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CURRENT LITERATURE

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

General and Historical

Al-Qubati, Y. and Al-Kubati, A. S. Multidrug therapy—the pathway for global leprosy elimination. Indian J. Lepr. **72** (2000) 477–490.

Introduction of dapsone therapy paved the way for a better and more humanitarian way of dealing with the leprosy victims who, prior to 1943, were simple rejected and segregated from society. Emergence of dapsone-resistant *M. leprae* and mycobacterial persistence provoked our quest for another solution. More drugs were discovered for treatment of leprosy. But the real breakthrough was the recommendation of regimens of multidrug therapy (MDT) for the treatment of leprosy by the WHO Study Group on Chemotherapy of Leprosy in October 1981. Subsequent world-wide development of leprosy control activities was phenomenal. The impact of MDT has led to the cure of over 8 million leprosy sufferers and the saving of 1 million patients from becoming crippled. Leprosy prevalence has decreased by 80% in 10 years. By the end of May 1999 the leprosy burden remained concentrated in only 12 countries of the world. These achievements are mainly attributed to the development and world-wide adoption of the MDT program.—Authors' Abstract

Maia, M. A. C., Alves, A., de Oliveira, R. and Barbosa, L. M. [Knowledge of leprosy by the nursing team and hand laborers.] Hansen. Int. **25** (2000) 26–30. (in Portuguese)

The objective of the present study was to determine the knowledge about leprosy among hand laborers and nursing professionals in two bordering municipalities of the state of Minas Gerais. The study included 132 individuals, 78 of them nursing professionals and 54 hand laborers. The research instrument used was a structured interview. The results showed that the "Hansen's disease" term is still little known among hand laborers; only 18.5% of them responded that they knew the disease as Hansen's disease and 35.2% of the nursing professionals and 31.1% of the hand laborers stated that they knew the disease as a blood disease. With respect to the mode of transmission, 15.3% of the nursing professionals responded that they did not know how the disease is transmitted, a percentage close to that of hand laborers (18.5%). With respect to a cure for the disease, 47.5% of the nursing professionals stated that there is no cure, as did 48% of the hand laborers. Wounds and physical deformities are still present in the perception of the disease by part of the interviewers. Thus, we noted that great efforts are needed to educate the population about this endemic disease and the same should be done with the various categories of the nursing team.—Authors' English Summary

Ranganadha Rao, P. V., Bhuskade, R. A. and Desikan, K. V. Modified leprosy elimination campaign (MLEC) for case detection in a remote tribal area in the state of Orissa, India. Lepr. Rev. **71** (2000) 377–381.

A leprosy project was established in a difficult-to-reach area under guidelines of the government of India. The leprosy services were provided by Koraput Leprosy Eradication Project (KORALEP) and general health services by primary health care (PHC). Leprosy elimination campaigns (LECs) were suggested by WHO to detect more cases in the community. A modified leprosy elimination campaign (MLEC), carried out utilizing the services of primary

health care workers, is discussed in this paper. Apart from the trained health workers, Anganwadi workers along with some literate people from the district were also included in the search teams. In all, 1543 cases were shortlisted from the suspects identified and on re-examination 576 cases were confirmed as active cases. Sixty percent of the cases detected were very early cases with two to three skin lesions. This could be achieved with a very brief training of health workers and involving village voluntary workers. MLEC was found to be a useful tool for case finding in such areas.—Authors' Summary

Chemotherapy

Gupta, U. D., Katoch, K., Singh, H. B., Natrajan, M., Sharma, V. D. and Katoch, V. M. Assessment of viability by normal mouse foot-pad and bacillary ATP bioluminescence assay in multibacillary cases treated with an MDT regimen using conventional as well as newer drugs like minocycline and ofloxacin. Indian J. Lepr. **72** (2000) 437–440.

The therapeutic effect of a drug regimen of conventional drugs as well as newer drugs like ofloxacin and minocycline in smear-positive multibacillary (MB) leprosy cases was assessed by mouse foot-pad and ATP bioluminescence methods. Biopsies were taken before starting treatment and after 1 year of treatment. They were processed for viability assessment by normal mouse foot-pad inoculation and bacillary ATP assay techniques. The test regimen was quite effective in its antibacterial effect as it was found to result in loss of bacillary viability in all the cases, as assessed by both methods.—Authors' Abstract

Jackson, C. J., Lamb, D. C., Kelly, D. E. and Kelly, S. L. Bactericidal and inhibitory effects of azole antifungal compounds on *Mycobacterium smegmatis*. FEMS Microbiol. Lett. **192** (2000) 159–162.

Azole antifungals are central to therapy and act by inhibiting a cytochrome P450, sterol 14-demethylase and blocking normal sterol synthesis. Our recent identification of a mycobacterial sterol biosynthetic pathway led us to probe the efficacy of a range of these compounds against *Mycobacterium smegmatis*. Several showed equivalent or greater inhibitory effects to those against *Candida albicans*, and bactericidal activity was demonstrated for four compounds, clotrimazole, econazole, miconazole and tebuconazole. The major drug used clinically, fluconazole, was ineffective. The results are discussed in light of the worldwide spread of tuberculosis, including drug-resistant forms and the requirement for new drugs.—Authors' Abstract

Jaggarajamma, K., Thomas, A. and Nagarajan, M. Status of long absentees among multibacillary leprosy patients admitted to a controlled clinical study. Indian J. Lepr. **72** (2000) 469–475.

Of 210 multibacillary leprosy patients who were admitted to a trial of two drug regimens, 57 were excluded from efficacy analysis at 120 months for various reasons; 33 of these patients were identified as long absentees. Efforts were made to contact these patients through letters and home visits to assess their clinical and bacteriological status and to find out the reasons for default; 21 patients were thus retrieved. Only one patient was found to be having active disease requiring treatment; the rest were clinically inactive and bacteriologically negative.—Authors' Abstract

Katoch, K., Katoch, V. M., Natrajan, M., Sharma, V. D., Singh, H. B. and Gupta, U. D. Chemotherapy trials in MB leprosy using conventional and newer drugs pefloxacin and minocycline. Indian J. Dermatol. Venereol. Leprol. **66** (2000) 18–25.

One hundred, untreated, smear-positive BB, BL and LL patients were treated with a regimen comprising of once a month, supervised, 600 mg of rifampin-400 mg ofloxacin-100 mg of minocycline in addition to self-administered 100 mg dapsone and 50 mg of clofazimine daily for 12 months. The treatment was then stopped and patients were followed up on placebo. This study reports the preliminary results after 2.5 to 3.5 years of post-treatment follow up. The drugs were well tolerated, the clinical response to the treatment was very good, and there was no case of treatment failure. Bacteriologically 25 out of the total 70 patients available for follow up were still positive at the end of 1 year of treatment. These patients continued to progress satisfactorily and four patents were still positive at the end of 2 years. No growth was observed in the normal mouse foot pad after 1 year of therapy. No bacillary ATP was detected in the biopsy tissues after 1 year. While no *M. leprae*-specific rRNA was detectable in any of the specimens after 1 year of treatment, weak PCR signals were detectable in 3/57 specimens at that period. In the follow up available no patient has relapsed. The patients are being followed up on placebo and longer follow up is required to draw firm conclusions.—Authors' Abstract

Mahajan, P. M., Jadhav, V. H., Jogiakar, D. G. and Mehta, J. M. Intranasal administration of fusidic acid cream in leprosy. Indian J. Lepr. **72** (2000) 451–455.

The effect of local treatment of nostrils with fusidic acid cream was investigated in 30 previously untreated, lepromatous leprosy patients. The cream was applied in the nostrils, after flushing the nostrils with normal saline, twice a day for a period of 4 weeks. It was found that 20 mg/g of sodium fusidate was effective in reducing the morphological index of the nose-blow smear to zero in 2 weeks in a majority of the patients. No untoward side effect was seen in any of the patients. Such nasal treatment along with multidrug therapy may help in reducing the patient's level of infectiousness to their contacts, since the nose is recognized to be an important portal of exit of *M. leprae*.—Authors' Abstract

Clinical Sciences

Amenu, A., Saunderson, P., Desta, K. and Byass, P. The pattern of decline in bacillary index after 2 years of WHO recommended multiple drug therapy: the AMFES cohort. Lepr. Rev. **71** (2000) 332–337.

With effective antibiotic treatment, the bacillary index (BI) in multibacillary leprosy patients declines over a number of years. This can be quantified as a rate of decline in log-units per year or as the time until smear negativity is reached. In the AMFES cohort 220 cases had data on the changes in their BI over time, while 170 cases are documented until smear negativity. The average BI at the start was 3.3 (S.D. 1.5; range 0.3–5.5) and the mean rate of decline was 0.85 units per year (median 0.7 units per year); in the first 2 years after diagnosis, the mean rate of decline was 1.15 units per year. The rate of decline was not related to any clinical features of the disease except delay in diagnosis: patients presenting for treatment early had a significantly faster rate of clearing the bacilli (adjusted relative risk 2.3; 95% CI 1.0–5.1). Fifty-eight percent of the cases took longer than 3 years to reach smear negativity, but this time interval is largely determined by the initial BI and classification, making it a less useful indicator of bacterial clearance. More severe impairment at the start of treatment was associated with a faster return to smear negativity, for which no obvious explanation can be given. Reversal reactions, which occurred in 25% of the cases reviewed, are not associated with a more rapid clearance of bacilli.—Authors' Summary

Brandsma, J. W. Monitoring motor nerve function in leprosy patients. Lepr. Rev. **71** (2000) 258–267.

Manual muscle strength testing has an important function in the management of leprosy patients. Its importance was first recognized in the 1960s, especially when following patients who were started on steroid treatment to monitor the nerve function and the effect of treatment. In those days, and still in many centers today, many or all muscles were tested that are innervated by the nerves that can be at risk in leprosy. The author argues that not all muscles innervated by the nerves at risk need to be tested and also that many muscles cannot be tested in isolation. A muscle charting form is presented which is suitable for screening purposes, and that also allows for more detail when motor function is impaired.—Author's Summary

Broekhuis, S. M., Meima, A., Koelewijn, L. F., Richardus, J. H., Benbow, C. and Saunderson, P. R. The hand-foot impairment score as a tool for evaluating prevention of disability activities in leprosy: an exploration in patients treated with corticosteroids. Lepr. Rev. **71** (2000) 344–354.

The hand-foot (HF) impairment score in leprosy patients is the sum of the WHO disability grades for hands and feet. This retrospective study explored the possibility of using the HF score for evaluation of the effectiveness of corticosteroid treatment programs for nerve function impairment (NFI). Changes in the score were compared with changes in sensory testing (ST) and voluntary muscle testing (VMT) for 42 leprosy patients who received corticosteroid treatment. The WHO grade did not change in 30/60 (50%) of extremities gaining, and in 4/10 (40) extremities losing sensation and/or muscle strength. However, 18/24 (75%) patients with a definite gain in function improved in HF score, while the HF score remained unchanged in 10/11 (91%) patients with no change in nerve function. Five patients with impairment in multiple extremities showed both gain and loss of sensation and/or muscle strength in the same or different extremities. Overall, improvement, deterioration and absence of change in NFI, as indicated by changes in ST and VMT, were reflected correctly by the HF score in 28 (76%) of the remaining 37 patients. It was also shown that the HF score does not give appropriate information on the extent of the effect of corticosteroid treatment. This study illustrates that the HF score cannot be used to support management of corticosteroid treatment of individual patients, but indicates this score to be a promising device for the evaluation of the effectiveness of corticosteroid treatment programs. This study used the HF score because information on (changes in) eye impairment was not considered reliable. However, in principle, we consider the EHF score, which is the sum of the WHO disability grades for hands, feet and eyes, preferable for evaluation purposes. We strongly recommend further validation of the EHF score as a tool for evaluation of corticosteroid treatment programs for patient groups with different distributions of NFI through prospective studies.—Authors' Summary

Dacas, P., Picanso, M., Mouchaileh, G., Percegona, L., Schultz, M. T., Silva, M. G. B. and Skare, T. L. [Autoantibodies and rheumatic manifestations in patients with Hansen's disease.] An. Bras. Dermatol. **75** (2000) 553–561.

Background. Autoantibodies, in the form of rheumatoid factor (RF) and antinuclear factor (ANF), are found in varying proportions in patients with Hansen's disease (HD) depending on the population under study. Articular manifestations are also very common among these patients and rank third in the list of most frequent complaints.

Objectives. Evaluate the incidence of rheumatoid factor and antinuclear factor among the local population (southern Brazil) of HD patients and study its possible correlation with articular complaints.

Material and methods. In all, 120 patients with HD there studied (77 with the lepromatous form and 43 with the tuberculoid form) together with a control group of 60 healthy individuals. The population studied completed a questionnaire and underwent a

physical examination with regard to articular complaints (arthralgia, arthritis, tendinitis and nodose erythema), plus tests for rheumatiod factor (RF) (by nephelometry) and antinuclear factor ANF (by indirect immunofluorescence).

Results. With regard to the ANF, 55.8% of the patients with HD and 16.7% of the controls tested positive for ANF ($p < 0.0001$), while for rheumatoid factor 35% of the HD carriers and 10% of the control group tested positive ($p < 0.0007$). Of those patients with HD, 68.3% presented inflammatory articular complaints compared to 1.7% among the control group. Among the HD group, 15.8% had articular complaints and were positive for both the autoantibodies against 5.8% in the control group that were also positive for both antibodies though without the articular complaints.

Conclusions. The population of HD carriers in the south of Brazil has significantly higher levels of autoantibodies than the normal population. However there is no correlation between the presence of these autoantibodies and rate of articular complaints.—Authors' English Summary

Gebre, S., Saunderson, P. and Byass, P. Relapses after fixed duration multiple drug therapy: the AMFES cohort. Lepr. Rev. **71** (2000) 325–331.

Relapse rates after multiple-drug therapy (MDT) have been low, although there remains a concern about the possibility of late relapse in those with an initially high bacterial load. In all, 502 patients in the AMFES cohort completed fixed-duration MDT and are included in this report. There have been no confirmed relapses in the AMFES cohort in a follow-up period of up to 8 years after completion of treatment, even in the 57 cases with an initial average bacillary index of ≥4.0, 20 of whom have been followed for more than 5 years after ceasing MDT. Methods of diagnosing a relapse are discussed.—Authors' Summary

Gebre, S., Saunderson, P., Messele, T. and Byass, P. The effect of HIV status on the clinical picture of leprosy: a prospective study in Ethiopia. Lepr. Rev. **71** (2000) 338–343.

No major interaction between HIV infection and leprosy has been documented. The ALERT MDT Field Evaluation Study (AMFES) has allowed the examination of possible interactions in a prospective manner, although the total number of HIV-positive individuals was not high at 22 (3.8%) of 581 patients tested. There was an excessive number of deaths in the HIV-positive group: 27% compared with 5.7% in the HIV-negative group, although the causes of death were not recorded (relative risk 4.8; 95% CI 2.2–10.2). HIV-positive individuals had a higher risk of ENL reactions (relative risk 5.2; 95% CI 1.7–15.9). Reversal reactions and neuritis (both acute and chronic) were not significantly influenced by HIV status, although there was a possible increase in recurrent reversal reactions in HIV-positive cases (relative risk 2.2; 95% CI 0.98–4.7). There was no evidence to suggest an increased risk of developing leprosy or of developing multibacillary rather than paucibacillary disease. There was no association between HIV positivity and the development of impairment.—Authors' Summary

Heynders, M. L., Meijs, J. J. and Anderson, A. M. Towards an understanding of non-compliance; an assessment of risk factors for defaulting from leprosy treatment. Lepr. Rev. **71** (2000) 369–376.

Within the Eastern Leprosy Control Project of Nepal, a retrospective case control study looked for simple factors that might be used operationally to predict noncompliant behavior in patients. Patients with these factors would then become the targets of measures such as intensified health education messages and home visits in order to reduce the risk of defaulting. A study of 1442 patient cards (half defaulters, half treatment completed) revealed occasional small but significant demographic and clinical differences, but none was of a sufficient magnitude to be operationally useful. Review of the attendance of patients in the first few months of treatment suggested that eventual defaulting was strongly associated with irregularity from the commencement of treatment. It is possible that an early indicator based on attendance over the first

months can be used to target patients who are in danger of noncompletion of treatment.—Authors' Summary

Illarramendi, X., Jardim, M. R., Sales, A. M., Nery, J. A. C. and Sarno, E. N. Acro-osteolysis prior to diagnosis of leprosy. Lepr. Rev. **71** (2000) 382–387.

Acro-osteolysis (bone resorption) has been observed in a heterogeneous group of congenital and acquired bone disorders. Leprosy is the main cause of peripheral neuropathy leading to acro-osteolysis in endemic countries. Pure neuritic leprosy, a less common form of the disease, is difficult to diagnose. Two unrelated leprosy patients with acropathy whose disease began as pure neuritic are discussed.—Authors' Summary

Kunst, H. Predisposing factors for recurrent skin ulcers in leprosy. Lepr. Rev. **71** 2000) 363–368.

This study was designed to determine the factors associated with recurrence of leprosy ulcers. Between April and August 1992, 55 consecutive leprosy patients admitted with skin ulcers were studied. Factors predisposing to recurrence, e.g., patient's age, disease duration, ulcer site, ulcer depth and physical deformity (taking into account neuromuscular and skeletal damage) were evaluated. Ulcer recurrence occurred in 40/55 (75%) patients. Recurrent ulceration was associated with location in the lower extremity ($p = 0.02$), where recurrences were more common in the midfoot and heel ($p = 0.01$). Recurrence was also associated with severity of physical deformity ($p = 0.01$), which increased the odds of recurrent ulceration by 4.2 times (95% confidence interval, 1.01—18.3). The severity of physical deformity itself was associated with the age of the patient ($p = 0.04$) and the disease duration ($p = 0.02$). In conclusion, there is a need to focus on identification of risk factors for recurrent leprosy ulceration. Targeted prevention strategies would be required if morbidity associated with recurrent skin ulceration is to be avoided.—Author's Summary

Naafs, B. Leprosy and HIV: an analysis. Hansen. Int. **25** (2000) 63–66.

After the introduction of HIV in the community, the number of patients with tuberculosis increased. Many of the HIV-infected patients suffer from clinical tuberculosis. Other mycobacterial infections too have an increased incidence among the HIV-infected patients, but not so leprosy. Many researchers have looked into this observation, however, with conflicting results. But a major increase in leprosy prevalence among HIV-infected patients was never encountered, nor a significant increase of HIV seroprevalence among leprosy patients.

In Africa during the past 30 years a continuous fall in the leprosy incidence was seen. However in recent years the decline seems to come to a halt and in some areas an increase is observed. The author speculates that *M. leprae* does not cause clinical disease in already HIV-infected patients, since *M. leprae* is virtually nontoxic and needs a more-or-less functioning CMI to cause clinical disease. However, the bacterium will multiply, the patient becoming a multibacillary carrier contributing to the infective mycobacterial pool. The non-HIV-infected persons then have more chance to be infected and may develop clinical leprosy since they have a functioning CMI.

The author therefore forecast an increase in leprosy incidence over the coming decade in countries like Brazil, with endemic leprosy, where at present HIV finds a foothold.—Author's Summary

Nabakumar Singh, T., Nandakishore, T. and Lokendro Singh, K. Leprosy with HIV infection in Manipur. Indian J. Dermatol. Venereol. Leprol. **66** (2000) 39–40.

A 27-year-old, unmarried male presented with typical clinical features of lepromatous leprosy which was confirmed by slit-skin smear and histopathological examinations. He also had a history of intravenous use of heroin and tested positive for HIV-I antibody by ELISA and Western blot. This is the first case report of co-infection of leprosy and HIV from Manipur.—Authors' Abstract

Nery, J. A. C., Perisse, A. R. S., Sales, A. M., Vieira, L. M. M., Souza, R. V., Sampaio, E. P. and Sarno, E. N. The use of pentoxifylline in the treatment of type 2 reactional episodes in leprosy. Indian J. Lepr. **72** (2000) 457–467.

It has been suggested that erythema nodosum leprosum (ENL) is associated with enhanced production of tumor necrosis factor-alpha (TNF-α), resulting in increased inflammation of the skin and nerve function impairment. Thalidomide and steroids are the major drugs used in the treatment of ENL, but due to the serious problems associated with their use, alternative therapeutic interventions are being considered. In the present retrospective study, the authors report their clinical observations on the effect of pentoxifylline (PTX) in the treatment of ENL. Parameters, such as the clinical involution of reactional lesions, the regression of the inflammatory symptoms associated with the lesions, and the impact on the systemic symptoms common to ENL were assessed at regular intervals during PTX therapy.

It was found that PTX therapy led to total elimination of systemic symptoms within the first week of treatment. This improvement was maintained until the end of the study (60 days of treatment). Moreover, the evolution of nodular lesions showed a 100% improvement within the first 14 days of treatment. However, by the 60th day, worsening of the lesions was noted in 20% of the cases.

The impression is that PTX is well tolerated, and it may be used for improving the patient's clinical condition during ENL reaction. Nevertheless, a randomized, double-blind, controlled trial to compare the effects of the widely accepted thalidomide and the yet untested pentoxifylline for treatment of type 2 reaction is still necessary.—Authors' Abstract

Nunez Marti, J. M. [Dental occlusion in leprosy patients.] Rev. Leprol. Fontilles **22** (2000) 577–582. (in Spanish)

Dental occlusion is the reciprocal position in which the teeth from both arches are left when they come into contact, develop maximum strength, exert pressure on the molars and the mandibular condylus is in the normal position. In this position, the teeth have the maximum amount of contact and mastication is in line with the temporomandibular articulation and neuralmuscular system that together form the stomatological system. If this unit is to be functional it must be completely balanced. Patients with Hansen's disease present disturbance in this balance because there is atrophy in the nasal septum that originates oral breathing with descompensation in muscular growth and development that affects the stomatological system together with severe problems.—Author's English Summary

Opromolla, D. V. A., Tonello, C. J. S. and Fleury, R. N. [Borderline leprosy and HIV infection.] Hansen. Int. **25** (2000) 54–59. (in Portuguese)

A young Caucasian woman presented with pain in the left leg and an erythematous plaque on the left upper arm with paresthesia and a positive BI (bacilloscopic index). She was diagnosed BT Hansen's disease (HD) and treatment was initiated with WHO/MDT for MB. Three months after the start of treatment she presented with a reactional episode (type 1 reaction) with increased infiltration and more severe edema of the initial lesion, and appearance of several other erythematous plaques disseminated over the body. On that occasion, pain in the leg had persisted and did not seem to be related to HD. Since the electromyography was inconclusive, an MRI was done; it showed a neurinoma at the level of the fourth lumbar vertebra. The tumor was surgically excised and the pain disappeared. Histopathology showed it to be a benign schwannoma.

Shortly after the reactional episode, the patient was found to be HIV positive with a marked decrease in CD4+. In spite of that she did well on both treatments and the HD lesions disappeared. After being released from leprosy treatment, the patient suffered a new reversal reaction coinciding with changes in the therapeutic scheme for the HIV infection. The treatment for HD was started again, with rifampin, ofloxacin and dapsone. These were given for 4 months and the patient improved.

The authors point out that it is usually taken for granted that nerve disturbances in a leprosy patient are due to leprosy. If careful examinations are not done, other causes, like in this patient will be missed. They also comment on the appearance and pathogenesis of type 1 reaction in this and in other cases. According to the authors, these reactional episodes are frequently related to intercurrent conditions, such as lung cancer, tuberculosis, diabetes and hepatitis, which change the immune status of the individual. In this case, the association with HIV infection fits this statement because of the changes in immune status it causes. The type 1 reaction in this case would be the result of multiplication of persistent bacilli in the tissue, which survived treatment and the immune system. These bacilli will expose antigens and give rise to a hypersensitivity reaction. When bacillary multiplication and reaction occur after treatment, the bacilli are usually easily eliminated by the host because of its small number, so that treatment is not needed. In cases in which the cellular immunity is not sufficient, some bacilli may survive and those remaining bacilli would, as a consequence, be responsible for more reactional episodes. This would most likely have happened in the patient presented, who was immunosuppressed because of the HIV infection. For that reason, her treatment was reinitiated. The rifampin and ofloxacin used in the new therapeutic regimen, both bactericidal drugs, were chosen to eliminate bacilli that could eventually multiply. The 4 months' duration of treatment is based on the assumption that rifampin, a highly bactericidal drug with rapid action, would destroy most of the bacilli but only temporarily interrupts multiplication of the remaining ones, thus preventing the slow action of ofloxacin. If treatment was discontinued right after the first month, the persistent bacilli not killed by rifampin would start to multiply sooner or later. Dapsone was added to the regimen because the authors intended to keep it indefinitely but, nonetheless, they decided to withdraw it and wait for the evolution of the patient.

Because little is known about the influence of AIDS on the response to treatment and clinical changes in HD, this patient is demonstrated as an association between the two diseases.—Authors' English Summary

Rodriques A. L. P., de Almeida. A. P., Rodrigues, B. de F., Pinheiro, C. A., Borges, D. S., de Mendonca, M. L. H., da Silva, V. E. F. and Goulart, I. M. B. Occurrence of late lepra reaction in leprosy patients: subsidies for implementation of a specific care program. Hansen. Int. **25** (2000) 17–25.

Leprosy would be an ordinary disease; however, it is not due to its reactive episodes with risk of disability maintaining the stigma related to the leprosy. These reactions and the potential loss of the neural function may happen before, during and after treatment through multidrug therapy (MDT). Release from treatment results from the number of doses and regularity of the treatment, when the patient leaves the coefficients of prevalence. The aim was to evaluate the magnitude of late leprosy reactions and the operational subjects referring to attendance quality. Charts of the 149 patients that received discharge for leprosy from 1994 to 1999 in CSE Jaraguá–UFU, Brazil were revised using the Record of Inquiry of Alterations After Cure of the Brazilian Ministry of Health. Of these patients, 34 (23%) presented late reaction, of which 11.76% were paucibacillary (PB) and 88.23% were multibacillary (MB). An average of 3 reactive episodes for borderline patients and 4 episodes for lepromatous patients occurred. Of PB patients, 100 presented with reversal reaction (RR). Among MB, 50% presented with RR, 40% erythema nodosum leprosum (ENL), 7% isolated neuritis and 3% mixed reaction. In 91% of the cases, the first reactive episode happened in the first year after treatment. There was a positive correlation among medium bacillary index (BI) at diagnosis and the number of reaction episodes during treatment and after release. Among patients with a late reaction, 97% used prednisone and 32% thalidomide, meaning 22% and 8% from the total, respectively. Grades 2 and 3 disability happened in MB patients of the economically active age. The need of implementing leprosy control programs for that new group of patients with warranty of treatment, personnel training for simplified monitoring of neuritis and handling of the adverse effects of corticosteroids therapeutics, seeking the prevention of disabilities is discussed.—Authors' Summary

Ruaro, R. T., Mesquita, L. A. F., Tokarski, M. C., Ioshii, S. O., Tarastchuck, A. V. and Kluppel, E. T. [Leprosy associated with non-Hodgkin's lymphoma: a case report.) An. Bras. Dermatol. **75** (2000) 595–598. (in Portuguese)

The authors report a case of a 55-year-old woman with a 5-month history of low-grade non-Hodgkin's lymphoma presenting with erythematous-violaceous hypoesthetic plaques on her right thigh. Histologic evaluation showed granulomatous lymphohistiocitic dermal infiltrate with acid-fast, red-staining bacilli. The diagnosis of borderline leprosy in association to non-Hodgkin's lymphoma was made. The rarity of this association which has an immunologic basis in common motivated us to publish this case.—Authors' English Summary

Saunderson, P., Gebre, S. and Byass, P. ENL reactions in the multibacillary cases of the AMFES cohort in central Ethiopia: incidence and risk factors. Lepr. Rev. **71** (2000) 318–324.

Erythema nodosum leprosum (ENL), or type 2 leprosy reactions, are an important complication of multibacillary leprosy. The AMFES cohort includes 300 new multibacillary cases that have been followed for up to 10 years from the start of treatment in central Ethiopia. Sixteen (5.3%) patients had ENL reactions. The incidence of ENL was maximal in the second and third years after the start of treatment, reaching 6.9 episodes per 100 person-years at risk. Factors associated with being lepromatous [LL classification and a high bacillary index (BI)] gave an increased risk of developing ENL; in the univariate analysis, LL classification gave a relative risk of 3.6 (95% CI 1.3–10) and a BI of 6 gave a relative risk of 8.6 (95% CI 2.3–32) for the development of ENL. HIV co-infection was found to be a risk factor in this cohort, but as the numbers involved are small (only two HIV-positive patients had ENL), this finding must be confirmed in larger studies. Ten of the 16 cases had recurrent episodes and five had at least five episodes occurring over a period of more than 2 years. The management and prognosis of ENL reactions are discussed.—Authors' Summary

Saunderson, P., Gebre, S. and Byass, P. Reversal reactions in the skin lesions of AMFES patients: incidence and risk factors. Lepr. Rev. **71** (2000) 309–317.

Reversal reactions affect the skin and/or nerves of leprosy patients. This paper looks at reversal reactions involving the skin in 594 new patients in central Ethiopia followed for between 6 and 11 years after the start of treatment. The incidence of reversal reaction declines steadily after the start of treatment, but the first episode may occur as long as 5 years after diagnosis in both paucibacillary (PB) and multibacillary (MB) patients. Recurrent episodes occurred up to 6 years after diagnosis. PB patients were at greatest risk for reversal reaction in the first year after diagnosis and MB patients in the first 4 years. The highest incidence rate was 18 episodes per 100 person-years in MB patients during the first year after diagnosis. The ratio of the incidence rates for the first 3 years in MB versus PB patients is 2.4 (95% CI 1.6–3.8). This study confirms that starting effective treatment and borderline classification are risk factors for reversal reactions. Pregnancy/delivery in the 6 months prior to diagnosis was a significant risk factor for presenting with a reversal reaction [relative risk (RR) 5.9 (95% CI 2.1–16.5)], but later pregnancies were not associated with an increased risk. Being female was a significant risk factor for the late appearance of the first episode of reversal reaction. Having a reversal reaction in the first year after diagnosis was a highly significant risk factor for the development of later reactions [RR in PB cases 11.9 (95% CI 3.4–41.7); in MB cases 6.4 (95% CI 3.8–10.6)]. Being HIV positive was a risk factor for developing recurrent reversal reactions, although only 3 out of 29 recurrent cases were HIV positive [RR 2.7 (95% CI 1.4–5.1)].—Authors' Summary

Saunderson, P., Gebre, S., Desta, K. and Byass, P. The ALERT MDT Field Evaluation Study (AMFES): a descriptive study of leprosy in Ethiopia. Patients, methods and baseline characteristics. Lepr. Rev. **71** (2000) 273–284.

The ALERT MDT Field Evaluation Study (AMFES) is a long-term prospective study of 650 patients (594 new cases and 56 relapses after dapsone monotherapy) treated with fixed-duration multiple-drug therapy (MDT) as recommended by WHO. Follow up has continued for up to 11 years from the start of treatment. This paper presents the methodology of the study and the baseline characteristics of the cohort, while accompanying papers examine the incidence of, and possible risk factors for, the various complications of leprosy, including relapse, reactions and nerve function impairment. The methods of diagnosis, classification and treatment with MDT are described; nerve function was assessed at every visit to the clinic using a standardized methodology so that reactions and new impairment could be detected early and treated. Eighty-four percent of new cases had at least one thickened nerve, with the ulnar nerve most commonly involved. Seventy-seven percent of cases completed treatment and only one adverse reaction to the MDT drugs was noted. Twenty-eight percent of all patients were given steroids at one time or another, almost always for new nerve function impairment, and 3% of these developed significant complications of steroid treatment. Twenty-nine patients (5%) received hospital care, including 14 patients who underwent major surgery. Sixty-one percent of the women over 19 years of age had at least one pregnancy, but pregnancies were much less common after leprosy was diagnosed.—Authors' Summary

Saunderson, P., Gebre, S., Desta, K., Byass, P. and Lockwood, D. N. J. The pattern of leprosy-related neuropathy in the AMFES patients in Ethiopia: definitions, incidence, risk factors and outcome. Lepr. Rev. **71** (2000) 285–308.

The ALERT MDT Field Evaluation Study (AMFES) began in 1988 and followed patients prospectively for up to 10 years after release from treatment (RFT). This paper presents the findings from this cohort with regard to neuropathy and nerve damage. Five-hundred-ninety-four new cases of leprosy are included in the study, 300 multibacillary (MB) and 294 paucibacillary (PB) cases. Fifty-five percent of patients had some degree of impairment at diagnosis and a further 73 (12%) developed new nerve function impairment (NFI) after starting multiple drug therapy (MDT). The overall incidence rate for neuropathy was 39 episodes per 100 PYAR in the first year after diagnosis, gradually declining to 12 episodes per 100 PYAR in the sixth year. In those patients without impairment at diagnosis, the incidence rate of neuropathy was 25 episodes per 100 PYAR for MB cases and 11 per 100 PYAR for PB cases in the first year; in 33% of MB cases whose first episode of neuropathy occurred after diagnosis, that first episode took place after the first year, or after the normal period of treatment with MDT. Seventy-three patients with neuropathy developing after diagnosis are reported more fully: 34 (47%) had only one nerve involved and of these 25 (73%) had a single, acute episode of neuropathy. Nine (27%) had further episodes. Thirty-nine (53%) had more than one nerve involved and of these 16 (41%) had a single, acute episode, while 23 (59%) had further episodes. The terms "chronic" and "recurrent" neuropathy are defined and used to describe the pattern of neuropathy in those with repeated attacks. In patients with no impairment at the start of the study, treatment with steroids resulted in full recovery in 88% of nerves with acute neuropathy but in only 51% of those with chronic or recurrent neuropathy. The median time to full recovery from acute neuropathy was approximately 6 months, but in a few cases recovery occurred gradually over 2–3 years. Severe neuropathy was less likely to be followed by a complete recovery than mild or moderate neuropathy. Forty-two percent of nerves with acute neuropathy that were not treated with steroids also fully recovered. In the group of patients who were thought to have old, permanent impairments at diagnosis, full recovery of nerve function occurred in 87/374 (23%) of the nerves involved. The overall outcome is illustrated by examining the average EHF score for groups of patients. Patients with no new neuropathy after diagnosis show a gradual improvement in their EHF score, while those with any episodes of neuropathy after diagnosis show a gradual deterioration after completion of MDT. Possible explanations

for these findings are discussed. Risk factors for neuropathy, for chronic and recurrent neuropathy, and for a poor outcome 5 years after release from treatment are examined. Impairment at diagnosis was the main risk factor for a poor outcome, accompanied by the occurrence of chronic/recurrent neuropathy or a reversal reaction.—Authors' Summary

Schon, R., Leekassa, R., Gebre, N., Sundqvist, T., Bizuneh, E. and Britton, S. High dose prednisolone treatment of leprosy patients undergoing reactions is associated with a rapid decrease in urinary nitric oxide metabolites and clinical improvement. Lepr. Rev. **71** (2000) 355–362.

Evidence is accumulating that nitric oxide (NO) produced by macrophages has a role in the pathogenesis of reactions in leprosy. We followed the urinary levels of the metabolites of NO [nitrite (NO_2^-) and nitrate (NO_3^-)] and the clinical response to prednisolone treatment in leprosy patients (N = 9) admitted to ALERT leprosy hospital, Addis Ababa, Ethiopia, because of reversal reaction (RR) or erythema nodosum leprosum (ENL). In untreated reactional leprosy patients, the levels of urinary NO metabolites (1645 ± 454 μM, N = 9, ENL = 4, RR = 5) decreased significantly 2 weeks after high-dose prednisolone treatment (1075 ± 414 μM, $p < 0.05$), and remained stable for 4 (895 ± 385 μM, $p < 0.02$) and 6 weeks following treatment initiation (1048 ± 452 μM, $p < 0.02$). This decrease was also present when the reactional patients were subdivided according to the type of reaction (ENL, RR) and coincided with a clinical improvement. In patients showing a poor clinical response to steroids, no or minor effects on the urinary NO metabolite levels were observed. We conclude that there is a correlation between the decrease in urinary NO metabolites and a favorable clinical response after high-dose prednisolone treatment of reactional leprosy patient.—Authors' Summary

Immuno-Pathology

Cobelens, P. M., Heijnen, C. J., Nieuwenkuis, E. E. S., Kramer, P. P. G., vander Zee, R., van Eden, W. and Kavelaars, A. Treatment of adjuvant-induced arthritis by oral administration of mycobacterial hsp65 during disease. Arthritis Rheum. **43** (2000) 2694–2702.

Objectives. Oral administration of antigen prior to disease induction has been shown to induce peripheral tolerance in several experimental autoimmune diseases. However, the clinical benefit of pretreatment with antigens is limited. The aim of this study was to investigate whether adjuvant-induced arthritis (AIA) could be treated by oral administration of mycobacterial heat-shock protein 65 (hsp65) during ongoing disease.

Methods. AIA was induced in Lewis rats by immunization with *Mycobacterium tuberculosis* in Freund's incomplete adjuvant. Oral feeding of hsp65 in the presence or absence of soybean trypsin inhibitor (SBTI) was started on day 11 after immunization. Arthritis was monitored visually, and joint pathology was examined radiologically.

Results. Oral treatment with hsp65 during ongoing disease significantly reduced the activity of AIA. However, treatment with hsp65 was only successful when SBTI was co-administered to prevent breakdown of the hsp65. The beneficial effect of hsp65/SBTI treatment during AIA was also represented by a clear reduction of articular destruction, as visualized by radiography. Moreover, feeding hsp65/SBTI resulted in a lower number of both spleen and mesenteric lymph node (MLN) cells expressing the costimulatory molecule CD80 (B7-1). The number of cells expressing CD86 (B7-2) was not altered. Furthermore, MLN cells from AIA animals treated with hsp65/SBTI contained a lower number of T cells expressing the activation marker CD134 (Ox-40). In addition, treatment with

hsp65/SBTI was accompanied by an increased proliferative response of spleen cells to the hsp65 antigen *in vitro*. Moreover, hsp65/SBTI-treated rats showed less hsp65-specific interferon-gamma and increased production of interleukin-10.

Conclusion. Ongoing AIA activity can be seduced by oral administration of hsp65 only when protein breakdown in the gastrointestinal tract is inhibited.—Authors' Abstract

Doffinger, R., Altare, F. and Casanova, J. L. Genetic heterogeneity of Mendelian susceptibility to mycobacterial infection. Microbes Infect. **2** (2000) 1553–1557.

Mendelian susceptibility to poorly virulent mycobacterial species, such as bacillus Calmette-Guerin (BCG) and environmental nontuberculous mycobacteria (NTM), is a phenotypically heterogeneous syndrome. It has therefore long been suspected to be genetically heterogeneous. In the past 5 years, this prediction has been confirmed and different types of mutations (dominant or recessive, nonfunctional or hypofunctional) in four genes (IFNGR1, IFNGR2, IL12B, IL12RB1) have revealed both allelic and nonallelic heterogeneity. The eight disorders resulting from these mutations are genetically different but immunologically related, since impaired IFN-gamma-mediated immunity is the common pathogenic mechanism accounting for mycobacterial infection in all patients. The severity of the phenotype depends on the genotype. Complete IFN-gamma R1 and IFN-gamma R2 deficiencies predispose patients to a more severe clinical course than partial IFN-gamma R1 and IFN-gamma R2 deficiencies and complete IL-12 p40 and IL-12R beta-1 deficiencies.—Authors' Abstract

Goulart, I. M. B., Mineo, J. R. and Foss, N. T. Production of transforming growth factor-beta 1 (TGF-beta 1) by blood monocytes from patients with different clinical forms of leprosy. Clin. Exp. Immunol. **122** (2000) 330–334.

In the present study, the concentration of TGF-beta1 secreted by adherent cells isolated from human peripheral blood mononuclear cells (PBMC) and either stimulated with phenolic glycolipid-I (PGL-I) or lipopolysaccharide (LPS) or left unstimulated was determined by ELISA. The cells were isolated from untreated patients with different clinical forms of leprosy and healthy individuals. The adherent cells exhibited spontaneous release of TGF-beta1 in all clinical forms of leprosy and in healthy individuals; however, lepromatous leprosy/borderline leprosy (LL/BL) patients presenting erythema nodosum leprosum (ENL) displayed significantly higher concentrations of TGF-beta1 than either the other patients studied or the controls. These high TGF-beta1 levels were consistently observed when LL/BL ENL cells were stimulated with PGL-I or LPS, and even in the absence of a stimulus ($p < 0.01$). The most significant differences in TGF-beta1 levels were observed when comparing the results in the presence of PGL-I from ENL with, in order of significance: tuberculoid leprosy (TT) patients ($p < 0.001$), LL/BL patients without ENL ($p < 0.01$), healthy individuals ($p < 0.01$) and borderline-borderline/borderline-tuberculoid (BB/BT) patients with reversal reaction (RR) ($p < 0.01$). The BB/BT patients produced equivalent levels of TGF-beta1 compared with LL/BL patients without ENL, for all types of stimuli ($p > 0.05$). In contrast, TT patients produced the lowest levels of TGF-beta1 among all the subjects studied (both patients and healthy controls), especially following PGL-I stimulation ($p < 0.001$, and $p < 0.05$, respectively). In conjunction with our previous data regarding TGF-beta1 expression in dermal lesions, it appears that TGF-beta1 probably plays different roles in leprosy: (i) to mediate a suppressive action locally, associated with the presence of PGL-I , and (ii) to induce proinflammatory effects when secreted systemically by monocytes, thereby acting as a modulatory cytokine in the acute inflammatory reactions of ENL and associated with the Th2 immune response in multibacillary forms of leprosy.—Authors' Abstract

Guerin, I. and de Chastellier, C. Disruption of the actin filament network affects delivery of endocytic contents marker to phagosomes with early endosome char-

acteristics: the case of phagosomes with pathogenic mycobacteria. Eur. J. Cell Biol. **79** (2000) 735–749.

Phagosomes containing live virulent mycobacteria undergo fusion with early endosomes, but they are unable to mature normally. Accordingly, they do not fuse with lysosomes. Although *M. avium*-containing phagosomes retain fusion and intermingling characteristics of early endosomes indefinitely, fusions with early endosomes are increasingly restricted as bacteria multiply. In addition, when endocytic tracers, such as horseradish peroxidase (HRP), are added to *M. avium*-infected macrophages at 1 or up to 15 days after infection, an atypical time course of acquisition of the tracer by the phagosomes is observed, i.e., a 10 to 20 min lag, instead of immediate acquisition as is typical for early endosomes (and phagosomes with early endosome characteristics). These events coincide with a marked disorganization of the actin filament network in *M. avium*-infected macrophages. In the present study, we have therefore addressed the following question: Do actin filaments play a role in fusion and intermingling of contents between early endosomes and immature phagosomes that undergo homotypic fusion with early endosomes? We examined the time course of acquisition of subsequently internalized endocytic marker (HRP) by early endosome-like pre-existing phagosomes, i.e., 2 hour-old phagosomes with either hydrophobic latex particles, virulent or avirulent *M. avium*, after depolymerization of the actin filament network with cytochalasin D or after repolymerization of the actin filament network with jasplakinolide, in cases where the network had been depolymerized (macrophages infected with *M. avium*, at 1 or up to 7 days after infection). By direct morphological observation at the electron microscope level and by a kinetic approach, we show here that depolymerization of the actin filament network with cytochalasin D delays acquisition of HRP; whereas repolymerization restores immediate acquisition of the marker. We conclude that the actin filament network is involved in fusion and intermingling of endocytic contents between early endosomes and early endosome-like phagosomes, and that disruption of this network by *M. avium* is the cause for the atypical acquisition of content marker by phagosomes containing these pathogenic mycobacteria.—Authors' Abstract

Holland, S. M. Treatment of infections in the patient with Mendelian susceptibility to mycobacterial infection. Microbes Infect. **2** (2000) 1579–1590.

Cytokines are increasingly used for the therapy of infections in patient populations with special defects in immunity (chemotherapy, bone marrow transplantation, chronic granulomatous disease). The recognition of multiple defects in the systems of the interferon-gamma (IFN-γ) receptor, interleukin-12 (IL-12) receptor and IL-12 p40 emphasizes the critical roles that cytokines play in preventing and clearing infection. The cases of patients with partially responsive IFN-γ receptors (autosomal dominant and partial defects) are ideal candidates for successful cytokine prophylaxis and therapy. Better understanding of the critical elements of the cytokine pathways may show us ways to circumvent these defects with complementary cytokine cascades.—Author's Abstract

Levin, M. and Newport, M. Inherited predisposition to mycobacterial infection: historical considerations. Microbes Infect. **2** (2000) 1549–1552.

Although one third of the world's population is estimated to be infected with *Mycobacterium tuberculosis*, only one tenth of infected individuals develop clinical disease. There is substantial epidemiological evidence that host genetic factors are important determinants of susceptibility to mycobacterial disease. This paper gives a historical context to the recent exciting advances in the field which have led to the identification of a number of human mycobacterial susceptibility genes.—Authors' Abstract

Marques, M. A., Mahapatra, S., Nandan, D., Dick, T., Sarno, E. N., Brennan, P. J. and Pessolani, M. C. V. Bacterial and host-derived cationic proteins

bind alpha 2-laminins and enhance *Mycobacterium leprae* attachment to human Schwann cells. Microbes Infect. **2** (2000) 1407–1417.

It has recently been demonstrated that laminin alpha2 chains present on the surface of Schwann cells are involved in the process of attachment of *Mycobacteria leprae* to these cells. In this study, a protein in the *M. leprae* cell wall that was found to be capable of binding alpha2-containing laminins (merosin) was isolated and characterized. The *M. leprae* laminin-binding protein was identified as a 21-kDa histone-like protein (hlp), a highly conserved cationic protein present in other species of mycobacteria. The gene that encodes this protein was PCR amplified, cloned, and expressed, and the recombinant protein was shown to bind alpha2-laminins. More significantly, when added exogenously, hlp, was able to greatly enhance the attachment of mycobacteria to ST88-14 human Schwann cells. The capacity to bind alpha2-laminins and to enhance mycobacterial adherence to Schwann cells was also found in other cationic proteins such as host-derived histones. Moreover, mutation in the hlp gene was shown not to affect the capacity of mycobacteria to bind to ST88-14 cells, suggesting that alternative adhesins and/or pathways might be used by mycobacteria during the process of adherence to Schwann cells. The potential role of hlp as a fortuitous virulence factor contributing to the pathogenesis *M. leprae*-mediated nerve damage is discussed.—Authors' Abstract

Moraes, M. O., Sarno, E. N., Teles, R. M. B., Almeida, A. S., Saraiva, B. C. C., Nery, J. A. C. and Sampaio, E. P. Anti-inflammatory drugs block cytokine mRNA accumulation in the skin and improve the clinical condition of reactional leprosy patients. J. Invest. Dermatol. **115** (2000) 935–941.

The aim of this study was to investigate in what ways *in vivo* anti-inflammatory treatment affects cytokine mRNA expression *in situ* in both erythema nodosum leprosum and reversal reaction patients. Serial biopsies were collected from the patients undergoing leprosy reactions before and during pentoxifylline (N = 7) or thalidomide (N = 3) treatment for erythema nodosum leprosum and prednisone (N = 3) for reversal reaction. Clinical evolution of the skin lesion was assessed during the study and semiquantitative reverse transcription-polymerase chain reaction was used to investigate cytokine mRNA expression at the lesion site. Results showed expression of interferon-gamma (IFN-γ), interleukin-6 (IL-6), IL-10, IL-12 p40, and tumor necrosis factor-alpha (TNF-α) in all patients tested at the onset of reactional episodes, but IL-4 mRNA was rarely detected in the lesions (N = 4). Follow-up analysis showed that, irrespective of the drugs used, TNF-α mRNA was diminished in 10 of the 13 patients tested. A concomitant decrease of mRNA accumulation was also observed for IFN-γ (9 of 11 patients), IL-6 (9 of 11), and IL-12 p40 (6 of 8). An inhibitory effect on IL-10 mRNA was likewise seen after thalidomide and pentoxifylline, but not subsequent to prednisone treatment. The data also demonstrated that cytokine mRNA inhibition correlates to the resolution of the inflammatory response *in situ* (N = 10); whereas the persistence/enhancement of cytokine message expression after treatment was associated with worsening of the skin condition, as seen in three erythema nodosum leprosum patients whose maintenance of local inflammation was accompanied by the appearance/persistence of IL-1 gene expression *in situ* subsequent to anti-inflammatory treatment. In summary, the participation of cytokines in leprosy inflammatory episodes seems to be directly associated with the patients' clinical evolution following therapy for reaction.—Authors' Abstract

Nath, I., Vemuri, N., Reddi, A. L., Jain, S., Brooks, P., Colston, M. J., Misra, R. S. and Ramesh, V. The effect of antigen presenting cells on the cytokine profiles of stable and reactional lepromatous leprosy patients. Immunol. Lett. **75** (2000) 69–76.

In view of varied reports on the Th1/Th2 paradigm in leprosy, we used a novel real time (RT) fluorogenic reverse transcriptase

based PCR (RT-PCR) to measure cytokine expression in peripheral blood cells from lepromatous leprosy patients with stable disease and those suffering from erythema nodosum leprosum (ENL; type 2) reactions. To evaluate the role of accessory cells in Th cell differentiation, co-expression of Th cytokines interferon gamma (IFN-γ) and interleukin (IL)-4 and regulatory cytokines IL-10 and IL-12 was compared in antigen-stimulated peripheral blood mononuclear cells (PBMC), cultures containing T cells reconstituted with autologous monocytes (MO) and cultures containing T cells reconstituted with autologous dendritic cells (DC); 7/8 stable lepromatous leprosy patients showed co-expression of both IFN-γ and IL-4, suggesting a Th0 or a combination of Th1 + Th2 subsets in PBMC. The RT-PCR demonstrated that stable lepromatous patients and patients in ENL had significantly higher levels of IFN-γ mRNA molecules compared to IL-4. In fact, 5/8 ENL patients had undetectable levels of IL-4 mRNA, with a skewing of the cytokine response toward a Th1-like profile. Consistent with this, IL-12 p40 mRNA molecules were significantly higher in the PBMC of ENL patients compared to stable lepromatous patients ($p < 0.01$). Reconstitution of purified T cells with autologous DC and MO from the stable lepromatous group resulted in downregulation of IL-4 ($p < 0.03$ for DC and $p < 0.02$ for MO) and IL-10 ($p < 0.01$ for DC and $p < 0.02$ for MO), and a consequent skewing toward a Th1 profile similar to that seen in ENL patients. The fact that accessory cells could alter the cytokine profile in the reconstituted cultures suggests that they may play a role in determining Th subset differentiation in chronic diseases, and may influence the immunological stability of such diseases.—Authors' Abstract

Ng, V., Zanazzi, G., Timpl, R., Talts, J. F., Salzer, J. L., Brennan, P. J. and Rambukkana, A. Role of the cell wall phenolic glycolipid-I in the peripheral nerve predilection of *Mycobacterium leprae*. Cell **103** (2000) 511–524.

The cell wall of pathogenic mycobacteria is abundant with complex glycolipids whose roles in disease pathogenesis are mostly unknown. Here, we provide evidence for the involvement of the specific trisaccharide unit of the phenolic glycolipid-I (PGL-I) of *Mycobacterium leprae* in determining the bacterial predilection to the peripheral nerve. PGL-I binds specifically to the native laminin-2 in the basal lamina of Schwann cell-axon units. This binding is mediated by the alpha 2LG1, alpha 2LG4, and alpha 2LG5 modules present in the naturally cleaved fragments of the peripheral nerve laminin alpha2 chain, and is inhibited by the synthetic terminal trisaccharide of PGL-I. PGL-I is involved in the *M. leprae* invasion of Schwann cells through the basal lamina in a laminin-2-dependent pathway. The results indicate a novel role of a bacterial glycolipid in determining the nerve predilection of a human pathogen.—Authors' Abstract

Sampaio, E. P., Oliveira, R. B., Warwick Davies, J., Neto, R. B. F., Griffin, G. E. and Shattock, R. J. T cell-monocyte contact enhances tumor necrosis factor-alpha production in response to *Mycobacterium leprae*. J. Infect. Dis. **182** (2000) 1463–1472.

Tumor necrosis factor-alpha (TNF-α) has been implicated as a key factor in inflammatory processes occurring in erythema nodosum leprosum (ENL). In the present study, the roles of soluble factors and contact-mediated interaction in the induction of enhanced TNF-α secretion in leprosy have been investigated. *In vitro* studies have demonstrated that *Mycobacterium leprae per se* is a poor stimulus for TNF-α production by purified monocytes obtained from normal subjects, although this could be enhanced by either exogenous interferon-gamma or cell contact with fixed activated T lymphocytes. Further investigations demonstrated that monocyte-T cell contact enhanced *M. leprae*-induced TNF-α production by peripheral brood mononuclear cells of ENL patients and was modulated by blocking antibodies to CD40L, CD69, and CD18. These results suggest that physical contact with T cells isolated from patients in a particular disease state (ENL) modulates monocyte function and may contribute

to the secretion of proinflammatory cytokines described in ENL.—Authors' Abstract

Senthil Kumar, K. S., Raja, A., Uma Devi, K. R. and Paranjape, R. S. Production and characterization of monoclonal antibodies to *Mycobacterium tuberculosis*. Indian J. Med. Res. **112** (2000) 37–46.

Background and objectives: Monoclonal antibodies (mAbs) against *Mycobacterium tuberculosis* H37Rv culture filtrate (CF) were raised by immunizing BALB/c mice and characterization was done. Attempts have been directed toward identifying mycobacterial antigens in biological fluids by employing polyclonal and monoclonal antibodies specific for *M. tuberculosis*. Immunohistologic studies, using mAbs for the localization of whole or fragmented bacilli in the biopsy specimens were also carried out.

Methods: Intrasplenic IS and intraperitoneal IP routes of immunization were compared. The mAbs were characterized for their isotype, binding specificity, nature of binding epitope, reactivity in immunoassays, etc.

Results: IS and IP routes of immunization were compared and IP was found supèrior. Ten mAbs designated TRC 1–10 were produced. Of these, 7 mAbs, TRC 1–7 reacted with the 30/31-kDa doublet (antigen 85 complex), TRC 8 with 12 kDa in addition to 30/31 kDa and TRC 9 and 10 with the 24 and 12 kDa antigens, respectively. Six mAbs were classified as broadly crossreactive and 2 showed limited crossreactivity. TRC 8 and 10 showed species specificity. Employing TRC 8 in sandwich ELISA, antigen was detected in sera from 17 of 25 pulmonary tuberculosis patients and 3 of 20 controls. TRC 8 was found to be useful in detecting antigens specifically in *M. tuberculosis*- and *M. leprae*-infected tissues by immunoperoxidase staining.

Interpretation and conclusion: TRC 8 was found to be restricted in its reactivity to the *M. tuberculosis* complex and *M. leprae*. TRC 8 may prove useful in immuno-diagnosis of tuberculosis.—Authors' Abstract

Xing, Z., Zganiacz, A. and Santosuosso, M. Role of IL-12 in macrophage activation during intracellular infection: IL-12 and mycobacteria synergistically release TNF-alpha and nitric oxide from macrophages via IFN-gamma induction. J. Leuk. Biol. **68** (2000) 897–902.

Interleukin-12 (IL-12) is believed to play an important role in cell-mediated immunity against intracellular infection primarily by acting on T and NK cells. Recent evidence has suggested, however, that IL-12 has broader cellular targets than previously thought. In this study, we examined the role of IL-12 in macrophage TNF-alpha and nitric oxide (NO) release by using an *in vitro* model of intracellular infection. IL-12 alone released relatively little TNF-alpha and NO; whereas live mycobacteria alone released TNF-alpha markedly but little NO from murine alveolar macrophages. However, IL-12 and mycobacteria together enhanced TNF-alpha and NO release synergistically. Because IL-12 and mycobacteria also released IFN-gamma from macrophages synergistically, and exogenous IFN-gamma with mycobacteria enhanced TNF-alpha and NO release synergistically, we examined the role of endogenous IFN-gamma in IL-12/mycobacteria-stimulated macrophage activation. Using macrophages from mice deficient in IFN-gamma, we found that IL-12/mycobacteria-enhanced macrophage TNF-alpha and NO release was mediated through endogenous IFN-gamma. We further demonstrated that IFN-gamma and mycobacteria together had a selective effect on macrophage cytokine release because they released TNF-gamma synergistically but not macrophage chemotactic protein-1 (MCP-1). These findings reveal that IL-12 can activate macrophages potently during intracellular infection, and this activating effect is mediated primarily through its effect on macrophage IFN-gamma release.—Authors' Abstract

Microbiology

Cho, S. N., Cellona, R. V., Villahermosa, L. G., Fajardo, T. T., Balagon, M. V. F., Abalos, R. M., Tan, E. V., Walsh, G. P., Kim, J. D. and Brennan, P. J. Detection of phenolic glycolipid I of *Mycobacterium leprae* in sera from leprosy patients before and after start of multidrug therapy. Clin. Diagn. Lab. Immunol. **8** (2000) 138–142.

A total of 100 untreated new leprosy patients were recruited prospectively and examined for the presence of phenolic glycolipid I (PGL-I) antigen in their serum specimens by dot enzyme-linked immunosorbent assay (ELISA) using rabbit anti-PGL-I antiserum. The presence of circulating PGL-I antigen was closely related to the bacterial indices (BI) of the patients. The PGL-I antigen was detectable in 27 (93.1%) of 29 patients with a BI of 4.0 or above and in 15 (68.2%) of 22 patients with a BI of 3.0 to 3.9. However, none of the 37 patients with a BI of less than 1.9 had detectable PGL-I antigen by the methods used in this study. The level of PGL-I in serum declined rapidly by about 90% 1 month after the start of multidrug therapy. This study showed clearly that anti-PGL-I IgM antibodies and circulating PGL-I antigen levels reflect the bacterial loads in untreated leprosy patients. The serological parameters based on the PGL-I antigen may therefore be useful in the assessment of leprosy patients at the time of diagnosis and possibly in monitoring patients following chemotherapy.—Authors' Abstract

Fischer, O., Matlova, L., Bartl, J., Dvorska, L., Melicharek, I. and Pavlik, I. Findings of mycobacteria in insectivores and small rodents. Folia Microbiol. **45** (2000) 147–152.

The organs of 30 insectivorous mammals and 62 rodents from areas inhabited by people or livestock where cattle paratuberculosis or mycobacterial infections of swine had been found to occur were examined by cultivation during the monitoring of occurrence and spread of mycobacterioses in cattle and swine. Mycobacteria were found in the organs of 3 insectivores (10%) and 6 rodents (9.7%). *Mycobacterium chelonae* was isolated from the organs of the lesser white-toothed shrew (*Crocidura suaveolens*) and the common vole (*Microtus arvalis*), and *M. vaccae* and *M. avium* subsp. *avium* (IS901+, serotype I) from the organs of the common shrew (*Sorex araneus*). *M. avium* subsp. *avium* (IS901+, serotype 1) was also isolated from the organs of the yellow-necked mouse (*Apodemus flavicollis*). Slow-growing mycobacteria of group III (according to Runyon) were isolated from the organs of the mouse (*Mus musculus sensu late*) and the yellow-necked mouse (*A. flavicollis*). These findings had no connection with the epizootological situation in the nearby livestock. *M. fortuitum* was isolated from the organs of the common vole (*M. arvalis*) caught in a field within easy reach of a swine breeding herd. *M. fortuitum* was also identified in the lymph nodes and droppings of this swine herd, as well as in the straw, scrapings from the floor of stalls, troughs and banisters, as well as from larvae and imagoes of dipterous insects. These results demonstrate the possibility that insectivores and small rodents can spread the causative agents of mycobacteria in wild and domestic animals.—Authors' Abstract

Horwitz, M. A., Harth, G., Dillon, B. J. and Maslesa Galic, S. Recombinant bacillus Calmette-Guerin (BCG) vaccines expressing the *Mycobacterium tuberculosis* 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. Proc. Acad. Sci. U.S.A. **97** (2000) 13853–13858.

Tuberculosis (TB) continues to ravage humanity, causing 2 million deaths per year. A vaccine against TB more potent than the current live vaccine, bacillus Calmette-Guerin (BCG), is desperately needed. Using two commercially avail-

able strains of BCG as host strains, BCG Connaught and Tice, we have constructed two recombinant BCG vaccines stably expressing and secreting the 30-kDa major secretory protein of *Mycobacterium tuberculosis* (M. Tb.), the primary causative agent of TB. We have tested the efficacy of the two strains in the highly susceptible guinea pig model of pulmonary TB, a model noteworthy for its close resemblance to human TB. Animals immunized with the recombinant BCG vaccines and challenged by aerosol with a highly virulent strain of M. Tb. had 0.5 logs fewer M. Tb. Bacilli in their lungs and 1 log fewer bacilli in their spleens on average than animals immunized with their parental conventional BCG vaccine counterparts. Statistically, these differences were highly significant. Paralleling these results, at necropsy, animals immunized with the recombinant BCG vaccines had fewer and smaller lesions in the lung, spleen, and liver and significantly less lung pathology than animals immunized with the parental BCG vaccines. The recombinant vaccines are the first vaccines against TB more potent than the current commercially available BCG vaccines, which were developed nearly a century ago.—Authors' Abstract

Marques, M. A., Antonio, V. L., Sarno, E. N., Brennan, P. J. and Pessolani, M. C. V. Binding of alpha 2-laminins by pathogenic and nonpathogenic mycobacteria and adherence to Schwann cells. J. Med. Microbiol. **50** (2001) 23–28.

The ability of *Mycobacterium leprae* to specifically bind alpha2-laminins of Schwann cells has been described recently as being an important property of the leprosy bacillus, which could explain the neural tropism of *M. leprae*. Therefore, the extent of the expression of alpha2-laminin-binding properties among mycobacteria was investigated. In an ELISA-based assay, all three species of *Mycobacterium* tested (*M. tuberculosis, M. chelonae* and *M. smegmatis*) expressed laminin-binding capacity, suggesting that the ability to bind alpha2-laminins is conserved within the genus *Mycobacterium*. This report also demonstrated that not only *M. leprae* but all the mycobacterial species tested readily interacted with the ST88-14 cells, a human schwannoma cell line, and that the addition of soluble alpha2-laminins significantly increased their adherence to these cells. These results failed to demonstrate the presence in *M. leprae* of a unique system based on alpha2-laminins for adherence to Schwann cells.—Authors' Abstract

Epidemiology and Prevention

Atrio Mourino, N., Sifontes Mejias, R. D., Rivero Sanchez, M., de Armas Fernandez, J. R. and Vidal Camero, I. [The behavior of leprosy; city of Camaguey, 1984–1988.] Rev. Leprol. Fontilles **33** (2000) 561–576. (in Spanish)

This is a descriptive study of the epidemiological behavior of leprosy in the township of Camagüey during the last 15 years (1984–1998). The study takes into account the 260 cases diagnosed by their family and individual clinical records. As primary register the information obtained from the annual reports of the statistical department of Camagüey together with the epidemiological questionnaire of leprosy patients were used and with all the information formed the basis of the primary register of data. Several different aspects were taken into account including: incidence of disease (×100,000 population), the distribution according to sex, color of skin, level of education, housing, clinical forms, first symptoms and signs and their distribution, detection methods, grade of disabilities, source of infection, interval of time for diagnosis and situation of the patient with relation to the source of infection.

Among all the results obtained a decline in the rate of incidence, slight predominance of females infected, poor education, higher number of unemployed and patients over 35 years and a higher incidence of multibacillary forms (BL and LL). In the LL forms there is a predominance of skin

nodules and in the other different clinical types (borderline, indeterminate and tuberculoid), anesthetic patches. The detection method was spontaneous with late diagnosis in the majority of cases; the source of infection went unknown for most cases. Most of the cases did not present disabilities at the moment of diagnosis and the few that did were of grade I.—Authors' English Summary

Goncalves, A., Goncalves, G. and Padovani, C. R. [Retrospective cohort study on retreated leprosy patients, with special reference to multidrug therapy.] Hansen. Int. **25** (2000) 31–38. (in Portuguese)

Considering previous figures from leprosy control, epidemiology and local health services, a retrospective cohort study was developed on 377 leprosy patients, registered in a sanitary unit in the southeast region of São Paulo State, Brazil. Exposition was considered anterior treatment referred to 71 (18.83%) of them; frequency distributions by sex, category of reingress, clinical form (on clinical and histologic bases), physical impairments (grade and localization in upper and lower limbs), number of communicants and ocurrence of reactions were taken as possible effects. Data, presented on descriptive and analytical grounds, focus on the difficulties in attaining the World Health Organization goal of eliminating the disease as a public health problem in 2000.—Authors' English Summary

Gupte, M. D., Kishore Kumar, B., Elangovan, A. and Arokiasamy, J. Modelling epidemiology of leprosy. Indian J. Lepr. **72** (2000) 305–316.

A simulation model for leprosy transmission and control has been developed with specific objectives. Several sensitivity experiments have been carried out by altering the various inputs based on empirical data combined with intelligent guessing. The outputs generated through these exercises were on the expected lines. While incremental exercises would improve the model, it can be used even at the existing stage as a tool for program managers.—Authors' Abstract

Prata, P. B., Bohland, A. K. and Vinhas, S. A. [Epidemiological characteristics of leprosy in localities of northeastern Brazil during the period 1994–1998.] Hansen. Int. **25** (2000) 49–53. (in Portuguese)

Although leprosy is endemic in the state of Sergipe (Brazil), there are few reports on the disease in this state, especially in the interior communities. This research reports an epidemiological study in patients registered in the Leprosy Control Program (1994–1998) in the cities of Aracaju and Itabaiana. During this period, 624 patients were registered in Aracaju and 525 in Itabaiana. The incidence was high in both cities. It was found that 58.6% of the patients were female, 54.4% were between 20 and 50 years old, and 65.2% had tuberculoid or virchowian leprosy. The high incidence of leprosy and the predominance of the polar forms, suggest that is necessary to intensify the control program.—Authors' English Summary

Rao, P. S. A., Mozhi, N. M. and Thomas, M. V. Leprosy affected beggars as a hidden source of transmission of leprosy. Indian J. Med. Res. **112** (2000) 52–55.

Background and objectives: Despite the large-scale implementation of multidrug therapy (MDT), the incidence rates of leprosy have not declined in several hyperendemic countries. Before searching for non-human reservoirs of leprosy it would be necessary to look for hidden human sources. This would include destitute leprosy-affected persons who resort to begging and operate in congested areas. Hence, this study was undertaken.

Methods: One major town and three semi-urban areas in Vellore district of Tamil Nadu and Chittoor town in Andhra Pradesh were purposefully selected for the study. All beggars in these towns were systematically identified and examined by allopathic doctors. Skin smears were examined for bacteriological index.

Results: Among the 193 beggars screened, 58 had leprosy. Of these, 10 were smear positive. Several beggars, although living sepa-

rately, were in touch with their relatives. Most beggars were pavement dwellers and regularly begged at places of worship, bus stands and shopping centers.

Interpretation and conclusion: The fact that nearly 20% of the leprosy-affected beggars were skin-smear positive highlights the need for regular screening and treatment of such beggars. Those positive should be actively treated and their close contacts frequently screened. This hidden reservoir should be completely eliminated.—Authors' Abstract

Smith, C. M. and Smith, W. C. S. Chemoprophylaxis is effective in the prevention of leprosy in endemic countries; a systematic review and meta-analysis. J. Infect. **41** (2000) 137–142.

Objective: To quantify the efficacy of chemoprophylaxis against leprosy.

Method: Literature searching of Medline and Embase databases, hand-searching of references and correspondence with investigators.

Study selection: Published papers relating to the prevention of leprosy and the use of chemotherapy in leprosy were identified for critical appraisal. Trials were selected and grouped into three categories according to study design and control groups.

Data analysis: the relative risks (RR) with 95% confidence intervals were calculated from the original data using a random effects model. To assess the cost-effectiveness of chemoprophylaxis, a further analysis of the rates of disease in the trial and control groups was done based on the numbers needed to be treated (NNT) to prevent one new case of leprosy.

Results: A total of 14 trials were identified from 127 published papers on chemoprophylaxis of leprosy. The trials were categorized into randomized controlled trials, nonrandomized controlled trials, and uncontrolled trials. The overall results of the meta-analysis shows that chemoprophylaxis gives around 60% protection against leprosy. The NNT are low in trials of household contacts.

Conclusions: The evidence shows that chemoprophylaxis against leprosy is an effective way to reduce the incidence of leprosy particularly in household contacts. The role of chemoprophylaxis needs to be re-examined using newer drugs given the continuing case-detection rates globally.—Authors' Abstract

Visschedijk, J., van de Broek, J., Eggens, H., Lever, P., van Beers, S. and Klatser, P. *Mycobacterium leprae*—millennium resistant! Leprosy control on the threshold of a new era. Trop. Med. Int. Health **5** (2000) 388–399.

Over the past decade the conditions of leprosy control implementation have changed dramatically. Introduction of multidrug therapy, together with the global effort of the World Health Organization to eliminate leprosy as a public health problem, had a tremendous impact on leprosy control, particularly by decreasing the registered prevalence of the disease. At the beginning of the new millennium, leprosy control programs face several new challenges. These relate not only to changes in the prevalence of the disease, but also to changes in the context of leprosy control, such as those created by health sector reforms and other disease control programs. This review discusses current knowledge on the epidemiology of *Mycobacterium leprae* and some important aspects of leprosy control. It is argued that our understanding is still insufficient and that, so far, no consistent evidence exists that the transmission of leprosy has been substantially reduced. Sustainable leprosy control, rather than elimination, should be our goal for the foreseeable future, which also includes care for patients on treatment and for those released from treatment. This, however, requires new strategies.—Trop. Dis. Bull. **97** (2000) 1235

Rehabilitation

Carvalho. G. A. and Alvarez, R. R. A. [Physical disabilities evaluation in Hansen's disease patients.] Hansen. Int. **25** (2000) 39–48. (in Portuguese)

Leprosy is a well-known disease for generating physical disabilities due to the *Mycobacterium leprae* peculiarities and preferences. This study has as its objective the prevalence of these disabilities in hands and feet in Hansen's disease patients treated of MDT. The used methodology was of a descriptive tranverse study, with registration of the physical exam on its own protocol, in 81 Hansen's disease patients in Distrito Federal, in its several clinical forms, treated in the Brasilia University Hospital between July 1996 and August 1997. It was observed that the degrees 1 and 2 of inabilities were the most frequent (19.8%), and that 56.6% did not have physical disabilities. There was a prevalence of lesions in the feet and the nerves were: the right and left posterior tibial nerve (22.2%), right common peroneal nerve (18.5%) and right ulnar nerve (12.3%). The sensitive loss found was isolated attack (19.8%) and clawhand, the most frequent deformity (9.8%). Associated deformities were present in larger amount in inferior members than in superior members or both. The clinical forms that presented a larger degree of physical disability were lepromatous and boderline leprosy. It is important that a meticulous evaluation of the hands and feet segments be made in order to avoid or to reduce this high prevalence of disabilities in leprosy patients through pertinent education and healing measures through physical therapy and rehabilitation.—Authors' English Summary

Cornielje, H., Nicholls, P. G. and Velema, J. Making sense of rehabilitation projects: classification by objectives. Lepr. Rev. **71** (2000) 472–485.

Rehabilitation of disabled persons can take many different forms according to the socio-cultural and political context in which it is undertaken. Some approaches have emphasized the restoration of the physical function of the client, while others have looked beyond to psychological and social well-being. Some have built on the expertise of professionals, while others have emphasized the caring capacity available in the family and the community and sought to reinforce it. Besides providing a wide range of possible services to disabled persons, rehabilitation seeks to change the attitudes that prevail in society as a whole and promote the integration of disabled people into society with equal rights and opportunities. This paper reviews a range of models and approaches which have been put forward in the international debate on rehabilitation. Furthermore, four dimensions are described which can be used to characterize and define classes of rehabilitation projects based on the objectives that are defined for them. Thus, types of rehabilitation projects can be distinguished. Management, evaluation and technical support for rehabilitation projects need to take these essential characteristics into account.—Authors' Summary

de Stigter, D. H., de Geus, L. and Heynders, M. L. Leprosy: between acceptance and segregation. Community behaviour towards persons affected by leprosy in eastern Nepal. Lepr. Rev. **71** (2000) 492–498.

This study describes community behavior toward persons affected by leprosy in the eastern Terai districts of Nepal. The results show that 95% of the persons affected by leprosy recognized by the community have visible signs such as wounds, swellings and deformed feet or hands. Persons affected by leprosy still experience negative behavior. Motives for negative community behavior are mostly found in the fact that people fear infection by germs, but fear of a curse from God is also mentioned. This study shows that negative community behavior is still present in eastern Nepal. Leprosy is more than a disease; the disease can nowadays be medically cured, but the sickness of leprosy still remains. Leprosy control programs should

focus on prevention of impairments and disabilities, because it seems that a visible sign is an important trigger for negative community behavior.—Authors' English Summary

Dong, L., Li, F., Jiang, J. and Zhang, G. Transplantation of fibula with vascular pedicle for fusion of ankle in leprotic drop-foot. Indian J. Lepr. **72** (2000) 431–442.

Devascularized bone grafts are pieces of dead bone that simply serve as scaffolds for new bone to grow and fill the gap, taking a long time when they succeed in doing so. In contrast, vascularized grafts being living tissues have a short healing time, great vitality and strong infection-resisting capacity. We report here the successful use of vascularized grafts of the lower end of the fibula for fusing the ankle in five leprosy patients.—Authors' Abstract

Ebenezer, G., Daniel, S., Suneetha, S., Reuben, E., Partheebarajan, S. and Solomon, S. Bacteriological study of pus isolates from neuropathic plantar ulcers associated with acute inflammatory phase. Indian J. Lepr. **72** (2000) 443–449.

In this retrospective study, sensitivity of organisms cultured from ulcers of leprosy patients without and with diabetes mellitus, diabetic patients without leprosy, and patients with ulcers from other causes was examined. The profile of organisms grown from these groups of patients did not differ significantly. However, there was a high prevalence of organisms like *Proteus, E. coli* and *Enterococcus* in the ulcers of leprosy patients, indicating fecal contamination of the ulcers. Co-trimaxazole and tetracycline were of little value in the treatment of these ulcers. We therefore recommend that in situations where there is no culture facility, the patients be started on a course of penicillin and gentamycin. If these antibiotics fail, it would be necessary to use more advanced antibiotics like norfloxacin, amikacin and ciprofloxacin.—Authors' Abstract

Floyd-Richard, M. and Gurung, S. Stigma reduction through group counselling of persons affected by leprosy—a pilot study. Lepr. Rev. **71** (2000) 499–504.

Stigmatization of persons with leprosy causes the emotional harm of social, economic and spiritual deprivation. Individual counselling has benefits in addressing these psychosocial problems but is a slow process and affects few people at any one time. Our experience of group counselling of stigmatized persons achieved the following: addressing common issues to more than one person at a time, encouraging the unity of sufferers, developing compassion for others, understanding the common effects of stigmatization, and beginning to overcome its harmful effects.—Authors' Summary

Prabhakara Rao, V., Rao, I. R. and Palande, D. D. Socio-economic rehabilitation programmes of LEPRA India—methodology, results and application of needs-based socio-economic evaluation. Lepr. Rev. **71** (2000) 466–471.

There is now a better understanding of the scope and process of rehabilitation. The approach recognizes the impact of leprosy on the individual, aims to understand the needs and concerns of those affected, their families and community in the rehabilitation process, and that aims to restore the person to a normal social life. LEPRA India has undertaken socio-economic rehabilitation (SER) activities in its projects in Andrah Pradesh and Orissa states in India with a holistic approach that has been evolutionary, developmental and participatory. An SER Officer (SERO) was posted to each project. A plan was formulated by the SERO with participation of all project staff. The main emphasis of the program was on active participation of the affected person in the rehabilitation process. A needs-assessment study was conducted in the target population using a semi-structured questionnaire. Information was elicited about social and economic status, before and after the disease, and the current rehabilitation needs of the persons affected. The next step was meeting the needs through interventions by the SER staff. The impact of the

program on restoration of social and economic status of the affected persons was analyzed. The paper stresses the importance of assessing the needs of persons affected by leprosy, structuring a rehabilitation program with the active participation of the affected person and evaluating the impact of the interventions in restoring normal social and economic life.—Authors' Summary

Robertson, L. M., Nicholls, P. G. and Butlin, R. Delay in presentation and start of treatment in leprosy: experience in an out-patient clinic in Nepal. Lepr. Rev. **71** (2000) 511–516.

Delayed presentation is a recognized risk factor for disability in leprosy but is the result of complex interactions between physical, social, economic and psychological factors. The present study is a response to the situation in an outpatient clinic in Nepal where the wide variation in delay in presentation was a cause for concern. A purpose-written questionnaire was used to collect information on 166 consecutive outpatient admissions. The data included demographics, the first symptom of leprosy, first actions, initial help-seeking behavior, the reasons for finally seeking treatment and experience with professional health services. Initial analysis found a relationship between delay in presentation and age, rural environment, leprosy classification, walking time, housing not shared with another person affected by leprosy, and an inappropriate first action. The relationship with lack of education and total travel time just failed to reach significance. Further analysis identified that for the study population initial lack of awareness of leprosy and an inappropriate first action were the primary contributors to delay. Extensive and effective health education is needed to address this situation.—Authors' Summary

Virmond, M. and Pereira, H. da R. Surgical correction of deformities and disabilities in leprosy patients. Indian J. Lepr. **72** (2000) 401–412.

Despite some positive experiences, surgical rehabilitation presently is minimally available in endemic countries, and the contingent of patients in need of this modality of treatment is enlarging constantly. With the reduction of prevalence of leprosy and the progressive integration of care of leprosy-affected persons into the general health services, surgical rehabilitation should be made available in these centers. Training health personnel of the general health services in leprosy surgery is mandatory and urgent. There is a need for the settlement of a multi-organizational task-force (WHO-ILEP-ILA) to deal with this problem. The aim of this task-force should be the definition of strategies to organize such training and to increase awareness among medical societies and schools regarding this issue. This group should also remember that the referral centers in Africa, Asia, North and South America should act as reservoirs of this knowledge and, as such, should be fully supported and enhanced since they have an important and unique role to play in the training of general health services personnel.—Authors' Abstract

Zodpey, S. J., Tiwari, R. R. and Salodkar, A. D. Gender differentials in the social and family life of leprosy patients. Lepr. Rev. **71** (2000) 505–510.

A study was carried out at the Leprosy Control Unit, Government Medical College, Nagpur, India, to investigate gender differentials in the social and family life of leprosy patients. The study included 486 (268 males and 218 females) leprosy patients who were diagnosed and registered at least 1 year prior to the data collection. It was observed that leprosy patients were isolated and refrained from various activities in the family. However, the effect of disease on this isolation was significantly greater in females as compared to males. Similarly, although men and women were both affected in terms of their social life, women suffered more isolation and rejection from the society. The current study describes the gender differentials in the social and family life of leprosy patients in central India.—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Bermudez, L. E., Inderlied, C. B., Kolonoski, P., Petroksky, M., Aralar, P., Wu, M. and Young, L. S. Activity of moxifloxacin by itself and in combination with ethambutol, rifabutin, and azithromycin *in vitro* and *in vivo* against *Mycobacterium avium*. Antimicrob. Agents Chemother. **45** (2001) 217–222.

Moxifloxacin activity against *Mycobacterium avium* complex (MAC) was evaluated *in vitro* against 25 strains. The MIC was determined to range from 0.125 to 2.0 μg/ml. In addition, U937 macrophage monolayers infected with MAC strain 101 (serovar 1) were treated with moxifloxacin (0.25 to 8 μg/ml) daily, and the number of intracellular bacteria was quantitated after 4 days. Moxifloxacin showed inhibitory activity at 0.5 μg/ml and higher. To assess the activity of moxifloxacin containing regimens *in vivo*, we infected C57BL bg + /bg + mice with 3×10^7 MAC strain 101 bacteria intravenously. One week later treatment was begun with the following: (i) moxifloxacin (50 mg/kg/day or 100 mg/kg/day), ethambutol (100 mg/kg/day), or a combination of moxifloxacin and ethambutol; or (ii) moxifloxacin (100 mg/kg/day), azithromycin (200 mg/kg/day), or rifabutin (40 mg/kg/day) as oral monotherapy; or (iii) all permutations of two-drug therapy or all three drugs in combination. All groups contained at least 14 animals, and the control group received the drug vehicle. After 4 weeks, quantitative blood cultures were obtained and the number of bacteria in liver and spleen was quantitated. Moxifloxacin, ethambutol, and azithromycin were active as single agents in liver, spleen, and blood. Rifabutin showed inhibitory activity only in the blood. Two-drug combinations containing azithromycin were no more active than azithromycin alone. Similarly, the three-drug combination was not more active than azithromycin alone in the spleen. Rifabutin did not add to the activity of any other single agent or two-drug combination. Moxifloxacin at both concentrations in combination with ethambutol was significantly more active than each drug alone.—Authors' Abstract

Biketov, S., Mukamolova, G. V., Potapov, V., Gilenkov, E., Vostroknutova, G., Kell, D. B., Young, M. and Kaprelyants, A. S. Culturability of *Mycobacterium tuberculosis* cells isolated from murine macrophages: a bacterial growth factor promotes recovery. FEMS Immunol. Med. Microbiol. **29** (2000) 233–240.

Very little is known about the culturability and viability of mycobacteria following their phagocytosis by macrophages. We therefore studied populations of the avirulent "Academia" strain of *Mycobacterium tuberculosis* isolated from murine peritoneal macrophage lysates several days postinfection *in vivo*. The resulting bacterial suspensions contained a range of morphological types including rods, ovoid forms and coccoid forms. Bacterial viability measured using the MPN method (dilution to extinction in liquid medium) was often much higher than that measured by CFU (plating on solid medium). Viability in the MPN assay was further enhanced when the *Micrococcus luteus* protein, Rpf, was incorporated into the liquid culture medium at picomolar concentrations. Rpf is an example of a family of autocrine growth factors found throughout the high G+C cohort of gram-positive bacteria including *M. tuberculosis*. *M. tuberculosis* cells obtained from macrophages had altered surface properties, as compared with bacteria grown *in vitro*. This was indicated by loss of the ability to adsorb bacteriophage DS6A, a reduced tendency to form clumps, acquisition of ethidium bromide stainability following heat treatment, and loss of Rpf-mediated resuscitation following freezing and thawing. These results indicate that a proportion of "unculturable" *M. tuberculosis* cells obtained from macrophages is either injured or dormant and that these cells may be recovered or resuscitated using Rpf in liquid medium.—Authors' Abstract

Cho, S., Mehra, V., Thoma Uszynski, S., Stenger, S., Serbina, N., Mazzaccaro, R. J., Flynn, J. L., Barnes, P. F., Southwood, S., Celis, E., Bloom, B. R., Modlin, R. L. and Sette, A. Antimicrobial activity of MHC class I-restricted CD8+ T cells in human tuberculosis. Proc. Natl. Acad. Sci. U.S.A. **97** (2000) 12210–12215.

Studies of mouse models of tuberculosis (TB) infection have indicated a central role for MHC class I-restricted CD8+ T cells in protective immunity. To define antigens and epitopes of *Mycobacterium tuberculosis* (MTB) proteins that are presented by infected cells to CD8+ T cells, we screened 40 MTB proteins for HLA class I A*0201-binding motifs. Peptides that bound with high affinity to purified HLA molecules were subsequently analyzed for recognition by CD8+ cytotoxic T lymphocytes. We identified three epitopes recognized by CD8+ T cells from patients recovering from TB infection. Those three epitopes were derived from three different antigens: thymidylate synthase [ThyA (30–38)], RNA polymerase beta-subunit [RpoB (127–135)], and a putative phosphate transport system permease protein A-1 [PstA1 (75–83)]. In addition, CD8+ T cell lines specific for three peptides [ThyA (30–38), PstA1 (75–83), and 85B (15–23)] were generated from peripheral blood mononuclear cells of normal HLA-A*0201 donors. These CD8+ T cell lines specifically recognized MTB-infected macrophages, as demonstrated by production of IFN-gamma and lysis of the infected target cells. Finally, CD8+ cytotoxic T lymphocytes reduced the viability of the intracellular MTB, providing evidence that CD8+ T cell recognition of MHC class I-restricted epitopes of these MTB antigens can contribute to effective immunity against the pathogen.—Authors' Abstract

Dhople, A. M. Antimicrobial activities of dihydrofolate reductase inhibitors, used singly or in combination with dapsone, against *Mycobacterium ulcerans*. J. Antimicrob. Chemother. **47** (2001) 93–96.

Development of new treatments against *Mycobacterium ulcerans* infection has become crucial because of its wide-scale prevalence throughout the world. The effects of dihydrofolate reductase inhibitors, used either singly or in combination with dapsone, against *M. ulcerans* were evaluated *in vitro*. When used singly, epiroprim was the most potent with MICs between 0.5 and 1.0 mg/l, while trimethoprim was totally ineffective. The MICs of K-130 and brodimoprim ranged from 1.0–2.0 mg/l for the former to 2.0–16.0 mg/l for the latter. When combined with dapsone, synergic effects were observed with epiroprim. These results indicate the great potential of epiroprim in treating *M. ulcerans* infection.—Author's Abstract

Dhople, A. M. *In vitro* activity of KRM-1648, either singly or in combination with ofloxacin, against *Mycobacterium ulcerans*. Int. J. Antimicrob. Agents **17** (2001) 57–61.

The antimicrobial effect of a benzoxazinorifamycin, KRM-1648, either alone or in combination with ofloxacin, was evaluated *in vitro* against two type strains and six clinical isolates of *Mycobacterium ulcerans*. Growth of *M. ulcerans* was measured by plate counts and the BACTEC radiometric method. The minimal inhibitory concentration as well as minimal bactericidal concentration of KRM-1648 against *M. ulcerans* was between 0.012 and 0.025 mg/l, while corresponding values for rifampin and rifabutin were in the range of 0.1–0.8 mg/l and 0.1–0.4 mg/l, respectively. When combined with ofloxacin, KRM-1648 exhibited strong synergistic activity while only additive effects were observed with the combination of rifampin (or rifabutin) and ofloxacin. These results suggest that KRM-1648 has a great potential in the treatment of *M. ulcerans* infection.—Author's Abstract

Geluk, A., van Meijgaarden, K. E., Franken, K. L. M. C., Drijfhout, J. W., DSouza, S., Necker, A., Huygen, K. and Ottenhoff, T. H. M. Identification of major epitopes of *Mycobacterium tuberculosis* Ag85B that are recognized by HLA-A*0201-restricted CD8+ T cells in HLA-transgenic mice and humans. J. Immunol. **165** (2000) 6463–6471.

CD8+ T cells are thought to play an important role in protective immunity to tuberculosis. Although several nonprotein ligands have been identified for CD1-restricted CD8+ CTLs, epitopes for classical MHC class I-restricted CD8+ T cells, which most likely represent a majority among CD8+ T cells, have remained ill-defined. HLA-A*0201 is one of the most prevalent class I alleles, with a frequency of over 30% in most populations. HLA-A2/K-b transgenic mice were shown to provide a powerful model for studying induction of HLA-A*0201-restricted immune responses *in vivo*. The Ag85 complex, a major component of secreted *Mycobacterium tuberculosis* proteins, induces strong CD4+ T cell responses in *M. tuberculosis*-infected individuals, and protection against tuberculosis in Ag85-DNA-immunized animals. In this study, we demonstrate the presence of HLA class I-restricted, CD8+ T cells against Ag85B of *M. tuberculosis* in HLA-A2/K-b transgenic mice and HLA-A*0201+ humans. Moreover, two immunodominant Ag85 peptide epitopes for HLA-A*0201-restricted, *M. tuberculosis*-reactive CD8+ CTLs were identified. These CD8+ T cells produced IFN-gamma and TNF-alpha and recognized Ag-pulsed or bacillus Calmette-Guerin-infected, HLA-A*0201-positive, but not HLA-A*0201-negative or uninfected human macrophages. This CTL-mediated killing was blocked by anti-CD8 or anti-HLA class I mAb. Using fluorescent peptide/HLA-A*0201 tetramers, Ag85-specific CD8+ T cells could be visualized in bacillus Calmette-Guerin-responsive, HLA-A*0201+ individuals. Collectively, our results demonstrate the presence of HLA class I-restricted CD8+ CTL against a major Ag of *M. tuberculosis* and identify Ag85B epitopes that are strongly recognized by HLA-A*0201-restricted CD8+ T cells in humans and mice. These epitopes thus represent potential subunit components for the design of vaccines against tuberculosis.—Authors' Abstract

George, A., Marziniak, M., Schafers, M., Toyka, K. V. and Sommer, C. Thalidomide treatment in chronic constrictive neuropathy decreases endoneurial tumor necrosis factor-alpha, increases interleukin-10 and has long-term effects on spinal cord dorsal horn met-enkephalin. Pain **88** (2000) 267–275.

Thalidomide reduces thermal hyperalgesia and mechanical allodynia in chronic constrictive sciatic nerve injury (CCI). Since thalidomide mainly inhibits tumor necrosis factor alpha (TNF-α) synthesis with less well-defined effects on other cytokines, we investigated the effect of the drug on the expression of the proinflammatory cytokines TNF-α, interleukin-1 beta (IL-1β) and (IL-6), and of the antiinflammatory cytokine (IL-10) in the lesioned rat sciatic nerve. The increase of endoneurial TNF-α during the first week after CCI was reduced after thalidomide treatment, as shown with immunohistochemistry and enzyme-linked-immunosorbent assay. In contrast, endoneurial IL-1β-immunoreactivity (LR) and IL-6-IR were not altered by thalidomide treatment, nor was macrophage influx. Recruitment of epineurial IL-10 immunoreactive macrophages as well as the recovery of injury-induced depletion of endoneurial IL-10-IR was enhanced by thalidomide treatment. To control for central plasticity as another factor for the effects of thalidomide, the spinal cord was analyzed for changes in neurotransmitters. The decrease in CGRP-IR and SP-IR in the dorsal horn of operated animals was not influenced by treatment. In contrast, the increase in met-enkephalin observed in the dorsal horn of operated animals was further enhanced in the thalidomide-treated animals. The study elucidates some of the complex alterations in CCI and its modulation by thalidomide, and provides further evidence for a possible therapeutic benefit of cytokine-modulating substances in the treatment of neuropathic pain.—Authors' Abstract

Hernandez Pando, R., Jeyanathan, M., Mengistu, G., Aguilar, D., Orozco, H., Harboe, M., Rook, G. A. W. and Bjune, G. Persistence of DNA from *Mycobacterium tuberculosis* in superficially normal lung tissue during latent infection. Lancet **356** (2000) 2133–2138.

Background. A third of the world's population has latent infection with *Mycobacte-*

rium tuberculosis, and in areas of low endemicity most cases of active tuberculosis arise as a result of reactivation of latent bacilli. We sought to establish the cellular location of these latent organisms to facilitate their elimination.

Methods. We applied *in-situ* PCR to sections of macroscopically normal lung tissue from 13 individuals from Ethiopia and 34 from Mexico who had died from causes other than tuberculosis. Sections of lung tissue from six Norwegian individuals (i.e., individuals from a nonendemic population) acted as negative controls, and six Ethiopian tuberculosis cases acted as positive controls.

Findings. Control necropsy samples from the Norwegian individuals were all negative by *in-situ* PCR and conventional PCR; whereas all samples from known Ethiopian tuberculosis cases were positive by both methods. However, in macroscopically normal lung tissue from Ethiopian and Mexican individuals without tuberculous lesions, the *in-situ* PCR revealed 5 of 13 and 10 of 34 positive individuals, respectively. These results were confirmed by conventional PCR with extracted DNA. Positive cells included alveolar and interstitial macrophages, type II pneumocytes, endothelial cells, and fibroblasts.

Interpretation. *M. tuberculosis* can persist intracellularly in lung tissue without histological evidence of tuberculous lesions. *M. tuberculosis* DNA is situated not only in macrophages but also in other nonprofessional phagocytic cells. These findings contradict the dominant view that latent organisms exist in old classic tuberculous lesions, and have important implications for strategies aimed at the elimination of latent and persistent bacilli.—Authors' Abstract

Holland, S. M. Nontuberculous mycobacteria. Am. J. Med. Sci. **321** (2001) 49–55.

The nontuberculous mycobacteria are for the most part ubiquitous environmental organisms that only rarely cause disease in humans. Therefore, the normal host defense against these organisms must be quite robust, as exposure is universal and disease is rare. The organisms that are most commonly encountered in clinical practice, *Mycobacterium avium*, *M. intracellulare*, *M. kansasii*, *M. fortuitum*, *M. abscessus*, and *M. chelonae*, are frequently found in water sources and soil. These organisms share significant structural and biochemical similarities with their more pathogenic relative, *M. tuberculosis* (MTB). Because they are of significantly lower pathogenicity than MTB, patients with abnormal susceptibility to these infections should include those with defects that may be identifiable. Study of these patients should lead to determination of the mechanisms underlying resistance to these organisms, which in turn are likely to be highly informative regarding host defense against these infections and their more virulent relative MTB. Furthermore, recognition of host factors that permit infection with nontuberculous mycobacteria in otherwise normal hosts will identify pathways that can be targeted for therapeutic intervention. Thus, the search for genetic and acquired susceptibility to nontuberculous mycobacteria is also a search for susceptibility factors for MTB as well as an opportunity to recognize endogenous pathways that can be exploited therapeutically.—Author's Abstract

Hu, Y. M., Mangan, J. A., Dhillon, J., Sole, K. M., Mitchison, D. A., Butcher, P. D. and Coates, A. R. M. Detection of mRNA transcripts and active transcription in persistent *Mycobacterium tuberculosis* induced by exposure to rifampin and pyrazinamide. J. Bacteriol. **182** (2000) 6358–6365.

Mycobacterium tuberculosis can persist in an altered physiological state for many years after initial infection, and it may reactivate to cause active disease. An analogous persistent state, possibly consisting of several different subpopulations of bacteria, may arise during chemotherapy; this state is thought to be responsible for the prolonged period required for effective chemotherapy. Using two models of drug-induced persistence, we show that both microaerophilic stationary-phase *M. tuberculosis* treated with a high dose of rifampin *in vitro* and pyrazinamide-induced persistent bacteria in mice are nonculturable yet still contain 16S rRNA and mRNA transcripts. Also, the *in*

vitro persistent, plate culture-negative bacteria incorporate radioactive uridine into their RNA in the presence of rifampin and can rapidly upregulate gene transcription after the replacement of the drug with fresh medium and in response to heat shock. Our results show that persistent *M. tuberculosis* has transcriptional activity. This finding provides a molecular basis for the rational design of drugs targeted at persistent bacteria.—Authors' Abstract

Mert, A., Ozturk, R., Sipahi, S., Fresko, I., Aslan, M., Hamuryudan V., Yurdakul, S., Dirincan, A. and Yazici, H. [Antimycobacterial antibodies in Behcet's syndrome.] Turkish J. Infect. **13** (1999) 527–529. (in Turkish)

A study was designed to determine the presence of specific anti-mycobacterial antibodies in Behçet's syndrome (BS) by means of ELISA using both homemade crude (adsorbed mycobacterial sonicated antigen, AMSA) and commercially available highly purified plus recombinant antigens (Pathozyme-Myco). Turkish patients with BS (N = 49), tuberculosis (N = 28, 16 active and 12 inactive), connective tissue disorders (N = 32) and healthy controls (N = 15) were included in the study. The positivity rates were 40.8% with AMSA and 20.4% with purified and recombinant antigens in Behçet's patients, but positivity rates in healthy controls were 6.6% with both antigens. The antibody levels in BS patients as a whole did not differ from those of healthy controls. However, subgroup analysis revealed that BS patients who were inactive (N = 21) or who had large vessel involvement (N = 8) had higher antibody titers compared to healthy controls (p = 0.009 and 0.003). It is suggested that mycobacterial specific antibodies are seen in higher titers and more frequently in BS when compared with the normal population depending on immune system stimulation by peptides present in patients with BS.—Trop. Dis. Bull. **97** (2000) 1137

Murugasu Oei, B. and Dick, T. Bactericidal activity of nitrofurans against growing and dormant *Mycobacterium bovis* BCG. J. Antimicrob. Chemother. **46** (2000) 917–919.

Depletion of oxygen triggers the shift-down of *Mycobacterium bovis* BCG to a state of dormancy. Bacilli in their dormant state are resistant to standard anti-mycobacterials. The nitroimidazole metronidazole was the first compound identified to show bactericidal activity against dormant tubercle bacilli, in contrast to metronidazole's selective toxicity for dormant bacilli. We report here that the nitrofurans nitrofurantoin, furaltadone and nitrofurazone showed bactericidal activity against dormant and growing bacteria. Importantly, the bactericidal effect of nitrofurans on dormant bacilli was 35- to 250-fold higher compared with metronidazole.—Authors' Abstract

Oliver, S. J., Moreira, A. and Kaplan, G. Immune stimulation in scleroderma patients treated with thalidomide. Clin. Immunol. **97** (2000) 109–120.

Scleroderma (SSc) is a fibrosing connective tissue disease that is poorly responsive to any treatment, including immune suppression. SSc shares many characteristics with chronic graft-versus-host disease (GVHD). Because the immunomodulatory drug thalidomide has proven beneficial in chronic GVHD, we studied the immune response and clinical effects of thalidomide in SSc patients. We treated 11 SSc patients with thalidomide in an open-label, dose-escalating, 12-week study. Histologic comparison of skin biopsies showed changes in skin fibrosis and an increase in epidermal and dermal infiltrating CD8+ T cells with thalidomide treatment. In thalidomide-treated SSc patients, plasma levels of IL-12 and TNF-alpha increased, while plasma IL-5 and IL-10 levels remained unchanged. These changes were associated with clinical effects, including dry skin, dermal edema, transient rashes, decreased gastroesophageal reflux symptoms, and healing of digital ulcers. When SSc PBMCs activated by anti-CDS mAb were exposed to thalidomide, increases in both production of IL-2, IL-3, GM-CSF, and IFN-gamma and T-cell expression of CD40L were observed. Thalidomide therefore appears to induce

immune stimulation in SSc patients in association with clinical changes. However, it remains to be shown whether long-term enhancement of immune responses in SSc patients is clinically beneficial.—Authors' Abstract

Ridder, G. J., Strohhacker, H., Lohle, E., Golz, A. and Fradis, M. Laryngeal sarcoidosis: treatment with the antileprosy drug clofazimine. Ann. Otol. Rhinol. Laryngol. **109** (2000) 1146–1149.

Sarcoidosis is a chronic systemic granulomatous disease that occasionally affects the larynx. When the larynx is affected, the symptoms are frequently mild, but severe airway obstruction can occur. Although systemic corticosteroids are helpful, patients may become refractory to further drug administration. The current methods of treatment are here summarized, and the patient literature is reviewed. We also report a case of a young patient suffering from laryngeal sarcoidosis successfully treated by the antileprosy agent clofazimine, and propose it as an alternative treatment of laryngeal sarcoidosis in patients refractory to corticosteroids.—Authors' Abstract

Rosenkrands, I., King, A., Weldingh, K., Moniatte, M., Moertz, E. and Andersen, P. Towards the proteome of *Mycobacterium tuberculosis.* Electrophoresis **21** (2000) 3740–3756.

Human tuberculosis is caused by the intracellular pathogen *Mycobacterium tuberculosis.* Sequencing of the genome of *M. tuberculosis* strain H37Rv has predicted 3924 open reading frames, and enabled identification of proteins from this bacterium by peptide mass fingerprinting. Extracellular proteins from the culture medium and proteins in cellular extracts were examined by two-dimensional gel electrophoresis using immobilized pH gradient technology. By mass spectrometry and immunodetection, 49 culture filtrate proteins and 118 lysate proteins were identified, 83 of which were novel. To date, 288 proteins have been identified in *M. tuberculosis* proteome studies, and a list is presented which includes all identified proteins (available at http://www.ssi.dk/publichealth/tbimmun). The information obtained from the *M. tuberculosis* proteome so far is discussed in relation to the information obtained from the complete genome sequence.—Authors' Abstract

Smith, S. M., Brookes, R., Klein, M. R., Malin, A. S., Lukey, P. T., King, A. S., Ogg, G. S., Hill, A. V. S. and Dockrell, H. M. Human CD8+ CTL specific for the mycobacterial major secreted antigen 85A. J. Immunol. **165** (2000) 7088–7095.

The role of CD8+ CTL in protection against tuberculosis in human disease is unclear. In this study, we stimulated the peripheral blood mononuclear cells of bacillus Calmette-Guerin (BCG)-vaccinated individuals with live *Mycobacterium bovis* BCG bacilli to establish short-term cell lines and then purified the CD8+ T cells. A highly sensitive enzyme-linked immunospot (ELISPOT) assay for single cell IFN-gamma release was used to screen CD8+ T cells with overlapping peptides spanning the mycobacterial major secreted protein, Ag85A. Three peptides consistently induced a high frequency of IFN-gamma responsive CD8+ T cells, and two HLA-A*0201 binding motifs, P48–56 and P242–250, were revealed within the core sequences. CD8+ T cells responding to the 9-mer epitopes were visualized within fresh blood by ELISPOT using free peptide or by binding of HLA-A*0201 tetrameric complexes. The class I-restricted CD8+ T cells were potent CTL effector cells that efficiently lysed an HLA-A2-matched monocyte cell line pulsed with peptide as well as autologous macrophages infected with *M. tuberculosis* or recombinant vaccinia virus expressing the whole Ag85A protein. Tetramer assays revealed a six-fold higher frequency of peptide-specific T cells than IFN-gamma ELISPOT assays, indicating functional heterogeneity within the CD8+ T cell population. These results demonstrate a previously unrecognized, MHC class I-restricted, CD8+ CTL response to a major secreted Ag of mycobacteria and supports the use of Ag85A as a candidate

vaccine against tuberculosis.—Authors' Abstract

Smyth, A. J., Welsh, M. D., Girvin, R. M. and Pollock, J. M. *In vitro* responsiveness of gamma delta T cells from *Mycobacterium bovis*-infected cattle to mycobacterial antigens: predominant involvement of WC1+ cells. Infect. Immun. **69** (2001) 89–96.

It is generally accepted that protective immunity against tuberculosis is generated through the cell-mediated immune (CMI) system, and a greater understanding of such responses is required if better vaccines and diagnostic tests are to be developed. Gamma delta T cells form a major proportion of the peripheral blood mononuclear cells (PBMC) in the ruminant system and, considering data from other species, may have a significant role in CMI responses in bovine tuberculosis. This study compared the *in vitro* responses of alpha beta and gamma delta T cells from *Mycobacterium bovis*-infected and uninfected cattle. The results showed that, following 24 hr of culture of PBMC with *M. bovis*-derived antigens, the majority of gamma delta T cells from infected animals became highly activated (upregulation of interleukin-2R), while a lower proportion of the alpha beta T-cell population showed activation. Similar responses were evident to a lesser degree in uninfected animals. Study of the kinetics of this response showed that gamma delta T cells remained significantly activated for at least 7 days in culture, while activation of alpha beta T cells declined during that period. Subsequent analysis revealed that the majority of activated gamma delta T cells expressed WC1, a 215-kDa surface molecule which is not expressed on human or murine gamma delta T cells. Furthermore, in comparison with what was found for CD4+ T cells, *M. bovis* antigen was found to induce strong cellular proliferation but relatively little gamma interferon release by purified WC1+ gamma delta T cells. Overall, while the role of these cells in protective immunity remains unclear, their highly activated status in response to *M. bovis* suggests an important role in antimycobacterial immunity, and the ability of gamma delta T cells to influence other immune cell functions remains to be elucidated, particularly in relation to CMI-based diagnostic tests.—Authors' Abstract

Stinear, T. P., Jenkin, G. A., Johnson, P. D. R. and Davies, J. K. Comparative genetic analysis of *Mycobacterium ulcerans* and *Mycobacterium marinum* reveals evidence of recent divergence. J. Bacteriol. **182** (2000) 6322–6330.

Previous studies of the 16S RNA genes from *Mycobacterium ulcerans* and *M. marinum* have suggested a very close genetic relationship between these species (99.6% identity). However, these organisms are phenotypically distinct and cause diseases with very different pathologies. To investigate this apparent paradox, we compared 3306 nucleotides from the partial sequences of eight housekeeping and structural genes derived from 18 *M. ulcerans* strains and 22 *M. marinum* strains. This analysis confirmed the close genetic relationship inferred from the 16S rRNA data, with nucleotide sequence identity ranging from 98.1% to 99.7%. The multilocus sequence analysis also confirmed previous genotype studies of *M. ulcerans* that have identified distinct genotypes within a geographical region. Single isolates of both *M. ulcerans* and *M. marinum* that were shown by the sequence analysis to be the most closely related were then selected for further study. One- and two-dimensional pulsed-field gel electrophoresis was employed to compare the architecture and size of the genome from each species. Genome sizes of approximately 4.4 and 4.6 Mb were obtained for *M. ulcerans* and *M. marinum*, respectively. Significant macrorestriction fragment polymorphism was observed between the species. However, hybridization analysis of DNA cleaved with more frequently cutting enzymes identified significant preservation of the flanking sequence at seven of the eight loci sequenced. The exception was the 16S rRNA locus. Two high-copy-number insertion sequences, IS2404 and IS2606, have recently been reported in *M. ulcerans* and, significantly, these elements are not present in *M. marinum*. Hybridization of the AseI restriction fragments

from *M. ulcerans* with IS2404 and IS2606 indicated widespread genome distribution for both of these repeated sequences. Taken together, these data strongly suggest that *M. ulcerans* has recently diverged from *M. marinum* by the acquisition and concomitant loss of DNA in a manner analogous to the emergence of *M. tuberculosis*, where species diversity is being driven mainly by the activity of mobile DNA elements.—Authors' Abstract

Sugawara, I., Yamada, H., Mizuno, S. and Iwakura, Y. IL-4 is required for defense against mycobacterial infection. Microbiol. Immunol. **44** (2000) 971–979.

Although the involvement of T helper (Th1) cells is central to protection against intracellular bacteria, including *Mycobacterium tuberculosis*, the involvement of Th2 cells, characterized by potent interleukin (IL)-4 secretion in mycobacterial infection is still unclear. In order to clarify the role of IL-4 in murine tuberculosis, IL-4-deficient mutant mice, IL-4 knockout (IL-4 KO) mice were utilized. The mice were infected with H37Rv, Kurono or BCG Pasteur via an airborne infection route by placing them in the exposure chamber of a Middlebrook airborne infection apparatus. Their capacity to control mycobacterial growth, granuloma formation, cytokine secretion, and nitric oxide (NO) production were examined. These mice developed large granulomas, but not necrotic lesions in the lungs, liver or spleen ($p < 0.05$). This was consistent with a significant increase in lung colony-forming units (CFU). Compared with levels in wild-type mice, upon stimulation with mycobacteria, splenic IL-10 levels were low and IL-6 levels were intermediate, but interferon (IFN)-gamma and IL-12 levels were significantly higher. IL-18 levels were within the normal range. The level of NO production by alveolar macrophages of the IL-4 KO mice was similar to that of the wildtype mice. Granulomatous lesion development by IL-4 KO mice was inhibited significantly by treatment with exogenous recombinant IL-4. These findings were not specific to the IL-4 KO mice used. Our data show that IL-4 may play a protective role in defense against mycobacteria, although IFN-gamma and TNF-alpha play major roles in it. Our data do not rule out an IFN-gamma-independent function of IL-4 in controlling tuberculosis.—Authors' Abstract

Swaminathan, S., Nandini, K. S., Hanna, L. E., Somu, N., Narayanan, P. R. and Barnes, P. F. T-lymphocyte subpopulations in tuberculosis. Indian Pediatrics **37** (2000) 489–495.

Tuberculosis is associated with both qualitative and quantitative defects in the cell-mediated immune response. The changes that occur in the lymphocyte profile in blood in children with tuberculosis are not well understood. A prospective study was conducted during July 1996 and February 1997 at referral hospitals in Chennai, India. Lymphocyte subpopulations were determined by flow cytometry in 17 healthy tuberculin-positive children, in 22 children with newly diagnosed pulmonary tuberculosis and in 8 of these children after antituberculosis therapy. Absolute numbers and percentages of CD3+ and CD4+ T cells were reduced in children with tuberculosis compared to controls. CD4+ counts increased significantly following antituberculosis treatment compared to baseline values. In contrast, the proportion of T cells expressing the γδT cell receptor was similar in tuberculosis patients and controls. It is concluded that children with tuberculosis have a systemic decrease in the proportion and number of CD3+ and CD4+ T cells which reverses during therapy.—Trop. Dis. Bull. **97** (2000) 1237

Thomas Uszynski, S., Stenger, S. and Modlin, R. L. CTL-mediated killing of intracellular *Mycobacterium tuberculosis* is independent of target cell nuclear apoptosis. J. Immunol. **165** (2000) 5773–5779.

Two subsets of human CTL have been defined based upon phenotype and function: CD4– CD8– double-negative (DN) CTL lyse susceptible targets via Fas-Fas ligand interaction and CD8+ CTL via the granule exocytosis pathway. CD8+ CTL, but not DN CTL, can mediate an antimicrobial activity against *Mycobacerium tuberculosis*-infected target cells that is depen-

dent on cytotoxic granules that contain granulysin. We investigated the role of nuclear apoptosis for the antimicrobial effector function of CD1-restricted CTL using the caspase inhibitor N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone. We found that DN CTL-induced target cell lysis was completely dependent on caspase activation; whereas the cytolytic activity of CD8+ CTL was caspase independent. However, both DN and CD8+ CTL-induced nuclear apoptosis required caspase activation. More important, the antimicrobial effector function of CD8+ CTL was not diminished by inhibition of caspase activity. These data indicate that target cell nuclear apoptosis is not a requirement for CTL-mediated killing of intracellular *M. tuberculosis*.—Authors' Abstract

Vankayalapati, R., Wizel, B., Samten, B., Griffith, D. E., Shams, H., Galland, M. R., von Reyn, C. F., Girard, W. M., Wallace, R. J. and Barnes, P. F. Cytokine profiles in immunocompetent persons infected with *Mycobacterium avium* complex. J. Infect. Dis. **183** (2001) 478–484.

To evaluate the immunologic factors that contribute to protection against *Mycobacterium avium* complex (MAC), cytokine production by peripheral blood mononuclear cells (PBMC) from human immunodeficiency virus-negative persons with pulmonary MAC (MAC patients) and healthy control subjects with a delayed hypersensitivity skin test response to *M. avium* sensitin (MAS-positive control subjects) was measured. In MAC patients, mycobacterium-stimulated PBMC produced higher concentrations of interleukin (IL)-10 but lower concentrations of gamma interferon (IFN-γ), IL-12, and tumor necrosis factor-alpha (TNF-α) compared with PBMC from MAS-positive control subjects. Immunolabeling for intracellular IL-10 revealed that this cytokine was produced by both monocytes and T cells. Alveolar macrophages produced TNF-α and IL-10 in response to MAC, which suggests that these cytokines are produced in the lungs of patients with pulmonary disease caused by this pathogen. Our findings suggest that IFN-γ, TNF-α, and IL-12 contribute to protection against MAC; whereas IL-10 is immunosuppressive.—Authors' Abstract

van Pinxteren, L. A. H., Cassidy, J. P., Smedegaard, B. H. C., Agger, E. M. and Andersen, P. Control of latent *Mycobacterium tuberculosis* infection is dependent on CD8 T cells. Eur. J. Immunol. **30** (2000) 3689–3698.

It is estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis*, but that only 10% of infected people break down with the disease. In the remaining 90% the infection remains clinically latent. In the present study, the immune mechanisms controlling the latent phase of tuberculosis infection were evaluated in a mouse model of latency and reactivation. Mice aerosol-infected with *M. tuberculosis* were treated with anti-mycobacterial drugs resulting in very low, stable bacterial numbers (<500 CFU in the spleen and lung) for 10–12 weeks followed by reactivation of the disease with increasing bacterial numbers. During latency, pathological changes in the lung had almost completely resolved and lymphocyte numbers and turnover were at the pre-infection level. The CD4 subset was highly active during the acute phase of infection and could be detected by intracellular staining for gamma interferon (IFN-γ) as well as after antigen-specific stimulation with mycobacterial antigens. The CD8 subset was not involved in the acute stage of infection, but this subset was active and produced IFN-γ during the latent phase of infection. *In vivo* depletion of T-cell subsets supported these findings with a 6–7-fold increase in bacterial numbers in the lung following anti-CD4 treatment during the acute phase, while anti-CD8 treatment did not have an effect. The opposite was found during the latent phase where anti-CD8 treatment as well as anti-IFN-γ treatment both resulted in a 10-fold increase in bacterial numbers in the lung, while anti-CD4 treatment induced only a modest change.—Authors' Abstract