequence and physical map data, indicates that the transpositions have occurred randomly in diverse genes in every quadrant of the genome. Using this system, it has been readily possible to generate libraries containing thousands of independent mutants of *M. phlei,* BCG, and *M. tuberculosis.*—Authors' Abstract

Behr, M. A. and Small, P. M. Molecular fingerprinting of *Mycobacterium tuberculosis*: how can it help the clinician? Clin. Infect. Dis. **25** (1997) 806–810.

In just a few years, molecular fingerprinting of *Mycobacterium tuberculosis* has provided clinicians with significant insight into the epidemiology of tuberculosis. This methodology has allowed for a new understanding of the extent of new transmission of tuberculosis among residents of various communities and within institutions. It has also allowed for differentiation between episodes of reinfection and relapse, a task hitherto almost impossible to accomplish. In addition, molecular fingerprinting has allowed assessment of situations where laboratory cross-contamination is suspected. Thus, this technology has in many ways made clinicians reexamine many of their long-held beliefs regarding tuberculosis. In this report, Drs. Behr and Small provide a lucid description of molecular fingerprinting of *M. tuberculosis,* its current uses, and its future potential value.—Authors' Abstract

Boireau, A., Bordier, F., Dubedat, P., Peny, C. and Imperato, A. Thalidomide reduces MPTP-induced decrease in striatal dopamine levels in mice. Neurosci. Lett. **234** (1997) 123–126.

The effects of thalidomide, a sedative, antiinflammatory and immunosuppressive agent, were studied in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) murine model of Parkinson's disease. The striatal levels of dopamine (DA) and of its main metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured both in the MPTP control group (3 × 15 mg/kg intraperitoneally) and in the thalidomide groups (repeated treatments at 25 mg/kg or 50 mg/kg postoperatively). For mice treated with thalidomide, a dose-dependent protection was observed against the MPTP-induced decrease in DA. The decrease in HVA levels was totally antagonized by thalidomide at both doses. That thalidomide has activity in this model suggests that an inflammatory process may be involved in the induction of lesions by MPTP in DAergic neurons.—Authors' Abstract

Caceres, N. E., Harris, N. B., Wellehan, J. F., Feng, Z. Y., Kapur V. and Barletta, R. G. Overexpression of the D-ala-

nine racemase gene confers resistance to D-cycloserine in *Mycobacterium smegmatis.* J. Bacteriol. **179** (1997) 5046–5055.

Mycobacterium avium and *M. tuberculosis.* To analyze the genetic determinants of D-cycloserine resistance in mycobacteria, a library of a resistant *M. smegmatis* mutant was constructed. A resistant clone harboring a recombinant plasmid with a 3.1-kb insert that contained the glutamate decarboxylase (gadA) and D-alanine racemase (alrA) genes was identified. Subcloning experiments demonstrated that alrA was necessary and sufficient to confer a D-cycloserine resistance phenotype. The D-alanine racemase activities of wild-type and recombinant *M. smegmatis* strains were inhibited by D-cycloserine in a concentration-dependent manner. The D-cycloserine resistance phenotype in the recombinant clone was due to the overexpression of the wild-type alrA gene in a multicopy vector. Analysis of a spontaneous resistant mutant also demonstrated overproduction of wild-type AlrA enzyme. Nucleotide sequence analysis of the overproducing mutant revealed a single transversion (G → T) at the alrA promoter, which resulted in elevated beta-galactosidase reporter gene expression. Furthermore, transformants of *M. intracellulare* and *M. bovis* BCG carrying the *M. smegmatis* wild-type alrA gene in a multicopy vector were resistant to D-cycloserine, suggesting that AlrA overproduction is a potential mechanism of D-cycloserine resistance in clinical isolates of *M. tuberculosis* and other pathogenic mycobacteria. In conclusion, these results show that one of the mechanisms of D-cycloserine resistance in *M. smegmatis* involves the overexpression of the alrA gene due to a promoter-up mutation.—Authors' Abstract

Cirillo, J. D., Falkow, S., Tompkins, L. S. and Bermudez, L. E. Interaction of *Mycobacterium avium* with environmental amoebae enhances virulence. Infect. Immun. **65** (1997) 3759–3767.

Environmental mycobacteria are a common cause of human infections. Recently, contaminated domestic water supplies have been suggested as a potential environmental source of several mycobacterial diseases. Since many of these mycobacterial species replicate best intracellularly, environmental hosts have been sought. In the present study, we examined the interaction of *Mycobacterium avium* with a potential protozoan host, the water-borne amoeba *Acanthamoeba castellanii.* We found that *M. avium* enters and replicates in *A. castellanii.* in addition, similar to that shown for mycobacteria within macrophages, *M. avium* inhibits lysosomal fusion and replicates in vacuoles that are tightly juxtaposed to the bacterial surfaces within amoebae. In order to determine whether growth of *M. avium* in amoebae plays a role in human infections, we tested the effects of this growth condition on virulence. We found that growth of *M. avium* in amoebae enhances both entry and intracellular replication compared to growth of bacteria in broth. Furthermore, amoeba-grown *M. avium* was also more virulent in the beige mouse model of infection. These data suggest a role for protozoa present in water environments as hosts for pathogenic mycobacteria, particularly *M. avium.*—Authors' Abstract

Cooper, A. M., Saunders, B. M., D'Souza, C. D., Frank, A. A. and Orme, I. M. *Mycobacterium tuberculosis*-driven processes in gene-disrupted mice. Bull. Inst. Pasteur **95** (1997) 85–96.

Mice which have disrupted genes for important components of the immune system have been used to study the role of these components in the immune response to infection with *Mycobacterium tuberculosis.* This has resulted in the identification of interleukin-12 (IL-12), interferon gamma (IFN-gamma), tumour necrosis factor-alpha (TNF-alpha) and inducible nitric oxide synthase (iNOS) as being essential to the protective response. Less crucial but perhaps more intriguing roles for other molecules such as intercellular adhesion molecule (ICAM) and IL-6 have also been suggested by this kind of analysis.—Authors' Abstract

Cywes, C., Hoppe, H. C., Daffe, M. and Ehlers, M. R. W. Nonopsonic binding of

Mycobacterium tuberculosis to complement receptor type 3 is mediated by capsular polysaccharides and is strain dependent. Infect. Immun. **65** (1997) 4258–4266.

The choice of host cell receptor and the mechanism of binding (opsonic versus nonopsonic) may influence the intracellular fate of *Mycobacterium tuberculosis.* We have identified two substrains of *M. tuberculosis* H37Rv, designated H37Rv-CC and -HH, that differed in their modes of binding to complement receptor type 3 (CR3) expressed in transfected Chinese hamster ovary (CHO-Mac-1) cells: H37Rv-CC bound nonopsonically, whereas H37Rv-HH bound only after opsonization in fresh serum. H37Rv-CC also bound nonopsonically to untransfected CHO cells, whereas H37Rv-HH binding was enhanced by serum and was mediated by the 1D1 antigen, a bacterial adhesin previously identified as a polar phosphatidylinositol mannoside. H37Rv-CC and -HH had identical IS6110 DNA fingerprint patterns. Of five *M. tuberculosis* clinical isolates examined, four displayed the same binding phenotype as H37Rv-CC, as did the Erdman strain, whereas one isolate, as well as *Mycobacterium smegmatis,* behaved like H37Rv-HH. Nonopsonic binding of H37Rv-CC to CHO cell-expressed CR3 was apparently to the beta-glucan lectin site, as it was cation independent and inhibited by laminarin (seaweed beta-glucan) and N-acetylglucosamine; laminarin also inhibited the binding of H37Rv-CC to monocyte-derived macrophages. Further, binding of H37Rv-CC to CHO-Mac-1 cells was inhibited by prior agitation of bacteria with glass beads (which strips outer capsular polysaccharides) and by preincubation with amyloglucosidase, as well as by the presence of capsular D-glucan and D-mannan from *M. tuberculosis* Erdman, but not by Erdman D-arabino-D-mannan, yeast mannan, or capsular components from H37Rv-HH. Analysis of capsular carbohydrates revealed that H37Rv-CC expressed 5-fold more glucose and 2.5-fold more arabinose and mannose than H37Rv-HH. Flow cytometric detection of surface epitopes indicated that H37Rv-CC displayed twofold less surface-exposed phosphatidylinositol mannoside and bound complement C3 less efficiently than H37Rv-HH; these differences were eliminated after treatment of H37Rv-CC with glass beads. Thus, outer capsular polysaccharides mediate the binding of H37Rv-CC to CR3, likely to the beta-glucan site. Moreover, there are strain-dependent differences in the thickness or composition of capsular polysaccharides that determine the mode of binding of *M. tuberculosis* to mammalian cells.—Authors' Abstract

Dumonceaux, M., Dufaux, M. F., Ooms, J., DeWit, L., Sonck, P. and Content, J. Cloning of the antigen 85A from *Mycobacterium gordonae* and its use for the specific PCR identification of these mycobacteria. Mol. Cell. Probes **11** (1997) 251–258.

The complete nucleotide sequence of 85A antigen of *Mycobacterium gordonae* was determined. This gene encodes 339 amino acids, including 43 amino acids for the signal peptide, followed by a mature protein of 296 amino acids.

A polymerase chain reaction (PCR) assay for the rapid detection of *M. gordonae* DNA using two pairs of oligonucleotide primers, derived from our sequence, is described. This one-step PCR has been used successfully to amplify 38 strains of *M. gordonae.* Conversely, the primers did not amplify DNA from any of the 25 mycobacterial species tested. The results suggest that this PCR assay could be a good alternative to existing commercial assays for the specific identification of *M. gordonae* on early culture on solid medium or on early BACTEC broth culture.—Authors' Abstract

Gatti, G., Hossein, J., Malena, M., Cruciani, M. and Bassetti, M. Penetration of dapsone into cerebrospinal fluid of patients with AIDS. J. Antimicrob. Chemother. **40** (1997) 113–115.

It has been proposed that dapsone in combination with pyrimethamine could be used for prophylaxis of both *Pneumocystis carinii* pneumonia and encephalitis due to

Toxoplasma gondii. Ten patients with AIDS undergoing lumbar puncture for diagnostic purposes were studied in order to assess the penetration of dapsone into CSF. Blood and CSF samples were obtained between 3 and 72 hr following administration. Six patients had received oral dapsone for at least 1 month at the dosage regimen of 100 mg twice or three times weekly and four patients had received a single oral 100 mg dose. Dapsone concentration in CSF ranged from 0.013 to 0.296 mg/L, while concentrations in plasma ranged from 0.018 to 1.231 mg/L. The CSF : plasma concentration ratio ranged from 0.21 to 2.01. The MIC of dapsone in combination with pyrimethamine against *T. gondii* is unknown, and further data are required to confirm whether the CSF concentrations of dapsone found in our study are sufficient to inhibit *T. gondii* growth in patients infected with human immunodeficiency virus (HIV). The high interpatient variability of dapsone CSF concentrations warrants further studies in selected categories of patients with HIV infection.—Authors' Abstract

Harth, G. and Horwitz, M. A. Expression and efficient export of enzymatically active *Mycobacterium tuberculosis* glutamine synthetase in *Mycobacterium smegmatis* and evidence that the information for export is contained within the protein. J. Biol. Chem. **272** (1997) 22728–22735.

We have investigated the expression and extracellular release of active, recombinant *Mycobacterium tuberculosis* glutamine synthetase (EC 6.3.1.2), an enzyme that is a potentially important determinant of *M. tuberculosis* infection and whose extracellular release is correlated with pathogenicity. The *M. tuberculosis* glutamine synthetase gene encodes a polypeptide of 478 amino acids; 12 such subunits comprise the active enzyme. Northern blot, nuclease S1, and primer extension analyses revealed glutamine synthetase-specific transcripts of similar to 1550 and 1650 nucleotides produced under low and high nitrogen conditions, respectively. Expression of recombinant *M. tuberculosis* glutamine synthetase in *Escherichia coli* YMC21E, a glutamine synthetase deletion mutant, led to transcomplementation of the mutant but not to release of active enzyme. Expression in *M. smegmatis* 1-2c, from the gene's own promoter, resulted in the release of >95% of all recombinant enzyme. No hybrid molecules containing *M. tuberculosis* and *M. smegmatis* glutamine synthetase subunits were detected. Native and recombinant exported and intracellular glutamine synthetase molecules were indistinguishable from one another by mass, N-terminal amino acid sequence, antibody reactivity, and enzymatic activity. Since *M. tuberculosis* glutamine synthetase is similar to other, strictly intracellular, bacterial glutamine synthetases and the DNA sequence upstream of the structural gene does not encode a leader peptide, the information to target the protein for export must be contained in its amino acid sequence and/or conformation. —Authors' Abstract

Haslett, P., Hempstead, M., Seidman, C., Diakun, J., Vasquez, D., Freedman, V. H. and Kaplan, G. The metabolic and immunologic effects of short-term thalidomide treatment of patients infected with the human immunodeficiency virus. AIDS Res. Hum. Retrovir. **13** (1997) 1047–1054.

Thalidomide therapy has been shown to cause increase in body weight in patients with HIV and tuberculosis infections. To examine the nature of this weight gain and its immunological correlates in patients with HIV infection, we studied a cohort of 13 patients with minimally symptomatic HIV disease. Patients were admitted to the Rockefeller University General Clinical Research Center and maintained on strict isocaloric diets to achieve weight stabilization before a 14-day course of thalidomide treatment. Mean percentage weight increase was 3.6% on day 14 of thalidomide therapy ($p = 0.002$). Weight gain was associated with a reduction in mean daily urinary nitrogen excretion of 1.81 g ($p = 0.017$). Resting energy expenditure was unaffected by thalidomide. Body composition analysis suggested some extracellular fluid retention in the first week of thalidomide

therapy, followed by an expansion of lean tissue mass during the second week. Remarkably, total lymphocyte counts and CD8+ T-cell counts increased following treatment with the drug from 1578 ± 185 to 2617 ± 265 and from 938 ± 146 to 1369 ± 231, respectively. Modest increases in CD4+ T-cell counts were also observed. Levels of circulating TNF-alpha were not elevated at baseline. A significant increase in mean plasma levels of soluble interleukin 2 receptor (sIL-2r), from 1918 ± 250 to 3816 ± 411 pg/ml ($p = 0.0022$), occurred in response to thalidomide, suggesting drug-induced immunological activation. In conclusion, thalidomide treatment of patients with HIV infection caused weight gain and lean tissue anabolism, even when caloric intake was kept constant. The nature of the association between thalidomide treatment-induced metabolic changes and the immunomodulatory effects of the drug has yet to be elucidated.—Authors' Abstract

Hu, F. R., Chang, S. C., Luh, K. T. and Hung, P. T. The antimicrobial susceptibility of *Mycobacterium chelonae* isolated from corneal ulcer. Curr. Eye Res. **16** (1997) 1056–1060.

Purpose: To determine the *in vitro* susceptibility of *Mycobacterium chelonae* isolates from corneal ulcers to various traditional and newly developed antimicrobial agents, alone or in combination.

Methods: Fifteen strains of *M. chelonae* isolated from corneal ulcers were collected at the National Taiwan University Hospital from 1989 to 1993. Susceptibility to antimicrobial agents was tested by the broth microdilution method to determine the minimum inhibitory concentration (MIC). The antimicrobial effects of combinations of antimicrobial agents were assessed by the checkerboard titration method to determine the fractional inhibitory concentration (FIC) index.

Results: The MIC results showed that traditional antituberculous drugs had poor activity against *M. chelonae.* In the aminoglycoside group, tobramycin and amikacin had better activity than gentamicin. Among macrolides, clarithromycin was especially effective, with an MIC ranging from 0.125 to 1 μg/ml. Among various beta-lactam antibiotics, imipenem was the only one to demonstrate good anti-mycobacterial activity. Of the quinolone group, ciprofloxacin was the most effective, with an MIC ranging from 0.5 to 16 μg/ml. Combination of an aminoglycoside with imipenem, ciprofloxacin or clarithromycin all showed antagonistic effect.

Conclusions: The results suggested that amikacin, clarithromycin, imipenem and ciprofloxacin had good *in vitro* antimicrobial activity against *M. chelonae.* However, no synergistic effect could be demonstrated for combinations of an aminoglycoside with other effective drugs.—Authors' Abstract

Hughes, M. S., Ball, N. W., Beck, L. A., deLisle, G. W., Skuce, R. A. and Neill, S. D. Determination of the etiology of presumptive feline leprosy by 16S rRNA gene analysis. J. Clin. Microbiol. **35** (1997) 2464–2471.

PCR-amplified 16S rRNA gene sequences were obtained directly from tissue specimens from eight cats with presumptive feline leprosy. Acid-fast bacilli were observed in sections from all eight specimens, but culture for mycobacteria was successful for one specimen only. Analysis of the V2 variable region of each 16S rRNA PCR product identified a sequence with 100% nucleotide identity to the sequences of *M. lepraemurium, M. avium,* and *M. paratuberculosis* in four of the specimens from cats with feline leprosy. Separate *M. paratuberculosis*- and *M. avium*-specific PCR amplifications of the four specimens were negative, thus substantiating the identification of *M. lepraemurium* in these specimens from cats with feline leprosy. Further sequence analysis of the V3 variable region of one of the four specimens provided conclusive evidence of the presence of *M. lepraemurium.* This is the first report of the definitive identification of *M. lepraemurium* in cats with feline leprosy by molecular biology-based analyses. *M. avium,* which is rarely reported in cats, and *M. chitae,* a reported nonpathogenic, rapidly growing mycobacterial species found in the environment, were identified in the specimen from which acid-fast bacilli were cultured. Two

of the specimens from cats were infected with a potentially novel species of mycobacteria which had a 16S rRNA gene sequence sharing the closest nucleotide sequence identity with that of *M. malmoense*. Molecular biology-based analyses provided for the accurate and rapid diagnosis of mycobacterial infections in cats and circumvented the problems of culture and misdiagnosis of feline leprosy associated with traditional methods.—Authors' Abstract

Iivanainen, E. K., Martikainen, P. J., Raisanen, M. L. and Katila, M. L. Mycobacteria in boreal coniferous forest soils. FEMS Microbiol. Ecol. **23** (1997) 325–332.

The occurrence of mycobacteria was studied in organic horizons of coniferous forest soils in Finland and related to environmental variables, i.e., plate counts of other heterotrophic bacteria, microbial respiration rate, chemical soil characteristics, vegetational characteristics and climatic conditions in the study period. Mycobacteria were isolated from all samples (N = 47), with plate counts varying from 4.5×10^4 to 1.2×10^6 cfu g^{-1} dry soil. The plate counts of mycobacteria correlated positively with those of other heterotrophic bacteria, microbial respiration rate and the contents of Ca and Mn. In factor analysis, the viable counts of mycobacteria and other heterotrophic bacteria, and respiration rate were grouped in the same factor emphasizing that mycobacteria and other heterotrophic bacteria had similar associations with environmental characteristics. The plate counts of mycobacteria and other heterotrophic bacteria and microbial respiration rate were similar in organic horizons of pine and spruce dominated forests. The large number of mycobacteria in all organic horizons indicates that boreal coniferous forest soils are important sources for these bacteria.—Authors' Abstract

Koga, H., Miyamoto, J., Ohno, H., Ogawa, K., Tomono, K., Tashiro, T. and Kohno, S. A rapid drug susceptibility test for *Mycobacterium tuberculosis* using the hybridization protection assay. J. Antimicrob. Chemother. **40** (1997) 189–194.

The conventional drug susceptibility tests for *Mycobacterium tuberculosis* are time-consuming and the results are available only after 2–4 weeks. We have recently reported a new, simple and fast *M. tuberculosis* drug susceptibility test, using the hybridization protection assay (HPA), that allows the detection of isoniazid- or rifampin-resistant strains of *M. tuberculosis* within 24 hr of incubation. In the present study, the scope of application of our new test was extended to another two first-line antimycobacterial agents, namely, ethambutol and streptomycin, and a quinolone antimicrobial agent, ciprofloxacin. The ethambutol-, streptomycin- and ciprofloxacin- resistance characteristics of *M. tuberculosis* were also delineated within 72 hr of incubation with or without the drug. The results of our novel and rapid drug susceptibility test for *M. tuberculosis* were not only comparable to those determined by the conventional method, but became available within a few days of incubation. Our results also suggest that the drug susceptibility test using HPA might also be useful for detecting organisms resistant to antimicrobial agents other than antimycobacterials.—Authors' Abstract

Lammas, D. A., Stober, C., Harvey, C. J., Kendrick, N., Panchalingam, S. and Kumararatne, D. S. ATP-induced filling of mycobacteria by human macrophages is mediated by purinergic P2Z($P2X^7$) receptors. Immunity **7** (1997) 433–444.

The death of BCG-infected human macrophages induced *in vitro* by ligation of surface CD95 (Fas), CD69, or complement-mediated lysis was shown not to result in the death of intracellular mycobacteria; whereas exposure to extracellular ATP initiated both macrophage death and killed the intracellular bacteria. ATP acted via P2Z receptors because these effects were mimicked by benzoylbenzoic ATP (a known agonist of P2Z receptors) and blocked by oxidized ATP, DIDS, suramin, amiloride, and KN62 (known inhibitors of P2Z-mediated responses). ATP-mediated bacterial killing was independent of reactive nitrogen and oxygen intermediates and of actinomycin D or cycloheximide inhibition. ATP-induced macrophage cell death, BCG killing, and

Lucifer yellow dye incorporation were minimal in 2 out of 19 healthy donors. The results suggest possible genetic heterogeneity of this mechanism of mycobacterial killing associated with P2Z-mediated pore formation.—Authors' Abstract

May, T., Brel, F., Beuscart, C., Vincent, V., Perrone, C., Doco Lecompte, T., Saint Marc, T., Dautzenberg, B., Grosset, J., *et al.* Comparison of combination therapy regimens for treatment of human immunodeficiency virus-infected patients with disseminated bacteremia due to *Mycobacterium avium.* Clin. Infect. Dis. **25** (1997) 621–629.

We conducted a randomized, open-label trial in 42 French hospitals to compare the clinical and bacteriologic efficacy of combination therapy with clarithromycin/clofazimine (Clm/Clof) with that of combination therapy with clarithromycin/rifabutin/ethambutol (Clm/Rib/Eth) as treatment for *Mycobacterium avium* bacteremia. One-hundred-forty-four human immunodeficiency virus-seropositive patients older than 18 years of age who had CD4 lymphocyte counts of $<100/mm^3$ and a blood culture positive for *M. avium* were enrolled in the study. The main measures of outcome were blood cultures, abatement of clinical symptoms (fever), and survival. Treatment success (defined as patient living, either no fever or a reduction of $\geq 1°C$ in initial body temperature, and a blood culture negative for *M. avium*) was similar in both treatment groups at months 2 and 6. However, following initial resolution of infection, relapse of *M. avium* bacteremia occurred in more patients in the Clm/Clof group than in the Clm/Rib/Eth group (22 vs. 6, respectively; $p < 0.001$); these relapses were accompanied by emergence of strains resistant to clarithromycin in 21 and 2 patients, respectively. In conclusion, combination therapy with Clm/Rib/Eth prevented relapse of mycobacterial disease and, compared with combination therapy with Clm/Clof, was associated with a significant decrease in the emergence of resistant *M. avium* strains in HIV-infected patients treated for at least 28 weeks.—Authors' Abstract

McCarty, M. F. Thalidomide may impede cell migration in primates by down-regulating integrin beta-chains: potential therapeutic utility in solid malignancies, proliferative retinopathy, inflammatory disorders, neointimal hyperplasia, and osteoporosis. Med. Hypotheses **49** (1997) 123–131.

A growing number of human inflammatory disorders are reported to respond to treatment with thalidomide, and recently this drug has been shown to inhibit angiogenesis in the rabbit in doses which can elicit teratogenicity in this species. Studies in marmosets and humans indicate that thalidomide, and a teratogenic analog, decrease the expression of beta integrin subunits, most notably $beta^3$ and the $beta^2$ produced by leukocytes. Since integrins are crucial for cell-matrix interactions, and the $beta^2$ integrins of leukocytes mediate adhesion to endothelium, it is reasonable to postulate that thalidomide inhibits cell migration in susceptible species, and that this accounts for its anti-inflammatory, anti-angiogenic, and teratogenic activity. This perspective suggests that thalidomide will show utility in the prevention or treatment of a wide range of disorders, including solid tumors, proliferative retinopathies, many inflammatory diseases, neointimal hyperplasia and osteoporosis. It is likely that dietary fish oil—as well as selective inhibitors of urokinase, when and if they become clinically available—will complement the efficacy of thalidomide in most if not all of these applications.—Author's Abstract

Moore, M., Onorato, I. M., McCray, E. and Castro, K. G. Trends in drug-resistant tuberculosis in the United States, 1993–1996. JAMA **278** (1997) 833–837.

Context: With the resurgence of tuberculosis (TB) disease in the late 1980s and early 1990s in the United States, multidrug-resistant (MDR) TB emerged as a serious challenge to TB control. In response, the Centers for Disease Control and Prevention in 1993 added drug susceptibility test results to the information collected for the national surveillance system to monitor trends in drug resistance.

Objective: To determine the extent of drug-resistant tuberculosis (TB) in the United States.

Design: Descriptive analysis or TB surveillance data.

Study Population: Patients reported to the national TB surveillance system as confirmed TB cases with culture-positive disease from 1993 through 1996 by the 50 states, New York City, and the District of Columbia (DC).

Main Outcome Measure: Percentage of case patients with culture-positive disease whose isolates are resistant to specific anti-TB drugs.

Results: Overall resistance to at least isoniazid was 8.4%; rifampin, 3.0%; both isoniazid and rifampin (i.e., MDR TB), 2.2%; pyrazinamide, 3.0%; streptomycin, 6.2%; and ethambutol hydrochloride, 2.2%. Rates of resistance were significantly higher for case patients with a prior TB episode. Among those without prior TB, isoniazid resistance of 4% or more was found in 41 states, New York City, and DC. A total of 1457 MDR TB cases were reported from 42 states, New York City, and DC; however, 38% were reported from New York City. Rates of isoniazid and streptomycin resistance were higher for cases among U.S.-born compared with foreign-born patients, but rates of rifampin resistance and MDR TB were similar. Among U.S.-born patients, resistance to first-line drugs, particularly rifampin mono-resistance, was significantly higher among those with human immunodeficiency virus (HIV) infection.

Conclusions: Compared with recent U.S. surveys in 1991 and 1992, isoniazid resistance has remained relatively stable. In addition, the percentage of MDR TB has decreased, although the national trend was significantly influenced by the marked decrease in New York City. Foreign-born and HIV-positive patients and those with prior TB have higher rates of resistance. The widespread extent of isoniazid resistance confirms the need for drug susceptibility testing to guide optimal treatment of patients with culture-positive disease.—Authors' Abstract

Mor, N. and Esfandiari, A. Synergistic activities of clarithromycin and pyrazinamide against *Mycobacterium tuberculosis* in human macrophages. Antimicrob. Agents Chemother. **41** (1997) 2035–2036.

The combination of clarithromycin and pyrazinamide was found to be synergistic against *Mycobacterium tuberculosis* in human macrophages. MICs were four- to eightfold lower for this combination than they were for either drug alone. Clarithromycin and rifampin, however, had only an additive effect.—Authors' Abstract

Moreira, A. L., Wang, J., Sarno, E. N. and Kaplan, G. Thalidomide protects mice against LPS-induced shock. Braz. J. Med. Biol. Res. **30** (1997) 1199–1207.

Thalidomide has been shown to selectively inhibit TNF-alpha production *in vitro* by lipopolysaccharide (LPS)-stimulated monocytes. TNF-alpha has been shown to play a pivotal role in the pathophysiology of endotoxic shock. Using a mouse model of LPS-induced shock, we investigated the effects of thalidomide on the production of TNF-alpha and other cytokines and on animal survival. After injection of 100–350 μg LPS into mice, cytokines including TNF-alpha, IL-6, IL-10, IL-1 beta, GM-CSF and IFN-gamma were measured in the serum. Administration of 200 mg/kg thalidomide to mice before LPS challenge modified the profile of LPS-induced cytokine secretion. Serum TNF-alpha levels were reduced by 93%, in a dose-dependent manner, and TNF-alpha mRNA expression in the spleens of mice was reduced by 70%. Serum IL-6 levels were also inhibited by 50%. Thalidomide induced a twofold increase in serum IL-10 levels. Thalidomide treatment did not interfere with the production of GM-CSF, IL-1 beta or IFN-gamma. The LD50 of LPS in this model was increased by thalidomide pretreatment from 150 μg to 300 μg in 72 hr. Thus, at otherwise lethal doses of LPS, thalidomide treatment was found to protect animals from death.—Authors' Abstract

Nicolai, S., Sies, H. and Stahl, W. Stimulation of gap junctional intercellular communication by thalidomide and thalido-

mide analogs in human skin fibroblasts. Biochem. Pharmacol. **53** (1997) 1553–1557.

It has been speculated that gap junctional intercellular communication (GJIC), an intercellular signalling pathway, is involved in embryogenesis by coupling compartments of the same developmental potential. We found that thalidomide induces GJIC in human fibroblasts after activation by liver microsomes in concentrations as low as 10^{-7} M. Treatment of cells with the thalidomide analog EM-12 increased GJIC without prior activation. No alteration of GJIC was detected with phthalimide and glutamate, the components of thalidomide.

However, 2 phthalimido glutaric acid (PGA), a hydrolysis product of thalidomide, stimulated GJIC without activation at concentrations between 10^{-10} M and 10^{-5} M. We suggest modification of GJIC as a biochemical mechanism responsible for pharmacological and toxicological properties of thalidomide and related compounds.—Authors' Abstract

Paramasivan, C. N., Venkataraman, P. and Herbert, D. Minimal inhibitory concentrations of sulbactam/ampicillin against drug sensitive and drug resistant isolates of *Mycobacterium tuberculosis.* Microbios **89** (1997) 135–141.

A total of 92 isolates of *Mycobacterium tuberculosis* consisting of equal numbers of sensitive and resistant strains was tested for their susceptibility to sulbactam and ampicillin (in the ratio of 1:2) on Lowenstein-Jensen (LJ) and 7H11 agar media. The geometric mean MIC was 63.97 μg/ml for the drug sensitive strains and 65.92 μg/ml for the resistant strains, and the overall mean was 65.01 μg/ml. The high MIC on LJ medium could be attributed to the higher protein content which resulted in greater binding of sulbactam/ampicillin. On the other hand, the geometric mean MIC on 7H11 medium was 26.73 μg/ml for sensitive strains and 23.82 μg/ml for resistant strains; the overall mean being 25.23 μg/ml. Although these MICs of sulbactam-ampicillin are higher than those reported earlier, they can be easily achieved in serum. Further studies on experimental tuberculosis and in humans will be needed to prove the efficacy of sulbactam/ampicillin in the treatment of patients with multidrug resistant tuberculosis.—Authors' Abstract

Pelicic, V., Jackson, M., Reyrat, J. M., Jacobs, W. R., Gicquel, B. and Guilhot, C. Efficient allelic exchange and transposon mutagenesis in *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U.S.A. **94** (1997) 10955–10960.

A better understanding of *Mycobacterium tuberculosis* virulence mechanisms is highly dependent on the design of efficient mutagenesis systems. A system enabling the positive selection of insertional mutants having lost the delivery vector was developed. It uses ts-sacB vectors, which combine the counterselective properties of the sacB gene and a mycobacterial thermosensitive origin of replication and can therefore be efficiently counterselected on sucrose at 39°C. This methodology allowed the construction of *M. tuberculosis* transposition mutant libraries. Greater than 10^6 mutants were obtained, far exceeding the number theoretically required to obtain at least one insertion in every nonessential gene. This system is also efficient for gene exchange mutagenesis as demonstrated with the purC gene: 100% of the selected clones were allelic exchange mutants. Therefore, a single, simple methodology has enabled us to develop powerful mutagenesis systems, the lack of which was a major obstacle to the genetic characterization of *M. tuberculosis.*—Authors' Abstract

Picardeau, M., Varnerot, A., Lecompte, T., Brel, F., May, T. and Vincent, V. Use of different molecular typing techniques for bacteriological follow-up in a clinical trial with AIDS patients with *Mycobacterium avium* bacteremia. J. Clin. Microbiol. **35** (1997) 2503–2510.

One-hundred-ninety-six *Mycobacterium avium* isolates from blood samples recovered from 93 AIDS patients for several months were typed by serotyping, by IS1245 restriction fragment length poly-

morphism (RFLP) analysis and in some cases RFLP analysis with plasmids pVT2 and pLR7 as probes, and by pulsed-field gel electrophoresis (PFGE). PCR typing of single colonies was also used to detect polyclonal infections. Strains belonged mainly to serotypes 1, 4, and 8. PVT2- and pLR7-related plasmids were detected in strains from 49% of the patients. The IS1245 RFLP and PFGE analyses showed a 96.8% diversity of the *M. avium* strains from the 93 patients. The vast majority (95.2%) of infections were monoclonal, indicating that recent infection is unlikely, even at an advanced stage of AIDS. For one patient, sequential isolates gave divergent patterns of sensitivity and resistance to clarithromycin, but all were identified as the initial clone. RFLP analysis and PCR typing of single colonies allowed for the detection of three polyclonal infections during the bacteriological follow up. Among strains from patients whose samples were positive by culture after treatment for 2 to 15 months, 97.4% were the same as the initial strain. In conclusion, relapses and failures were mostly due to the initial strain. These relapses and failures resulted either from the selection of resistant mutants or the reappearance of sensitive strains, suggesting the persistence of nonsterilized tissue reservoirs.—Authors' Abstract

Plum, G., Brenden, M., Clark Curtiss, J. E. and Pulverer, G. Cloning, sequencing and expression of mig gene of *Mycobacterium avium,* which codes for a secreted macrophage-induced protein. Infect. Immun. **65** (1997) 4548–4557.

Mycobacterium avium is an intracellular pathogen that has evolved to be a frequent cause of disseminated infection in immunocompromised patients. Although these bacilli are readily phagocytized, they are able to survive and even multiply within human macrophages. The process whereby mycobacteria circumvent the lytic functions of the macrophages is currently not well understood, but this is a key aspect in the pathogenicity of all pathogenic mycobacteria. Previously, we identified a gene in *M. avium,* designated mig (for macrophage-induced gene), the expression of which is induced when the bacilli grow in human macrophages (G. Plum and J. E. Clark-Curtiss, Infect. Immun. 62:476–483, 1994). In the present study we show that a) the nucleotide sequence of the mig gene has an open reading frame of 295 amino acids with a strong bias for mycobacterial codon usage, b) the mig gene also codes for a putative signal peptide of 19 amino acid residues, c) mig is induced by acidity to be expressed as an early-secreted 30-kDa protein, and d) the Mig protein exhibits an AMP-binding domain signature. However, beyond this motif which is common to enzymes that activate a large variety of substrates, no homologies to known sequences are found. We also show that e) *M. smegmatis* strains expressing the Mig protein have a limited advantage for survival in macrophages. These findings may be concordant with a role of the mig gene in the virulence of *M. avium.*—Authors' Abstract

Realini, L., Vander Stuyft, P., DeRidder, K., Hirschel, B. and Portaels, F. Inhibitory effects of polyoxyethylene stearate, PANTA, and neutral pH on growth of *Mycobacterium genavense* in BACTEC primary cultures. J. Clin. Microbiol. **35** (1997) 2791–2794.

We report on the influences of polyoxyethylene stearate (POES), PANTA, and pH on primary cultures of *Mycobacterium genavense* in BACTEC vials. As a model for primary cultures from tissue, seven different strains first isolated from AIDS patients (five from Switzerland and two from the United States) were inoculated into nude mice in order to obtain large amounts of bacilli to test different conditions simultaneously. Our results demonstrate that the size of the inoculum (10^6 acid-fast bacilli/vial), an acid pH (pH 6.0), and the absence of additives (FOES and PANTA) significantly ($p < 0.001$) increased the probability of a successful culture in 1 month, considering growth index (GI) of ≥100 or a GI of ≥999 as criterion of success. In logistic regression analysis, all factors maintained a significant ($p < 0.001$) independent effect, and no interactions were observed between them. The best conditions for the primary cultures of *M. genavense* were the use of Middlebrook 7H12 medium at pH 6.0 without any additives.—Authors' Abstract

Robert, B. and Hirst, R. Immunomagnetic separation and PCR for detection of *Mycobacterium ulcerans.* J. Clin. Microbiol. **35** (1997) 2709–2711.

We have developed a technique based on the use of monodisperse magnetic beads to isolate *Mycobacterium ulcerans* from heterogenous mixtures prior to PCR amplification. Using this method, we were able to detect *M. ulcerans* in water samples taken from Phillip Island, Australia, the site of several outbreaks of *M. ulcerans* disease in recent times.—Authors' Abstract

Ross, B. C., Johnson, P. D. R., Oppedisano, F., Marino, L., Sievers, A., Stinear, T., Hayman, J. A., Veitch, M. G. K. and Robins Browne, R. M. Detection of *Mycobacterium ulcerans* in environmental samples during an outbreak of ulcerative disease. Appl. Environment. Microbiol. **63** (1997) 4135–4138 .

Mycobacterium ulcerans is an environmental bacterium which causes chronic skin ulcers. Despite significant epidemiological evidence to suggest that water is the source of infection, the organism has never been identified in the environment. Environmental water samples were collected from a small town in which an outbreak of 29 cases had occurred in a 3-year period. These were examined by mycobacterial culture and PCR amplification. Similar to previous studies, *M. ulcerans* was not cultured from the water samples. However, five samples were positive for *M. ulcerans* by PCR. These samples were collected from a swamp and a golf course irrigation system within the outbreak area. This is the first time that *M. ulcerans* has been demonstrated to be present in the environment and supports the postulated epidemiology of disease due to this organism.—Authors' Abstract

Rossau, R., Traore, H., de Beenhouwer, H., Mijs, W., Jannes, G., de Rijk, P. and Portaels, F. Evaluation of the INNO-LiPA Rif. TB assay, a reverse hybridization assay for the simultaneous detection of *Mycobacterium tuberculosis* complex and its resistance to rifampin. Antimicrob. Agents Chemother. **41** (1997) 2093–2098.

Mycobacterium tuberculosis resistance to rifampin results from nucleotide changes in the gene encoding the β-subunit of the RNA polymerase (*rpoB*). We developed a reverse hybridization-based line probe assay (LiPA; the INNO-LiPA Rif. TB) carrying one oligonucleotide probe for the detection of *M. tuberculosis* complex strains and nine probes designed to detect nucleotide changes in the relevant part of *rpoB.* This assay was evaluated with 107 *M. tuberculosis* isolates with known *rpoB* sequences, 52 non-*M. tuberculosis* complex strains, and 61 and 203 clinical isolates found to be sensitive and resistant, respectively, by *in vitro* testing. The results indicated that (i) the *M. tuberculosis* complex probe was 100% specific, (ii) when compared to the results of nucleotide sequencing, no discrepancies with the results of INNO-LiPA Rif. TB were observed, (iii) all strains sensitive by *in vitro* susceptibility testing were correctly identified, and (iv) among the strains resistant by *in vitro* susceptibility testing, only 4 (2%) yielded conflicting results. The INNO-LiPA Rif. TB is therefore a reliable and widely applicable assay and a valuable tool for routine diagnostic use, given its simplicity and rapid performance.—Authors' Abstract

Schneider, J., Bruckmann, W. and Zwingenberger, K. Extravasation of leukocytes assessed by intravital microscopy: effect of thalidomide. Inflamm. Res. **46** (1997) 392–397.

Objective and Design: Thalidomide is very effective in the treatment of idiopathic aphthous stomatitis, characterized by recurrent focal intramucosal leukocytic vasculitis. The mode of action of thalidomide in this clinical entity may include inhibition of the extravasation of leukocytes. Therefore, we studied the effect of thalidomide on different steps of leukocyte migration by intravital microscopy.

Material: Male Syrian golden hamsters were used.

Treatment: Leukocyte migration in buccal mucosa of the hamster cheek pouch was

elicited by the local application of lipopolysaccharide (LPS, 20 μg/ml) or murine tumor necrosis factor-alpha (muTNF-alpha, 10 ng/ml). (+)-Thalidomide (20–200 mg/kg i.p.) was administered 60 min before the local application of LPS or muTNF-alpha. Dexamethasone (2 × 1.0–10 mg/kg i.p.) was administered 18 hr and 60 min before topical LPS application.

Methods: The numbers of rolling, firmly adherent and migrating leukocytes were estimated by intravital microscopy up to 165 min after the topical applications of LPS or muTNF-alpha and evaluated by an interactive image analysis software.

Results: Thalidomide (20–200 mg/kg i.p.) dose-dependently inhibited LPS-stimulated perivenular leukocyte migration by up to 87 ± 5% and muTNF-alpha-induced leukocyte migration by up to 78 ± 4%. Dexamethasone (2 × 1.0–10 mg/kg i.p.) inhibited LPS-stimulated rolling by 80 ± 5% and reduced the number of firmly adherent leukocytes by about 40%. Dexamethasone (2 × 10 mg/kg i.p.) did not reduce the number of rolling leukocytes but inhibited leukocyte adherence by 72 ± 9%.

Conclusions: These results show that (+)-thalidomide predominantly inhibits leukocyte rolling and thus differs from the glucocorticoid dexamethasone. The inhibition of LPS- or muTNF-alpha-induced leukocyte extravasation by thalidomide may account for some of its clinical activities.—Authors' Abstract

Sechi, L. A., Pinna, M. P., Sanna, A., Pirina, P., Ginesu, F., Saba, F., Aceti, A., Turrini, F., Zanetti, S. and Fadda, G. Detection of *Mycobacterium tuberculosis* by PCR analysis of urine and other clinical samples from AIDS and non-HIV-infected patients. Mol. Cell. Probes **11** (1997) 281–285.

A number of different clinical specimens, such as sputum, cerebrospinal fluid and blood, have been reported to be good substrates for the detection of *Mycobacterium tuberculosis* by PCR assay. We wanted to search for the presence of mycobacteria in other body fluids, such as urine. Urine samples and other samples obtained from AIDS patients and nonHIV-infected patients were analyzed by PCR. The results were compared with those obtained using conventional methods [BACTEC 460 TB and AFB (acid fast bacilli stain)]. We analyzed 412 urine samples and 210 different other samples (sputum and cerebrospinal fluid) obtained from AIDS patients by PCR; almost identical levels of PCR-positive (14%–17%) results were observed in all samples analyzed. The results were then compared with those obtained with the BACTEC 460 TB and AFB. PCR, BACTEC 460 TB, and acid fast stain were also used to analyze 190 urine samples and 230 other samples from nonHIV-infected patients in the consumption ward of Sassari Hospital. The number of urine samples positive by PCR (6.3%) and BACTEC 460 TB (2.1%) was half that obtained from samples taken from the AIDS patients. As expected, an increase in the number of positive sputum samples was observed with all methods. The results indicate that PCR analysis of urine samples represents a valid alternative for fast and sensitive detection of *M. tuberculosis.* This method can be routinely used in the clinical laboratory, especially in HIV-infected patients.—Authors' Abstract

Sodhi, A., Gong, J. H., Silva, C., Qian, D. J. and Barnes, P. F. Clinical correlates of interferon gamma production in patients with tuberculosis. Clin. Infect. Dis. **25** (1997) 617–620.

To determine if the capacity to produce interferon (IFN) gamma is related to the clinical manifestations of tuberculosis, we correlated *Mycobacterium tuberculosis*-induced IFN-gamma production by peripheral blood mononuclear cells (PBMCs) with clinical, radiographic, and laboratory variables for 63 human immunodeficiency virus (HIV)-negative patients and 43 HIV-positive patients with tuberculosis. For HIV-negative patients whose chest radiographs showed moderately advanced disease, the mean IFN-gamma concentration ± S.D. was 1639 ± 388 pg/mL; whereas that for patients with far-advanced disease was 327 ± 100 pg/mL ($p = 0.0001$). For HIV-infected patients who had only pleuropulmonary disease, the mean IFN-gamma concentration was 1002 ± 257 pg/mL; whereas

that for patients with disease outside the lungs and pleura was 149 ± 55 pg/mL ($p = 0.0004$). Multivariate analysis confirmed that the radiographic extent of disease and the site of disease were the only independent predictors of IFN-gamma production in HIV-negative and HIV-infected patients ($p \leq 0.001$). We conclude that reduced IFN-gamma production by PBMCs is a marker of severe tuberculosis in both HIV-negative and HIV-infected patients with tuberculosis.—Authors' Abstract

Sreevatsan, S., Pan, X., Stockbauer, K. E., Connell, N. D., Kreiswirth, B. N., Whittam, T. S. and Musser, J. M. Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. Proc. Nat. Acad. Sci. U.S.A. **94** (1997) 9869–9874.

One-third of humans are infected with *Mycobacterium tuberculosis,* the causative agent of tuberculosis. Sequence analysis of two megabases in 26 structural genes or loci in strains recovered globally discovered a striking reduction of silent nucleotide substitutions compared with other human bacterial pathogens. The lack of neutral mutations in structural genes indicates that *M. tuberculosis* is evolutionarily young and has recently spread globally. Species diversity is largely caused by rapidly evolving insertion sequences, which means that mobile element movement is a fundamental process generating genomic variation in this pathogen. Three genetic groups of *M. tuberculosis* were identified based on two polymorphisms that occur at high frequency in the genes encoding catalase-peroxidase and the A subunit of gyrase. Group 1 organisms are evolutionarily old and allied with *M. bovis,* the cause of bovine tuberculosis. A subset of several distinct insertion sequence IS6110 subtypes of this genetic group have IS6110 integrated at the identical chromosomal insertion site, located between dnaA and dnaN in the region containing the origin of replication. Remarkably, study of approximately 6000 isolates from patients in Houston and the New York City area discovered that 47 of 48 relatively large case clusters were caused by genotypic group 1 and 2 but not group 3 organisms. The observation that the newly emergent group 3 organisms are associated with sporadic rather than clustered cases suggests that the pathogen is evolving toward a state of reduced transmissibility or virulence.—Authors' Abstract

Svensson, E., Hanberger, H., Nilsson, M. and Nilsson, L. E. Factors affecting development of rifampicin resistance in biofilm-producing *Staphylococcus epidermidis.* J. Antimicrob. Chemother. **39** (1997) 817–820.

Selection and regrowth of variants resistant to 0.016–32 mg/L of rifampin, which were present at a frequency of 10^{-7} in the initial inoculum, were seen when large inocula (>10^5 cfu/mL) of *Staphylococcus epidermidis* ATCC 35984 were incubated with the drug. Conventional MIC determinations using approximately 10^5 cfu/mL did not detect the resistant variants. Larger inocula increased the MIC by >8000-fold. Population analysis showed that rifampin concentrations above the MIC (measured at an inoculum of approximately 10^5 cfu/mL) select highly resistant variants (MIC >256 mg/L) when large inocula (≥10^5 cfu/mL) were incubated with rifampin. The resistant variants were stable through ten passages. It was not possible to prevent regrowth of the resistant variants by increasing the rifampin concentration further. At subinhibitory concentrations there was no development of rifampin resistance.—Authors' Abstract

Urguhart, B. L., Atsalos, T. E., Roach, D., Basseal, D. J., Bjellqvist, B., Britton, W. L. and Humphery Smith, I. "Proteomic contigs" of *Mycobacterium tuberculosis* and *Mycobacterium bovis* (BCG) using novel immobilised pH gradients. Electrophoresis **18** (1997) 1384–1391.

Tuberculosis remains a major health problem throughout the world and the failure of the existing bacille Calmette-Guerin (BCG) vaccine in recent trials has prompted a search for potential replacements. Recent advances in molecular and cell biology have cast doubts on the ability of genetic

analysis alone to predict polygenic human diseases and other complex phenotypes and have therefore redirected our attention to proteome studies to complement information obtained from DNA sequencing initiatives. Novel acidic (pH 2.3–5) and basic (pH 6–11) IPG gel gradients were employed in conjunction with commercially available pH 4–7 gradients to significantly increase (fourfold) the number of protein spots previously resolved on two-dimensional (2-D) gels of *Mycobacterium* species. A total of 772 and 638 protein spots were observed for *M. bovis* BCG and *M. tuberculosis* H37Rv, respectively, the latter corresponding to only the pH regions 4–7 and 6–11. Of interest was the bimodal distribution observed for proteins separated from *M. bovis* BCG across both M-r and pH ranges. Some differences in protein expression were observed between these two organisms, contrary to what may have been expected considering the high degree of conservation in gene order and sequence similarity between homologous genes. Further work will be directed toward a more detailed analysis of these differences, so as to allow more accurate diagnosis between vaccination and active tuberculosis. The latter is of major importance to epidemiological studies and for patient management.—Authors' Abstract

Van Soolingen, D., Hoogenboezem, T., deHaas, P. E. W., Hermans, P. W. M., Koedam, M. A., Teppema, K. S., Brennan, P. J., Besra, G. S., Portaels, F., Top, J., Schouls, L. M. and van Embden, J. D. A. A novel pathogenic taxon of the *Mycobacterium tuberculosis* complex, Canetti: characterization of an exceptional isolate from Africa. Int. J. System. Bacteriol. **47** (1997) 1236–1245.

In an attempt to characterize an unusual mycobacterial strain isolated from a 2-year-old Somali patient with lymphadenitis, we applied various molecular methods not previously used for the taxonomic classification of mycobacteria. This isolate, designated So93, did not differ from *Mycobacterium tuberculosis* in the biochemical tests and in its 16S rRNA sequence, but produced smooth and glossy colonies, which is highly exceptional for this species. This smooth phenotype was unstable and switched nonreversibly to a rough colony morphology with a low frequency. The two colony types were equally virulent for the guinea pig, exhibiting characteristic tuberculous disease. Both morphotypes had shorter generation times than the *M. tuberculosis* reference laboratory strain H37Rv and clinical isolates of *M. tuberculosis* and *M. bovis*. Furthermore, the So93 isolate differed from all *M. tuberculosis* complex strains described thus far by having only a single copy of insertion sequence IS1081, an unusual composition of the direct repeat cluster, and a characteristic phenolic glycolipid and lipooligosaccharide. This glycolipid had previously been observed only in a smooth isolate of *M. tuberculosis* obtained in 1969 by Canetti in France. Analysis of the Canetti strain showed that it shared virtually all genetic properties characteristic of So93, distinguishing these two strains from the known *M. tuberculosis* complex taxa, *M. tuberculosis, M. africanum, M. bovis,* and *M. microti.* The natural reservoir, host range, and mode of transmission of the group of bacteria described in this paper are presently unknown. This study, partly based on not previously used molecular criteria, supports the idea that the established members within the *M. tuberculosis* complex and the newly described Canetti grouping should be regarded as a single species, which likely will be designated "*M. tuberculosis.*"—Authors' Abstract

Vilagut, L., Pares, A., Vinas, O., Villa, J., DeAnta, T. and Rodes, J. Antibodies to mycobacterial 65-kDa heat shock protein crossreact with the main mitochondrial antigens in patients with primary biliary cirrhosis. Eur. J. Clin. Invest. **27** (1997) 667–672.

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease associated with autoimmune disorders. The etiology is unknown, although it has been suggested that the disease may be related to infectious agents. Previous studies revealed that sera from patients with PBC react against *Mycobacterium gordonae.* This specific reactivity, characterized by a recognition of two

membrane polypeptides of 70–65 and 55 kD crossreact with the two major mitochondrial autoantigens of PBC. Since the most immunogenic components of mycobacteria are the heat-shock proteins (hsp), which have been associated with autoimmunity, this study has been undertaken to characterize whether the reacting polypeptides in PBC are hsp from *M. gordonae.* Cultures of *M. gordonae* were incubated at 37°C and 46°C before sonication, protein extraction and separation by SDS-PAGE. Exposure of *M. gordonae* to heat-shock treatment resulted in membrane-protein overexpression, similar to the 70–65-kD polypeptide recognized by the sera from patients with PBC. Immunoprecipitation assays with a monoclonal antibody directed against the hsp65 kD of mycobacteria and with sera from patients with PBC revealed similar reacting profiles characterized by the precipitation of the overexpressed 65-kD polypeptide from *M. gordonae.* Competitive immunoblotting showed that binding of the monoclonal antibody to the hsp65-kD protein was prevented by preincubation with sera from patients with PBC, but not with sera from healthy subjects. Furthermore, monoclonal antibody to the hsp65-kD protein recognized the main mitochondrial autoantigens of PBC (PDH-E2 and BCKDH-E2). These data indicate the existence of crossreacting epitopes contained on *M. gordonae* hsp65 kD and the main mitochondrial antigens in patients with PBC.—Authors' Abstract

Vynnycky, E. and Fine, P. E. M. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. Epidemiol. Infect. **119** (1997) 183–201.

Many aspects of the natural history of tuberculosis are poorly understood. Though it is recognized that clinical tuberculosis may follow shortly after initial infection ("primary" disease), or many years thereafter through either endogenous reactivation or after reinfection, the relative importance of these mechanisms is often disputed. The issue is complicated by the fact that the risks of developing disease are age-dependent, and reflect infection risks which may change over time. This paper estimates the age-dependent risks of developing tuberculosis using an age-structured deterministic model of the dynamics of tuberculous infection and disease in England and Wales since 1900. The work extends the classical studies of Sutherland and colleagues. The best estimates of the risks of developing "primary" disease (within 5 years of initial infection) were approximately 4%, 9% and 14% for individuals infected at ages 0–10, 15 years and over 20 years, respectively, and a previous infection appeared to impart little protection against (further) reinfection, but 16%–41% protection against disease subsequent to reinfection for adolescents and adults. We also provide evidence that reinfection made an important contribution to tuberculous morbidity in the past, as (i) exclusion of exogenous disease from the model considerably worsened the fit to observed notification rates, and (ii) the dramatic decline in the risk of tuberculous infection from 1950 in England and Wales accelerated the decline in morbidity among all individuals, even among the older age groups with a high prevalence of tuberculous infection. We conclude that the risk of infection is the single most important factor affecting the magnitude of the tuberculous morbidity in a population since it determines both the age pattern of initial infection (and hence the risk of developing disease) and the risk of reinfection.—Authors' Abstract

Wiessler, R. E., Collart, J. P., McNerney, R. and Wilson, S. M. Cotton wool swabs provide a convenient medium for the collection, transport and storage of sputum for the subsequent molecular investigation of *Mycobacterium tuberculosis.* Trans. R. Soc. Trop. Med. Hyg. **90** (1996) 256–257.

In Burundi, 31 swabs were soaked in sputum, removed, dried, and stored at room temperature in plastic sleeves before being posted to London, U.K., for the detection of *Mycobacterium tuberculosis* complex by the polymerase chain reaction (PCR). Batch analysis, after at least 1 year of storage at room temperature, showed that cotton wool swabs can be successfully used to collect

sputum for subsequent molecular analysis. —Authors' Abstract

Zhu, X., Venkataprasad, N., Ivanyi, J. and Vordermeier, H. M. Vaccination with recombinant vaccinia viruses protects mice against *Mycobacterium tuberculosis* infection. Immunology **92** (1997) 6–9.

A number of subunit-based vaccine candidates have recently begun to erode the exclusive position of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG), which gives unpredictable and highly variable protection against tuberculosis. In this paper we investigated the protective capacity of the 19,000 MW and 38,000 MW glycolipoproteins of *M. tuberculosis* expressed by recombinant vaccinia viruses in a mouse *M. tuberculosis* infection model. Both proteins were expressed at high levels by recombinant vaccinia-infected cells. In addition, two inoculations of C57B/6 mice with either recombinant vaccinia virus significantly reduced the bacterial counts in the lungs of *M. tuberculosis* H37Rv-infected mice, when compared with the group infected with control virus. This is the first report of protection against tuberculous infection using recombinant vaccinia viruses with results that suggest that secreted glycolipoproteins in conjunction with the vaccinia vector represent suitable candidates for further vaccine-related studies.—Authors' Abstract