CURRENT LITERATURE

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

General and Historical

Croft, R. P. and Croft, R. A. Knowledge, attitude and practice regarding leprosy and tuberculosis in Bangladesh. Lepr. Rev. **70** (1999) 34–42.

A small survey was carried out in two areas of northern Bangladesh to assess and compare the level of knowledge, attitude and practice toward leprosy and tuberculosis (TB) among two communities that differed widely in the amount of health education received about these diseases. The results indicate that without a health education program levels of knowledge about the cause of treatability of the diseases are poor, worse for leprosy than TB, with correspondingly negative attitudes. Only 16% of the respondents in the "uninformed" area mentioned "skin patch" in a question about what they knew about leprosy; and only 44% mentioned "cough" as a symptom of TB. In the area that had received health education, 90% mentioned, respectively, "skin patch" and "cough." Seventy-eight percent of the respondents would not buy goods from a shopkeeper known to have leprosy, 76% if he had TB in the uninformed area; but in the community which had received health education the proportions were reversed, with three-quarters agreeing to purchase from a diseased shopkeeper. The implications of these findings for the Danish Bangladesh Leprosy Mission and national health education programs are discussed.—Authors' Summary

Ebenso, B. E. Results of a 1 year Special Action Project for the Elimination of Leprosy (SAPEL) in poorly accessible areas of Akwa Ibom State, Nigeria. Lepr. Rev. **90** (1999) 56–62.

This article reports the outcome of a Special Action Project for the Elimination of Leprosy (SAPEL), including the implementation of multidrug therapy (MDT) in difficult situations in Akwa Ibom state in Nigeria. Twenty-two fishing villages and 5 communities in areas of gully erosion participated in the project from August 1996 to September 1997. Seven new cases were detected and treated with MDT. Twenty-one out of 22 defaulters examined resided in the mainland part of the project area and not in the fishing villages. Considerable difficulties were encountered with regard to the exorbitant cost of transport, physical attacks on the teams and the lack of reliable information on population figures for the project area. The discussion includes attention to the cost-effectiveness of the SAPEL approach under the conditions described and the need to develop better monitoring of treatment and community participation in poorly accessible areas.—Author's Summary

Foss, N. T. [Hansen's disease: clinical, immunological and therapeutic aspects.] An. Bras. Dermatol. Rio de Janeiro **74** (1999) 113–119. (in Portuguese)

In this review, we present the correlation between clinical manifestations and the immunological alterations on the clinical spectrum of leprosy and the importance of the specific treatment on the clinical, immunological and epidemiological controls. Related are the more important aspects, especially results of immunological investigation to understand better the pathophysiologic mechanisms on the leprosy evolution. There are presented also the therapeutic plan for reactional episodes and the multidrug therapy efficacy on leprosy control and the low frequency of relapses.—Author's English Summary

Han, C., *et al.* [Leprosy control and its fund raising.] China Lepr. J. **14** (1998) 226–229. (in Chinese)

In Jining City, Shangdong Province, China, in the early 1960s there were 1361 leprosy patients and by the end of 1995 only 35 cases remained. The prevalence decreased from 0.74‰ to 0.007‰. Before the 1980s, all the funds were provided by the government at various levels, but in 1995 13.23% was raised by professional leprosy units. In the 1960s, the average outlay on a patient was RMB 136.9 yuan yearly, but in 1995 it was 17,941 yuan. The fund/year for a professional worker was 8101 yuan in the 1960s and 12,937.7 yuan in 1995. Before the 1970s, all professional workers in leprosy control units had been working for leprosy control and at present only 39% of them are working for leprosy. In the last years, although the funds allocated by the government greatly increased and the number of patients evidently decreased, the money spent for leprosy lessened in large part because of the rising prices and raises of personnel wages. And so leprosy control work has been affected. The authors appeal that the relevant policy should be formulated for changing such a situation.—Authors' English Abstract

Jacobson, R. R. and Krahenbuhl, J. L Leprosy. Lancet **353** (1999) 655–660.

Leprosy is an ancient disease which is still poorly understood and often feared by the general public and even by some health care professionals. Fortunately, improvements in the management of leprosy over the past three decades have diminished the stigma and greatly altered the outlook for patients. Public understanding of the disease has benefited from WHO's goal of eliminating leprosy as a public health problem by the year 2000. Unfortunately, that goal has also led many to believe that leprosy has been or will soon be eradicated. This will not happen in the near future because, despite a fall in registered cases, the incidence of the disease has changed very little, and eradication of a bacterial infectious disease such as this is unlikely with chemotherapy alone. Nevertheless, as a result of the WHO's efforts, patients nearly everywhere should have access to care, and the incidence may begin to diminish if adequate control efforts are maintained beyond the year 2000. Given the mobility of patients today a physician anywhere may occasionally see a case or be asked about the disease so a basic understanding of leprosy and its management should prove useful.—Authors' Abstract

Pedrazzani, E. S., Helene, L. M. F., Vieira, C. S. de C. A., Vieth, H., Bezerra, C. M. and Mendes, E. B. [Training multipliers in the nursing area in hanseniasis.] Hansen. Int. **23** (1998) 27–34. (in Portuguese)

Hansen's disease is an endemic disease in Brazil, where it shows the highest prevalence in Latin America. In 1994 the WHO and PAHO together with the Ministry of Health wrote an agreement to set up an elimination plan. The São Paulo Health Secretariat set up goals to be met until the year 2000, within them the training of health personnel. In the nursing area a technical group was formed with the objective of giving advice on specific nursing actions in Hansen's disease. This group held training for nurses from different areas of São Paulo state with the objective of training these professionals to be the regional supervisors in Hansen's disease. Firstly, a division of the State considering epidemiological indicators of the disease was done. The criteria for selecting participants were a) regions with the highest rates of prevalence and incidence and b) regions where other health workers have already been trained. The training course had 6 theoretical and practical parts and contained nurse consultation, epidemiological control, prevention and control of disabilities, treatment, dressing, bacilloscopy, Mitsuda's test and whatever considered paramedical attributions. The methodology involved pedagogical techniques using discussions and reflections based on the experience of the participants. There were two evaluations, one before and the other after the training. The first had a variation from 7 points to 127 points with a mean of 70 (55.1%). The evaluation after the training had a variation from 95 points

to 127 points with a mean of 115 points (90.5%). At the end of the training, all participants had to present a plan on how to multiply this program in his/her region. At this time, these nurses had already trained 10 other nurses, always supervised by the technical group.—Authors' English Summary

Rajaratnam, J., Abel, R. and Arumai, M. Is knowledge of leprosy adequate among teachers? A comparative study. Lepr. Rev. **70** (1999) 28–33.

A cross-sectional comparative study on the levels of knowledge and attitude on leprosy among teachers and students was carried out in a rural area of Vellore district in Tamil Nadu, India. A total of 30 teachers and 120 students participated in the study. It was found that knowledge about leprosy among teachers was inadequate. Only 23.4% of teachers stated that germs caused leprosy, while 23.4% mentioned immoral conduct, 20.0% marrying a leprosy patient, 6.6% insects and 26.6% did not know the causes of leprosy. While 80.0% of teachers knew that anesthetic hypopigmented patches were a sign of leprosy, enlarged painful nerves were not mentioned by a single teacher, although this sign was identified by 17.5% of students. Teachers had a more positive attitude towards leprosy than students and this was statistically significant ($p < 0.001$). This paper discusses the need for continuous education, especially for teachers and through them the students, using different media so as to ensure sustained knowledge for behavioral change in the community.—Authors' Summary

Scollard, D. M. and Skinsnes, O. K. Oropharyngeal leprosy in art, history, and medicine. Oral Sur. Oral Med. Oral Pathol. Oral Radiol. Endod. **87** (1999) 463–470.

Advanced lesions of the face, nasopharynx, and oropharynx have played an important role in the medical and social history of Hansen's disease. Renaissance artists included detailed portrayals of these lesions in some of their paintings, a testimony not only to their artistic skill and powers of observation but also to the common presence of these patients in European cities and towns of the period. The disease is now understood as a broad immunologic spectrum of host responses to *Mycobacterium leprae,* with a variety of clinical and pathologic manifestations in nerve, soft tissues, and bone. This review incorporates the findings of 2 extraordinary studies (one from Europe and the other from Japan) of pharyngeal and facial lesions. In the 1950s, studies of skeletal remains from the churchyard of a Danish leprosarium revealed a triad of maxillofacial lesions unique to leprosy and designated *facies leprosa.* In pre-World War II Japan, before effective treatment had been discovered, a prominent otorhinolaryngologist studying oropharyngeal and nasopharyngeal lesions prepared watercolor illustrations of the natural progression of untreated Hansen's disease. As a result of effective antimicrobial therapy, such advanced lesions are now rarely seen, but the presenting signs and symptoms of leprosy still occasionally arise in the nasal and oral mucosa. The nasopharynx and oropharynx may be important early sites of inoculation and infection by *M. leprae,* and they require additional emphasis in worldwide efforts toward early diagnosis and treatment of Hansen's disease.—Authors' Abstract

Zhou, H., *et al.* [A survey of knowledge on leprosy among medical workers in Huangdao Developing Area, Qingdao.] China Lepr. J. **14** (1998) 239–242. (in Chinese)

In Huangdao, an economic developing district of Qingdao City, China, knowledge on leprosy among doctors in departments of dermatology and neurology of regional hospitals and leprosy control units and medical workers in town medical centers and village health stations was examined. The result showed that medical workers at the town and village levels are relatively short of knowledge, and the authors pointed out that popularization of knowledge on diagnosing leprosy among them is essential after the basic eradication of leprosy.—Authors' English Abstract

Chemotherapy

Cynamon, M. H., Klemens, S. P., Sharpe, C. A. and Chase, S. Activities of several novel oxazolidinones against *Mycobacterium tuberculosis* in a murine model. Antimicrob. Agents Chemother. **43** (1999) 1189–1191.

The activities of linezolid, eperezolid, and PNU-100480 were evaluated in a murine model of tuberculosis. Approximately 10^7 viable *Mycobacterium tuberculosis* ATCC 35801 organisms were given intravenously to 4-week-old, outbred CD-1 mice. In the first study, treatment was started 1 day postinfection and was given by gavage for 4 weeks. Viable cell counts were determined form homogenates of spleens and lungs. PNU-100480 was as active as isoniazid. Linezolid was somewhat less active than PNU-100480 and isoniazid. Eperezolid had little activity in this model. In the next two studies, treatment was started 1 week postinfection. A dose-response study was performed with PNU-100480 and linezolid (both at 25, 50, and 100 mg/kg of body weight). PNU-100480 was more active than linezolid, and its efficacy increased with an escalation of the dose. Subsequently, the activity of PNU-100480 alone and in combination with rifampin or isoniazid was evaluated and was compared to that of isoniazid-rifampin. The activity of PNU-100480 was similar to that of isoniazid and/or rifampin in the various combinations tested. Further evaluation of these oxazolidinones in the murine test system would be useful prior to the development of clinical studies with humans.—Authors' Abstract

Dalpino, D., Magna, L. A. and Opromolla, D. V. A. [NADH-methemoglobin-reductase activity in the hemolysate and erythrocyte ghost cells from leprosy patients undergoing sulfone therapy.] Hansen. Int. **23** (1998) 14–26. (in Portuguese)

We measured in the blood samples of 72 adult leprosy patients, who were ingesting 100 mg of dapsone/day, the levels of erythrocytes, hemoglobin, methemoglobin, sulfone and reticulocytes. NADH-methemoglobin-reductase was measured both in the hemolysate and ghost cells. As a control, identical tests, except for sulfone measure, were applied to blood samples from 72 healthy individuals who did not ingest any oxidant drug. Significant statistical differences regarding the values in erythrocytes, methemoglobin, reticulocytes and activity of NADH-reductase in the erythrocyte ghosts were found among these two groups. The mean enzyme activity in the hemolysate of the leprosy patients and the healthy individuals had not differed significantly when expressed in Ul/g.hemoglobin/l. The enzyme activity in erythrocyte ghosts from leprosy patients was significantly smaller than that in erythrocyte ghosts from healthy individuals. The mean level of methemoglobin in the leprosy patients was higher than in the control group, probably due to oxidization of sulfone. We have found smaller levels of enzymatic activity in the erythrocyte membranes of the leprosy patients and coincident levels in the hemolysate compared to controls. We can affirm that probably this is due to an enzymatic displacement from the cellular membrane to the cytoplasm, with the objective of maintaining in the erythrocyte a constant hemoglobin balance, in spite of the sulfone oxidizer action.—Authors' English Summary

Dhople, A. M. *In vitro* and *in vivo* activity of K-130, a dihydrofolate reductase inhibitor, against *Mycobacterium leprae.* Arzneimittalforshung **49** (1999) 267–271.

The antimicrobial effects of a new dihydrofolate reductase inhibitor, K-130 (2,4-diaminodiphenyl sulfone substituted 2,4-diamino-5-benzylpyrimidine), alone and in combination with dapsone (CAS 80-08-0) against both dapsone-sensitive and dapsone-resistant strains of *Mycobacterium leprae* were evaluated *in vitro,* in a cell-free culture system, and *in vivo* in mouse foot pads. The minimal inhibitory concentration of K-130 against dapsone-sensitive as well as dapsone-resistant strains of *M. leprae* was 0.03 μg/ml, and the activity was bactericidal, in both cases. However, when combined with dapsone, K-130 exhibited syner-

gism in the case of dapsone-sensitive *M. leprae,* while in the case of dapsone-resistant *M. leprae* the effect was merely additive. Similar synergistic effects were also observed in the mouse foot pad system for both types of *M. leprae* strains.—Author's Abstract

Goto, M., Miyagi, S., Takizawa, H. and Kitajima, S. I. [Chemotherapy of Hansen's disease in Japan—present status.] Jpn. J. Lepr. **67** (1998) 305–311. (in Japanese)

In order to assess the current status of leprosy chemotherapy in Japan, 3 recently conducted, government-supported nationwide surveys, conducted in 1994, 1996 and 1997, were compared and analyzed. For most new and relapse cases, multidrug therapy was applied, including rifampin/dapsone or rifampin/dapsone/clofazimine combinations in about half of new cases and about one-third of relapse cases. In many cases, doses and intervals were based on the WHO protocol; however smaller dosages were used in some cases. Quinolone (ofloxacin) was used in 40% of cases during therapy. Reversal reactions were observed in 7/71 cases and erythema nodosum leprosum was observed in 6/71 cases. In order to prevent chemotherapy-induced deformities, the authors propose a modified protocol for new cases accompanied by reactional status which starts with clofazimine monotherapy and is followed by WHO/MB.—Trop. Dis. Bull. **96** (1999)

Habtemariam, H. Viability and drug sensitivity of *M. leprae* isolated from long-term WHO/MDT treated multibacillary leprosy patients. Lepr. Rev. **70** (1999) 43–46.

In this study, we observed that the isolation of viable bacilli is not necessarily related to the duration of MDT or to the average BI. It also appears that there was a rapid clearing of bacilli following the discontinuation of therapy in some of our patients. Viable bacilli can be isolated from some patients after completion of WHO/MDT, but most patients can eliminate these bacilli without further chemotherapy and do not seem to be at great risk of relapse.—From the Article

Hatano, K., Matsuki, T. and Makino, M. [The trend of leprosy treatment in which the prevision of peripheral nerve damage is the main objective.] Jpn. J. Lepr. **67** (1998) 353–360. (in Japanese)

An investigation was made into changes in the rate of peripheral nerve damage, before and after treatment, in leprosy patients in Bangladesh. In well-organized non-governmental organization (NGO) programs where early diagnosis was possible, the damage rate was found to improve slightly during treatment. But among MB cases, the damage rate at the time of a patient's first follow-up examination remained high. Furthermore, during the treatment of MB cases, 72% cases showed an episode of neuritis, and during 2 years of follow-up, 15% cases showed the same episodes. Among the recurrent cases of the last 10 years in Oku-Komyo-en, Japan, the drugs prescribed for the treatment of recurrent cases were studied. The amount of each dosage was found to be relatively small and the prescription period was rather long.—Trop. Dis. Bull. **96** (1999)

Hosokawa, A. [Treatment of patients with leprosy at the Ryukyu University Hospital.] Jpn. J. Lepr. **67** (1998) 313–327. (in Japanese)

Therapeutic regimens in patients with leprosy were analyzed at the Ryukyu University Hospital, Okinawa, Japan. Most of the medications were similar to that of multidrug therapy (MDT) recommended by the WHO. Some modified MDT was applied to some patients for prevention of the various sequelae caused by peripheral nervous disorders. In addition, some alternative treatments were also performed at the outpatient clinic, such as administration of drugs to improve the peripheral circulation (e.g., nicotinamide, tocopherol nicotinate), thermotherapy (raising the body temperature by 2–3°C to promote bacteriostasis), and steroid therapy to aid the recovery of dam-

aged peripheral nerves.—Trop. Dis. Bull. **96** (1999)

Phetsuksiri, B., Baulard, A. R., Cooper, A. M., Minnikin, D. E., Douglas, J. D., Besra, G. S. and Brennan, P. J. Antimycobacterial activities of isoxyl and new derivatives through the inhibition of mycolic acid synthesis. Antimicrob. Agents Chemother. **43** (1999) 1042–1051.

Isoxyl (ISO), a thiourea (thiocarlide; 4,4′-diisoamyloxythiocarbanilide), demonstrated potent activity against *Mycobacterium tuberculosis* H37Rv (MIC, 2.5 μg/ml), *M. bovis* BCG (MIC, 0.5 μg/ml), *M. avium* (MIC, 2.0 μg/ml), and *M. aurum* A+ (MIC, 2.0 μg/ml), resulting in complete inhibition of mycobacteria grown on solid media. Importantly, a panel of clinical isolates of *M. tuberculosis* from different geographical areas with various drug-resistance patterns were all sensitive to ISO in the range of 1 to 10 μg/ml. In a murine macrophage model, ISO exhibited bactericidal killing of viable intracellular *M. tuberculosis* in a dose-dependent manner (0.05 to 2.50 μg/ml). The selective action of ISO on mycolic acid synthesis was studied through the use of [1,2-^{14}C] acetate labeling of *M. tuberculosis* H337Rv, *M. bovis* BCG, and *M. aurum* A+. At its MIC for *M. tuberculosis,* ISO inhibited the synthesis of both fatty acids and mycolic acids (α-mycolates by 91.6%, methoxymycolates by 94.3%, and ketomycolates by 91.1%); at its MIC in *M. bovis* BCG, ISO inhibited the synthesis of α-mycolates by 87.2% and that of ketomycolates by 88.5%; and the corresponding inhibitions for *M. aurum* A+ were 87.1% for α-mycolates, 87.2% for ketomycolates, and 86.5% for the wax-ester mycolates. A comparison with isoniazid (INH) and ethionamide (ETH) demonstrated marked similarity in action, i.e., inhibition of the synthesis of all kinds of mycolic acids. However, unlike INH and ETH, ISO also inhibited the synthesis of shorter-chain fatty acids. ISO showed no acute toxicity against primary macrophage cell cultures as demonstrated by diminution of redox activity. A homologous series of ISO derivatives were synthesized. Most derivatives were as effective or more effective than the parent compound in the agar proportion assay. Thus, these thioureas, like INH and ETH, specifically inhibit mycolic acid synthesis and show promise in counteracting a wide variety of drug-sensitive and -resistant strains of *M. tuberculosis.*—Authors' Abstract

Reilly, T. P., Woster, P. M. and Svensson, C. K. Methemoglobin formation by hydroxylamine metabolites of sulfamethoxazole and dapsone: implications for differences in adverse drug reactions. J. Pharmacol. Exp. Ther. **288** (1999) 951–959.

Differences in the incidence of adverse drug reactions to trimethoprim-sulfamethoxazole and dapsone may result from differences in the formation, disposition, toxicity, and/or detoxification of their hydroxylamine metabolites. In this study, we examine whether differences in the biochemical processing of sulfamethoxazole hydroxylamine (SMX-NOH) and dapsone hydroxylamine (DDS-NOH) by erythrocytes [red blood cells (RBCs)] contribute to this differential incidence. The methemoglobin (MetHgb)-forming capacity of both metabolites was compared after a 60-min incubation with washed RBCs from 4 healthy human volunteers. DDS-NOH was significantly more potent ($p = 0.004$) but equally efficacious with SMX-NOH in its ability to form MetHgb. The elimination of potential differences in disposition by lysing RBCs did not change the MetHgb-forming potency of either hydroxylamine. At pharmacologically relevant concentrations, greater reduction to the parent amine occurred with DDS-NOH. Maintenance of MetHgb-forming potency was dependent on recycling with glutathione, but no difference in cycling efficiency was observed between DDS-NOH and SMX-NOH. In contrast, the pharmacodynamics of hydroxylamine-induced MetHgb formation were not changed by pretreatment with the glucose 6-phosphate dehydrogenase inhibitor epiandrosterone or by compounds that alter normal antioxidant enzyme activity. Methylene blue, which stimulates NADPH-dependent MetHgb reductase activity, decreased MetHgb levels but did not alter the differential potency of these hydroxylamines. DDS-NOH was also significantly

more potent when incubated with purified human hemoglobin A(o). Collectively, these data suggest that the inherently greater reactivity of DDS-NOH with hemoglobin, the greater conversion of DDS-NOH to its parent amine, and potential differences in disposition of hydroxylamine metabolites may contribute to the preferential development of dapsone-induced hemotoxicity and sulfamethoxazole-induced hypersensitivity reactions.—Authors' Abstract

Terencio de las Aguas, J. [Treatment of leprosy; new regimens.] Rev. Leprol. Fontilles **21** (1998) 689–697. (in Spanish)

The different stages of therapeutics in leprosy are analyzed: sulfones, clofazimine, rifampin, ofloxacin, clarithromycin and minocycline. The different regimens of multidrug therapy (MDT) and the last recommendations by WHO on treatment and leprosy reactions are discussed.—Author's English Summary

Zhang, Y., Scorpio, A., Nikaido, H. and Sun, Z. H. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. J. Bacteriol. **181** (1999) 2044–2049.

Pyrazinamide (PZA) is an important antituberculosis drug. Unlike most antibacterial agents, PZA, despite its remarkable *in vivo* activity, has no activity against *Mycobacterium tuberculosis in vitro* except at an acidic pH. *M. tuberculosis* is uniquely susceptible to PZA, but other mycobacteria as well as nonmycobacteria are intrinsically resistant. The role of acidic pH in PZA action and the basis for the unique PZA susceptibility of *M. tuberculosis* are unknown. We found that in *M. tuberculosis* acidic pH enhanced the intracellular accumulation of pyrazinoic acid (POA), the active derivative of PZA, after conversion of PZA by pyrazinamidase. In contrast, at neutral or alkaline pH, POA was mainly found outside *M. tuberculosis* cells. PZA-resistant *M. tuberculosis* complex organisms did not convert PZA into POA. Unlike *M. tuberculosis*, intrinsically PZA-resistant *M. smegmatis* converted PZA into POA, but it did not accumulate POA even at an acidic pH, due to a very active POA efflux mechanism. We propose that a deficient POA efflux mechanism underlies the unique susceptibility of *M. tuberculosis* to PZA and that the natural PZA resistance of *M. smegmatis* is due to a highly active efflux pump. These findings may have implications with regard to the design of new antimycobacterial drugs.—Authors' Abstract

Clinical Sciences

Britton, W. J. The management of leprosy reversal reactions. Lepr. Rev. **69** (1998) 225–234 (51 refs.).

This review discusses the prevalence of reversal reactions, immunopathology, clinical issues in management, predictive factors for reversal reaction, current management with corticosteroids, other therapies and future approaches to management.—Trop. Dis. Bull. **96** (1999) 157

de Lima, M. A., Rodrigues, V., Jr., Silva-Vergara, M. L., Nomelini, M. B., Paim, N., dos Santos, T. A. M. and dos Santos, V. M. [Generalized hanseniasis in Chagas' myocardiopathy: necropsy study.] Rev. Soc. Bras. Med. Trop. **31** (1998) 385–390. (in Portuguese)

The case is reported of a 34-year-old black man from Minas Gerais, Brazil, who died of heart failure. Post-mortem examination revealed chagasic cardiomyopathy together with mycobacterial infection. The pathogenesis of this case of mixed Chagas' disease (*Trypanosoma cruzi*) and leprosy (*Mycobacterium leprae*) is discussed.—Trop. Dis. Bull. **96** (1999) 382

Feliciano, K. V. de O., Kovacs, M. H. and Alzate, A. [Early diagnosis of Hansen's

disease: the case of health services in Recife (Pernambuco), Brazil.] Rev. Panam. Salud Publica **4** (1998) 6–13. (in Portuguese)

A descriptive study carried out in the city of Recife, Pernambuco, Brazil, during March–September 1994, to assess the health services available for performing early diagnosis of Hansen's disease, is presented with emphasis on accessibility and quality of the services provided. A total of 32 health clinics visited for diagnostic purposes by 183 patients with Hansen's disease were included in the study. Information on organizational infrastructures was collected by interviews with health clinic managers. Information regarding routine procedures in the 32 clinics was collected by observation, with special attention given to archival and inspection activities. A total of 1998 patients were interviewed to determine accessibility of services. Time spent in consultation with the physician was determined for 1000 patients who were seen by 123 physicians at the clinics during the interviews. To explore physicians' attitudes and knowledge regarding Hansen's disease, 133 were randomly selected from a list of names. The following factors were identified as hindering early diagnosis of Hansen's disease: the large number of people seeking service who could not be seen by a physician on the same day; the long time lapse between appointment scheduling and the actual visit (for those not seen on the same day); the long wait for the consultation; the brevity of the consultation; the low availability of trained personnel; the low proportion of physicians who examined all body surfaces; difficulties in the clinical recognition of the disease; and physicians not prepared to make a differential diagnosis.—Trop. Dis. Bull. **96** (1999)

Gao, X., *et al.* [Results of evaluating with SCL-90 measuring table for 34 children of persons affected with leprosy.] China Lepr. J. **14** (1998) 247–248. (in Chinese)

A survey with the SCL-90 table was completed in 34 young persons who are sons or daughters of those whose leprosy had been cured in Fei County, Shandong, China, a high leprosy-endemic area a few years ago. The results showed that psychological conditions among them were normal, compared with those of healthy controls. The authors regard it as a success in health education along with the campaign of basically eradicating leprosy.—Authors' English Abstract

Han, C., *et al.* [Changes in mentality and behavior of leprosy patients.] China Lepr. J. **14** (1998) 248–250. (in Chinese)

The mind and behavior were analyzed among 318 persons who had or have leprosy (266 males and 52 females with a mean age of 30.7 years) of which 96.9% showed some changes including: anxiety, inferiority complex, pessimism and unsociability, particularly during their hospitalization. Seven out of them suicided themselves.—Authors' English Abstract

Hari, L., Suryanarayanan, D., Thomas, A. and Joseph, P. An assessment of the value of midfinger smears in multibacillary leprosy patients. Lepr. Rev. **70** (1999) 47–51.

In view of the different opinions on fingers as sites for persisting bacilli in multibacillary (MB) leprosy patients, it was decided to examine the midfingers for the presence of acid-fast bacilli (AFB) and establish its usefulness. Sixty-nine MB leprosy patients [lepromatous (LL) and borderline lepromatous (BL)], treated with multidrug therapy for fixed duration (2 years) were analyzed. The bacillary load in the midfinger sites was lower when compared to that in the "compulsory" (both earlobes) and "optional" (four active lesions) sites. The midfinger bacterial index (BI) was higher among LL patients when compared to BL patients ($p < 0.001$). However, the difference in mean BI in "optional" and "compulsory" sites was not significant. The overall fall in BI was gradual and on expected lines for all sites, including midfingers, during treatment and follow-up periods. Except in one case, at no time were the smears from midfinger sites positive when all other sites were negative, and their inclusion did not contribute to the early detection of relapse. Furthermore, the collection of blood-free smears from this site is

technically difficult and often painful for the patient. The inclusion of midfinger smears in this study in patients in South India did not contribute useful information to that which is routinely available from smears of earlobes and other active sites.—Authors' Summary

Kato, L. E. and Nakandakari, S. [Specific nail alterations in Hansen's disease.] Hansen. Int. **23** (1998) 59–63. (in Portuguese)

Ungual alterations in Hansen's disease may be observed in over 60% of the patients and are characterized by subungual keratosis, onychogryphosis, mycronychia, anonychia, onychorrhexis, Beau's lines and ungual pterygium. Such alterations may be the consequence of the anesthesia caused by Hansen's disease peripheral neuropathy, repetitive trauma, peripheral vascular insufficiency, infections, and associated factors. The authors presented 4 lepromatous patients without previous treatment with diffuse infiltration in the fingers and toes, hyperplasia of the nail bed, thinned nail plate and onychorrhexis. Biopsy of the nail bed showed lepromatous infiltration with numerous acid-fast bacilli. The authors suggested that the nail distrophies may be caused by pressure of the specific infiltration on the nail matrix.—Authors' English Summary

Nery, J. A. C., Vieira, L. M. M., de Matos, H. J., Gallo, M. E. N. and Sarno, E. N. Reactional states in multibacillary Hansen's disease patients during multidrug therapy. Rev. Inst. Med. Trop. Sao Paulo **40** (1998) 363–370.

A cohort of 162, newly diagnosed, bacilloscopic-positive patients treated at the Leprosy Care Outpatient Clinic of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, during 1986–1991 was studied. While 46% of the multibacillary (MB) patients submitted to the 24 fixed-dose multidrug therapy regimen suffered reactions during treatment, it was found that all MBs were susceptible and that constant attention and care were required at all times; 14% were classified as BB, 52% as BL and 33% as LL. None of the variables studied, such as sex, age, clinical form, length of illness, length of dermatological lesions, bacillary index (BI) or degree of disability, were associated with reaction among these patients. Reversal reaction (RR) occurred in 45% of patients and erythema nodosum leprosum (ENL) occurred in 55%. Among BB patients who developed reactions (N = 15), 93% presented RR; while among the LL patients who developed reactions (N = 34), 91% presented ENL. ENL was very frequent among those with disseminate lesions, while RR was most often observed in patients with segmentary lesions. RR was also most likely to occur during the initial months of treatment. The recurrence rate of ENL was significantly higher than that of RR. Neither grade of disability nor BI was associated with RR and ENL reaction, although the RR rate was significantly higher among patients showing BI <3, while ENL predominated among those patients with BI >3.—Trop. Dis. Bull. **96** (1999) 494

Nubukpo, P. K., Edorh, K., David, M., Denis, F. and Grunitzky, E. K. [HTLV-1 in leprosy patients in Togo.] Med. Afr. Noire **45** (1998) 550–552. (in French)

To find out the relationship between human T-cell lymphotropic virus type I (HTLV-I) and leprosy, a cross-sectional survey was carried out in a group of leprosy patients from 3 leprosy care centers in Togo in 1991: 103 patients aged 30–50 years were included. There as a male predominance (59.2%). Blood samples were taken for serology for HTLV-I, HIV-1 and HIV-2. No risk factors for HTLV-I contamination were found in 30% of the patients. More than 50% had their disease diagnosed >10 years previously. The seroprevalence for HIV-1 was 1.94% while no HTLV-I or HIV-2 was found. This study found no relationship between HTLV-I and leprosy.—Trop. Dis. Bull. **96** (1999)

Nunez Marti, J.-M. [Bucodental lesions in leprosy patients.] Rev. Leprol. Fontilles **21** (1998) 655–663. (in Spanish)

The disease of leprosy presents itself in two forms: tuberculoid and lepromatous, both affecting skin and peripheral nerves.

The tuberculoid type presents a papule perfectly delimitated in the skin, including the face but there have never been reports of papules in the oral cavity; the lepromatous type presents the characteristic brown-reddish type of lesion nodules (lepromas) on the faces. In the oral cavity, the lesions are very infrequent in dimorphous and tuberculoid forms, but not in the lepromatous. In this study, I will try to describe these lesions in generic terms and also those observed personally by me with the support of my professional experience in the Hospital of Fontilles.—Author's English Summary

Nunez Marti, J.-M. [Oral hygiene in leprosy patients.] Rev. Leprol. Fontilles **21** (1998) 649–653. (in Spanish)

Bacterial plaque, calculus, food residues and impact are etiological factors that can be correlated with oral hygiene and proper conservations. Then, what happens with leprosy patients? Their dental hygiene is limited due to deformities or mutilations of fingers and hands together with loss of motivation and/or reduced general status of health and in the near future will suffer from periodontal disease. If we consider that leprosy is probably the most disabilitating disease in the world, the secondary chronic infections or traumas can lead to the loss of fingers or the distal portion of the limbs. In this study we discuss the proper oral hygiene and the difficulty that these patients have in implementing it and the different forms of motivating and helping them to compensate for these difficulties in the Hospital of San Francisco de Borja (Fontilles).—Author's English Abstract

Opromolla, D. V. A., Ura, S., Fleury, R. N., Daher, F. C. and Pagung, R. [Type 1 ulcerated hansenic reaction.] Hansen. Int. **23** (1998) 5–13. (in Portuguese)

In the thirties Rodriguez and Wade described a patient who they labeled as borderline who after an evolution of 10 years presented ulcerated lesions all over his body. Ryrie at Malaysia also wrote about ulcerated tuberculoid cases. Today all leprosy workers are familiar with these cases but so far there are not many studies about them. Reactional tuberculoid (RT) and reactional borderline (RB) cases with ulcerated lesions are not frequent. During a period of 10 years (1987 to 1998) admitted in our Institute were 316 reactional cases (1983 RT and 133 RB) and only 20 of such patients had ulcerated lesions. We studied 15 of these cases. There were 3 males and 12 females. The age ranged from 30 to 76 years, and all of them were white. All cases were reactional ones and showed several lesions all over their skin. Data about classification, number of reactional episodes, bacilloscopy and Mitsuda test were provided. The patients were classified as RT and RB based on clinical, bacterioscopic, histopathological and Mitsuda test results. RT cases showed Mitsuda tests equal to 6 mm or higher, and the bacilloscopy was negative or with few bacilli. There were 13 RT and 2 RB patients. All patients showed several erythematous papules, nodules and plaques. The RB ones showed more edema and more neural involvement. Characteristic lesions were seen on the face, around the eyes, nose and mouth, palms and soles. Some patients had many ulcerated lesions and others had only a few. Only 2 cases showed all the lesions with ulceration. Circular shallow ulcers involved all the lesions or were observed only near their edges, giving an annular configuration. From a histopathological point of view we observed a moderate epidermal hyperplasia. The tuberculoid granulomas were large and soft formed by epithelioid cells, foreign body giant cells, Langhans' giant cells and lymphocytes. Often the histiocytes spread to the neighboring stroma and sometimes they invaded the epidermis. There was interstitial and intracellular edema and the lymphocytes showed variations in distribution. Necrosis exhibited a fibrinoid pattern and in some places there was a caseous pattern of this necrosis. In general, we observed an uncharacteristic vascular proliferative reactivity and in rare cases we noted a granulomatous vasculitis. The majority of these ulcerated cases showed high levels of cell mediated immunity as showed by the values of Mitsuda reaction and the frequent negative bacilloscopy. The ulcerations occurred during the second or third reactional episodes in almost all the patients, and only in 3 of them were the lesions ulcerated during the first episode. We know that the acti-

vation of macrophages by lymphocytes may limit the infection, but continuous stimulation may lead to tissue damage through the release of macrophage products, including reactive oxygen intermediates and hydrolases, and this is due to an increase in TNF-α. In granulomatous reactions the activated macrophages become a major source of TNF-α and the granulomas develop by auto-amplification, with differentiation of macrophages into epithelioid cells which produce large amounts of TNF-α leading to tissue necrosis. This could be an explanation for the appearance of ulceration in our patients. In our opinion, in the reactional cases a multiplication of bacilli occurs, is destroyed by body defenses or treatment and consequently, a hypersensitivity reaction due to the antigens is released. If the immunity level is high the bacilli are destroyed and there will not be a another reactional episode. However, in cases where the immunity is not so efficient, some presister bacilli may remain and when conditions become more favorable for its multiplication there will be a new reactional episode. If the interval between these episodes is short the stimulation of the macrophages becomes continuous and there will be the possibility of ulceration by the mechanism discussed above. Another hypothesis to try to explain why ulceration occurs in some reactional tuberculoid and borderline cases could be to admit the possibility that these cases are a selected genetic group that show homozygous for the alellus $TNF\beta_2$ which is related with patients that show very high plasma concentrations of TNFα when compared to heterozygous and homozygous individuals for the alellus $TNF\beta_1$. TNF genes are polymorphic and are localized on the short arm of chromosome 6 in man, close to the genes of the major histocompatibility complex (MHC) in the class II region. This would explain the liaison among some MHC genotypes and the potential to produce serum TNF.—Authors' English Summary

Rivelli, V. B., Aldama, A. B., Correa, J. E. and Mendoza, M. G. [Aspects of the disease, leprosy, in patients in a general hospital.] Rev. Leprol. Fontilles **21** (1998) 665–675. (in Spanish)

This is a review performed at the Dermatology Department of the Hospital Nacional in the period from March 1993 to March 1998 on 30 inpatients with Hansen's disease. The patients were classified into 5 groups. This allowed us to know some interesting aspects related to these patients: differential diagnosis, intensity of reactional episodes with systemic demonstrations, complications and clinical intercurrencies. Most of the patients had the lepromatous form of the disease. In that period, 120 patients were registered at the Dermatology Department; 25% of them were inpatients. In 20 subjects the diagnosis was made at the moment of admittance. It is therefore important for clinicians from endemic countries to be aware about this diagnosis. In all cases, diagnosis was based on clinical, bacteriological and histological parameters.—Authors' English Summary

Samant, G., Shetty, V. P., Uplekar, M. W. and Antia, N. H. Clinical and electrophysiological evaluation of nerve function impairment following cessation of multidrug therapy in leprosy. Lepr. Rev. **70** (1999) 10–20.

Seventeen multibacillary (MB) and 15 paucibacillary (PB) cases of leprosy who had had regular and adequate multidrug therapy (MDT) were examined clinically and electrophysiologically at periodic intervals for 1 year following cessation of MDT. All of the major nerves were assessed for nerve function impairment (NFI). Overall, 2 MB (13.3%) and 3 PB (20%) cases showed signs of deterioration clinically and/or electrophysiologically. The nerve conduction (NC) follow-up studies revealed no significant improvement in the sensory conduction in both the MB and PB groups of nerves, while motor conduction showed a significant improvement at the first 6-monthly follow up among the MB group of nerves. At the study onset, sensory impairment (MB = 62%, PB = 25%) predominated over motor in terms of both severity and frequency. The lower extremity was more frequently and severely affected than the upper in both groups of patients. As an individual test, NC measurement proved to be more sensitive in detecting NFI, but the combination of physical palpitation for

nerve thickening and graded nylon test (GNT) was closely comparable to measurement of nerve conduction.—Authors' Summary

Selvasekar, A., Geetha, J., Nisha, K., Manimozhi, N., Jesudasan, K. and Sundar Rao, P. S. S. Childhood leprosy in an endemic area. Lepr. Rev. **70** (1999) 21–27.

A study was done on 794 new cases of leprosy among children (aged 0–14 years) detected and treated with MDT during 1990–1995 in Gudiyatham Taluk, South India. Incidence rates of leprosy and the proportion of multibacillary cases increased with age, while bacillary positive tuberculoid was most common. Over 80% had a single patch and most children were detected through surveys. Nearly 30% had history of household contacts with leprosy, mostly parents or grandparents. Reactions and relapses were not uncommon. The findings emphasize the need for more careful surveys for case detection and better follow up in case management.—Authors' Summary

Shen, J., *et al.* [Analysis of leprosy patients at the clinic of the Institute of Dermatology, CAMS.] China Lepr. J. **14** (1998) 233–234. (in Chinese)

The Institute of Dermatology, Chinese Academy of Medicine Sciences in Nanjing, China, received some 700 outpatients a day, of which 88 cases of leprosy were found, from January 1991 to December 1996, including TT/BT 44, BB 15 and BL/LL 29 [58 males and 30 females with a mean age of 39.9 years (9 to 70) and a mean disease duration of 24 months (1 to 100)]. Five had relapsed after being cured with DDS and 47 were smear positive with a mean BI of 3.29, making up 53.4%. On diagnosis, there was type I reaction in 9 (10.2%) and visible disability in 18 (20.5%). Most (39) of them were found in May to July and some (28) were from provinces other than Jiangsu. The authors pointed out that the departments of dermatology in hospitals still are one of the most important places for finding leprosy cases.—Authors' English Abstract

Srinivasan, S., Nehru, V. I., Bapuraj, J. R., Sharma, V. K. and Mann, S. B. S. CT findings in involvement of the paranasal sinuses by lepromatous leprosy. Br. J. Radiol. **72** (1999) 271–273.

The role of nasal infection in the transmission of leprosy has been extensively studied. Leprosy can affect the paranasal sinuses due to mucosal continuity and bacillemia. This prospective study was performed on 25 untreated patients with lepromatous leprosy. Five mm contiguous axial and coronal CT sections of paranasal sinuses, on soft tissue and bone windows, were obtained in all patients. Each sinus was examined for mucosal thickening, soft tissue densities and bony outlines. Representative biopsies were taken from ethmoid sinus to confirm the radiological diagnosis in 12 patients with multiple paranasal sinus involvement. Ethmoid air cells were involved in 20 patients (80%). Maxillary, frontal and sphenoid sinuses showed abnormalities in 12, 4 and 3 patients, respectively. The ethmoid biopsy showed involvement by lepromatous leprosy in 7 of 12 patients (58.3%). Involvement of paranasal sinuses is common in lepromatous leprosy and is of considerable epidemiological significance.—Authors' Abstract

Terencio de las Aguas, J. [Nasal, buccal, pharyngeal, and laryngeal lesions.] Rev. Leprol. Fontilles **21** (1998) 677–688. (in Spanish)

A study was carried out on the specific nasal, buccal, larynx and pharynx lesions. The nasal mucosa as entry site and at the same time site of dissemination of bacilli is discussed. The frequency and extension of these lesions in the pre-sulfone era with important complications, like obstructive laryngitis, is reviewed.—Author's English Summary

Torrelas, A., Solis, R. L., Perez, E., Medina, Y., Kerguelen, C. and Olaya, P. Anti-*M. leprae* IgM antibody determination by ultramicroimmunoenzymatic (UMELISA HANSEN) for the diagnosis and monitoring of leprosy. Rev. Inst. Med. Trop. Sao Paulo **40** (1998) 177–181.

The relationship between the IgM antibody response and antigenic load as well as clinical improvement after drug therapy was studied in 115 leprosy patients from Cuba and Colombia [date not given] in order to obtain useful data for the early diagnosis and monitoring of leprosy. In 82% of cases (94 of 115) agreement was obtained between IgM UMELISA HANSEN and slit-skin smear examination. Discrepant results were observed in 16 patients who showed positive IgM response despite being negative by the skin-smear examination. In these patients, the IgM response was associated with the early signal for bacilli recurrence in the skin. In one of these patients the presence of bacilli was demonstrated in the skin 2 months after IgM antibodies were detected by UMELISA HANSEN. Also in one of the treated patients, positive by both diagnostic techniques, a decrease in the IgM antibody levels was seen, correlating with a significant clinical improvement. A direct relationship was found between the IgM antibody response and bacterial antigenic load, regardless of the time elapsed in the disease's evolution.—Trop. Dis. Bull. **96** (1999) 383

Xu, M., *et al.* [On causes of death for persons affected with leprosy in Taizhou City, Jiangsu.] China Lepr. J. **14** (1998) 237–239. (in Chinese)

In Taizhou City, Jiangsu, China, among 218 registered leprosy patients 108 died from 1958 to 1997. The causes of death were malignant tumor in 37 (24.26%), cerebrovascular accident in 26 (24.04%), cor pulmonale in 16 (14.81%), cardiac infarct in 7 (4.62%), chronic bronchitis in 4 (3.7%), accident in 4 and other in 9 (8.3%); 67.59% of them died at the age of over 60 years.—Authors' English Abstract

Yan, L., *et al.* [A survey of ophthalmopathies in 405 persons affected with leprosy.] China Lepr. J. **14** (1998) 234–235. (in Chinese)

A survey of oculopathies in 405 cases of leprosy with a mean age of 60.2 (17–90) years and disease duration of 3 to 66 years has been conducted in Shanghai, China, including 231 inpatients and 174 outpatients (male 287 and female 122), and PB 163 and MB 242. Vision less than 0.05 is defined as blindness and that of 0.05 to 0.3 as low vision. The result showed that for unilateral and bilateral side, blindness was seen in 161 (39.8%) and 61 (15.1%), lagophthalmos in 286 (70.6%) and 102 (25.1%), corneal macula in 171 (42.2%) and 60 (14.8%), abnormal iris in 208 (50.1%) and 96 (23.7%), and pterygium in 92 (36.8%) and 28 (6.9%). The number of oculopathy in inpatients was far more than in outpatients ($p < 0.01$). The longer the disease duration, the more oculopathy. So, to prevent oculopathy in leprosy, early detection and treatment, education in self-care, and regular examination of the eye for the patients are necessary.—Authors' English Abstract

Immuno-Pathology

Apostolou, I., Takahama, Y., Belmant, C., Kawano, T., Huerre, M., Marchal, G., Cui, J., Taniguchi, M., Nakauchi, H., Fournie, J. J., Kourilsky, P. and Gachelin, G. Murine natural killer cells contribute to the granulomatous reaction caused by mycobacterial cell walls. Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 5141–5146.

Mice injected with deproteinized cell walls prepared from the strain H37Rv of *Mycobacterium tuberculosis* develop a granuloma-like lesion in which NKT cells are predominant. NKT cells play a primary role in the granulomatous response, because the latter does not occur in J alpha 281 (–/–) mice, which lack NKT cells. The glycolipidic fraction of the cell walls is responsible for the recruitment of NKT cells; the recruiting activity is associated with fractions containing phosphatidylinositol mannosides. These results define a powerful experimental set up for studying the *in*

vivo induction of NKT cell responses to microbial components.—Authors' Abstract

Barral Netto, M., Santos, S., Santos, I., van Sohsten, R., Bittencourt, A. L., Carvalho, E. M., Barral, A. and Waters, M. Immunochemotherapy with interferon-gamma and multidrug therapy for multibacillary leprosy. Acta Trop. **72** (1999) 185–201.

Treatment for multibacillary (MB) leprosy is presently performed with a multidrug therapy (MDT) scheme maintained for 2 years. Leprosy treatment however can benefit from the reduction of length. The lack of interferon-gamma (IFN-γ) production by lepromatous leprosy (LL) patients' lymphocytes led us to use this cytokine in the treatment of MB leprosy associated with MDT in the treatment of MB leprosy, and monitor several clinical and immunological parameters during the course of treatment. A total of 20 MB leprosy patients were evaluated, 10 treated with MDT alone, and 10 treated with MDT + 10 daily doses of 2×10^6 international units (IU) of recombinant human IFN-γ/m^2 followed by 10 daily doses of 10^7 IU IFN-γ/m^2, intramuscularly, during the first 20 days of MDT. IFN-γ was well tolerated and did not cause any increase in the rate of leprosy reactions development during treatment. Decrease of bacillary load, fall of anti-*Mycobacterium leprae* IgG serum antibodies, changes of histological pattern, as well as changes in lymphocyte proliferation assay in response to mitogens (PHA or PWM), *M. leprae* antigen or PPD was similar in both groups of patients. Among several soluble immunological markers measured before and 30 days after beginning of treatment, levels of soluble IL-2R receptor increased in patients treated with MDT plus IFN-γ whereas it decreased in patient treated with MDT alone. Soluble ICAM-1 levels decreased in the MDT group but did not change in the MDT + IFN-γ treated patients. Soluble CD4 and soluble CD8 markers did not change significantly in either group of patients. Neopterin, a marker of macrophage activation, increased in all but one patient treated with MDT + IFN-γ but in none treated with MDT alone, indicating that IFN-γ was active *in vivo*. Our findings indicate that despite being able to promote macrophage activation in MB leprosy patients a short course of systemically administered IFN-γ is not able to change the clinical course of a long standing disease such as leprosy.—Authors' Abstract

Deretic, V. and Fratti, R. A. *Mycobacterium tuberculosis* phagosome. Mol. Microbiol. **31** (1999) 1603–1609.

The arrest of *Mycobacterium tuberculosis* phagosome maturation in infected macrophages is a phenomenon of dual significance both for the pathogenesis of tuberculosis and as a model system to study interference of microbes with membrane trafficking and organelle biogenesis in host cells. Among other factors, compartment-specialized regulators of vesicular trafficking and other parts of membrane fusion machinery are likely to play a role in these processes. Here we summarize the emerging view of mycobacterial phagosome maturation arrest in the context of the dynamic processes of intracellular membrane trafficking.—Authors' Summary

Gupta, A., Sharma, V. K., Vohra, H. and Ganguly, N. K. Spontaneous apoptosis in peripheral blood mononuclear cells of leprosy patients: role of cytokines. FEMS Immunol. Med. Microbiol. **24** (1999) 49–55.

Peripheral blood mononuclear cells from leprosy patients underwent spontaneous apoptosis upon culture for 24 hr. The apoptosis was inhibited by anti-TNF-alpha antibodies and to a certain extent by anti-IL-1-alpha and IL-6, thus showing that Th2-type cytokines (mainly TNF-alpha) are responsible for inducing apoptosis. This cytokine-mediated apoptosis could be inhibited by ionomycin and zinc, thereby suggesting that these metal ions can be used to decrease the levels of these inflammatory cytokines in various diseases.—Authors' Abstract

Hussain, R., Dockrell, H. M. and Chiang, T. J. Dominant recognition of a crossreactive B-cell epitope in *Mycobacterium leprae* 10 K antigen by immunoglobulin

G1 antibodies across the disease spectrum in leprosy. Immunology **96** (1999) 620–627.

Mycobacterium leprae-specific immunoglobulin G_1 (IgG_1) antibodies in patients with leprosy show a direct correlation with bacterial load ($\rho = 0.748$; $p < 0.002$) suggesting that IgG_1 B-cell responses may be surrogate markers of disease progression. To investigate if this upregulation was a general feature of IgG_1 responses to all *M. leprae* (ML) antigens, we analyzed responses to several recombinant purified ML heat-shock proteins (HSP). Three recombinant HSPs (ML 10 K, ML 18 K and ML 65 K) were tested for their ability to induce various IgG subclasses in patients with either the lepromatous (LL/BL, N = 26) or tuberculoid form (BT/TT, N = 39) of the disease as well as in healthy household contacts (HC, N = 14) and endemic controls (EC, N = 19). Our major findings were: 1) selective augmentation of IgG_1 antibody responses to ML 10 K; 2) recognition of a restricted number of epitopes across the disease spectrum and healthy controls by IgG_1 antibodies; 3) dominant recognition of crossreactive epitopes which were common to both ML and MT 10 K. This response was not related to contamination with endotoxin. Epitope mapping using 15-mer overlapping peptides spanning the ML 10,000 MW revealed an immunodominant IgG_1 binding peptide (aa41–55) in patients as well as healthy controls. This peptide is a shared epitope with *M. tuberculosis* 10 K, suggesting that postswitched IgG_1 B cells recognizing this epitope rather than naive B cells are being expanded.—Authors' Abstract

Jouanguy, E., Lamhamedi Cherradi, S., Lammas, D., Dorman, S. E., Fondaneche, M. C., Dupuis, S., Doffinger, R., Altare, F., Girdlestone, J., Emile, J. F., Docoulombier, H., Edgar, D., Clarke, J., Oxelius, V. A., *et al.* A human INFGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nature Genet. **21** (1999) 370–378.

The immunogenetic basis of severe infections caused by bacille Calmette-Guerin vaccine and environmental mycobacteria in humans remains largely unknown. We describe 18 patients from several generations of 12 unrelated families who were heterozygous for 1 to 5 overlapping IFNCR1 frameshift small deletions and a wild-type IFNGR1 allele. There were 12 independent mutation events at a single mutation site, defining a small deletion hotspot. Neighboring sequence analysis favors a small deletion model of slipped mispairing events during replication. The mutant alleles encode cell-surface IFN-gamma receptors that lack the intracytoplasmic domain which, through a combination of impaired recycling, abrogated signaling and normal binding to IFN-gamma exert a dominant-negative effect. We thus report a hotspot for human IFNGR1 small deletions that confer dominant susceptibility to infections caused by poorly virulent mycobacteria.—Authors' Abstract

Kamath, A. T., Feng, C. G., Macdonald, M., Briscoe, H. and Britton, W. J. Differential protective efficacy of DNA vaccines expressing secreted proteins of *Mycobacterium tuberculosis.* Infect. Immun. **67** (1999) 1702–1707.

The development of more-effective antituberculosis vaccines would assist in the control of the global problem of infection with *Mycobacterium tuberculosis.* One recently devised vaccination strategy is immunization with DNA plasmids encoding individual microbial genes. Using the genes for the *M. tuberculosis* secreted proteins MPT64 (23 kDa), Ag85B (30 kDa), and ESAT-6 (6 kDa) as candidate antigens, DNA vaccines were prepared and tested for immunogenicity and protective efficacy in a murine model of aerosolized tuberculosis (TB). Intramuscular immunization with DNA-64 or DNA-85B resulted in the activation of CD4+ T cells, which produce gamma interferon (IFN-γ) and high titers of specific immunoglobulin G antibodies. Further, DNA-64 induced major histocompatibility complex class I-restricted CD8+ cytotoxic T cells. The addition of a eukaryotic leader sequence to mpt64 did not significantly increase the T-cell or antibody response. Each of the three DNA vectors stimulated a significant reduction in the

level of *M. tuberculosis* infection in the lungs of mice challenged 4 weeks after immunization, but not to the levels resulting after immunization with *M. bovis* BCG. The vaccines showed a consistent hierarchy of protection, with the most effective being Ag85B followed by ESAT-6 and then MPT64. Co-immunization with the three vectors resulted in a greater degree of protection than that induced by any single vector. This protective efficacy was associated with the emergence of IFN-γ-secreting T cells earlier than in infected animals immunized with a control vector. The efficacy of these DNA vaccines suggests that multisubunit vaccination may contribute to future vaccine strategies against TB.—Authors' Abstract

Oliveira, R. B., Moraes, M. O., Oliveira, E. B., Sarno, E. N., Nery, J. A. C. and Sampaio, E. P. Neutrophils isolated from leprosy patients release TNF-alpha and exhibit accelerated apoptosis *in vitro*. J. Leuk. Biol. **65** (1999) 364–371.

This study demonstrated that polymorphonuclear neutrophils (PMN) participate in the acute inflammatory response in leprosy as effector cells. Lepromatous patients present intense infiltrate of neutrophils in reactional (ENL) lesions. Circulating PMN of nonreactional patients, healthy donors, and reactional patients were purified and analyzed *in vitro*. The study confirmed the short lifespan of these cells in culture with progressive changes characteristic of apoptosis. Apoptosis was greatly accelerated in ENL patients as shown by cellular morphology, later confirmed by qualitative and quantitative analysis of fragmented DNA. It was observed that neutrophils stimulated with lipopolysaccharide, *Mycobacterium leprae,* and lipoarabinomannan secrete interleukin-8 and tumor necrosis factor-alpha (TNF-α). Thalidomide, a drug known to inhibit TNF-α synthesis on monocytes, also exerted an inhibitory effect on TNF-α secretion in neutrophils. These data suggest that PMN can participate in the regulation of the immune response in leprosy and can contribute to the amplification of TNF-α production at the site of ENL lesion.—Authors' Abstract

Pinho, J. R. R., de Andrade, H. R., Jr., and Schenberg, A. C. G. [Different skin tests utilized to follow up leprosy patients.] Hansen. Int. **23** (1998) 49–52. (in Portuguese)

Skin tests are utilized in the follow up of leprosy patients, constituting one of the parameters for the classification of the clinical forms of this disease, its prognosis and even for treatment indication and follow up. These tests have been developed in the first quarter of this century and are based on bacilli suspensions obtained from human lesions or from infected armadillos. In this paper, we review the characteristics of lepromin or Mitsuda antigen and other alternative tests. The potential or recombinant antigens in the preparation of skin tests is also highlighted as a future perspective for the preparation of improved, standardized and more specific reagents, in comparison from those tests obtained from human lesions or armadillos.—Authors' English Summary

Pinho, J. R. R., de Andrade, H. R., Jr. and Schenberg, A. C. G. [Vaccines against leprosy.] Hansen. Int. **23** (1998) 53–58. (in Portuguese)

Only *Mycobacterium bovis* BCG have already been approved for human use as a vaccine against leprosy although the major goal for its use is protection against tuberculosis. Furthermore, a large variability (20%–80%) in its efficacy has been reported. Many groups are studying new vaccines against leprosy in the search of preparations with better reproducibility and efficacy. In this paper, we review the alternative vaccines already studied and the recent new developments (other mycobacteria, inactivated *M. leprae,* recombinant antigens, DNA, and mechanisms to modify the antigenic presentation).—Authors' English Summary

Schon, T., Gebre, N., Sundqvist, T., H/Mariam, H. S., Engeda, T. and Britton, S. Increased levels of nitric oxide metabolites in urine from leprosy patients in reversal reaction. Lepr. Rev. **70** (1999) 52–55.

We measured the metabolites of NO [nitrite (NO_2^-) and nitrate (NO_3^-)] in urine from Ethiopian patients suffering from leprosy. The urinary level of NO_2^-/NO_3^- in a group of healthy Ethiopians was 1020 ± 471 μM (N = 22). Leprosy patients in reversal reaction had significantly higher levels of NO_2^-/NO_3^- (1817 ± 492 μM, $p < 0.001$, N = 12) than both the control group and leprosy patients who were not in reversal reaction (1079 ± 446 μM, N = 12). We conclude that the reversal reaction in leprosy is associated with increased urinary levels of nitric oxide metabolites.—Authors' Summary

Scollard, D. M., McCormick, G. and Allen, J. L. Localization of *Mycobacterium leprae* to endothelial cells of epineurial and perineurial blood vessels and lymphatics. Am. J. Pathol. **154** (1999) 1611–1620.

Infection of peripheral nerve by *Mycobacterium leprae,* the histopathological hallmark of leprosy, is a major factor in this disease, but the route and mechanisms by which bacilli localize to peripheral nerve are unknown. Experimentally infected armadillos have recently been recognized as a model of lepromatous neuritis; the major site of early accumulation of *M. leprae* is epineurial. To determine the epineurial cells involved, 1-cm segments of 44 nerves from armadillos were screened for acid-fast bacilli (AFB) and thin sections were examined ultrastructurally. Of 596 blocks containing nerve, 36% contained AFB. Overall, *M. leprae* were found in endothelial cells in 40% of epineural blood vessels and 75% of lymphatics, and in 25% of vessels intraneurally. Comparison of epineurial and endoneurial findings suggested that colonization of epineurial vessels preceded endoneurial infection. Such colonization of epineural nutrient vessels may greatly increase the risk of endoneurial *M. leprae* bacteremia, and also enhance the risk of ischemia following even mild increases in inflammation or mechanical stress. These findings also raise the possibility that early, specific mechanisms in the localization of *M. leprae* to peripheral nerve may involved adhesion events between *M. leprae* (or *M. leprae*-parasitized macrophages) and the endothelial cells of the vasa nervorum.—Authors' Abstract

Sharma, N., Sharma, V. K., Gupta, A., Kaur, I. and Ganguly, N. K. Immunological defect in leprosy patients: altered T-lymphocyte signals. FEMS Immunol. Med. Microbiol. **23** (1999) 355–362.

The early events of activation were studied in paucibacillary (TT/BT) and multibacillary (BL/LL) leprosy patients by stimulation of their lymphocytes with mitogenic agents (calcium ionophore A23187/PMA) and *Mycobacterium leprae* antigen (PGL-I). Maximum proliferation in response to PMA/A23187 and PGL-I was observed in the BT/TT patients and the control group, respectively. Inositol triphosphate (IP3) and calcium were constitutively elevated in BT/TT and LL/BL patients. PMA/A23187 caused an increase in both IP3 and [Ca2+] (i) in BT/TT patients and controls. PGL-I marginally increased IP3 levels in BT/TT patients. In the LL/BL patients, although PMA/A23187 increased IP3 levels but no change was seen in [Ca2+] (i), PGL-I had no effect. Protein kinase C levels were seen to be associated with particulate fractions in BT/TT patients and were found to increase further in response to PMA/A23187. PGL-I did not increase translocation of protein kinase C in controls or LL/BL patients. A preactivated and sensitized slate of T-lymphocytes was observed in BT/TT patients responsive to antigen and mitogens; whereas the cells of LL/BL patients were unresponsive to PGL-I. The altered signal transduction events characterized in the MB patients thus correlate well with the anergic state of their cells.—Authors' Abstract

Sreenivasan, P., Misra, R. S., Wilfred, D. and Nath, I. Lepromatous leprosy patients show T helper 1-like cytokine profile with differential expression interleukin-10 during type 1 and 2 reactions. Immunology **95** (1998) 529–536.

To further understand the development of Th-like responses during leprosy, 32 lepromatous patients undergoing reactions were recruited from the Hansen's disease

clinics of the Leprosy Mission Hospital, Shahadra, and Safdarjung Hospital, New Delhi, India [date not given]. Cytokine-specific RT-PCR and ELISA in peripheral blood and some skin biopsies were performed. Of interest was the evidence of a Th1-like response with the presence of interferon-gamma (IFN-γ) and the absence of interleukin-4 (IL-4) mRNA in the peripheral blood mononuclear cells (PBMC) of 85 and 64% of type 1 and 2 reaction patients, respectively, and in all reaction sites. Whereas a Th0-like response was seen in some, a Th2-like response was absent. IL-12p40 mRNA was seen in 21/25 erythema nodosum leprosum (ENL) and all type 1 reaction subjects irrespective of the Th phenotype. IL-12p40 and IFN-γ were detectable in unstimulated PBMC, suggesting an *in vivo* priming during reactions. IL-10 was mainly associated with adherent cells and showed a differential expression in the two reactions. It was present in the PBMC of ENL but not in reversal reaction patients. Moreover, it was not detectable in the skin lesions of either type of reactions. A Th1-like cytokine profile was associated with immunopathology and persisted up to 6–7 months after the onset of reactions.—Trop. Dis. Bull. **96** (1999) 495

Wang, J., Wakeham, J., Harkness, R. and Xing, Z. Macrophages are a significant source of type 1 cytokines during mycobacterial infection. J. Clin. Invest. **103** (1999) 1023–1029.

T-helper 1 (Th1) cells are believed to be the major producer of the type 1 cytokine interferon-gamma (IFN-γ) in cell-mediated immunity against intracellular infection. We have investigated the ability of macrophages to release type 1 cytokines and their regulatory mechanisms using both *in vivo* and *in vitro* models of pulmonary mycobacterial infection. During pulmonary infection by live *Mycobacterium bovis* bacilli Calmette-Guerin (BCG) in wild-type mice, lung macrophages released interleukin-12 (IL-12), IFN-γ, and tumor necrosis factor-alpha (TNF-α), and expressed surface activation markers. However, macrophages in infected IL-12 (–/–) mice released TNF-α but not IFN-γ and lacked surface activation makers. In freshly isolated lung macrophages from naive IL-2 (–/–) mice, mycobacteria alone released TNF-α but not IFN-γ, whereas exogenously added IL-12 alone released a minimum of IFN-γ. However, these macrophages released large quantities of IFN-γ upon stimulation with both mycobacteria and IL-12. In contrast, mycobacteria and exogenous IFN-γ released only a minimum of endogenous IFN-γ. Endogenous IL-18 (IFN-γ-inducing factor) played little role in IFN-γ responses by macrophages stimulated by mycobacteria and IL-12. Our data reveal that macrophages are a significant source of type 1 cytokines during mycobacterial infection and that both IL-12 and intracellular pathogens are required for the release of IFN-γ but not TNF-α. These findings suggest that macrophages regulate cell-mediated immunity by releasing not only IL-12 and TNF-α but also IFN-γ and that full activation of IFN-γ response in macrophages is tightly regulated.—Authors' Abstract

Weir, R. E., Butlin, C. R., Neupane, K. D., Failbus, S. S. and Dockrell, H. M. Use of a whole blood assay to monitor the immune response to mycobacterial antigens in leprosy patients: a predictor for type 1 reaction onset? Lepr. Rev. **69** (1998) 279–293.

Whole blood assays were used to assess the natural variation in the "normal" T-cell response to mycobacterial antigens in healthy U.K. donors and healthy Nepali donors, tested over 6 months. This was compared with variation in T-cell responses measured over 6 months in 22 leprosy patients In Nepal, including 8 who developed type 1 reactions during this time. The *in vitro* T-cell response to *Mycobacterium leprae* sonicate (MLS), *M. tuberculosis* PPD, the mitogen PHA and (in the U.K. study) recombinant mycobacterial antigens (70-kDa and 30/31-kDa proteins) was measured by lymphoproliferation and interferon-gamma (IFN-γ) responses, and variation in responses over time in each subject calculated as a coefficient of variation (CV). The baseline high, low or nonresponder status of the healthy U.K. donors remained stable. The magnitude of IFN-γ responses varied by mean CV ranging from 26% (to PPD) to 63% (to Mtb 70 kDa); proliferation re-

sponses showed less variation, ranging from mean CV of 18% (to PHA) to 47% (to Mtb 70 kDa). Response variation was independent of lymphocyte number in culture. Similar variation in lymphoproliferation responses to MLS, PPD and PHA was observed in the group of healthy Nepali subjects, and in Nepali leprosy patients who did not experience reactions during the study. Of the 8 leprosy patients who developed type 1 reactions, 4 showed significantly increased proliferation to MLS at the time of reaction (74%–300% above baseline); 4 remained low or nonresponders to MLS throughout. An alternative marker of immune response [anti-phenolic glycolipid-I (PGL-I) antibody titer] was not predictive of reaction onset in these patients. It is concluded that whole blood assays provide reproducible *in vitro* measurements that can be used to monitor changes in T-cell responses to *M. leprae* antigens; their practical use as a diagnostic marker of type 1 reaction onset is discussed.—Trop. Dis. Bull. **96** (1999) 159

Wilkinson, R. J., Wilkinson, K. A., Jurcevic, S., Hills, A., Sinha, S., Sengupta, U., Lockwood, D. N. J., Katoch, K., Altman, D. and Ivanyi, J. Specificity and function of immunogenic peptides from the 35-kDa proteins of *Mycobacterium leprae*. Infect. Immun. **67** (1999) 1501–1504.

We identified a T-cell determinant of the 35-kDa antigen of *Mycobacterium leprae* which is discriminatory against cross-sensitization by its closely related homolog in *M. avium*. From synthetic peptides covering the entire sequence, those with the highest affinity and permissive binding to purified HLA-DR molecules were evaluated for the stimulation of proliferation of peripheral blood mononuclear cells (PBMCs) from leprosy patients and healthy sensitized controls. Responses to the peptide pair 206–224, differing by four residues between *M. leprae* and *M. avium*, involved both species-specific and crossreactive T cells. Lymph node cell proliferation in HLA-DRB1*01 transgenic mice was reciprocally species specific, but only the response to the *M. leprae* peptide in the context of DR1 was immunodominant. Of the cytokines in human PBMC cultures, gamma interferon production was negligible, while interleukin 10 (IL-10) responses in both patients and controls were more pronounced. IL-10 was most frequently induced by the shared 241–255 peptide, indicating that environmental cross-sensitization may skew the response toward a potentially pathogenic cytokine phenotype.—Authors' Abstract

Microbiology

Christensen, H., Gargon, N. J., Horobin, R. W., Minnikin, D. E. and Barer, M. R. Lipid domains of mycobacteria studied with fluorescent molecular probes. Mol. Microbiol. **31** (1999) 1561–1572.

The complex mycobacterial cell envelope is recognized as a critical factor in our failure to control tuberculosis, leprosy and other nontuberculous pathogens. Although its composition has been extensively determined, many details regarding the organization of the envelope remain uncertain. This is particularly so for the noncovalently bound lipids, whose natural distribution may be disrupted by conventional biochemical or cytological techniques. In order to study the native organization of lipid domains in the mycobacterial envelope, we have applied a range of fluorescent lipophilic probes to live mycobacteria, including *Mycobacterium smegmatis, M. tuberculosis, M. avium, M. gadium* and *M. aurum,* and analyzed the resultant signals by fluorescence microscopy and digital image processing. Five key features were observed: a) the presence of both envelope and intracellular lipid domains; b) differen-

tial localization of probes into these domains influenced predominantly by their hydrophobicity, as modeled by their calculated octonal : water partition coefficients and by their amphiphilicities; c) uneven distribution of lipophilic material in the envelope; d) selective labeling of septal regions of the envelope; and e) modification of labeling patterns by additional treatments such as fluorescence quenching antibodies, detergents and solvents. Using this last approach, a coherent cell envelope lipid domain was demonstrated outside the cytoplasmic membrane and, for the first time, the proposed covalently linked mycolyl-arabinogalactan-peptidoglycan macromolecular complex was imaged directly. The use of fluorescent probes and high-resolution fluorescence microscopy has enabled us to obtain a coherent view of distinct lipid domains in mycobacteria. Further application of this approach will facilitate understanding of the role of lipids in the physiology of these organisms.—Authors' Abstract

Dhople, A. M. Factors influencing the *in vitro* growth of *Mycobacterium leprae*: effect of inoculum. Microbios **94** (1998) 103–113.

Factors responsible for the *in vitro* growth of *Mycobacterium leprae* in Dhople-Hanks (DH) medium, and also to improve the technique devised earlier, and the source of the *M. leprae* used as inoculum, were investigated. *M. leprae* were obtained from armadillos and nude mice, both inoculated earlier with human- or armadillo-derived *M. leprae*. The growth of *M. leprae* in DH medium was monitored using two biochemical indicators. Normal growth was obtained when inocula were from livers and spleens of *M. leprae*-infected armadillos. The *M. leprae* harvested from the foot pads of nude mice failed to multiply in the same medium. Using inocula from livers and spleens of infected armadillos, a gradual decrease in inoculum size resulted in a proportionately slower multiplication of *M. leprae*. When the DH medium was supplemented with whole *M. leprae,* or cell-free extracts of *M. leprae,* from irradiated livers and spleens of infected armadillos, nude mouse-derived *M. leprae* exhibited growth in the DH medium in accord with that obtained using armadillo-derived *M. leprae*. Similar results were obtained with cell-free extracts of *M. leprae* harvested from non-irradiated livers and spleens of infected armadillos, but no growth was obtained when the medium was supplemented with extracts from livers or spleens of normal armadillos. These results indicate the possible existence of a growth factor in armadillo-derived *M. leprae*.—Trop. Dis. Bull. **96** (1999) 57

Frothingham, R. Evolutionary bottlenecks in the agents of tuberculosis, leprosy, and paratuberculosis. Med. Hypotheses **52** (1999) 95–99.

Parasitic mycobacteria cause important human and animal diseases including tuberculosis, leprosy, and paratuberculosis. Several methods demonstrate a high degree of sequence conservation in three parasitic mycobacterial species (*Mycobacterium tuberculosis, M. leprae,* and *M. avium* subspecies *paratuberculosis*). Each of these species has a completely conserved deoxyribonucleic acid (DNA) sequence in an internal transcribed spacer. In contrast, several species of environmental mycobacteria (*M. intracellulare, M. kansasii, M. gordonae,* and *M. scrofulaceum*) have substantial strain-to-strain variation in this region. These data suggest that each of the parasitic species has gone through a recent evolutionary bottleneck. Comparisons of tandem-repeat DNA from ancient and modern mycobacterial strains may allow this hypothesis to be tested directly.—Author's Abstract

Gupta, S., Jain, S. and Tyagi, A. K. Analysis, expression and prevalence of the *Mycobacterium tuberculosis* homolog of bacterial virulence regulating proteins. FEMS Microbiol. Lett. **172** (1999) 137–143.

We have previously reported the identification of a gene from *Mycobacterium tuberculosis,* H37Rv, which on the basis of its nucleotide sequence encoded a protein product of 38 kDa. This 38-kDa mycobacterial protein designated as VirS exhibits homology with the VirF protein of *Shigella,* the VirFy protein of *Yersinia* and the Cfad,

Rns and FapR proteins from various enterotoxigenic *Escherichia coli* strains. In this communication, we show the close sequence and structural similarities of the VirS protein with VirF, VirFy, Cfad, Rns and FapR and describe the results of our studies on the characterization of the virS gene promoter and its expression in *E. coli* and mycobacteria. VirS was present exclusively in the species belonging to the *M. tuberculosis* complex as revealed by Southern blot and PCR analysis. Our findings suggest the involvement of virS in the regulation of pathogenesis of *M. tuberculosis*.—Authors' Abstract

Iivanainen, E., Martikainen, P., Vaananen, P. and Katila, M. L. Environmental factors affecting the occurrence of mycobacteria in brook sediments. J. Appl. Microbiol. **86** (1999) 673–681.

The occurrence of mycobacteria was studied in aerobic brook sediments from 39 drainage areas in Finland. The culturable counts of mycobacteria were related to climatic conditions, characteristic of the drainage area, chemical characteristics of the sediment and water, culturable counts of other heterotrophic bacteria, and microbial respiration rate in the sediment. The counts of mycobacteria varied from 1.1×10^2 to 15×10^4 cfu g^{-1} dry weight of sediment. They correlated positively with the proportion of the drainage area consisting of peatland, total content of C, content of Pb, microbial respiration rate in the sediment, and chemical oxygen demand of the water. The correlations of the mycobacterial counts with pH of sediment and alkalinity of water were negative. The results of the present sediment study and of the forest soil study published earlier strongly suggest that an increase in acidity increases the counts of mycobacteria and decreases the counts and activity of other heterotrophic bacteria. Mycobacterial counts were more than 100 times higher (per dry weight) in forest soils with pH 3.5–4.3 than in sediments with pH 4.5–6.3.—Authors' Abstract

Jackson, M., Raynaud, C., Laneelle, M. A., Guilhot, C., Laurent Winter, C., Ensergueix, D., Gicquel, B. and Daffe, M. Inactivation of the antigen 85C gene profoundly affects the mycolate content and alters the permeability of the *Mycobacterium tuberculosis* cell envelope. Mol. Microbiol. **31** (1999) 1573–1587.

The antigen 85 complex of *Mycobacterium tuberculosis* consists of three abundantly secreted proteins. The recent characterization of a mycoloyltransferase activity associated *in vitro* with each of these antigens suggested that they are potentially important for the building of the unusual cell envelope of mycobacteria. To define the physiological role of these proteins, the gene coding for antigen 85C was inactivated by transposon mutagenesis. The resulting mutant was shown to transfer 40% fewer mycolates to the cell wall with no change in the types of mycolates esterifying arabinogalactan or in the composition of non-covalently linked mycolates. As a consequence, the diffusion of the hydrophobic chenodeoxycholate and the hydrophilic glycerol, but not that of isoniazid, was found to be much faster through the cell envelope of the mutant than that of the parent strain. Taken together, these data demonstrate that: a) antigen 85C is involved directly or indirectly in the transfer of mycolates onto the cell wall of the whole bacterium; b) the enzyme is not specific for a given type of mycolate; and c) the cell wall-linked mycolate layer may represent a barrier for the diffusion of small hydrophobic and hydrophilic molecules.—Authors' Abstract

Marques, M. A. M., Chitale, S., Brennan, P. J. and Pessolani, M. C. V. Mapping and identification of the major cell wall-associated components of *Mycobacterium leprae*. Infect. Immun. **66** (1998) 2625–2631.

Mycobacterium leprae was fractionated into its major subcellular components, cell wall, cytoplasmic membrane and soluble cytosol. A number of biochemical markers, including diaminopimelic acid content, monosaccharide composition, mycolic acid and glycolipid distribution, were applied to their characterization, and 2-dimensional gel electrophoresis was used to map the component proteins. A total of 391 major

protein spots were resolved, and 8 proteins were identified based on their reactivity to a panel of monoclonal antibodies and/or relative pI size. Microsequencing of 6 protein spots present in the cell-wall fraction allowed identification of new proteins, including the protein elongation factor EF-Tu and a homolog for the *M. tuberculosis* MtrA response regulator.—Trop. Dis. Bull. **96** (1999) 56

Masso, F., Paez, A., Varela, E., de Leon, L. D., Zenteno, E. and Montano, L. F. Collagen degrading activity associated with *Mycobacterium* species. Thorax **54** (1999) 439–441.

Background. The mechanism of *Mycobacterium tuberculosis* penetration into tissues is poorly understood but it is reasonable to assume that there is a contribution from proteases capable of disrupting the extracellular matrix of the pulmonary epithelium and the blood vessels. A study was undertaken to identify and characterize collagen degrading activity of *M. tuberculosis.*

Methods. Culture filtrate protein extract (CFPE) was obtained from reference mycobacterial strains and mycobacteria isolated from patients with tuberculosis. The collagen degrading activity of CFPE was determined according to the method of Johnson-Wint using H-3-type I collagen. The enzyme was identified by the Birkedal-Hansen and Taylor method and its molecular mass determined by SDS-PAGE and Sephacryl S-300 gel filtration chromatography using an electroelution purified enzyme.

Results. CFPE from *M. tuberculosis* strain H37Rv showed collagenolytic activity that was four times higher than that of the avirulent strain H37Ra. The 75-kDa enzyme responsible was divalent cation dependent. Other mycobacterial species and those isolated from patients with tuberculosis also had collagen degrading activity.

Conclusions. *Mycobacterium* species possess a metalloprotease with collagen degrading activity. The highest enzymatic activity was found in the virulent reference strain H37Rv.—Authors' Abstract

Nakamura, M. and Matsuoka, M. [Morphological features to be considered as the growth of *Mycobacterium leprae* Thai-53 strain on a silicon coated slide in a cell-free liquid medium.] Jpn. J. Lepr. **67** (1998) 287–291. (in Japanese)

This paper describes the morphological features of *M. leprae* Thai-53 strain smeared on a silicon-coated slide, cultured in Kirchner liquid medium at pH 7.0, and enriched with adenosine, egg yolk, folinic acid, vitamin K_3, lecithin, and N-acetylglucosamine at 30°C. Demonstrated were exquisite morphological growth patterns and an increase in the amount of template DNA prepared from the cultured cells, indicating that *M. leprae* was capable of multiplication under cell-free *in vitro* conditions. However, the ATP content did not increase in parallel with morphological features and the increase in the DNA, presumably because the multiplication of *M. leprae* in this culture system was supported only by consuming the energy derived from the infected host cells.—Trop. Dis. Bull. **96** (1999)

Roberts, M. M., Coker, A. R., Fossati, C., Mascagni, P., Coates, A. R. M. and Wood, S. P. Crystallization, X-ray diffraction and preliminary structure analysis of *Mycobacterium tuberculosis* chaperonin 10. Acta Crystallogr. D **55** Part 4 (1999) 910–914.

Mycobacterium tuberculosis chaperonin 10 (Mtcpn10) has been crystallized by the sitting-drop vapor-diffusion method. The crystals belong to the monoclinic space group P2 (1), with unit-cell parameters a = 76.5, b = 87.9, c = 124.4 Å, beta = 106.8°. X-ray diffraction data were collected to 2.8 Å. The self-rotation function and the molecular-replacement solution show that the asymmetric unit contains a dimer of heptamers related by twofold non-crystallographic symmetry. The two heptamers interact through interleaving flexible loops in a similar fashion to *M. leprae* and Gp31 cpn10. In addition to its role in protein folding, Mtcpn10 has unique effects on the growth of host cells and is a major immunogen in tuberculosis infections. The structure determination will permit the analysis of the amino acids identified as important for the protein-folding and cell-sig-

naling activity of Mtcpn10.—Authors' Abstract

Shen, J., *et al.* [Impact of long-distance transportation of leprosy specimens on the vitality of *M. leprae.*] China Lepr. J. **14** (1998) 220–222. (in Chinese)

Twenty-five biopsy samples taken from untreated leprosy patients were preserved in small glass bottles with normal saline and kept at 0°C in crushed ice. The samples could withstand a transport duration of as long as 72 hr from field to central laboratory without reduction of the viability of *M. leprae.* The authors consider this transport method of the samples to be cheap and practical in leprosy research.—Authors' English Abstract

Yin, Y., *et al.* [On crossantigenicity of complex 85 of *M. leprae* and *M. tuberculosis.*] China Lepr. J. **14** (1998) 217–219. (in Chinese)

The crossreaction antigenicities of *M. leprae* 85 complex A and B with *M. tuberculosis* 85 complex were studied. The results showed that the *M. leprae* 85 complex B has higher specificity than the complex A which showed a crossreaction with the 85 complex of *M. tuberculosis.*—Authors' English Abstract

Zaborina, O., Li, X., Cheng, G., Kapatral, V. and Chakrabarty, A. M. Secretion of ATP-utilizing enzymes, nucleoside diphosphate kinase and ATPase, by *Mycobacterium bovis* BCG: sequestration of ATP from macrophage P2Z receptors? Mol. Microbiol. **31** (1999) 1333–1343.

Mycobacterium bovis BCG secretes two ATP-scavenging enzymes, nucleoside diphosphate kinase (Ndk) and ATPase, during growth in Middlebrook 7H9 medium. In synthetic Sauton medium without any protein supplements, there is less secretion of these two enzymes unless proteins such as bovine serum albumin (BSA), ovalbumin or extracts of macrophages are added to the medium. There is a gradient of activity among various proteins in triggering the induction of secretion of these two enzymes. Other mycobacteria, such as *M. smegmatis,* primarily secrete Ndk, while *M. chelonae* does not appear to secrete either of these two enzymes. Purification of the enzymes from the culture filtrate of 7H9-grown *M. bovis* BCG cells and determination of the N-terminal amino-acid sequence have demonstrated a high level of sequence identity of one of the ATPases with DnaK, a heat-shock chaperone, of *M. tuberculosis* and *M. leprae,* while that of Ndk shows significant identity with the Ndk of *Myxococcus xanthus.* As both Ndk and ATPase use ATP as a substrate, the physiological significance of the secretion of these two ATP-utilizing enzymes was explored. External ATP is important in the activation of macrophage surface-associated P2Z receptors, whose activation has been postulated to allow phagosome-lysosome fusion and macrophage cell death. We demonstrate that the presence of the filtrate containing these enzymes prevents ATP-induced macrophage cell death, as measured by the release of an intracellular enzyme, lactate dehydrogenase. *In vitro* complexation studies with purified Ndk/ATPase and hyperproduced P2Z receptor protein will demonstrate whether these enzymes may be used by mycobacteria to sequester ATP from the macrophage P2Z receptors, thereby preventing phagosome-lysosome fusion or macrophage apoptotic death.—Authors' Abstract

Epidemiology and Prevention

Brasil, M. T. L. R. F., de Oliveira, L. R., de Melo, C. S., Nakamura, P. M., Rimoli, N. S., Cavalari, F. S., Oliveira, M. B., Gattas, B. L., Goncalves, O. S. J. and Rotta, O. [Using ELISA anti-PGL-I test on an urban community with high leprosy endemicity in northern Sao Paulo state.] Hansen. Int. **23** (1998) 35–48. (in Portuguese)

Serum tests for the diagnosis of leprosy using the phenol-glycolipid-I (PGL-I) antigen had opened several possibilities to the study of the epidemiological behavior of this disease. This research aimed at the evaluation of the results of using ELISA anti PGL-I on an urban community with high leprosy endemicity in the state of São Paulo, Brazil. It presented, at the research time, detection and prevalence rates (27.1 and 167.2 cases per 10,000 inhabitants, respectively). A census revealed 8491 persons living in the urban area and 6666 of them were tested with the ELISA anti PGL-I. The serology was positive in 9.0% of the general population, 10.1% for the female populations and 7.6% for the male population. Positive serology observed among women was higher in almost all age levels, except for those 50 years old or more. Among the registered patients the serum positive rate reached 22.6%. The high rate of clinically normal persons testing positive may be indicative of subclinical infection. Some unsolved questions remain, such as: which of these persons will present the disease and when. It also remains to be evaluated how this serology will perform in communities in medium-to-low endemic areas. Low sensitivity of the test does not commend its general adoption as a diagnosis method in routine control program activities. Nevertheless, it is considered important not to discard it as a research object but to improve its sensitivity, specificity and lower its cost. Thus, another instrument for the control and elimination of leprosy could be established.—Authors' English Summary

Dan, Z., *et al.* [Analysis of 512 children having leprosy.] China Lepr. J. **14** (1998) 214–217. (in Chinese)

In Hunan Province, China, 512 child cases of leprosy have been detected during the period 1950 to 1995, including 470 (91.8%) before 1985 and 42 (8.2%) after 1985. Among them, 66.0% had disease duration of less than 2 years, and 59.6% were infected within their houses, of which the main infection source was untreated multibacillary (MB) cases. The first skin lesions mainly were erythema in 43.7% of MB and in 38.1% of paucibacillary (PB) cases, being mostly the face in MB (42.2%) and on the upper limbs in PB (30.7%). Grade II and III disability rates were 28.9%, 73.1% of those who were going to school were ordered to quit school after being diagnosed with leprosy. After 1985 most of them were detected during examination of contacts, with shortened disease duration and fewer disabilities.—Authors' English Abstract

Du, X., *et al.* [Disability of persons who have and had leprosy in Xinyi City, Jiangsu.] China Lepr. J. **14** (1998) 245–246. (in Chinese)

In Xinyi County, Jiangsu, China, there were accumulatively 972 registered cases of leprosy, of which 440 have been cured and were living, and 7 patients with active leprosy were under MDT by the end of 1995. The authors examined 444 persons out of those, including 336 men and 108 women, and found 318 (71.62%) among them to have disability, of which 282 have Grade II and III disability. Out of a total of 478 disabled sites, 437 (91.4%) had had the disability before they were diagnosed with leprosy and given treatment, showing fully the importance of early antileprosy treatment for the prevention of the disability.—Authors' English Abstract

Taylor, J. P., Vitek, I., Enriquez, V. and Smedley, J. W. A continuing focus of Hansen's disease in Texas. Am. J. Trop. Med. Hyg. **60** (1999) 449–452.

To describe epidemiologic and clinical characteristics of Hansen's disease cases in Texas, U.S.A., information was abstracted from records of 810 patients reported from 1973 through 1997. Annually, from 18 to 54 patients were reported. Average annual incidence rates ranged from 1.9 to 2.4 cases per million population. A majority of the patients were male (63%) and white (77%). More than half (53%) of the patients were born in the United States; a majority (83%) of the patients born in the United States were born in Texas. Most (76%) patients were diagnosed with multibacillary leprosy.

Foreign-born patients were more likely to be younger at onset and have multibacillary disease compared with patients born in the United States. Within Texas, an endemic focus of Hansen's disease exists along the Gulf of Mexico coast.—Authors' Abstract

World Health Organization. Elimination of leprosy as a public health problem (update). Wkly. Epidemiol. Rec. **73** (1998) 308–312.

This paper defines elimination and describes the current epidemiological situation regarding leprosy. Leprosy is considered to be a public health problem in 32 countries (population >1 million and prevalence rate >1 case per 10,000 population). A total of 804,396 leprosy cases were under treatment in the world at the beginning of 1998, almost all registered cases being treated with multidrug therapy (MDT). In 1997, approximately 693,000 cases were detected, as notified by 121 countries. Of these, approximately 661,000 cases (95%) were detected in 16 major endemic countries, 76% of the new cases living in India. Globally, during 1985–1997 the rate of leprosy prevalence was reduced by 85%, but the detection rate by only 3%. Data tables are presented showing the following: prevalence and detection of leprosy by WHO region and percentage change in prevalence between 1997 and 1998; registered cases of leprosy and coverage with MDT by WHO region in 1998; prevalence of leprosy, MDT coverage and detection rates in the top 16 endemic countries; and leprosy trends in 32 endemic countries combined during 1985–1997.—Trop. Dis. Bull. **96** (1999)

Wu, P., *et al.* [MDT has sped the process of basically eradicating leprosy in Guangxi Autonomic Region.] China Lepr. J. **14** (1998) 243–245. (in Chinese)

In Guangxi Region of China with a population of 15,300,000, from 1956 to 1995 there were 26,575 registered cases of leprosy in all, of which 22,121 cases have been cured and there were only 144 active cases under MDT by the end of 1995. MDT has been adopted since 1986 when the time estimated for reaching basic eradication of leprosy (the prevalence <0.1‰ and the incidence <0.5/100,000 by the county) will need 20 to 39 years if continuing to use DDS monotherapy, but because of using WHO's MDT, the goal was achieved by 1996.—Authors' English Abstract

Xie, C. [On benefit of leprosy control network.] China Lepr. J. **14** (1998) 242–243. (in Chinese)

In Huozhou City, Guangxi, China, with an area of 5174.2 km^2, 2839 villages and a population of 840,355, there accumulatively were 410 registered cases of leprosy, of which 363 have been cured and only 2 active cases were under MDT by the end of 1996. Since 1969, leprosy control has been made with a three-step network consisting of county-town-village medical workers, including full- and part-time ones. The trained part-time medical workers in the town and village were responsible for almost all of the tasks in leprosy control, and full-time specific doctors at the county level were mainly responsible for operational and technical guidance. Because the former are close to the masses, their expense for service to the people who have or had leprosy was equal only to 1/32.78 of the cost in the use of specific workers for the same condition.—Authors' English Abstract

Yan, X., *et al.* [Effects of a SAPEL in Qiubei County, Yunnan.] China Lepr. J. **14** (1998) 230–233. (in Chinese)

A SAPEL was completed in two towns of Qiubei County, Yunnan, China, from November 1997 to January 1998. In both the towns of Tianxing and Guanzhai (with a population of 37,012 and 19,916, respectively), there had been only 26 new cases found from 1992 to 1996. During the SAPEL, 58 new cases without any visible disability were detected, of which 18 were children below 15 years old, and the detection rates were 56/100,000 and 109/100,000. The authors point out that for the SAPEL the leadership of the government was a decisive factor.—Authors' English Abstract

Rehabilitation

de Oliveira M. H. P. and Romanelli, G. [The effects of leprosy on men and women: a gender study.] C. Salude Publica **14** (1998) 51–60. (in Portuguese)

On the basis of social representations, gender differences were detected in family and individual living experiences for people with Hansen's disease (HD, or leprosy) in a sample of 202 HD patients (132 men and 70 women) enrolled in a public treatment and control program in Ribeirão Preto, São Paulo state, Brazil, during 1994–1995. Information was gathered in 2 stages, First, the HD patients responded to a structured questionnaire, and second, reports were given by 10 men and 10 women on their daily living situations. Data indicated that the disease received different representations by men as compared to women in the different social groups. HD was found to be a source of gender imbalance, further aggravating existing sociocultural inequalities and creating new sources of biopsychosocial and economic harm, in addition to the stigma weighing on individual patients. It is suggested that the results should help reorient treatment and control programs aimed at the social rehabilitation of HD patients besides serving as a paradigm for future research.—Trop. Dis. Bull. **96** (1999) 257

Guo, Z., *et al.* [Socio-economic rehabilitation for leprosy patients.] China Lepr. J. **14** (1998) 236–237. (in Chinese)

Jinju Welfare Settlement in Dongguan city, Guangdong, China, possessing 3.000 mu of land and over 30,000 m^2 of buildings, is receiving 336 persons cured of leprosy, including 222 men and 114 women with a mean age of 61 years, of which only 6 are in the work force. They all have no family. Under support of preferential policy the government gave and guidance of agricultural experts, development of farming and breeding with higher techniques have made their life rich and the surrounding graceful as a community. The authors pointed out that social and economical rehabilitation might lighten their financial burden and allow society, family and residents to benefit.—Authors' English Abstract

Kazen, R. O. Management of plantar ulcers in leprosy. Lepr. Rev. **70** (1999) 63–69.

The aim of this article is to promote and encourage ulcer care at the lowest possible level, as near to the patient's home as possible, and to stimulate paramedical workers and physicians in peripheral units to take responsibility for such treatment.

Traditionally surgeons, often working full time in leprosy institutions, in vertical leprosy programs have given ulcer care. As integration proceeds, focusing on multidrug therapy, the priority for care after cure in programs gets a lower profile. There is then an increasing need for empowering patients with the responsibility for their own ulcer prevention, and empowering peripheral health units for basic surgical treatment of ulcers.

The focus of this article is on basic care of foot ulcers in leprosy. The principles and methods presented aim at solving the problems of ulcer formation by the simplest possible procedures, which can be easily taught to medical staff at different levels. The details of surgery are presented in a subsequent article.—Author's Introduction

Liao, Q., *et al.* [Personality analysis of leprosy patients.] China Lepr. J. **14** (1998) 222–226. (in Chinese)

A matched examination of 550 leprosy patients and 434 persons cured of leprosy, who were randomly selected, with EPQ personality questionnaire showed that the score of P and N all were higher in the PAL than in healthy controls, in men than in women, and in disabled PALs than in nondisabled ones. Their personality structure was analyzed in terms of the obtained data.—Authors' English Abstract

Oliver, M. Theories of disability in health practice and research. Lepr. Rev. **70** (1999) 3–9.

Implicit and explicit social theorizing, coupled with disabled people's insistence that their voices be heard, have begun to change the understanding of the nature of

impairment and disability. The new understandings pose key questions for health care and research if they are going to provide an appropriate knowledge base for both medical and social progress: What is the proper balance for investment between research into bodily impairment and into social disability? Who should be setting the research agenda? Who should be in control of the research process? What are the most appropriate methods for undertaking disability research? How should disability research be disseminated and evaluated? Such questions help us to identify both the common ground and fundamental differences between researches.—Author's Conclusion

Shi, Z., *et al.* [Needs of leprosy patients for health education.] China Lepr. J. **14** (1998) 250–251. (in Chinese)

Demand for health education in 1500 persons living in leprosaria with an age of 14 to 75 years, including 884 PB and 616 MB, was surveyed, showing that some 53.3% to 86.7% of them could obtain proper information through health education and believed them to be true and were willing to take action as they were told, but what they would most like to hear are stories of persons who had been cured of leprosy and who have returned to the community successfully.—Authors' English Abstract

Zhao, X., *et al.* [Situation of 365 persons cured of leprosy who still live in the colonies.] China Lepr. J. **14** (1998) 251–252. (in Chinese)

Among 365 persons cured of leprosy living in 20 colonies in various areas of Shandong Province, China (331 men and 34 women with a mean age of 67 years), only 19 have married (5.21%) and 346 had visible disability (94.8%), including 160 (25.24%) of the eyes, 225 of the hands (35.49%) and 249 of the feet (39.27%). The time when they lived in the colonies was 1 to 37 years, and the causes why they did not return home were that there was no home for them in 144 (39.45%), they were not willing in 148 (40.55%), their family refused reception in 48 (13.15%), and the villagers did not receive them in 25 (6.85%). The authors think that the medical and living conditions for them are in urgent need of betterment.—Authors' English Abstract

Zhao, Z. [Treatment of plantar ulcers with iso-tretinoin and nifepine plus dilantin.] China Lepr. J. **14** (1998) 251–253. (in Chinese)

Thirty-two plantar ulcers in 12 residents of a leprosy colony of Cixi City, Zhejiang, China, were treated by using oral isotretinoin and nifepidine 10 mg three times a day for 4 weeks and then two times a day for 4 weeks each, and topical dilantin powder per day or 2 days after debridement; 20 ulcers in 15 cases have healed and the others have all improved. During treatment the persons did not need to rest in bed and were allowed to walk as usual.—Author's English Abstract

Other Mycobacterial Diseases and Related Entities

Aronson, T., Holtzman, A., Glover, N., Boian, M., Froman, S., Berlin, O. G. W., Hill, H. and Stelma, G. Comparison of large restriction fragments of *Mycobacterium avium* isolates recovered from AIDS and non-AIDS patients with those of isolates from potable water. J. Clin. Microbiol. **37** (1999) 1008–1012.

We examined potable water in Los Angeles, California, U.S.A., as a possible source of infection in AIDS and non-AIDS patients. Nontuberculous mycobacteria were recovered from 12 (92%) of 13 reservoirs, 45 (82%) of 55 homes, 31 (100%) of 31 commercial buildings, and 15 (100%) of 15 hospitals. Large-restriction-fragment

(LRF) pattern analyses were done with AseI. The LRF patterns of *Mycobacterium avium* isolates recovered from potable water in 3 homes, 2 commercial buildings, 1 reservoir, and 8 hospitals had varying degrees of relatedness to 19 clinical isolates recovered from 17 patients. The high number of *M. avium* isolates recovered from hospital water and their close relationship with clinical isolates suggests the potential threat of nosocomial spread. This study supports the possibility that potable water is a source for the acquisition of *M. avium* infections.—Authors' Abstract

Arrieta, O., Ortiz Reyes, A., Rembao, D., Calvillo, M., Rivera, E. and Sotelo, J. Protective effect of pentoxifylline plus thalidomide against septic shock in mice. Int. J. Exp. Pathol. **90** (1999) 11–16.

Mortality caused by septic shock in experimental animals is reduced by thalidomide, an inhibitor of tumor necrosis factor-alpha. Another drug that could act on the pathophysiological mechanisms of septic shock is pentoxifylline, an inhibitor of platelet aggregation that increases the flexibility of the erythrocyte membrane and has fibrinolytic activity. We studied the effect of pentoxifylline alone and combined with thalidomide in septic shock; 97 NIH mice were injected with lipopolysaccharides of *Salmonella abortus equi* and D galactosamine. Animals were separated in 4 groups; group A (N = 20) was used as control, group B (N = 15) received thalidomide 50 mg/kg, group C (N = 20) received pentoxifylline 40 mg/kg, and group D (N = 15) received thalidomide plus pentoxifylline. Mortality was recorded every hour. Additionally, 5 animals from each group were sacrificed 8 hr after the induction of septic shock for histological analysis of heart, lung, brain, kidney, small intestine, adrenal glands and liver. Microscopic findings were rated as absent, mild, moderate and severe damage. In control animals histological analysis showed intense hemorrhage and necrosis in all organs studied. When compared with controls, treatment with pentoxifylline plus thalidomide reduced mortality ($p < 0.03$). The tissue damage was less severe in animals from the groups that received pentoxifylline or pentoxifylline plus thalidomide ($p < 0.05$). Pentoxifylline seems to potentiate the beneficial effects of thalidomide, reducing mortality and attenuating the pathological changes produced by septic shock.—Authors' Abstract

Arruda, S., Chalhoub, M., Cardoso, S. and Barral-Netto, M. Cell-mediated immune responses and cytotoxicity to mycobacterial antigens in patients with tuberculous pleurisy in Brazil. Acta Trop. **71** (1998) 1–15 (42 references).

Lymphocyte proliferation, cytokine production and natural killer cell cytotoxicity were evaluated as parameters to screen 4 mycobacterial recombinant antigens. Pleural fluid mononuclear cells (PFMC) and peripheral blood mononuclear cells (PBMC) from 13 HIV-negative patients with tuberculous pleurisy, living in a tropical region of Brazil, were used in these assays. Crude *Mycobacterium tuberculosis* antigen and recombinant 70-, 65- and 38-kDa mycobacterial antigens induced greater proliferation in PFMC than in PBMC. Interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-4, and IL-10 were evaluated in the PFMC supernatants stimulated by these antigens. Both crude and 70-kDa antigens induced higher levels of IFN-γ, TNF-α and IL-10. There was a significant positive correlation between IFN-γ and the proliferative response induced by crude *M. tuberculosis* antigen, and an inverse correlation was identified between IL-10 and cell proliferation. IL-4 was not detected in the supernatants of pleural fluid mononuclear cell cultures stimulated by either crude, or recombinant antigens. TNF-α was detected in variable amounts in supernatants of PFMC stimulated by all antigens tested. Natural killer cytotoxicity was induced by both crude and 70-kDa antigen. It is concluded that cells present at the site of disease recognized 3 of the antigens screened, as shown by lymphocyte proliferation and production of regulatory and inflammatory cytokines, and that the results obtained with PFMC were consistently higher than those obtained with homologous PBMC.—Trop. Dis. Bull. **96** (1999) 488

Barzilai, A., Rubinovich, B., Blank-Porat, D., Rubinstein, E., Keller, N. and Levi, I. Successful treatment of disseminated *Mycobacterium simiae* infection in AIDS patients. Scand. J. Infect. Dis. **30** (1998) 143–146.

A retrospective review was made of the medical records of HIV-infected patients who had positive blood or bone marrow cultures for *M. simiae* at the Sheba Medical Center, Tel-Hashomer, Israel, between January 1992 and December 1996. A case of disseminated *M. simiae* infection was defined as isolation of *M. simiae* in blood or bone marrow culture in an HIV-infected patient with a compatible, otherwise unexplained, systemic disease. *M. simiae* was isolated in blood and/or bone marrow cultures from 3 HIV-infected patients during the last 5 years. The first successful treatment in AIDS patients with disseminated *M. simiae* is described. The patients were alive and well 20 months after instituting a combination of 3 antimycobacterial agents: clarithromycin, ethambutol and ciprofloxacin plus intensive antiretroviral therapy.—Trop. Dis. Bull. **96** (1999) 161

Brooks, J. V., Furney, S. K. and Orme, I. M. Metronidazole therapy in mice infected with tuberculosis. Antimicrob. Agents Chemother. **43** (1999) 1285–1288.

The capacity of metronidazole to inhibit the growth of *Mycobacterium tuberculosis* was tested in *in vitro* and *in vivo* mouse models. *In vitro* addition of metronidazole to cultures of infected bone marrow-derived macrophages had no effect, nor did it increase the reduction in bacterial load due to isoniazid. *In vivo,* metronidazole did not reduce bacterial numbers in the lungs of aerosol-infected mice during the active stage of the disease, during a phase of containment, or after prolonged isoniazid therapy (Cornell model). A small but significant reduction was seen if metronidazole therapy was given during an established chronic disease state 100 days after aerosol administration. These data indicate that under most conditions *M. tuberculosis* organisms are not in a metabolic state in which they are susceptible to the action of metronidazole and, hence, that this drug would be of limited clinical value.—Authors' Abstract

Cao, Z., Joseph, W. R., Browne, W. L., Mountjoy, K. G., Palmer, B. D., Baguley, B. C. and Ching, L. M. Thalidomide increases both intra-tumoural tumour necrosis factor-alpha production and anti-tumour activity in response to 5,6-dimethylxanthenone-4-acetic acid. Br. J. Cancer **80** (1999) 716–723.

5,6-Dimethylxanthenone-4-acetic acid (DMXAA), synthesized in this laboratory and currently in phase I clinical trial, is a low molecular weight inducer of tumor necrosis factor-alpha (TNF-α). Administration of DMXAA to mice with established transplantable tumors elicits rapid vascular collapse selectively in the tumor, followed by extensive hemorrhagic necrosis mediated primarily through the production of TNF-α. In this report we have investigated the synthesis of TNF-α mRNA in hepatic, splenic and tumor tissue. Coadministration of thalidomide with DMXAA increased antitumor activity and increased intra-tumoral TNF-α production approximately tenfold over that obtained with DMXAA alone. Thalidomide increased splenic TNF-α production slightly but significantly decreased serum and hepatic levels of TNF-α induced with DMXAA. Lipopolysaccharide (LPS) induced 300-fold higher serum TNF-α than did DMXAA at the maximum tolerated dose, but induced similar amounts of TNF-α in spleen, liver and tumor. Splenic TNF-α activity induced with LPS was slightly increased with thalidomide, but serum and liver TNF-α levels were suppressed. Thalidomide did not increase intratumoral TNF-α production induced with LPS, in sharp contrast to that obtained with DMXAA. While thalidomide improved the antitumor response to DMXAA, it had no effect on the antitumor action of LPS that did not induce a significant growth delay or cures against the Colon 38 tumor. The increase in the antitumor action by thalidomide in combination with DMXAA corresponded to an increase in intra-tumoral TNF-α production. Co-administration of

thalidomide may represent a novel approach to improving selective intra-tumoral TNF-α production and antitumor efficacy of DMXAA.—Authors' Abstract

Das, S. D., Narayanan, P. R., Kolappan, C. and Colston, M. J. The cytokine response to bacille Calmette-Guerin vaccination in South India. Int. J. Tuberc. Lung Dis. **2** (1998) 836–843.

An evaluation of the immune response after BCG vaccination was studied in a population from South India (Tamil Nadu) where BCG vaccination had been a failure. Cell-mediated immune response (CMI) were analyzed *in vitro* by assessing skin test conversion, lymphocyte proliferation and cytokine patterns before and after BCG vaccination in 20 Mantoux-negative subjects. Results were compared with those of 20 naturally Mantoux-positive subjects. *In vitro* lymphocyte proliferation and cytokine responses to various mycobacterial antigens were studied in 12 subjects from each group. The cytokines interferon gamma (IFN-γ), interleukin (IL)-4 and IL-10 were measured by both reverse transcription (RT)-PCR and ELISA. All but one of those who were initially Mantoux-negative converted to positivity following vaccination, confirming that BCG vaccination does cause skin test conversion. However, *in vitro* proliferative responses to phytohemagglutinin (PHA), purified protein derivative (PPD) and *Mycobacterium tuberculosis* remained largely unaltered by vaccination. The production of IFN-γ was significantly higher in PPD-positive individuals compared to the PPD-negative group, and BCG vaccination of the latter did not change the levels of IFN-γ, IL-4 or IL-10. The finding that PPD-negative individuals did not produce IFN-γ even following vaccination and skin test conversion suggests that BCG had little effect in driving the immune response toward a protective Th1 type.—Trop. Dis. Bull. **96** (1999) 150

Ellison, E., Lapuerta, P. and Martin, S. E. Fine needle aspiration diagnosis of mycobacteria lymphadenitis—sensitivity and predictive value in the United States. Acta Cytol. **43** (1999) 153–157.

Objective: Fine needle aspiration (FNA) has proven valuable in diagnosing tuberculous lymphadenitis in countries with endemic mycobacterial infection (MI). Its role in developed countries, where sensitivity and positive predictive value are likely to be lower, has not been adequately explored.

Study design: This retrospective, 5-year study from a public hospital in the United States examined the predictiveness of 238 nodal FNAs in patients with MI; 59% of patients were also HIV+.

Results: Diagnostic results (stainable acid-fact bacilli or positive culture) were present in nearly half the aspirates; sensitivity was 46%, specificity 100%, positive predictive value (PPV) 100% and negative predictive value (NPV) 94%. IF granulomatous inflammation (GI) was also considered a positive result, sensitivity increased to 53%; false-positive cases of GI reduced PPV to 80%, while specificity (98%) and NPV (95%) changed little. Considered alone, GI had the lowest sensitivity (25%) and PPV (65%).

Conclusion: FNA was useful in this U.S. population with MI, identifying almost half the affected patients. However, nondiagnostic results, such as a granulomatous inflammation, should be interpreted with caution.—Authors' Abstract

Fang, Z., Doig, C., Rayner, A., Kenna, D. T., Watt, B. and Forbes, K. J. Molecular evidence for heterogeneity of the multiple-drug-resistant *Mycobacterium tuberculosis* population in Scotland (1990 to 1997). J. Clin. Microbiol. **37** (1999) 998–1003.

Multiple-drug-resistant *Mycobacterium tuberculosis* (MDR-MTB) has been well studied in hospitals or health care institutions and in human immunodeficiency virus-infected populations. However, the characteristics of MDR-MTB in the community have not been well investigated. An understanding of its prevalence and circulation within the community will help to estimate the problem and optimize the strategies for control and prevention of its development and transmission. In this study, MDR-MTB isolates from Scotland collected between 1990 and 1997 were charac-

terized, along with non-drug-resistant isolates. The results showed that they were genetically diverse, suggesting they were unrelated to each other and had probably evolved independently. Several new alleles of rpoB, katG, and ahpC were identified: rpoB codon 525 (ACC → AAC; Thr525Asn); katG codon 128 (CGG → CAG; Arg128Gln) and codon 291 (GCT → CCT; Ala291Pro); and the ahpC synonymous substitution at codon G (ATT → ATC). One of the MDR-MTB isolates from an Asian patient had an IS6110 restriction fragment length polymorphism pattern very similar to that of the MDR-MTR W strain and had the same drug resistance-related alleles but did not have any epidemiological connection with the W strains. Additionally, a cluster of *M. tuberculosis* isolates was identified in our collection of 715 clinical isolates; the isolates in this cluster had genetic backgrounds very similar to those of the W strains, one of which had already developed multiple drug resistances. The diverse population of MDR-MTB in Scotland, along with a low incidence of drug-resistant *M. tuberculosis,* has implications for the control of the organism and prevention of its spread.—Authors' Abstract

Gordon, S. V., Heym, B., Parkhill, J., Barrell, B. and Cole, S. T. New insertion sequences and a novel repeated sequence in the genome of *Mycobacterium tuberculosis* H37Rv. Microbiology **145** (1999) 881–892.

The genome sequence of *Mycobacterium tuberculosis* H37Rv was found to contain 56 loci with homology to insertion sequences (ISs). As well as the previously described IS6100, IS1081, IS1547 and IS-like elements, new ISs belonging to the IS3, IS5, IS21, IS30, IS110, IS256 and ISL3 families were identified. In addition, 6 ISs created a grouping of their own to form a new family (the IS1535 family). Elements with similarity to ISs in other actinomycetes were identified, suggesting the movement of ISs between related genera. The location of ISs on the chromosome revealed that an approximately 600-kb region close to the origin of replication lacks ISs, pointing to the possible detrimental effect of insertions in this area. Analysis of the distribution of ISs through the tubercle strains *Mycobacterium africanum, M. microti, M. bovis, M. bovis* BCG Pasteur, *M. tuberculosis* H37Ra, *M. tuberculosis* CSU#93 and 29 clinical isolates revealed that only IS1532, IS1533, IS1534, and IS1561′ were absent from some of the strains tested. A novel repeated sequence, the REP13E12 family, is described that is present in 7 copies on the *M. tuberculosis* H37Rv chromosome and which contains a probable phage attachment site. This study therefore offers an insight into the possible role of ISs and repetitive elements in the evolution of the *M. tuberculosis* genome, as well as identifying genetic markers that may be useful for phylogenetic and epidemiological analysis of the tubercle complex.—Authors' Abstract

Haydel, S. E., Dunlap, N. E. and Benjamin, W. H. *In vitro* evidence of two-component system phosphorylation between the *Mycobacterium tuberculosis* TrcR/TrcS proteins. Microb. Pathogen. **26** (1999) 195–206.

Two-component regulatory proteins, histidine kinases and response regulators, function in bacteria as sensing and adaptive factors in response to a wide range of environmental stimuli. Conserved histidine and glycine regions of histidine kinase sensor proteins were used to design degenerate oligonucleotide primers for amplification of DNA fragments from *Mycobacterium tuberculosis.* Two adjacent genes, *trcR* and *trcS,* which encode a response regulator and a histidine kinase, respectively, have been identified. Full-length and truncated TrcR and TrcS proteins have been expressed in *Escherichia coli.* Difficulties in expressing recombinant full-length TrcS and a truncated N-terminal form of TrcS reveal that the transmembrane domains are toxic to *E. coli.* Overexpressed truncated C-terminal transmitter domains of TrcS have been autophosphorylated *in vitro* and have transphosphorylated both the full-length recombinant TrcR protein and the N-terminal receiver/regulator domain of TrcR. *In vitro* autophosphorylation of TrcS requires the presence of Mn2+ or Ca2+ as a divalent cation cofactor and subsequent transphosphorylation of TrcR is evident in the pres-

ence of TrcS-phosphate and Ca2+. Transphosphorylation between these two proteins provides evidence that these *M. tuberculosis* genes encode functional two-component system regulatory proteins that are members of a signal transduction circuit.—Authors' Abstract

Hirsch, C. S., Toossi, Z., Vanham, G., Johnson, J. L., Peters, P., Okwera, A., Mugerwa, R., Mugyenyi, P. and Ellner, J. J. Apoptosis and T cell hyporesponsiveness in pulmonary tuberculosis. J. Infect. Dis. **179** (1999) 945–953.

Mycobacterium tuberculosis (MTB)-induced T-cell responses are depressed in peripheral blood mononuclear cells of persons with newly diagnosed pulmonary tuberculosis (TB), and levels of interferon (IFN)-gamma (γ) remain low even after completion of antituberculous therapy. Loss of MTB-reactive T cells through apoptotic mechanisms could account for this prolonged T-cell hyporesponsiveness. T-cell apoptosis was studied in TB patients and healthy control subjects. Both spontaneous and MTB-induced apoptosis (in CD4 and non-CD4 T cells) from TB patients was increased when compared with healthy control subjects; whereas coculture with control antigen (candida) had no effect on T-cell apoptosis in either group of study subjects. An inverse correlation existed between increased MTB-induced T-cell apoptosis and IFN-γ and interleukin 2 immunoreactivities. Successful antituberculous chemotherapy resulted in a 50% reduction in both spontaneous and MTB-induced apoptosis, which coincided with 3- and 8-fold increases in levels of MTB-stimulated IL-2 and IFN-γ, respectively. These data indicate that apoptotic pathways are operant during active MTB infection and may contribute to deletion of MTB-reactive T cells and the immunopathogenesis of this disease.—Authors' Abstract

Kamath, A. T., Hanke, T., Briscoe, H. and Britton, W. J. Co-immunization with DNA vaccines expressing granulocyte-macrophage colony-stimulating factor and mycobacterial secreted proteins enhances T-cell immunity, but not protective efficacy against *Mycobacterium tuberculosis.* Immunology **96** (1999) 511–516.

The development of more effective antituberculosis vaccines would assist in the control of the global problem of infection with *Mycobacterium tuberculosis.* One recent vaccination strategy is immunization with DNA plasmids encoding individual microbial genes. Using the genes for the *M. tuberculosis*-secreted proteins, MPT64 (23,000 MW) and Ag85B (30,000 MW) as candidate antigens, we previously prepared DNA vaccines and demonstrate their ability to stimulate T-cell responses and confer protection in a mouse model of aerosol tuberculosis (TB). The protective efficacy of the DNA vaccines was less than that promoted by the current vaccine *M. bovis* bacille Calmette-Guerin (BCG). To improve the immunogenecity and protective efficacy of these mycobacterial vectors, co-immunization of a plasmid expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) was investigated. Intramuscular immunization with DNA expressing MPT64 or Ag85B and GM-CSF enhanced the antigen-specific cellular immune response, with increased proliferative response and production of interferon-gamma (IFN-γ). The titer of antimycobacterial protein immunoglobulin G (IgG) antibodies was unchanged. Mice immunized with DNA vaccines showed reduced pulmonary bacterial load following an aerosol challenge of *M. tuberculosis,* but co-delivery of the plasmid expressing GM-CSF did not increase the protective effect. Therefore, despite modifying the cellular immune response to DNA vaccines, GM-CSF does not improve their protective efficacy at the peak of infection after an aerosol challenge with 100 cfu of *M. tuberculosis.*—Authors' Abstract

Lee, B. Y. and Horwitz, M. A. T-cell epitope mapping of the three most abundant extracellular proteins of *Mycobacterium tuberculosis* in outbred guinea pigs. Infect. Immun. **67** (1999) 2665–2670.

The three most abundant extracellular proteins of *Mycobacterium tuberculosis,* the 30-, 32-, and 16-kDa major extracellular

proteins, are particularly promising vaccine candidates. We have mapped T-cell epitopes of these three proteins in outbred guinea pigs by immunizing the animals with each protein and assaying splenic lymphocyte proliferation against a series of overlapping synthetic peptides covering the entire length of the mature proteins. The 30-kDa protein contained 9 immunodominant epitopes, the 32-kDa protein contained 2 immunodominant epitopes, and the 16-kDa protein contained a highly immunodominant region at its N terminus. The immunodominant epitopes of the 30- and 32-kDa proteins in outbred guinea pigs were frequently identified in healthy purified-protein-derivative-positive or BCG-vaccinated individuals in previous studies. The immunodominant epitopes of these major extracellular proteins have potential utility in an epitope-based vaccine against tuberculosis.—Authors' Abstract

Lim, A., Eleuterio, M., Hutter, B., Murugasu Oei, B. and Dick, T. Oxygen depletion-induced dormancy in *Mycobacterium bovis* BCG. J. Bacteriol. **181** (1999) 2252–2256.

Gradual depletion of oxygen causes the shift-down of aerobic-growing *Mycobacterium bovis* BCG to an anaerobic synchronized state of nonreplicating persistence. The persistent culture shows induction of glycine dehydrogenase and alpha-crystallin-like protein and is sensitive to metronidazole.—Authors' Abstract

Liu, Y.-C., Huang, T.-S., Huang, W.-K., Chen, C.-S. and Tu, H.-Z. Dideoxy fingerprinting for rapid screening of *rpoB* gene mutations in clinical isolates of *Mycobacterium tuberculosis.* J. Formos. Med. Assoc. **97** (1998) 400–404.

The accuracy of dideoxy fingerprinting (ddF) for rapid screening of rifampin resistance in *M. tuberculosis* isolates was examined; 72 *M. tuberculosis* isolates collected in 1994–1996 in Taipei, Taiwan, from patients with tuberculosis were analyzed by ddF. The results were compared with those of automated dideoxy sequencing and antibiotic resistance profiling (determined with the BACTEC system). Of the 72 isolates, 50 were rifampin resistant. The ddF findings were completely consistent with those of dideoxy sequencing in all isolates. In 68 (94%) isolates, the ddF findings were consistent with the rifampin resistance status determined with the BACTEC system; all 4 isolates with inconsistent results had no mutation in the 69-bp region, but were resistant to rifampin. These findings suggest that ddF accurately detects mutations in the rifampin resistance-associated 69-bp region of the *rpoB* gene in clinical isolates of *M. tuberculosis,* and may be a valuable screening tool for rifampin resistance.—Trop. Dis. Bull. **96** (1999) 52

N'Zi, K. P., N'Dri, K., Aka, B. R., Diabate, A. S., Ouattara, D. N. and Djedje, A. T. [Radiographic aspects of osteo-articular complications in Buruli ulcers.] Bull. Soc. Pathol. Exot. **91** (1998) 229–231. (in French)

Radiographic aspects of osteoarticular and soft tissue lesions were examined in 30 inpatients with Buruli ulcers at a hospital in Abidjan, Côte d'Ivoire [date not given]. The chronology and precociousness of certain symptoms, soft tissue lesions, bone demineralization, periosteal apposition 2 months after the onset of symptoms, and later osteolysis and joint complications were analyzed. It was concluded that these lesions are not specific and occur contiguously to soft tissue lesions. The role of secondary infections in the appearance of bone lesions is discussed.—Trop. Dis. Bull. **96** (1998)

Osorio, L. E., Villegas, M. V., Benitez, A. M., Hernandez, H., *et al.* Acquired multidrug-resistant tuberculosis—Buenaventura, Colombia, 1998. Morbid. Mortal. Wkly. Rep. **47** (1998) 759–761.

During October–November 1997, The International Center for Training and Medical Investigation in Cali, Colombia, performed sputum cultures for *Mycobacterium tuberculosis* and drug-susceptibility testing on isolates from 18 (75%) of 24 tuberculosis patients in Buenaventura who were known to be clinically unresponsive to standard tuberculosis treatment. Multidrug-resistant tuberculosis (MDR-TB; isolates re-

sistant to at least isoniazid and rifampin) was identified in 12 (67%) of the patients; 4 patients subsequently died. An investigation of the 12 patients with MDR-TB was conducted in March 1998. The median age of the 12 patients was 30 years (range 18–79 years); 9 were men and all were long-term residents of Buenaventura (median 29 years; range 17–80 years); 10 (83%) of the 12 had no known epidemiological link to another MDR-TB case. None of the 7 cases tested for human immunodeficiency virus infection were positive. Clinical charts of all MDR-TB cases were reviewed for possible factors associated with the development of MDR-TB. All had received a median of 3.5 years of tuberculosis treatment (range 2–13 years), but 11 (92%) had treatment interrupted and reinitiated several times. All 12 cases were found to have experienced at least 2 instances of incorrect treatment or management of their illness (median 3.9; range 2–6) based on World Health Organization and Colombian treatment protocols.—Trop. Dis. Bull. **96** (1999)

Ramirez Amador, V. A., Esquivel Pedraza, L., Ponce de Leon, S., Reyes Teran, G., Gonzalez Guevara, M., Ponce de Leon, S. and Sierra Madero, J. G. Thalidomide as therapy for human immunodeficiency virus-related oral ulcers: a double-blind placebo-controlled clinical trial. Clin. Infect. Dis. **28** (1999) 892–894.

A double-blind, randomized, placebo-controlled clinical trial was performed in Mexico City, Mexico, to evaluate the efficacy of thalidomide in treating oral recurrent aphthae in human immunodeficiency virus (HIV)-infected subjects. Sixteen HIV-infected patients with clinical and histological diagnosis of oral recurrent aphthous ulcerations received randomly an 8-week course of either thalidomide or placebo, with an initial oral dosage of 300 mg/d for 1 week, followed by 200 mg/d for 7 weeks. Ten subjects received thalidomide and 6 received placebo. At 8 weeks, 9 subjects (90%) in the thalidomide group had complete healing of their ulcers, compared with 2 (33.3%) of the 6 patients in the placebo group ($p = 0.03$). There was a significant reduction in largest ulcer diameter in the thalidomide group. Rash was observed in 80% of the thalidomide patients. Although thalidomide demonstrated an unquestionable benefit in treatment of oral ulcers in PW patients, caution must be taken given the frequent occurrence of side effects.—Authors' Abstract

Salman, M., Brennan, P. J. and Lonsdale, J. T. Synthesis of mycolic acids of mycobacteria: an assessment of the cell-free system in light of the whole genome. Biochim. Biophys. Acta **1437** (1999) 325–332.

Mycolic acids are 70–90 carbon, alpha-alkyl, beta-hydroxy fatty acids constituting a major component of the cell envelope of *Mycobacterium tuberculosis*. The fact that the mycolic acid biosynthetic pathway is both essential in mycobacteria and the target for many first-line anti-TB drugs necessitates a detailed understanding of its biochemistry. A whole cell-free, but cell particulate- and membrane-containing enzyme preparation for mycolic acid biosynthesis was developed a few years ago and studied extensively. This system was shown to catalyze the synthesis of mature mycolic acids from [C-14]acetate, but allows only minimal deposition into the cell wall proper. In the meantime the sequence of the entire genome of *M. tuberculosis* has been elucidated and its analysis using numerous protein sequence-based algorithms predicted cytoplasmic localization and a soluble, not a particulate, nature for the enzymes involved in the mycolic acid synthetic pathway. Accordingly, we re-assessed the "cell-free" system for mycolic acid synthesis and concluded that it is probably due to the presence of unbroken cells, since viable cells were recovered from the cell-wall preparation. The amount of whole cells depended upon the efficiency of the cell disruption method and conditions, and the amount of mycolic acid synthesized by the putative cell-free system correlated with the content of whole cells. Thus, accumulated results from the use of this "cell-free" cell wall-based system should be reevaluated in the light of these new data.—Authors' Abstract

Salman, M., Lonsdale, J. T., Besra, G. S. and Brennan, P. J. Phosphatidylinositol synthesis in mycobacteria. Biochim. Biophys. Acta **1436** (1999) 437–450.

The metabolism and synthesis of an important mycobacterial lipid component, phosphatidylinositol (PI), and its metabolites, was studied in *Mycobacterium smegmatis* and *M. smegmatis* subcellular fractions. Little is known about the synthesis of PI in prokaryotic cells. Only a cell-wall fraction (P60) in *M. smegmatis* was shown to possess PI synthase activity. Product was identified as PI by migration on TLC treatment with phospholipase C and ion exchange chromatography. PI was the only major product (92.3%) when both cells and P60 fraction were labeled with [H-3]inositol. Also, a neutral lipid inositol-containing product (4.1% of the total label) was identified in the P60 preparations. Strangely, PI synthase substrates, CDP-dipalmitoyl-DAG and CDP-NBD-DAG, added to the assay did not stimulate [H-3]PI and NBD-PI yield by *M. smegmatis*. At the same time, addition of both substrates to rat liver and *Saccharomyces cerevisiae* PI synthase assays resulted in an increase in the product yield. Upon addition of CHAPS to the mycobacterial PI synthase assay, both substrates were utilized in a dose-dependent manner for the synthesis of NBD-PI and [H-3]PI. These results demonstrate a strict substrate specificity of mycobacterial PI synthase toward endogenous substrates. K-m of the enzyme toward inositol was shown to be 25 μM. Mg2+ stimulated the enzyme to a greater degree than Mn2+. Structural analogs of myo-inositol, epi-inositol and scyllo-inositol and Zn2+ were shown to be more potent inhibitors of mycobacterial PI synthase than of mammalian analogs. Lack of sequence homology with mammalian PI synthases, different kinetic characteristics, existence of selective inhibitors and an important physiological role in mycobacteria suggest that PI synthase may be a good potential target for antituberculosis therapy.—Authors' Abstract

Sethna, K. B., Mistry, N. F., Dholakia, Y., Antia, N. H. and Harboe, M. Longitudinal trends in serum levels of mycobacterial secretory (30 kDa) and cytoplasmic (65 kDa) antigens during chemotherapy of pulmonary tuberculosis patients. Scand. J. Infect. Dis. **30** (1998) 363–369.

Antigen 85 (mol. wt. 30,000) (30 kD), secreted by actively growing mycobacteria under axenic conditions, and mol. wt. 65,000 (65 kD), a cytoplasmic antigen released during mycobacterial lysis, were used in a study in Bombay, India, to monitor the efficacy of chemotherapy in previously untreated pulmonary tuberculosis (UPTB) patients using ELISA. Sera from 125 UPTB patients were examined for each of the 2 antigens individually and for the ratio of secretory (30 kD) to cytoplasmic (65 kD) antigen (SCR), before commencement of treatment, after intensive phase (IP), completion of optimum period of treatment (COPT) and 6 months post-COPT; 116 controls (healthy individuals and contacts) were also checked for these antigens. The detection of 30-kD and 65-kD antigens in UPTB patients had a sensitivity ranging from 50%–57% (mean 30-kD value: 0.64 ± 1.24 ng/ml) to 20%–22% (mean 65-kD value: 0.51 ± 1.87 ng/ml), respectively; whereas in controls it ranged from 2%–8% (0.05 ± 0.28 ng/ml) to 14%–47% (0.09 ± 0.22 ng/ml), respectively. Although the decline in 30-kD positivity was more evident at COPT, computation of the SCR denoted efficacy of chemotherapy more readily at IP. Similarly, SCR resolved the ambiguity between individual antigen levels and the clinical status of a patient. Since significant numbers of patients demonstrated 30 kD at IP it may be computed that the lifespan of circulating 30 kD in serum could be at least 2 months after the start of treatment, declining gradually thereafter. It is concluded that although seromonitoring for secretory antigen generally reflects the efficacy of chemotherapy, the interpretation of findings clearly requires further elucidation.—Trop. Dis. Bull. **96** (1999) 488

Shimazawa, R., Miyachi, H., Takayama, H., Kuroda, K., Kato, F., Kato, M. and Hashimoto, Y. Antiangiogenic activity of tumor necrosis factor-alpha production regulators derived from thalidomide. Biol. Pharm. Bull. **22** (1999) 224–226.

Recently, we developed novel tumor necrosis factor (TNF)-alpha production regulators with a phthalimide skeleton derived from thalidomide. We show here that some of these compounds are more potent inhibitors than thalidomide of angiogenesis induced by basic fibroblast growth factor in a murine angiogenesis assay.—Authors' Abstract

Silva, C. L., Bonato, V. L. D. and Lima, V. M. F. DNA encoding individual mycobacterial antigens protects mice against tuberculosis. Bras. J. Med. Biol. Res. **32** (1999) 231–234.

Over the last few years, some of our experiments in which mycobacterial antigens were presented to the immune system as if they were viral antigens have had a significant impact on our understanding of protective immunity against tuberculosis. They have also markedly enhanced the prospects for new vaccines. We now know that individual mycobacterial protein antigens can confer protection equal to that from live BCG vaccine in mice. A critical determinant of the outcome of immunization appears to be the degree to which antigen-specific cytotoxic T cells are generated by the immune response. Our most recent studies indicate that DNA vaccination is an effective way to establish long-lasting cytotoxic T-cell memory and protection against tuberculosis.—Authors' Abstract

Stinear, T., Ross, B. C., Davies, J. K., Marino, L., Robins Browne, R. M., Oppedisano, F., Sievers. A. and Johnson, P. D. R. Identification and characterization of IS2404 and IS2606: two distinct repeated sequences for detection of *Mycobacterium ulcerans* by PCR. J. Clin. Microbiol. **37** (1999) 1018–1023.

Molecular analysis of *Mycobacterium ulcerans* has revealed two new insertion sequences (ISs), IS2404 and IS2606. IS2404 was identified by complete sequencing of a previously described repetitive DNA segment from *M. ulcerans*. This element is 1274-bp long, contains 12-bp inverted repeats and a single open reading frame (ORF) potentially encoding a protein of 327 amino acids (aa), and apparently generates 7-bp direct repeats upon transposition. Amino acid similarity was found between the putative transposase and those encoded by ISs in other bacterial sequences from *Aeromonas salmonicida* (AsIs1), *Escherichia coli* (N repeat element), *Vibrio cholerae* (VcIS1), and *Porphyromonas gingivalis* (PGIS2). The second IS, IS2606, was discovered by sequence analysis of a HaeIII fragment of *M. ulcerans* genomic DNA containing a repetitive sequence. This element is 1404-bp long, with 12-bp inverted repeats and a single ORF potentially encoding a protein of 445 aa. Database searches revealed a high degree of amino acid identity (70%) with the putative transposase of IS1554 from *M. tuberculosis*. Significant amino acid identity (40%) was also observed with transposases from several other microorganisms, including *Rhizobium meliloti* (ISRm3), *Burkholderia cepacia* (IS1356), *Corynebacterium diphtheriae*, and *Yersinia pestis*. PCR screening of DNA from 35 other species of mycobacteria with primers for IS2404 confirm that this element is found only in *M. ulcerans*. However, by PCR, IS2606 was also found in *M. lentiflavum*, another slow-growing member of the genus *Mycobacterium* that is apparently genetically distinct from *M. ulcerans*. Testing the sensitivity of PCR based on IS2404 and IS2606 primers demonstrated the ability to detect 0.1 and 1 *M. ulcerans* genome equivalents, respectively. The ability to detect small numbers of cells by using two gene targets will be particularly useful for analyzing environmental samples, where there may be large concentrations of *M. ulcerans* among large numbers of other environmental mycobacteria.—Authors' Abstract

Sun, Z. H. and Zhang, Y. Reduced pyrazinamidase activity and the natural resistance of *Mycobacterium kansasii* to the anti-tuberculosis drug pyrazinamide. Antimicrob. Agents Chemother. **43** (1999) 537–542.

Pyrazinamide (PZA), an analog of nicotinamide, is a prodrug that requires conversion to the bactericidal compound pyrazinoic acid (POA) by the bacterial pyrazinamidase (PZase) activity of nicotinamidase to show activity against *My-*

cobacterium tuberculosis. Mutations leading to a loss of PZase activity cause PZA resistance in *M. tuberculosis. M. kansasii* is naturally resistant to PZA and has reduced PZase activity along with an apparently detectable nicotinamidase activity. The role of the reduction in PZase activity in the natural PZA resistance of *M. kansasii* is unknown. The MICs of PZA and POA for *M. kansasii* were determined to be 500 and 125 μg/ml, respectively. Using [C-14]PZA and [C-14]nicotinamide, we found that *M. kansasii* had about 5-fold less PZase activity and about 25-fold less nicotinamidase activity than *M. tuberculosis.* The *M. kansasii* pncA gene was cloned on a 1.8-kb BamHI DNA fragment, using *M. avium* pncA probe. Sequence analysis showed that the *M. kansasii* pncA gene encoded a protein with homology to its counterparts from *M. tuberculosis* (69.9%), *M. avium* (65.6%), and *Escherichia coli* (28.5%). Transformation of naturally PZA-resistant *M. bovis* BCG with *M. kansasii* pncA conferred partial PZA susceptibility. Transformation of *M. kansasii* with *M. avium* pncA caused functional expression of PZase and high-level susceptibility to PZA, indicating that the natural PZA resistance in *M. kansasii* results from a reduced PZase activity. Like *M. tuberculosis, M. kansasii* accumulated POA in the cells at an acidic pH; however, due to its highly active FOG efflux pump, the naturally PZA-resistant species *M. smegmatis* did not. These findings suggest the existence of a weak POA efflux mechanism in *M. kansasii.*—Authors' Abstract

Sugawara, I., Yamada, H., Kaneko, H., Mizuno, S., Takeda, K. and Akira, S. Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice. Infect. Immun. **67** (1999) 2585–2589.

Immunity to mycobacterial infection is closely linked to the emergence of T cells that secrete cytokines, gamma interferon (IFN-γ), interleukin-12 (IL-12), and tumor necrosis factor-alpha (TNF-α), resulting in macrophage activation and recruitment of circulating monocytes to initiate chronic granuloma formation. The cytokine that mediates macrophage activation is IFN-γ, and, like IL-12, IL-18 was shown to activate Th1 cells and induce IFN-γ production by these cells. In order to investigate the role of IL-18 in mycobacterial infection, IL-18-deficient mice were infected with *Mycobacterium tuberculosis* and *M. bovis* BCG Pasteur, and their capacities to control bacterial growth, granuloma formation, cytokine secretion, and NO production were examined. These mice developed marked granulomatous, but not necrotic, lesions in their lungs and spleens. Compared with the levels in wild-type mice, the splenic IFN-γ levels were low but the IL-12 levels were normal in IL-18-deficient mice. The reduced IFN-γ production was not secondary to reduced induction of IL-12 production. The levels of NO production by peritoneal macrophages of IL-18-deficient and wild-type mice did not differ significantly. Granulomatous lesion development by IL-18-deficient mice was inhibited significantly by treatment with exogenous recombinant IL-18. Therefore, IL-18 is important for the generation of protective immunity to mycobacteria, and its main function is the induction of IFN-γ expression.—Authors' Abstract

Taylor, G. M., Goyal, M., Legge, A. J., Shaw, R. J. and Young, D. Genotypic analysis of *Mycobacterium tuberculosis* from medieval human remains. Microbiology **145** Part 4 (1999) 899–904.

Three medieval bone samples with osteological evidence of tuberculosis infection were analyzed for the presence of DNA sequences from *Mycobacterium tuberculosis* using a series of PCRs. In each case amplification of IS6110 and part of the beta-subunit of RNA polymerase identified infection with a bacterium belonging to the *M. tuberculosis* complex. Amplification of the mtp40 genome fragment and the presence of a guanine residue at position 285 in the oxyR pseudogene, demonstrate the infecting strain to be similar to present-day *M. tuberculosis* isolates rather than to *M. bovis.* Spoligotyping, based on amplification of the direct repeat (DR) region of the mycobacterial genome, provided further evidence of similarity to *M. tuberculosis* and indicated a close relationship between isolates associated with two separate medieval burials. The study demonstrates the feasi-

bility of amplifying multiple *M. tuberculosis* loci in ancient human remains and suggests important applications in the study of the palaeoepidemiology and virulence of tuberculosis in past populations.—Authors' Abstract

Vynnycky, E. and Fine, P. E. M. Interpreting the decline in tuberculosis: the role of secular trends in effective contact. Int. J. Epidemiol. **28** (1999) 327–334.

Background. The dramatic decline in tuberculosis (TB) in developed countries during the past century has been attributed to many factors, including improvements in living and social conditions and, more recently, effective treatment. Each of these changes should have reduced the average number of individuals "effectively contacted" (i.e., sufficiently to transmit infection) by each infectious TB case.

Method. Estimates of the average number of individuals effectively contacted by each infectious TB case, for each year since 1900 in England and Wales, are derived as the ratio between published estimates of the annual risk of infection and estimates of the prevalence of infectious cases, as derived using a published model of the epidemiology of TB.

Results. The results suggest that each infectious case contacted, on average, about 22 individuals in 1900 sufficiently to transmit *Mycobacterium tuberculosis* infection, and that this number declined to about 10 by 1950 and to approximately 1 by 1990.

Conclusions. Although several factors contributed to the decline in TB in developed countries during this century, a major contributor has been the decline in the number of effective contacts by each case over time. Similar declines have doubtless occurred over the past century for many infections in developed countries.—Authors' Abstract

Wallis, R. S., Perkins, M., Phillips, M., Joloba, M., Demchuk, B., Namale, A., Johnson, J. L., Williams, D., Wolski, K., Teixeira, L., Dietze, R., Mugerwa, R. D., Eisenach, K. and Ellner, J. J. Induction of the antigen 85 complex of *Mycobacterium tuberculosis* in sputum: a determinant of outcome in pulmonary tuberculosis treatment. J. Infect. Dis. **178** (1998) 1115–1121.

Sputum quantitative culture, acid-fast smear, days-to-positive by BACTEC, and *M. tuberculosis* antigen 85 complex were monitored during therapy in 42 patients with pulmonary tuberculosis (TB) from Uganda and Brazil [date not given]. By BACTEC, 4 patients were persistently positive on days 90–180, and treatment ultimately failed in 2 of these. Antigen 85 expression increased in subjects in whom disease persisted (persisters) during days 0–14 when the difference between persisters and nonpersisters was statistically significant ($p = 0.002$). Only antigen 85 complex values at day 14 suggested TB persistence at or after day 90. All subjects with day 14 antigen 85 complex values <60 pg/ml responded rapidly to treatment and were cured. Of those with values >60 pg/ml, in 33% TB persisted at or after day 90 and treatment failed in 17%. It is suggested that biological factors expressed early in therapy, not related to compliance or resistance, may exert a substantial influence on outcome. The antigen 85 complex is critical in cell-wall biosynthesis and is induced by isoniazid *in vitro*. Its induction may represent an adaptive transition to a persistent state during therapy.—Trop. Dis. Bull. **96** (1999) 251

Wild, I., Hoal-van Helden, E., Hon, D., Lombard, C. and van Helden, P. Potentiation of isoniazid activity against *Mycobacterium tuberculosis* by melatonin. Antimicrob. Agents Chemother. **43** (1999) 975–977.

The limited number of effective antituberculosis drugs available necessitates optimizing current treatments. We show that melatonin, which is synthesized in the pineal gland, can cause at least a threefold increase in the efficacy of isoniazid. This suggests that tuberculosis chemotherapy can be improved by innate molecules such as melatonin.—Authors' Abstract

Wiker, H. G., Michell, S. L., Hewinson, R. G., Spierings, E., Nagai, S. and Harboe, M. Cloning, expression and significance of MPT53 for identification

of secreted proteins of *Mycobacterium tuberculosis*. Microb. Pathogen. **26** (1999) 207–219.

Based on our N-terminal amino acid sequence of MPT53 and a deduced DNA sequence, we searched for the corresponding gene in the *Mycobacterium tuberculosis* genomic sequence at the Sanger center, localizing mpt53 close to mpt70 and mpt83. The gene was cloned and expressed, followed by purification of MPT53 to homogeneity from recombinant *M. smegmatis* culture fluid. In MPT53 there is 60% identity with the active site of thioredoxin of *M. tuberculosis* (MPT46) with two cysteins in a CXXC motif, but MPT53 could not serve as an alternative substrate for thioredoxin reductase. Testing for IgM and IgG_1 anti-MPT53 in cattle sera showed that MPT53 is immunogenic following natural and experimental infection with *M. bovis*. Cloning of mpt53 represents cloning of the last of the 10 proteins originally defined as "secreted proteins" of *M. tuberculosis* and *M. bovis* based on determination of their "Localization Index" (LI) (J Gen Microbiol 1991; 137: 875–84). The need for a precise definition of the term "secreted protein" is discussed. So far we have observed full concordance between occurrence of an LI value indicating secretion of a protein and occurrence of a signal sequence in the corresponding gene. Signal sequence independent protein secretion in mycobacteria may occur for a limited number of proteins and remains to be established.—Authors' Abstract

Wilkinson, D. and Gilks, C. F. Increasing frequency of tuberculosis among staff in a South African district hospital: impact of the HIV epidemic on the supply side of health care. Trans. R. Soc. Trop. Med. Hyg. **92** (1998) 500–502.

To describe the changing frequency of tuberculosis among staff in a South African hospital, and to compare incidence in health workers with that in ancillary staff, the number and type of cases of tuberculosis among staff diagnosed between 1991 and 1996 were ascertained. The incidence rate of tuberculosis among health workers and ancillary staff was compared with the age-specific rate in the community (aged 20–59 years). In 1991–1992, 2 cases of tuberculosis were diagnosed among hospital staff; however in 1993–1996 there were 20 cases diagnosed (annualized incidence rates 138/100,000 and 690/100,000, respectively; $p < 0.0001$). The mean age of the 22 cases was 29.6 years and 12 of 14 cases tested were infected with HIV. Most cases (18) successfully completed treatment, but 4 died. The incidence of tuberculosis amongst health workers (558/100,000 person-years of observation [PYO]) and ancillary staff (445/100,000 PYO) was not significantly different, but it was lower than the incidence rate among people in the community aged 20–59 years (1543/100,000). It is concluded that tuberculosis has increased among hospital staff, secondary to the impact of HIV.—Trop. Dis. Bull. **96** (1999) 249

World Health Organization. Antituberculosis drug resistance worldwide. Wkly. Epidemiol. Rec. **73** (1998) 249–254.

This paper describes the results from the first phase of the global project on antituberculosis drug resistance surveillance started in 1994 by the WHO and the International Union Against Tuberculosis and Lung Disease (IUALD). In includes results from 35 countries in 5 continents, with surveillance or surveys conducted on approximately 50,000 tuberculosis cases sampled from areas representing 20% of the world's population. Studies enrolled between 59 and 14,344 tuberculosis patients. Primary drug resistance was determined in cases with effectively no previous treatment, the prevalence of resistance to any drug ranging from 2% in the Czech Republic to 40.6% in the Dominican Republic, with a median value of 9.9%. Primary resistance to all 4 drugs tested (isoniazid, rifampin, ethambutol and streptomycin) was observed in a median of 0.2% of the cases (range 0–4.6%). Primary multidrug-resistant tuberculosis (MDR-TB) was found in all countries surveyed except Kenya, with a median prevalence of 1.4% and a range of 0% (Kenya) to 14.4% (Latvia). Acquired resistance to any drug (in patients who had been treated for 1 month or longer in the past) exhibited prevalence rates ranging

from 5.3% in New Zealand to 100% in Ivanovo Oblast, Russian Federation, with a median value of 36%. Resistance to all 4 drugs among previously treated patients was reported in a median of 4.4% of the cases (range 0%–17%). Acquired MDR-TB had a median prevalence of 13% with a range of 0% (Kenya) to 54.4% (Latvia). Recommendations for surveillance, management and research are given.—Trop. Dis. Bull. **96** (1999) 54

Zhao, B. Y., Pine, R., Domagala, J. and Drlica, K. Fluoroquinolone action against clinical isolates of *Mycobacterium tuberculosis*: effects of a C-8 methoxyl group on survival in liquid media and in human macrophages. Antimicrob. Agents Chemother. **43** (1999) 661–666.

When the lethal action of a C-8 methoxyl fluoroquinolone against clinical isolates of *Mycobacterium tuberculosis* in liquid medium was measured, the compound was found to be three to four times more effective (as determined by measuring the 90% lethal dose) than a C-8-H control fluoroquinolone or ciprofloxacin against cells having a wild-type gyrA (gyrase) gene. Against ciprofloxacin-resistant strains, the C-8 methoxyl group enhanced lethality when alanine was replaced by valine at position 90 of the GyrA protein or when aspartic acid 94 was replaced by glycine, histidine, or tyrosine. During infection of a human macrophage model by wild-type *M. bovis* BCG, the C-8 methoxyl group lowered survival 20- to 100-fold compared with the same concentration of a C-8-H fluoroquinolone. The C-8 methoxyl fluoroquinolone was also more effective than ciprofloxacin against a gyrA Asn94 mutant of *M. bovis* BCG. In an *M. tuberculosis*-macrophage system the C-8 methoxyl group improved fluoroquinolone action against both quinolone-susceptible and quinolone-resistant clinical isolates. Thus, a C-8 methoxyl group enhances the bactericidal activity of quinolones with N1-cyclopropyl substitutions; these data encourage further refinement of fluoroquinolones as antituberculosis agents.—Authors' Abstract

Zhu, X. Y., Zhang, Y. P., Klopman, G. and Rosenkranz, H. S. Thalidomide and metabolites: indications of the absence of "genotoxic" carcinogenic potentials. Mutat. Res. **425** (1999) 153–167.

Because of the reintroduction into human therapeutics of thalidomide, a recognized developmental toxicant in humans, there has been concern about its potential for inducing other health effects as well. The present study is concerned with the possible mutagenicity and carcinogenicity of this chemical. Using the expert system, META, a series of putative metabolites of thalidomide was generated. In addition to the known or hypothesized metabolites of thalidomide (N = 12), a number of additional putative metabolites (N = 131) were identified by META. The structures of these chemicals were subjected to structure-activity analyses using predictive CASE/MULTICASE models of developmental toxicity, rodent carcinogenicity and mutagenicity in *Salmonella*. While thalidomide and some of its putative metabolites were predicted to be developmental toxicants, none of them predicted to be a rodent carcinogen. Putative metabolites containing the hydroxamic acid or hydroxylamine moieties were predicted to be mutagens. None of the "known" metabolites of thalidomide contained these reactive moieties. Whether such intermediates are indeed generated or whether they are generated and are either unstable in the presence of oxygen or react rapidly with nucleophiles is unknown.—Authors' Abstract

Zwarenstein, M., Schoeman, J. H., Vundule, C., Lombard, C. J. and Tatley, M. Randomised controlled trial of self-supervised and directly observed treatment of tuberculosis. Lancet **352** (1999) 1340–1343.

Direct observation (DO) was compared with self-supervision, in which patients on the same drug regimen are not observed taking their pills, to assess the effect of each on the success of tuberculosis treatment. The study involved an unblinded, randomized controlled trial in 2 communities in South Africa (Khayelitsha and Elsies River)

with large tuberculosis case loads. Patients were recruited during 1994–1995 and included 216 adults who started pulmonary tuberculosis treatment for the first time, or who had a second course of treatment (retreatment patients). No changes to existing treatment delivery were made other than randomization. Analysis was by intention to treat. Individual patient data from the 2 communities were combined. Treatment for tuberculosis was more successful among self-supervised patients (60% of patients) than among those on DO (54% of patients; difference between groups, 6% (90% CI –5.1–17.0)). Retreatment patients had significantly more successful treatment outcomes if self-supervised (74% of patients) than on DO [42% of patients, difference between groups 32% (11–52%)]. At high rates of treatment interruption, self-supervision achieved equivalent outcomes to clinic DO at lower cost. Self-supervision achieved better outcomes for retreatment patients. It is suggested that supportive patient-career relations, rather than authoritarian surveillance implicit in DO, may improve treatment outcomes and help to control tuberculosis.—Trop. Dis. Bull. **96** (1999)