CURRENT LITERATURE

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

General and Historical

Campos-Outcalt, D. An evaluation of a microcomputer information system for leprosy control two years post-implementation. Lepr. Rev. **62** (1991) 65–71.

As part of a national program to improve the management of health services in Papua New Guinea, a microcomputerized information system was designed and implemented in seven provinces. Four other provinces later adopted this system. One component of this information system was a program to assist disease control officers to monitor the treatment received by leprosy and tuberculosis patients. In contrast to other components of the information system, the leprosy and TB computer program was not maintained nor used after 2 years. This article describes the computer program developed and discusses possible reasons for its nonuse.—Author's Summary

Kumar, R. P., Keystone, J. S., Christian, M. and Jesudasan, K. Transmission of health information on leprosy from children to their families: another approach to health education. Lepr. Rev. **62** (1991) 58–64.

A controlled study was carried out in the North Arcot District of Tamil Nadu, South India, to determine whether health information given to schoolchildren would influence the knowledge and attitudes of their families concerning leprosy. A total of 41 children and almost all of their household members participated in the study. The study, conducted by questionnaire, involved a pre-test of knowledge and attitude about leprosy of seventh standard students and their families. After one group of children received health education about leprosy and the other received information about tuberculosis, an identical post-test questionnaire was administered to all participants. Although significant improvement in knowledge about leprosy was detected in the leprosy-educated group of children compared with controls, no transmission of information on leprosy was detected in the family members of either group. The attitudes of the children who had been educated about leprosy may have been adversely affected by the health education session. The reasons for our failure to detect significant transfer of information about leprosy in this setting are discussed, as well as the need for additional research in this area.—Authors' Summary

Revankar, C. R., Pawer, P. L., Belurkar, L. S., Pai, R. R. and Ganapati, R. Reduction in caseload after multidrug therapy in an urban leprosy control programme—a retrospective study in Bombay. Lepr. Rev. **62** (1991) 44–48.

A fall in the active registered case prevalence rate together with a fall in the active caseload per worker after the introduction of multidrug therapy (MDT) is becoming a managerial issue in leprosy control. A retrospective analysis was undertaken to assess the caseload per paramedical worker with reference to active cases for treatment (3341), cases for surveillance (2227), and cases for care after cure (165) at the end of December 1989. All these cases were under the care of 24 paramedical workers. The analysis showed that the caseload per worker was 239 (active cases 139, plus surveillance cases 93, plus care after cure cases 7), although active registered case prevalence rate declined from 1.82/1000 (before starting MDT) to 0.79/1000 by the end of December 1989. The case detection rate was 0.49/1000 by the end of 1989. So, although the active registered case prevalence rate declines, the worker will have enough to do because of the need for surveillance and the detection of relapses, early neuritis, early

disabilities, and care after cure. Simultaneously, new case detection and treatment must be continued. All these aspects need to be considered when program managers are reviewing leprosy control strategy.—Authors' Summary

Chemotherapy

Abhayambika, K., Chacko, A., Mahadevan, K. and Najeeb, O. M. Peripheral neuropathy and haemolytic anaemia with cherry red spot on macula in dapsone poisoning. J. Assoc. Physicians India **38** (1990) 564–565.

A young female presented with hemolytic anemia due to dapsone overdosage. She developed peripheral neuropathy and marked visual impairment with a cherry red spot on the macula, possibly due to toxic retinal vascular damage; both of these resolved in due course. Cherry red spot due to dapsone poisoning has not been reported previously.—Authors' Summary

Choy, A. M. and Lang, C. C. Gall-bladder perforation after long-term dapsone therapy. J. Intern. Med. **228** (1990) 409–410.

A 65-year-old man on maintenance dapsone therapy for dermatitis herpetiformis for 30 years was admitted to the hospital with acute abdominal pain and vomiting. Investigations revealed a Heinz body hemolytic anemia. Worsening symptoms prompted an emergency laparotomy that revealed a perforated gallbladder with pigmented biliary calculi. In previous reviews of the hematological abnormalities associated with dapsone therapy, life-threatening cholecystitis has not been described.—Authors' Abstract

Coleman, M. D., Scott, A. K., Breckenridge, A. M. and Park, B. K. The use of cimetidine as a selective inhibitor of dapsone *N*-hydroxylation in man. Br. J. Clin. Pharmacol. **30** (1990) 761–767.

The *N*-hydroxylation of dapsone is thought to be responsible for the methemoglobinemia and hemolysis associated with this drug. We wished to investigate the effect of concurrent administration of cimetidine (400 mg three times per day) on the disposition of a single dose (100 mg) of dapsone in seven healthy volunteers in order to inhibit selectively *N*-hydroxylation. The AUC of dapsone (31.0 $\pm$ 7.2 μg ml^{-1} h) was significantly increased ($p < 0.001$) in the presence of cimetidine (43.3 $\pm$ 8.8 μg ml^{-1} h). Peak methemoglobin levels observed after dapsone administration (2.5 $\pm$ 0.6%) were significantly ($p < 0.05$) reduced in the presence of cimetidine (0.98 $\pm$ 0.35%). The percentage of the dose excreted in urine as the glucuronide of dapsone hydroxylamine was significantly ($p < 0.05$) reduced in the presence of cimetidine (34.2 $\pm$ 9.3 vs 23.1 $\pm$ 4.2%). Concurrent cimetidine therapy might reduce some of the hematological side effects of dapsone.—Authors' Abstract

Govoni, M., Moretti, M., Menini, C. and Fiocchi, F. Rifampicin-induced immune hemolytic anemia: therapeutic relevance of plasma exchange. Vox Sang. **59** (1990) 246–247.

The development of immune hemolytic anemia (IHA) during intermittent rifampin administration has been well described in the literature. The mechanism of this drug-induced hemolysis may be similar to that of quinidine-induced hemolytic anemia (formation of antibody-drug complexes). Theoretically, plasma exchange (PE) should be of benefit in diseases induced by immune complexes such as drug-induced hemolysis. The efficacy of PE in IHA has not been clearly defined yet, although a few investigators reported some benefits. This report describes the positive application of PE in a specific case of IHA induced by rifampin therapy.—Authors' Abstract

Ji, B., Perani, E. G. and Grosset, J. H. Effectiveness of clarithromycin and minocycline alone and in combination against experimental *Mycobacterium leprae* in-

fection in mice. Antimicrob. Agents Chemother. **35** (1991) 579–581.

As determined by the proportional bactericide method, clarithromycin had strong bactericidal activity against *Mycobacterium leprae*. Clarithromycin was administered to mice by gavage as 20 daily doses at dosages of 12.5 to 50 mg/kg of body weight. At a dosage of 25 mg/kg, minocycline was more active than clarithromycin at a dosage of 50 mg/kg. Additive effects were displayed with the combination of clarithromycin (50 mg/kg) and minocycline (25 mg/kg), both of which were administered daily be gavage, and of clarithromycin and minocycline, both of which were administered daily by gavage at dosages of 25 mg/kg each, with rifampin at a single oral dose of 10 mg/kg.—Authors' Abstract

Orege, P. A. Obura, M. Okelo, C., Okuku, P., Makokha, S. and Nyawalo, J. Multidrug therapy for treatment of paucibacillary leprosy in western Kenya—preliminary communications. E. Afr. Med. J. **67** (1990) 632–639.

A prospective study is being undertaken in western Kenya to evaluate the effectiveness and tolerability of WHO-MDT, while at the same time comparing it to a modified multidrug regimen, which is rifampin 1500 mg at the onset supervised, and repeated after 3 months and dapsone 100 mg daily for 6 months. Preliminary analysis done on 127 cases admitted into the study are presented. The inactivity index observed between 0–12 weeks was 20% for WHO-MDT and 47% for modified-MDT ($p < 0.01$). The inactivity index observed between 0–24 weeks was 63.3% for WHO-MDT and 82.3% for modified-MDT ($p < 0.05$). The inactivity index observed between 0–32 weeks was 83% for WHO-MDT, and 88% for modified-MDT. Type 1 reaction was noted in 23.3% of those on WHO-MDT, and 20.3% of those cases on modified-MDT ($p > 0.1$). Compliance rate was 93.8% for those on WHO-MDT and 95.2% for those on modified MDT. All regimens were well tolerated. These preliminary results indicate that MDT is effective in treatment of paucibacillary leprosy, and also that clinical cure can be achieved in much shorter duration, particularly with higher dosage of rifampin.—Authors' Summary

Rastogi, N. and Labrousse, V. Extracellular and intracellular activities of clarithromycin used alone and in association with ethambutol and rifampin against *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **35** (1991) 462–470.

Mycobacterium avium complex bacteria are opportunistic human pathogens, and their chemotherapy remains a challenge since these organisms are resistant to a majority of routine antituberculous drugs. Recently, a wide range of new macrolide antibiotics has been developed, among which the drug clarithromycin appears to have a selective action against *M. avium* bacteria. In the present study, we have investigated the action of clarithromycin alone (MIC and MBC determinations) and in association with the routine antimycobacterial drugs ethambutol and rifampin at sublethal concentrations (1 μg/ml; below concentrations obtainable in human serum) against *M. avium*. Our viable count data showed that clarithromycin was bactericidal against all 10 strains of *M. avium* studied and that its activity was enhanced by ethambutol (in 8 of 9 strains) and rifampin (in 3 of 9 strains). The use of all three drugs in association resulted in higher bactericidal effects than found with any of the drugs used alone or in two-drug combinations in 7 of 9 strains. The bactericidal effects of various drugs used alone and in combination at concentrations obtainable in human serum were investigated against the type strain ATCC 15769 by using 7H9 broth and BACTEC radiometry (extracellular action) and a J-774 macrophage cell line (intracellular action). A good agreement between the extracellular and intracellular activities was found. Electron microscopy using a ruthenium red cytochemical staining of the bacteria showed that clarithromycin disorganized the outer wall layer and the cytoplasmic membrane in the mycobacterial cell envelope and resulted in formation of large vacuoles inside the cytoplasm, with solubilization of ribosomal structures and consequent plasmolysis. Its association with ethambutol and rifampin resulted in more drastic alterations in the bacterial morphology than were seen

with any of the drugs used alone, leading to the removal of the bacterial outer layer, homogenization of cytoplasm, complete cell lysis, and formation of ghosts.—Authors' Abstract

Roche, P. W., Failbus, S. and Britton, W. Self-administered drug compliance in Nepali leprosy patients. Trop. Doct. **19** (1989) 56–61.

The self-administration of dapsone by Nepali leprosy patients receiving multidrug therapy was assessed by a colorimetric and a filter-paper spot test. Overall 45 out of 337 (13.3%) patients were found to be noncompliant. The relation of noncompliance to sex, age, leprosy classification, therapy type, and length of therapy was investigated. The spot test was compared with the colorimetric assay and found to have a relative sensitivity of 99.3% and specificity of 95.4%. Follow up of patients was successful in that two thirds of noncompliant patients were compliant on their follow-up test.—Authors' Summary

Saito, H., Tomioka, H., Sato, K., Emori, M., Yamane, T., Yamashita, K., Hosoe, K. and Hidaka, T. In vitro antimycobacterial activities of newly synthesized benzoxazinorifamycins. Antimicrob. Agents Chemother. **35** (1991) 542–547.

Newly synthesized rifamycin derivatives, KRM-1648, KRM-1657, KRM-1668, KRM-1686, and KRM-1687, having the chemical structures of 3′-hydroxy-5′-(4-alkylpiperazinyl)-benzoxazinorifamycins (alkyl residues: isobutyl, propyl, *sec*-butyl, *sec*-butyl [*R* configuration], and *sec*-butyl [*S* configuration], respectively), were studied for their *in vitro* antimycobacterial activities. Representative (KRM-1648) MICs for 90% of the strains tested, determined by the agar dilution method on 7H11 medium, of various pathogenic mycobacteria (9 species, 174 strains) were as follows (in micrograms per milliliter): *Mycobacterium tuberculosis* (rifampin [RMP]-susceptible strains), ≤ 0.0125; *M. tuberculosis* (RMP-resistant strains), 12.5; *M. kansasii,* 0.05; *M. marinum,* ≤ 0.0125; *M. scrofulaceum,* 0.1; *M. avium,* 1.56; *M. intracellulare,* 0.1; *M. fortuitum,* >100; and *M. chelonae* subsp. *abscessus* and *M. chelonae* subsp. *chelonae,* >100. These values are more than 64 times lower than those of RMP, except for the values against RMP-resistant *M. tuberculosis* (8 times lower) and those against rapid growers, including *M. fortuitum* and *M. chelonae* (the same as those of RMP). The other derivatives had similar levels of *in vitro* activity against these mycobacteria. When murine peritoneal macrophages in which *M. intracellulare* was phagocytosed *in vitro* were cultured in the presence of the benzoxazinorifamycins (1 μg/ml), much more rapid killing of the organisms ingested in the macrophages was seen compared with when the same amount of RMP was added to the medium. The addition of benzoxazinorifamycins at the concentration of 0.05 μg/ml caused more marked suppression of intracellular growth of the organisms compared with addition of RMP. KRM-1648 and KRM-1657 inhibited intracellular growth of *M. tuberculosis,* and their efficacies were much greater than that of RMP.—Authors' Abstract

van Trier, Y. D. M. and de Soldenhoff, R. Self-administered dapsone compliance of leprosy patients in Eastern Nepal. Lepr. Rev. **62** (1991) 53–57.

Self-administered dapsone intake by leprosy patients in eastern Nepal was monitored with a urine spot test. Of 341 outpatients 55 (16.1%) were found to be noncompliant. A significant relationship was found between noncompliance and age and between noncompliance and caste. Sex, disease classification, type of treatment, duration of treatment, history of leprosy reactions and travel time to the clinic did not influence the compliance. In remote areas the urine spot test can be useful in leprosy control programs.—Authors' Summary

Clinical Sciences

Agarwal, U. S., Handa, A. K., Mathur, D., Mehta, R. D., Mittal, A., Dhar, N. and Mathur, N. K. Hypopigmented lesions in early leprosy—a clinical and histological study. Indian J. Lepr. **62** (1990) 416–421.

Twenty-six leprosy patients presenting with hypopigmented lesions were divided on morphological grounds into three subgroups: Group I (9 patients) with well-defined single patches with moderate to complete sensory loss; Group II (8 patients) with single ill-defined lesions having partial sensory loss; Group II (8 patients) with single ill-defined lesions having partial sensory loss; and Group III (9 patients) having multiple hypopigmented patches with mild-to-moderate sensory loss. Epidermal atrophy was a conspicuous histological finding in all groups. Only patients in Group I showed epithelioid cells in the dermal infiltrate with erosion of the epidermis in one case. This group may be labelled as maculoanesthetic leprosy. Patients in Groups II and III showed mononuclear cell infiltrates in the dermis, around neurovascular bundles and appendages. They were histologically consistant with indeterminate leprosy. Follow-up biopsies after 6–8 months of treatment showed healing of the lesion and reduction in the infiltrate in most cases.—Authors' Abstract

Awasthi, S. K., Singh, G., Dutta, R. K. and Pahuja, O. P. Audiovestibular involvement in leprosy. Indian J. Lepr. **62** (1990) 429–434.

One-hundred leprosy patients were studied for audiovestibular involvement. Conductive hearing loss was detected in 6 cases of BT leprosy, all of them having coincidental chronic middle ear infection. Sensorineural hearing loss was detected in 10 cases, of which 6 had LL, 2 BT and 2 pure neuritic type of disease, respectively. All the cases of lepromatous leprosy having sensorineural hearing loss had evidence of ENL reaction. Vestibular involvement was not detected in any of the cases. Evaluation of audiovestibular function was also carried out in 50 fresh cases of leprosy and after 3 months, 6 months and 1 year of multidrug therapy to ascertain any ototoxic side effects of antileprosy drugs. No audiovestibular dysfunction was detected in these patients at any time during follow-up.—Authors' Abstract

Bechelli, L. M. and Pagnano, P. M. G. Reflections on some aspects of leprosy among children in Brazil and in other countries. Acta Leprol. (Genève) **7** (1990) 229–237.

The authors discuss the classification, clinical aspects, lepromin reactivity, and epidemiologic features of leprosy among children. The most frequent characteristics of each form of leprosy are described. Lepromatous leprosy is less frequent among children in countries of low endemicity and more frequent, even in the most advanced forms, in hyperendemic regions. Borderline forms are rare. In a large number of cases, the initial manifestations are those of the indeterminate form and, in an even larger number of cases, of the tuberculoid pole. The evolution from indeterminate to the tuberculoid pole very frequently occurs in a few months or within less than 1 year. With respect to epidemiology, the authors consider the general frequency of leprosy among children and the frequency of each form of leprosy among children and adults. Data from surveys carried out in Brazil and other countries are presented. Children have a potential for the rapid development of immunoresistance and consequently of lepromin-positivity, and therefore only a few of them eventually develop lepromatous leprosy. This explains the low indices of lepromatous leprosy (approximately 5‰) even in hyperendemic areas. Finally, they discuss the factors that may be responsible for the clinical manifestations of leprosy and their characteristics among children.—Authors' Summary

ffytche, T. J. Residual sight-threatening lesions in leprosy patients completing multidrug therapy and sulphone monotherapy. Lepr. Rev. **62** (1991) 35–43.

An analysis of data derived from standardized surveys of the ocular findings in

cross-sections of the leprosy population in 23 areas is presented. It shows that 24.3% of the patients completing multidrug therapy and 32.9% of those completing sulfone monotherapy have on-going eye problems which have the potential to lead to blindness or severe visual impairment. Most of the ocular complications involve the lids, cornea, and anterior uveal tract, but a significant proportion of patients had cataract threatening vision. If left unsupervised, many of these patients will develop major visual problems which could have been avoided. It is important that completion of systemic leprosy therapy should not be regarded as a guarantee that the eyes are safe, and that regular ocular supervision should be continued long after the patient has been classified as "cured."—Author's Summary

ffytche, T. J. The continuing challenge of ocular leprosy. Br. J. Ophthalmol. **75** (1991) 123–124.

The greatest current tragedy in this disease is that most of the ocular complications that lead to visual loss can be avoided by simple therapeutic measures requiring little more than adequate supervision, patient education, and common sense. The role of the ophthalmologists should therefore be extended to one of a teacher, persuading leprologists and field workers and even fellow ophthalmologists that leprosy is an ocular disease, that the eyes need supervision, not necessarily with sophisticated instruments, and that a mechanism for rapid referral to an ophthalmic center should be available when serious sight-threatening complications develop.

Most of the visual impairment and blindness results from four main causes which may be isolated but often occur together: lagophthalmos leading to exposure keratopathy, corneal hypoesthesia leading to ulceration, acute or chronic iridocyclitis, and secondary cataract.—From the article

Goodless, D. R., Ramos-Caro, F. A. and Flowers, F. P. Reactional states in Hansen's disease: practical aspects of emergency management. So. Med. J. **84** (1991) 237–241.

Hansen's disease (leprosy), though not a common condition in the United States, can be found in some localities among patients who come to the emergency room for treatment. Hansen's disease (HD) is a chronic, systemic, infectious granulomatous disease involving principally the skin, mucosa, nerves, and eyes. The causative organism, *Mycobacterium leprae,* is neither highly contagious nor aggressive, but rapid alterations in the immunologic response to *M. leprae* in affected tissues can result in acute exacerbations termed "reactions." Since most of the symptoms and morbidity in HD are a consequence of these reactional states, they must be recognized and treated early to prevent permanent sequelae, especially neurologic and ophthalmologic. Drug therapy, physical therapy, and sometimes surgery all play a role in minimizing the injury caused by reactions.—Authors' Abstract

Karaçorlu, M. A., Cakiner, T. and Saylan, T. Corneal sensitivity and correlations between decreased sensitivity and anterior segment pathology in ocular leprosy. Br. J. Ophthalmol. **75** (1991) 117–119.

Leprosy is one of the leading causes of corneal hyposensitivity. In this article the corneal sensitivity of 143 leprosy patients was examined, and correlations between corneal hyposensitivity and anterior segment pathology were detected. Twenty-four healthy volunteers were examined as controls. Various degrees of corneal loss of sensitivity were found in 46.2% of leprosy patients. Lagophthalmos, chronic lepromatous granulomatous uveitis, iris atrophy, and social blindness were found 4.5–16.6 times more frequently in eyes which developed severe corneal hyposensitivity.—Authors' Abstract

Karaçorlu, M. A., Cakiner, T. and Saylan, T. Influence of untreated chronic plastic iridocyclitis on intraocular pressure in leprosy patients. Br. J. Ophthalmol. **75** (1991) 120–122.

The intraocular pressure of a total of 286 eyes of patients with lepromatous and borderline lepromatous leprosy who never had regular ophthalmological care or local eye treatment were measured. The patients were categorized according to the type of leprosy they had, and the eyes were categorized as without or with chronic plastic iridocyclitis.

In patients with lepromatous and borderline lepromatous types of leprosy the intraocular pressure was significantly lower in eyes with chronic plastic iridocylitis 10·1 (3·6) mmHg than in both unaffected eyes 11·0 (3·2) mmHg and control eyes 13·5 (2·5) mmHg. It has been shown that chronic plastic iridocyclitis which remains untreated for years results in a lower intraocular pressure than normal.—Authors' Abstract

Karaçorlu, M. A., Surel, Z., Cakiner, T., Hanyaloğlu, E., Saylan, T. and Mat, C. Pupil cycle time and early autonomic involvement in ocular leprosy. Br. J. Ophthalmol. **75** (1991) 45–48.

Ocular complications of leprosy patients often develop insidiously and with few if any symptoms. This study involves measurement of the pupil cycle time (PCT) to evaluate the autonomic nerve system of the iris to determine the presence of subclinical intraocular involvement. The study included 19 lepromatous (LL), 19 borderline lepromatous (BL), and 5 borderline tuberculoid (BT) leprosy patients, and involved 83 eyes. The control group included 25 healthy volunteers, 10 patients with pulmonary tuberculosis and 8 with Duhring disease. The PCT was measured in these groups. In all leprosy groups included in the study the PCT was higher than in the control groups. Moreover, the PCT of the leprosy patients without any intraocular involvement was higher than in the controls. These results show that in the ophthalmic examination of leprosy patients without any symptoms the fact that the autonomic nerve system of the eye is affected by leprosy can often be determined by measuring the PCT.—Authors' Abstract

Klenerman, P., Hammond, C., Kulkarni, V. N. and Mehta, J. M. Vibration sense and trasal disintegration. Indian J. Lepr. **62** (1990) 422–428.

The extent of loss of vibration and pressure sensations was assessed in 21 leprosy patients with disintegration of the tarsus. Feet which had and did not have tarsal disintegration both showed severe impairment of pressure sensation, but the loss of vibration sense was more severe in feet which had undergone the destructive process. It appears that loss of deep sensation is an important factor in the process of tarsal disintegration in feet which are already anesthetic. Measurement of vibration sense using a biosthesiometer may be a valuable clinical test in the investigation and follow-up of the patient with the insensitive foot to identify those at risk of developing tarsal disintegration.—Authors' Abstract

Mezebish, D. S., Yeager, J. K. and Vidmar, D. A. Tuberculoid leprosy: a case report. Cutis **47** (1991) 116–118.

A case of tuberculoid leprosy [in a 28-year-old Filipino immigrant], undiagnosed and consequently untreated for 4 years, is presented. This case report is presented in an attempt to alert the clinician to the occurrence of leprosy in the American population and to raise the index of suspicion in the proper clinical setting.—Authors' Abstract

Premkumar, R., Pannikar, V. K. and Fritschi, E. P. Foot soaks for callosities and fissures. Indian J. Lepr. **62** (1990) 478–482.

A study to assess the effect of soap soaks and plain water soaks on the dry anesthetic soles of 15 leprosy patients bearing multiple fissures and callouses is reported. A callous scraper devised by us was found effective. It is recommended that a hypotonic keratolytic solution, such as toilet soap or plain water, be used for soaking which has the effect of softening the keratin. It may be better to use soap solution for this purpose.—Authors' Abstract

Sarno, E. N., Grau, G. E., Vieira, L. M. M. and Nery, J. A. Serum levels of tumour necrosis factor-alpha and interleukin-1β during leprosy reactional states. Clin. Exp. Immunol. **84** (1991) 103–108.

The possible role of cytokines in leprosy reactions was investigated by analyzing the levels of tumor necrosis factor (TNF) and interleukin-1 (IL-1) in serum samples from 39 leprosy patients, 22 of them presenting either type 1 (upgrading) or type 2 (ENL) reactions. Fifty percent of the patients showed elevated concentrations of TNF and IL-1 in at least one of the serum samples

tested. This included all four patients undergoing type 1 reversal reaction and nine (50%) of the ENL patients studied. Concentrations of TNF above 1000 pg/ml were found in four patients with ENL. Development of erythema multiforme in these ENL patients represented an aggravating factor, and all four patients suffering from this type of lesion demonstrated increased serum TNF levels. All BT patients tested presented elevated IL-1 levels, while only half of them presented elevated levels of TNF. No correlation was found between any particular systemic symptoms and the levels of TNF and IL-1. These results suggest that TNF and IL-1 may be implicated in leprosy reactions, either acting directly or in synergism with other cytokines.—Authors' Summary

Shaw, M. A., Turner, A. C., Blackwell, J. M., Fine, P. E. M. and Ponnighaus, J. M. Setting up HIV serology for the Karonga leprosy vaccine trial in Malawi. Lepr. Rev. **62** (1991) 87–104.

As part of the leprosy vaccine trial taking place in Karonga District, Northern Malawi, it is essential to establish whether the presence of HIV infection in the population is affecting the incidence rate or clinical presentation of leprosy or the effectiveness of the trial vaccines. To obtain the appropriate information, a rapid and economical HIV testing protocol, which could be performed in a rural laboratory and would be robust under variable environmental conditions, had to be developed. This paper reports on the development/evaluation phase of a multitest protocol based on commercially available particle agglutination and ELISA anti-HIV antibody detection kits. The protocol was devised by first evaluating a range of kits in London using a battery of African and non-African sera and then field testing 1455 sera in Malawi, which included 184 sera from leprosy patients and 60 sera from syphilis patients to check for crossreactivity. According to the protocol developed, all sera are screened initially both by indirect ELISA (Organon) and using a rapid and economical modification of the Serodia particle agglutination test. Positives are retested using both a competitive ELISA (Wellcome or Behring) and the standard Serodia particle agglutination test. The validity of this multitest protocol was confirmed by Western blotting a large sample of the positive and negative Malawian sera in London. Factors affecting kit selection, and problems associated with individual kits, are discussed. While the specific multitest protocol developed for Malawi might not be suitable for every project, the principle of developing economical alternatives to Western blotting is an important consideration for any field investigation of HIV.—Authors' Summary

Shi, Z.-R., *et al.* [Histological findings in the iris of DDS treated patients.] China Lepr. J. **6** (1990) 203–207. (in Chinese)

Forty-nine iris specimens, obtained during cataract extraction operations from 43 leprosy patients with no sign of active iritis, negative skin smears for *Mycobacterium leprae* and long treatment with dapsone, were studied histologically. Mild atrophy of the iris was observed in 84% of the specimens, and lymphocyte, plasma cell and macrophage infiltration to various degrees were seen in 85%. The latter causes may be related to why more complications were usually seen after the cataract extraction in leprosy patients. In 5 specimens of 5 patients (2 LL, 2 BL, and 1 BT, with leprosy disease history of 26, 40, 28, 40 and 16 years, respectively), *M. leprae* were found. Some *M. leprae* remaining in the iris of the long-treated patients with negative skin smears are interesting both theoretically and practically.—Authors' English Abstract

Singh, K., Raina, V., Narulla, A. K. and Singh, R. Sarcoidosis masquerading as leprosy, pulmonary tuberculosis and urolithiasis. J. Assoc. Physicians India **38** (1990) 657–659.

A middle-aged man presented as a recalcitrant case of borderline leprosy with concomittant pulmonary tuberculosis and urolithiasis which continued to progress relentlessly despite adequate multidrug antileprosy and antitubercular treatment. After detailed and relevant workup, the diagnosis of sarcoidosis was made. Rapid clinical improvement occurred with steroid therapy.—Authors' Summary

Taneja, K., Khanna, N. V., Shiv, V. K., Pandhi, R. K. and Bhargava, S. K. Hepatic ultrasonography in patients with lepromatous leprosy. Indian J. Lepr. **62** (1990) 443–447.

Hepatic sonography was done in 36 patients with lepromatous leprosy and 3 patients with borderline lepromatous leprosy with the view to assess abnormalities of size, changes in the echotexture, and to observe the presence of any nodules and calcification in the liver. Routine liver function tests were also done in these patients. No definite abnormal sonographic findings were seen in the liver in a large majority of these patients. One patient, however, showed nodular changes in the liver.—Authors' Abstract

Tekle-Haimanot, R., Frommel, D., Tadesse, T., Verdier, M., Abebe, M. and Denis, F. A survey of HTLV-I and HIVs in Ethiopian leprosy patients. AIDS **5** (1991) 108–109.

Recent studies have shown that there is a strong association between human T-cell lymphotropic virus (HTLV) and endemic tropical spastic paraparesis (TSP) as well as the nontropical syndrome of HTLV-I-associated myelopathy (HAM) described in Japan. In Africa, the distribution of HTLV-I infection has not yet been extensively investigated but there are reports to indicate that the prevalence of HTLV-I infection is high in West Africa, as well as in endemic foci of TSP, such as the Seychelles Islands. A high rate of HTLV-I infection has also been observed in West African leprosy patients. We therefore studied the HTLV-I and HIV status in Ethiopian leprosy patients.

Antibodies to HTLV-I were found in three women: 1 among the leprosy patients (0.4%), and 2 from the dermatological control patients (0.8%), 1 of whom was co-infected with HIV. Antibodies to HIV-1 were found at higher prevalence in the three cohorts: 8 of the leprous patients (3.2%); 7 of the nonleprous control patients of the ALERT hospital, excluding hospital personnel (3.3%); and 9 of the second group of controls (2.5%). Three of the seropositive leprosy patients were MB, 3 were PB, and 2 presented with an indeterminate form of leprosy. None were in reaction state. Unlike the report of Meeran in Zambia, and of Léonard, *et al.* in the Ivory Coast, we did not find a significantly higher HIV infection rate in leprous patients compared with controls. The HIV infection in the leprous patients studied is likely to be too recent to modify, as in *Mycobacterium tuberculosis* infection, the course and spread of the disease. However, the superimposition of HIV infection on *M. leprae* contagion, still widespread, is a matter of great concern.—From the article

Thankappan, T. P. and Sulochana, G. Keratosis spinulosa developing in borderline-tuberculoid lesions during type I lepra reaction: two case reports. Lepr. Rev. **62** (1991) 49–52.

Two cases of borderline-tuberculoid leprosy which developed keratosis spinulosa over the anesthetic areas alone during type 1 lepra reactions are described. Both patients only developed spiny papules during the period of reaction and subsided with control of the reaction. The probable mechanism of this peculiar phenomenon might be due to the generation of epidermal growth factors by local T-cell activation during the type 1 lepra reaction.—Authors' Summary

Immuno-Pathology

Anderson, D. C., Van Schooten, W. C. A., Janson, A., Barry, M. E. and de Vries, R. R. P. Molecular mapping of interactions between a *Mycobacterium leprae*-specific T cell epitope, the restricting HLA-DR2 molecule, and two specific T cell receptors. J. Immunol. **144** (1990) 2459–2464.

A systematic series of 89 single residue substitution analogs of the *Mycobacterium leprae* 65-kDa protein-derived peptide LQAAPALDKL were tested for stimulation of two HLA-DR2 restricted 65-kDa-reactive T-cell clones from a tuberculoid leprosy patient. Some analogs with substi-

tutions outside a "core" region showed enhanced stimulation of the T-cell clones. This core region of seven or eight residues was essential for recognition; whereas substitution of amino acids outside this region did not affect T-cell recognition although these residues could not be omitted. Thus, these core residues interact directly with the presenting HLA-DR2 molecule and/or the TCR. Except for analogs of position 419 for clone 2B6, the majority of the nonstimulatory substitution analogs did not inhibit the presentation of LQAAPALDKL and, thus, probably failed to bind to the HLA-DR2 molecule. Unless all of the core residues are physically involved in binding to DR2, substitution at a position not directly involved in binding appears to have an influence on other residues that do bind to the DR2 molecule. Active peptide analogs with two or more internal prolines suggest that not all analogs need to be helical for activity with clone 2F10.—Authors' Abstract

Chugh, K. S. and Sakhuja, V. Glomerular diseases in the tropics. Am. J. Nephrol. **10** (1990) 437–450.

An attempt has been made to focus attention on features of tropical glomerulopathies with which the majority of the Western-trained nephrologists, pathologists and immunologists have been quite unfamiliar. There is no denying the fact that advances in the understanding of these conditions have been made only through collaborative research with some of the Western centers. Hospital data, though not fully representative, have been cited since population surveys are not available. The prevalence of glomerular disease in the tropics is strikingly higher compared with the temperate regions, and appears to be related to the exposure of the populations in these areas to a number of environmental agents not encountered elsewhere.

P. malariae and *S. mansoni* infections undeniably play a major role in the causation of glomerulonephritis; however, their distribution varies widely. Leprosy, filariasis and sickle cell disease are the other important causes of tropical glomerular disease.

Among the primary glomerular diseases, available evidence indicates that steroid-responsive minimal change nephropathy is only rarely seen in most African populations, but its incidence in Asian countries is similar to that in the U.S.A. and Europe. Whereas poststreptococcal glomerulonephritis appears to have declined significantly from the economically advanced countries, it continues to be a frequent cause of glomerular disease in Asia and Africa. IgA nephropathy has emerged as the commonest primary glomerular disease in Southeast Asia. Systemic lupus erythematosus, which was at one time considered rare in African and Asian populations, is seen commonly, but racial susceptibility accounts for an uneven distribution. Hepatitis-B-virus-associated nephropathy is now being increasingly recognized in both children and adults in several parts of Southeast Asia and Africa.—Authors' Conclusions

Clark-Curtiss, J. E., Thole, J. E. R., Sathish, M., Bosecker, B. A., Sela, S., de Carvalho, E. F. and Esser, R. E. Protein antigens of *Mycobacterium leprae.* Res. Microbiol. **141** (1990) 859–871.

Protein antigens of *Mycobacterium leprae* have been identified by screening the λgt11, pYA626 and pHC79::*M. leprae* genomic libraries with pooled sera from leprosy patients and with antiserum to *M. leprae* cell-wall protein (CWP) aggregate. Immunological screening of the λgt11 library with pooled sera from 21 lepromatous (LL) leprosy patients resulted in the identification of 19 antigens that are apparently different from previously identified *M. leprae* antigens. Five additional antigens were identified by screening the λgt11 library with pooled sera from 30 borderline tuberculoid or tuberculoid patients. Four other antigens were identified by screening the λgt11 library with anti-CWP. Two groups of recombinant cosmids were identified by screening the pHC79 library with LL patients' sera: one group specified proteins that reacted with monoclonal antibodies (mAb) against the 65-kDa protein and against the 18-kDa protein; the other group specified a 15-kDa protein that did not react with any of the mAb that were tested. One pYA626 clone also specified a 15-kDa protein that reacted with LL patients' sera, but did not react with any mAb. Genes specifying sev-

eral of these antigens have been subcloned into the Asd$^+$ plasmid vector pYA292 and have been introduced into a Δ*caya* Δ*crp* Δ*asd* *Salmonella typhimurium* strain to evaluate the ability of individual *M. leprae* proteins to elicit immune responses against *M. leprae* infection.—Authors' Summary

Denis, M. Modulation of *Mycobacterium lepraemurium* growth in murine macrophages: beneficial effect of tumor necrosis factor alpha and granulocyte-macrophage colony-stimulating factor. Infect. Immun. **59** (1991) 705–707.

Mycobacterium lepraemurium grew progressively in monolayers of proteose peptone-elicited macrophages from C57BL/6 mice. Treatment of macrophage monolayers with gamma-interferon led to an enhancement of growth of *M. lepraemurium* in macrophages. Treatment with tumor necrosis factor alpha or granulocyte-macrophage colony-stimulating factor led to restriction of mycobacterial growth in macrophages.—Author's Abstract

Doherty, T. M., Bäckström, B. T., Prestidge, R. L., Love, S. G., Harding, D. R. K. and Watson, J. D. Immune responses to the 18-kDa protein of *Mycobacterium leprae*; similar B cell epitopes but different T cell epitopes seen by inbred strains of mice. J. Immunol. **146** (1991) 1934–1940.

Antibody responses to the 18-kDa protein of *Mycobacterium leprae* have been analyzed in different strains of mice. High, intermediate, and low responder strains have been identified and these response patterns show clear linkage to genes encoded in the H-2 complex. Three peptides, residues 1–50, 51–100, and 101–148, have been synthesized, as well as a series of 20-mer peptides, which span the entire 18-kDa protein. Repeated immunization of different strains of mice with the 18-kDa protein resulted in IgG responses to epitopes found on all three synthetic peptides. Immunization of BALB/cJ and B10.BR mice, two high responder strains, with 18-kDa protein resulted in high levels of IgG antibody to epitopes found on peptides 1–20, 16–35, 31–50, 46–65, and 76–95. B10.BR mice also contained IgG that bound peptide 61–80, and BALB/cJ mice produced IgG that bound peptide 91–110. Although B10.BR mice produced IgG that bound the 50-mer peptide 101–148, this IgG was not detected by binding to peptides 91–110, 106–125, 121–140, and 131–148. Immunization of B10.BR mice with individual overlapping 20-mer peptides as antigen revealed that peptides 1–20, 16–35, 31–50, and 76–95 elicited high titers of IgG that bound both the immunizing peptide as well as 18-kDa protein. Since these peptides induce antibody synthesis they must contain both B-cell and T-cell epitopes. By contrast, immunization of BALB/cJ mice with the same 20-mer peptides, all of which contain B-cell epitopes for this strain, failed to elicit IgG responses with one exception. Peptide 91–110 induced IgG that bound peptide 91–110, but not the intact 18-kDa protein. We conclude that peptides 1–20, 16–35, 31–50, and 76–95 either lack T-cell epitopes for BALB/cJ mice, or activate different T-cell subpopulations in the two strains. We suggest that the induction of IgG responses to small peptide antigen is an *in vivo* assay of the activity of Th2 cell subpopulations.—Authors' Abstract

Esaguy, N., Aguas, A. P., van Embden, J. D. A. and Silva, M. T. Mycobacteria and human autoimmune disease: direct evidence of cross-reactivity between human lactoferrin and the 65-kilodalton protein of tubercle and leprosy bacilli. Infect. Immun. **59** (1991) 1117–1125.

We document here by Western immunoblotting and immunogold ultracytochemistry that polyclonal antibodies against human lactoferrin (Lf) bind to tubercle and leprosy bacilli. *In situ* immunogold labeling of *Mycobacterium leprae* (present in armadillo liver and in human skin) and of *M. tuberculosis* indicated that receptors for anti-Lf antibodies were present both on the cytoplasm and on the envelope of the bacilli. We found by immunoblotting that the 65-kDa heat-shock protein is the major component of *M. leprae* and *M. tuberculosis* that is responsible for the binding of the anti-Lf probe. Furthermore, we show that anti-Lf immunoglobulin G eluted from the nitrocellulose-transferred mycobacterial 65-kDa

protein band did bind back to Lf. Ultracytochemistry of biopsy samples of human lepromas showed that dead or severely damaged *M. leprae* was strongly marked by the anti-Lf antibodies; a similar pattern of immunogold marking was observed on *M. leprae* when antibodies against the 65-kDa mycobacterial protein were used. Our results offer direct evidence that the 65-kDa protein of leprosy and tubercle bacilli is recognized with specificity by antibodies against the human protein Lf. The Lf-65-kDa protein antigenic crossreactivity may contribute to the formation of autoantibodies and immune complexes as well as to other autoimmune events that are frequent in tuberculosis and leprosy. Our immunocytochemical data also suggest that the crossreactivity may persist for some time after the death of mycobacteria in infected hosts.—Authors' Abstract

Hancock, G. E., Molloy, A., Ab, B. K., Kiessling, R., Becx-Bleumink, M., Cohn, Z. A. and Kaplan, G. *In vivo* administration of low-dose human interleukin-2 induces lymphokine-activated killer cells for enhanced cytolysis *in vitro.* Cell. Immunol. **132** (1991) 277–284.

We have examined the effect of the intradermal administration of IL-2 on the generation of natural killer (NK) cell and lymphokine-activated killer (LAK) cell activity. Peripheral blood mononuclear cells (PBMC) obtained from borderline lepromatous (BL) and lepromatous leprosy (LL) patients and normal volunteers prior to and after IL-2 injection were stimulated *in vitro* with IL-2 and their cytolytic activities compared against ^{51}Cr-labeled target K562 cells, Daudi cells, and monocytes. Before IL-2 administration, PBMC obtained from BL/LL patients and normal volunteers possessed similar levels of NK-cell activity indicating that the NK-cell activity of the BL/LL patients was intact. LAK-cell activity was induced with IL-2 *in vitro* in both BL/LL patients and in normal volunteers. The level of LAK-cell activity in BL/LL patients was, however, suboptimal. A single intradermal dose of 25 μg IL-2 had no effect on the phenotype of circulating mononuclear cells in either patients or normal volunteers. However, 6–12 days after IL-2 injection and subsequent restimulation of the PBMC with IL-2 *in vitro,* cytolytic activity of LAK cells obtained from the BL/LL patients was enhanced while cells from normal volunteers expressed the same high levels of activity as observed before IL-2 injection.—Authors' Abstract

Iyer, R. R., Prasad, H. K., Bhutani, L. K. and Rao, D. N. Effect of tuftsin stimulation on the microbicidal activity exerted by blood monocyte-macrophages of leprosy patients. Int. J. Immunopharmacol. **12** (1990) 859–869.

The ability of blood monocyte/macrophages from normal donors, tuberculoid leprosy (BT/TT), and lepromatous leprosy (BL/LL) patients to exert enhanced microbicidal activity was assayed after stimulating with 0.8 μM tuftsin, as a function of the duration of cultures *in vitro.* Normal and BT/TT macrophage cultures showed a statistically significant increase in microbacterial activity against *Staphylococcus aureus* at all ages of culture (6 hr to 14 days), although the overall magnitude of the enhancement shows a decrease with increasing culture age in the same populations. However, 14-day-old BL/LL macrophage cultures were unable to undergo tuftsin-mediated stimulation of microbicidal activity against *S. aureus* and even, fresh 6 hr-old cultures exhibited a tuftsin-stimulated response profile similar to 14-day old normal and BT/TT cultures. Also, 7- and 14-day cultures of normal, BT/TT, and BL/LL macrophages were unable to inhibit/kill intracellular *Mycobacterium leprae* after a single stimulation with 0.8 μM tuftsin. However, serial, daily stimulation with 0.8 μM tuftsin resulted in 77%–140% inhibition of ^{3}H-thymidine uptake by the 12th day of cultures *in vitro* in the three groups. These results suggest that BL/LL macrophages exhibit a premature inability to undergo tuftsin stimulated microbicidal activity, which may possibly be reversed by serial dosage of tuftsin.—Authors' Abstract

Iyer, R. R., Prasad, H. K., Bhutani, L. K. and Rao, D. N. Modulation of human lepromatous monocyte-macrophage functions *in vitro* by tuftsin. Int. J. Immunopharmacol. **12** (1990) 847–858.

Human peripheral blood monocytes/macrophages derived from normal donors, patients of tuberculoid leprosy (BT/TT) and lepromatous leprosy (BL/LL) were assayed for stimulated phagocytic responses to the potent macrophage stimulator "Tuftsin" (NH_2 Thr-Lys-Pro-Arg-OH) after varying periods (6 hr to 14 days) of culture *in vitro.* The assays consisted of visual scoring of ingested *Mycobacterium leprae* and radiometric measurement of ingested ^{14}C-acetate-labeled *Staphylococcus aureus* and *M. tuberculosis* (H37Ra). While normal and BT/TT macrophages showed a progressively increasing ability for tuftsin-stimulated phagocytosis with increasing age of culture *in vitro,* BL/LL macrophages showed the opposite response so that 14-day cultures were refractory to a stimulatory dose of up to 7.0 μM (10 to 20 times the optimal dose for normal and BT/TT macrophages). The 14-day BL/LL macrophage cultures were, however, responsive to 35 μM tuftsin (100 times the optimal dose for normal macrophages). Analysis of the dose-response curves also indicates that BT/TT cultures despite exhibiting an apparent similarity to normal macrophages demonstrate a rightward shift for a maximal stimulated phagocytosis. Finally SEM photomicrographs of 14-day macrophage cultures of the three groups revealed that while normal and BT/TT cultures demonstrated an increase in membrane ruffling and filopodia on stimulation with 0.8 μM tuftsin, BL/LL cultures exhibited none of the features associated with stimulation. From the above findings, we conclude that lepromatous macrophages may display an aberrant differentiation profile leading to a terminal state of unresponsiveness, and that the defect may possibly lie at the level of tuftsin receptor expression on transmembrane signal transduction.—Authors' Abstract

Kaleab, B., Ottenhoff, T., Converse, P., Halapi, E., Tadesse, G., Rottenberg, M. and Kiessling, R. Mycobacterial-induced cytotoxic T cells as well as nonspecific killer cells derived from healthy individuals and leprosy patients. Eur. J. Immunol. **20** (1990) 2651–2659.

Little information is available about the generation and specificity of the cytotoxic cells that eliminate human monocytes/macrophages infected with mycobacteria. To address this, we have developed a cytotoxicity assay in which ^{51}Cr-labeled monocytes pulsed with bacillus Calmette Guérin (BCG) or *Mycobacterium leprae,* were used as target cells in overnight cytotoxicity assays. As effector cells, peripheral blood mononuclear cells from healthy occupational contacts or from leprosy patients stimulated with antigen for 7 days were used. Cytotoxicity against antigen-pulsed monocytes that could be induced by mycobacterial antigens was proportional to the degree of antigen responsiveness in each individual, as measured in lymphocyte transformation tests. The lepromatous leprosy patients tested were often poor responders to BCG as well as *M. leprae,* both with regard to induction of cytotoxicity as well as in lymphoproliferation. Killing was significantly higher against antigen-pulsed vs nonpulsed monocytes, although significant killing was induced against the latter as well and paralleled by induction of natural killer activity against the K-562 target cell. Crossreactivity was observed between BCG and *M. leprae,* but not with unrelated antigen (tetanus toxoid) or with endogenous stress proteins induced by heat shock. *M. leprae*- and BCG-activated cytotoxic cells were found in both the CD4−CD8+ and CD4+CD8− populations; whereas in contrast the soluble antigen, purified protein derivative of *M. tuberculosis,* generated cytotoxic cells that were exclusively of the CD4+ phenotype. The involvement of both specific T cells as well as nonspecific cells in the killing of human macrophages may be important with respect to protection and immunopathology induced by mycobacterial antigens.—Authors' Abstract

Kaklamani, E., Koumandaki, Y., Katsouyanni, K. and Trichopoulos, D. BCG, tuberculosis, and leprosy. (Letter) Lancet **1** (1991) 304.

One-hundred-sixteen patients with lepromatous leprosy (LL) and 73 patients with tuberculoid leprosy (TT) were studied who were seen as outpatients at the Center for Hansen's Disease in Athens, Greece. Only cases with either of the two polar types of leprosy were included; 382 patients of low

socioeconomic class, admitted to nearby hospitals for other reasons, acted as a control group. All subjects were of caucasian origin, a unique feature of this study. For patients with LL and TT, 10 (8.6%) and 0 subjects had a history of tuberculosis, respectively ($\chi^2 = 6.64$, $p = 0.01$). The frequency of tuberculosis among control patients was 10%. We conclude that there is a negative correlation between tuberculosis and the TT form of leprosy, which is not found in the LL form.—From the letter

Kaplan, G., Britton, W. J., Hancock, G. E., Theuvenet, W. J., Smith, K. A., Job, C. K., Roche, P. W., Molloy, A., Burkhardt, R., Barker, J., Pradhan, H. M. and Cohn, Z. A. The systemic influence of recombinant interleukin 2 on the manifestations of lepromatous leprosy. J. Exp. Med. **173** (1991) 993–1006.

Fourteen patients with lepromatous leprosy received twice daily injections of 10 μg recombinant interleukin 2 (rIL-2), by the intradermal route, in the skin of the back for 8 days (total dose, 160 μg). Lymphokine administration was accomplished without drug toxicity, or the development of acute nerve damage. The majority of patients developed nontender axillary lymphadenopathy during the course of treatment. Local injection sites showed progressively larger zones of induration, peaking at 24 hr and persisting for many days. Early 12-hr reactions were of a macular, erythematous nature and exhibited an increasingly striking diurnal variation. The morning injections sites were three- to fourfold larger in diameter than those placed in the evening (9 a.m. to 9 p.m.). Systemic manifestations of intradermal rIL-2 administration were noted. Peripheral blood T cells, including CD4+ and CD8+ phenotypes, increased 2–2.5-fold and NK cells increased sixfold. Elevations in [^{3}H]TdR incorporation into peripheral blood mononuclear cells occurred to a variety of mycobacterial antigens, but not to those of *Mycobacterium leprae*. Within 2 weeks, biopsies at sites far removed from the back showed increased infiltration of mononuclear cells in 12 of 14 patients. Immunocytochemistry revealed the presence of newly emigrated CD4+ T cells, monocytes, and dermal CD1+ Langerhans' cells. Endothelial cells of small dermal vessels expressed major histocompatibility complex class II determinants on their surface. Transmission electron microscopy of these specimens revealed markedly enlarged endothelial cells with many surface projections extending into the lumen as well as extravasating lymphoid cells. The numbers of acid-fast *M. leprae* in the peripheral sites were examined by slit smear and in biopsies of matched leprosy lesions taken before and after IL-2 administration. Within 2 months, slit smears showed a 0.5 log or greater reduction in 12 of 14 patients, with a mean for all patients tested of 0.5 log units. Biopsy specimens showed a 1 log unit or greater reduction in the bacterial index (BI) in 6 of 14 patients. Historical controls in this Nepalese population showed a 0.5 log unit reduction after multidrug therapy over a period of 12 months. Thus, after 8 days of IL-2 injections, a fivefold reduction in BI was observed during the first 2 months of the study. Antibody levels against *M. leprae* phenolic glycolipid I (PGL-I) and lipoarabinomannan-B were markedly elevated after IL-2 injections, while PGL-I antigen levels were reduced. We conclude that the administration of rIL-2 has had a significant effect in decreasing the total body burden of *M. leprae*. This is accomplished by the influx of mononuclear leukocytes from the circulation, the enhancement of cell-mediated immunity, and the degradation of leprosy bacilli. This occurs much more rapidly compared with multidrug chemotherapy alone.—Authors' Summary

Kaufmann, S. H. E. Immunity to mycobacteria. Res. Microbiol. **141** (1990) 765–768.

How do these different T-cell sets interact with each other in a mycobacterial granuloma? Although much remains hypothetical, we can envisage the following scenario. A granuloma is formed of mononuclear phagocytes of different differentiation and maturation stages. At least some mycobacteria reside in "aged" macrophages which are insufficiently equipped for intracellular killing. Activation of these macrophages with IFN-γ, IL-4 and IL-6 may help to confine these microbes to distinct loci but may not always be sufficient for sterile elimina-

tion of the pathogens. In addition, blood-derived monocytes enter the lesion and these phagocytes are better equipped for killing. To facilitate optimal uptake by blood monocytes, it may be necessary to first lyse infected granulomatous macrophages. As long as the released bacteria are taken up by monocytes effectively, this event is to the benefit of the host. However, extensive lysis may allow for uncontrolled release of bacteria and, as a corollary, mycobacterial dissemination may occur. This, as well as tissue destruction caused by cytolytic T-cell mechanisms, is rather detrimental to the host. Although the scenario envisaged is highly speculative, it illustrates that the host-parasite relationship comprises a multitude of different elements which may both threaten and benefit the host.—Author's Conclusions

Klatser, P. R., de Wit, M. Y. L., Kolk, A. H. J. and Hartskeerl, R. A. Characterization of murine B-cell epitopes on the *Mycobacterium leprae* proline-rich antigen by use of synthetic peptides. Infect. Immun. **59** (1991) 433–436.

Using synthetic peptides representing overlapping sequences of the 100-amino-acid-long N-terminal region of the proline-rich antigen of *Mycobacterium leprae* (PRA), we have mapped the epitopes in the primary structure of PRA recognized by four monoclonal antibodies. The *M. leprae*-specific monoclonal antibody F47-9 recognized the amino-acid sequence LGSAYP (residues 34 to 39). Both monoclonal antibodies F67-1 and F67-5 recognized the sequence YPPP within the repeated sequence of PRA at four sites (residues 38 to 41, 50 to 53, 60 to 63, and 70 to 73). Monoclonal antibody F126-5 recognized the sequence SYPPP, also within the repeat, at three sites (residues 49 to 53, 59 to 63, and 69 to 73). All three epitopes appeared to be linear as far as can be determined by this approach.—Authors' Abstract

Kleinau, S., Söderström, K., Kiessling, R. and Klareskog, L. A monoclonal antibody to the mycobacterial 65kDa heat shock protein (ML 30) binds to cells in normal and arthritic joints of rats. Scand. J. Immunol. **33** (1991) 195–202.

A monoclonal antibody reactive with the mycobacterial 65 kDa heat-shock protein (hsp) (ML 30) was investigated for reactivity with biopsies from normal rat joints and with inflamed joints due to adjuvant arthritis (AA) or collagen induced arthritis (CIA). Immunohistochemical stainings with the anti-hsp 65 antibody on paraffin sections from normal rat joints revealed a weak but exclusive staining of cells within the synovial lining. Also normal chondrocytes and bone-marrow cells showed occasional staining. In biopsies from inflamed joints obtained from rats suffering from AA or CIA, an intense staining with ML 30 was seen within the cartilage-pannus junction as well as sites of bone erosion. An increased staining, compared with the normal, was also seen in chondrocytes of the eroded cartilage and in some bone-marrow cells. No staining with ML 30 was seen in biopsies from inflammatory lesions due to delayed-type hypersensitivity reactions in the skin of rats. Reactivity of ML 30 was also seen in a Western blot assay performed on lysates from inflamed synovia from rats with CIA, preferentially with a component slightly below 60 kDa in molecular weight.

The demonstration of epitopes crossreactive with hsp 65 of mycobacteria in normal and, in higher quantity, in arthritic rat joints, suggests, together with our preliminary biochemical findings, that a recently identified mammalian counterpart to bacterial hsp 65 is both preferentially expressed in normal joints and subject to increased expression in arthritis of different etiologies.—Authors' Abstract

Krahenbuhl, J. L., Sibley, L. D. and Chae, G.-T. Gamma interferon in experimental leprosy. Diagn. Microbiol. Infect. Dis. **13** (1990) 405–409.

We have shown that preactivated macrophages (MACs) are able to cope with newly acquired leprosy bacilli, and a high intracellular burden of live *Mycobacterium leprae* induces a refractory response of the host MAC to activation by IFN-γ. Our studies underscore the fact that MAC function in LL is dependent on localized conditions, influenced by the high intracellular burden of leprosy bacilli and, in part, involving the production of prostanoids. These

findings preclude inferences about the function of granuloma MACs in leprosy based on the responses of MACs from other easily accessible anatomical compartments such as the peritoneal cavity or peripheral blood. We feel that the clearance of bacilli from the LL lesion as a consequence of local immunotherapeutic measures or chemotherapy likely depends on the influx of new competent MAC rather than the activation of resident lepromatous MACs.—Authors' Summary

Laal, S., Sharma, Y. D., Prasad, H. K., Murtaza, A., Singh, S., Tangri, S., Mishra, R. S. and Nath, I. Recombinant fusion protein identified by lepromatous sera mimics native *Mycobacterium leprae* in T-cell responses across the leprosy spectrum. Proc. Natl. Acad. Sci. U.S.A. **88** (1991) 1054–1058.

Pooled polyvalent sera from lepromatous leprosy patients were used to screen a λgt11 recombinant DNA expression library of *Mycobacterium leprae* in order to identify the relevant antigens recognized by the human immune response. Of the 300,000 phages screened, 4 clones were identified that coded for fusion proteins of the same molecular mass. The fusion protein from clone LSR2 was tested for immunoreactivity in assays using peripheral blood cells and sera from 11 laboratory personnel and 105 patients across the leprosy spectrum. LSR2 protein appears to be predominantly a T-cell antigen. It evokes similar lymphoproliferative responses as the native bacillus both at the individual level and in the leprosy spectrum as a whole. Although only 50% of patient sera with anti-*M. leprae* antibodies reacted with the fusion protein, the pattern of reactivity in the antibody responses was also similar for the various clinical types. The coding regions of clones LSR1 and LSR2 are identical. They show no homology with sequences stored in data banks and encode a protein of 89 amino acids with a calculated molecular mass of ≈ 10 kDa.—Authors' Abstract

Launois, P., Blum, L., Dieye, A., Millan, J., Sarthou, J. L. and Bach, M.-A. Phenolic glycolipid-I from *M. leprae* inhibits oxygen free radical production by human mononuclear cells. Res. Immunol. **140** (1989) 847–855.

We studied the effect of PGL-I, a phenolic glycolipid unique to *Mycobacterium leprae,* on the activation of the phagocyte oxidative respiratory burst, by measuring the chemiluminescence (CL) generated by normal mononuclear cells. PGL-I induced a decrease in oxygen-free radical production stimulated by mycobacteria (*M. leprae,* BCG and *M. kansasii*) or by phorbol myristate acetate, but did not prevent the binding or ingestion of fluorescein-conjugated mycobacteria. In contrast, mycoside A from *M. kansasii,* a structurally related compound, did not alter the CL response. In addition, treatment of *M. leprae* with anti-PGL-I antibodies failed to restore the response to this microorganism. PGL-I could act as an oxygen species scavenger and protect *M. leprae* from killing by toxic oxygen metabolites.—Authors' Summary

Laver, W. G., Air, G. M., Webster, R. G. and Smith-Gill, S. J. Epitopes on protein antigens: misconceptions and realities. Cell **61** (1990) 553–556.

Structural data establish that epitopes on native proteins consist of 15–22 residues in a discontinuous array. Energetic calculations suggest that a smaller subset of 5–6 of these residues contributes most of the binding energy, with the surrounding residues merely indulging in complementarity. It should be stressed that the residues proposed to contribute most of the binding energy are not arranged in a linear sequence but are scattered over the epitope surface; in no sense can they be considered equivalent to unfoldons identified with antisera against short peptides. Ultimately, definition of the precise relationship between the structure and the function of the epitope will require detailed kinetic and structural analysis of site-directed mutants of both antigen and antibody.—From the article

Levine, S. and Saltzman, A. Induction of arthritis in rats by aqueous suspensions of mycobacteria without the use of oil. Arthritis Rheum. **34** (1991) 63–67.

We report for the first time the induction of arthritis by an aqueous, rather than oil,

suspension of killed tubercle bacilli. This was accomplished in the highly susceptible dark Agouti strain of rats, by intraperitoneal injection during the healing phase of chemically induced peritonitis. The same procedure (injection after the induction of peritonitis) augmented the incidence of arthritis produced by bovine type II collagen and Freund's complete adjuvant. Enhanced delivery of antigen from the peritoneal cavity to regional lymph nodes in the postinflammatory state was responsible for this increase in the induction of arthritis.—Authors' Abstract

Li, M.-H., *et al.* [Determination of antibodies to leprosy by MTPA.] China Lepr. J. **6** (1990) 214–217. (in Chinese)

Antibody against phenolic glycolipid specific to *Mycobacterium leprae* in the sera of 61 leprosy patients including 41 MB and 20 PB, 128 household contacts with leprosy, 30 cases of tuberculosis, and 31 healthy controls was determined quantitatively and qualitatively using the microtiter particle agglutination (MLPA) test. The result showed that the test's sensibility is higher, i.e., positivity over 67% in MB cases, and the specificity is over 95%. The antibody level being higher in MB and lower in PB indicates that there is a correlation between the antibody level and the form of leprosy. The positivity in the household contacts and negativity in tuberculosis cases both proved that there is no crossreaction between the serum of tuberculosis and the PGL. The authors consider that the MLPA test without need for special instrumentation is reliable for determining antibody against *M. leprae* and is easy to use.—Authors' English Abstract

Macedo, H. W. and Quirico-Santos, T. Nonspecific esterase staining of lymphocyte subsets in leprosy. Exp. Pathol. **41** (1991) 50–52.

A comparative study of lymphocyte subpopulations from leprosy patients were carried out using nonspecific esterase (ANAE) staining and monoclonal antibodies. Regardless of the methodology used, an intense reduction in the total number of the T-helper/inducer subset and an inversion of the helper/suppressor ratio could be observed. These alterations were more evident with the progress of the disease from the tuberculoid (TT) to the lepromatous (LL) pole. Thus ANAE may be considered an economical and reliable method to be used in the study of the immune status of leprosy patients.—Authors' Abstract

Mahon, A. C., Nurlign, A., Kebede, B., Becx-Bleumink, M. and Lefford, M. J. Urinary phenolic glycolipid 1 in the diagnosis and management of leprosy. J. Infect. Dis. **163** (1991) 653–656.

A simplified assay to measure the phenolic glycolipid I (PGL-I) of *Mycobacterium leprae* in the urine was applied to the diagnosis of leprosy and the monitoring of antileprosy chemotherapy. One-hundred-seventy-nine previously untreated patients and 25 normal controls were tested. The specificity of the assay was 100%. There were no false-positive results. The sensitivity of the assay varied with the type of leprosy from 92% for lepromatous leprosy to 56% for borderline lepromatous and 18% for borderline tuberculoid patients. After the onset of chemotherapy in lepromatous leprosy patients, there was often a transient increase in urinary PGL-I, followed by a steady decline. Within 3 months of multiple drug therapy, urinary PGL-I levels were reduced by 90%–99% and were often undetectable. This assay appears to have considerable potential for monitoring chemotherapy and detecting treatment failure and relapse in patients with Hansen's disease.—Authors' Abstract

Parkash, O., Katoch, K. and Sengupta, U. Terminal complement complex in plasma of leprosy patients. Acta Leprol. (Genève) 7 (1990) 221–224.

A study was carried out to find out the difference in the levels of circulating terminal complement complex between healthy volunteers and the untreated leprosy patients by using double antibody sandwich ELISA. In addition, the levels of terminal complement complex in lepromatous patients with and without reaction were also compared. None of the group showed any significant difference in the levels of circulating ter-

minal complement complex. These findings suggest that complement may not play a direct role in tissue damage in leprosy.—Authors' Summary

Pope, R. M., Wallis, R. S., Sailer, D., Buchanan, T. M. and Pahlavani, M. A. T cell activation by mycobacterial antigens in inflammatory synovitis. Cell. Immunol. **133** (1991) 95–108.

To define which mycobacterial antigens were responsible for the activation of synovial fluid T lymphocytes, acetone-precipitated *Mycobacterium tuberculosis* (AP-MT) antigens were separated into five fractions following polyacrylamide gel electrophoresis and added to the mononuclear cell cultures of patients with inflammatory synovitis. Fractions 2 (50 to 70 kDa) and 5 (< 28 kDa) resulted in significantly more proliferation than that of fractions 1, 3, and 4. The response to a purified mycobacterial 65-kDa heat-shock protein (hsp), which migrated in fraction 2, was highly correlated ($r = 0.89$, $p < 0.001$) with the response to the crude AP-MT. The proliferative response to a different hsp, the *Escherichia coli* DnaK, by synovial fluid lymphocytes was marginal. Analysis of the synovial fluid T-cell response to mycobacterial culture filtrates by T-cell Western blotting revealed dominant responses to antigen(s) in the range of 31 to 21 kDa in each responding patient, although no other consistent pattern of T-cell activation was noted. Three lines of evidence suggested that the response to the low molecular weight fractions was directed against degradation fragments of the 65-kDa protein. These observations suggest that the activation of T lymphocytes obtained from inflammatory synovial fluids by crude mycobacterial antigens was due in large part to recognition of the 65-kDa mycobacterial hsp.—Authors' Abstract

Rasheed, F. N., Locniskar, M., McCloskey, D. J., Hasan, R. S., Chiang, T. J., Rose, P., de Soldenhoff, R., Festenstein, H. and McAdam, K. P. W. J. Specificity of lymphocytotoxic autoantibodies (LCAbs) found in the serum of leprosy patients: class I MHC antigens. Lepr. Rev. **62** (1991) 13–20.

Lymphocytotoxic autoantibodies (LCAbs) of the IgM class have been identified in patients with borderline tuberculoid (BT) and borderline lepromatous (BL) leprosy with type 1 reactions (I) as well as lepromatous leprosy (LL) patients with erythema nodosum leprosum reactions (ENL). The observation that lymphocytotoxic activity (LCA) was reduced in the presence of platelets led us to determine whether LCAbs had specificities for class I major histocompatibility complex (MHC) determinants. Absorption of LCA positive sera with platelets, classically used to deplete class 1-specific lymphocytotoxic antibodies, reduced LCA toward autologous as well as allogeneic target cells. This was true for LCA positive sera from all patient classifications (group BT in the autologous system, $p < 0.01$; in all other patient groups, $p < 0.001$). Introducing B-2m to cytotoxicity assays only marginally reduced LCA when added at high concentrations (5 mg/ml). An anti-class I MHC antiserum which blocked the lytic activity of class I tissue typing sera did not inhibit lymphocytotoxic activity. The data indicate that LCAbs while absorbed by platelets, are not specific for the class I MHC antigens. The autoantigen recognized by these autoantibodies therefore remains to be identified.—Authors' Summary

Rav, S. D., Pratap, V. K., Sharma, N. K. and Dayal, S. S. Mast cell in leprosy. Indian J. Lepr. **62** (1990) 467–472.

Mast cell distribution in the affected skin and in the apparently normal skin at least 10 cm away from the lesion was studied in 250 leprosy patients. These cells were found and were more numerous in the apparently normal skin of established cases of leprosy as well as in the indeterminate group. Absence of mast cells was conspicuous in 16.7% LL, 41.7% BB, 40.9% BT, and 68.0% TT lesions. It is suggested that mast cells might play a role in the early stages of the disease and in postreactional connective tissue proliferation.—Authors' Abstract

Roy, A., Agarwal, A. and Ralhan, R. Anti-arabinogalactan IgM/IgG ratio: a screening index for leprosy patients. Indian J. Lepr. **62** (1990) 435–442.

The serological activities of arabinogalactan from *Mycobacterium smegmatis* and phenolic glycolipid-I (PGL-I) from *M. leprae* were examined by enzyme-linked immunosorbent assay using sera from 88 patients with leprosy (44 treated and 44 untreated) and 45 normal healthy individuals. Both IgM and IgG type of antibodies were measured against these antigens. The results confirmed the previous observation that antiPGL-I IgM antibodies are higher in lepromatous leprosy cases than in normal individuals. However, with arabinogalactan, the ratio of IgM/IgG was more than one in normal individuals and less than one in untreated LL patients. Treated patients fell in both categories. Moreover, a reverse relationship was found between antiPGL-I IgM titers and anti-arabinogalactan IgM/IgG ratio.—Authors' Abstract

Salgame, P., Convit, J. and Bloom, B. R. Immunological suppression by human $CD8^+$ T cells is receptor dependent and HLA-DQ restricted. Proc. Natl. Acad. Sci. U.S.A. **88** (1991) 2598–2602.

Mechanisms of specific immunologic unresponsiveness or tolerance and their regulation by the major histocompatibility complex remain central issues in immunology. Recent findings that potentially reactive anti-self T cells are not completely clonally deleted in the thymus and that specific immunological unresponsiveness can be acquired in certain infectious diseases, such as leprosy, suggest that peripheral unresponsiveness can be developed and maintained in adults. Human antigen-specific T-suppressor cells represent one mechanism of peripheral tolerance. Clones of CD8+ T-suppressor cells have been derived from blood or lesions of patients with lepromatous leprosy who are selectively unable to mount cellular immunity to *Mycobacterium leprae.* Using a panel of *M. leprae*-specific CD4+ and CD8+ T-cell clones of differing major histocompatibility complex class II haplotypes, suppression *in vitro* was found to be restricted by HLA-DQ and not by HLA-DR and inhibited by antibodies to HLA-DQ. In addition, antigen-induced suppression could be inhibited by antibodies specific to appropriate polymorphic T-cell receptor β chains of the CD8+ clones. The results establish that activation of specific T-suppressor cells is dependent on their polymorphic T-cell receptors and suggest that HLA-DQ serves as the preferred restricting element for suppression.—Authors' Abstract

Sheela, R., Ilangumaran, S. and Muthukkaruppan, V. R. Flow cytometric analysis of CD2 modulation on human peripheral blood T lymphocytes by Dharmendra preparation of *Mycobacterium leprae.* Scand. J. Immunol. **33** (1991) 203–209.

It has been reported previously that *Mycobacterium leprae* modulated CD2 on human peripheral blood T lymphocytes and that this modulation was accompanied by a marked reduction in the proliferative response of these cells to mitogens and antigens. In this study, we report that treatment of peripheral blood mononuclear cells from healthy individuals with Dharmedra preparation of *M. leprae* inhibited their ability to form rosettes with sheep red blood cells. Flow cytometric analysis of Dharmendra lepromin-treated cells showed that, in addition to CD2, CD4 and CD8 were modulated while the surface expression of CD3 was not affected. The specificity of CD2 modulation was confirmed by similar effects of Dharmedra lepromin on thymocytes and lymph node cells from human CD2 transgenic mice. The modulatory effect of Dharmendra lepromin was not observed at lower temperatures. Dharmendra lepromin treatment of activated T cells resulted in reduced binding of monoclonal antibodies to IL-2R and D66 epitope of CD2. The modulatory effects were not observed with Dharmendra preparation of BCG or other preparations of *M. leprae.* Our results indicate that certain *M. leprae* factor(s) specifically modulate(s) CD2, CD4, CD8 and IL-2R but not CD3 on T lymphocytes. The suppressive effect of Dharmendra lepromin on the T-cell proliferative response reported earlier may be explained by its modulatory effect on the number of T-cell surface molecules.—Authors' Abstract

Stanford, J. L., Rook, G. A. W., Bahr, G. M., Dowlati, Y., Ganapati, R., Ghazi Saidi, K., Lucas, S., Ramu, G., Torres, P., Ly, H. M. and Anstey, N. *Mycobacterium*

vaccae in immunoprophylaxis and immunotherapy of leprosy and tuberculosis. Vaccine **8** (1990) 525–530.

Both leprosy and tuberculosis present continuing problems in their control, especially in the developing world, despite the availability of drugs effective in producing a bacteriological cure. Improved immunoprophylaxis and an effective immunotherapy to be used with chemotherapy are urgently required. Intradermal injection of a suspension of killed *Mycobacterium vaccae* promotes cell-mediated responses to antigens common to all mycobacteria, and switches off the tissue-necrotizing aspects of the Koch phenomenon. These properties led to the use of the suspensions as an improved vaccine, either alone or in combination with BCG. The same properties led to the employment of the suspension in immunotherapy as an adjunct to chemotherapy in the treatment of both leprosy and tuberculosis. The evidence leading to these conclusions is reviewed and discussed.—Authors' Abstract

Steinhoff, U., Wand-Württenberger, A., Bremerich, A. and Kaufmann, S. H. E. *Mycobacterium leprae* renders Schwann cells and mononuclear phagocytes susceptible or resistant to killer cells. Infect. Immun. **59** (1991) 684–688.

Acquired resistance to *Mycobacterium leprae,* the etiologic agent of leprosy, crucially depends on cellular immune mechanisms. In addition to interleukin-mediated helper functions, killer mechanisms seem to be involved. This study addresses the question of how *M. leprae* renders mononuclear phagocytes and Schwann cells, its natural targets, susceptible or resistant to killer cells. Killer activities were stimulated in peripheral blood mononuclear cells from healthy individuals by incubation with mycobacteria plus interleukin-2. These cells lysed Schwann cells and mononuclear phagocytes which had been pulsed with dead *M. leprae,* while unpulsed targets remained virtually unaffected. Importantly, targets infected with viable *M. leprae* were not lysed; furthermore, infection with viable *M. leprae* as well as gamma-interferon stimulation or heat shock caused resistance in otherwise susceptible targets which had been pulsed with dead *M. leprae.* Thus, *M. leprae* markedly influenced the effect of killer cells on Schwann cells and mononuclear phagocytes.—Authors' Abstract

Suri Babu, S. S., Kannan, K. B., Katoch, V. M. and Bharadwaj, V. P. Adenosine deaminase activity in leprosy. Indian J. Lepr. **62** (1990) 473–477.

Adenosine deaminase (ADA) activity was studied in serum and peripheral blood lymphocytes of leprosy patients and healthy controls. Serum ADA levels were found to be elevated in tuberculoid as well as lepromatous cases compared to control subjects. Serum ADA activity was significantly higher in tuberculoid cases than in the lepromatous group. Lymphocyte adenosine deaminase activity showed a similar trend. These results suggest that, since the overall activity of the enzyme is not deficient in leprosy, the cellular immune aberration seen in the different types of leprosy may be due to abnormal proliferation of different subsets of lymphocytes in response to *Mycobacterium leprae.*—Authors' Abstract

Vallat, J. M., Leboutet, M. J., Henry, P., Millan, J. and Dumas, M. Endoneurial proliferation of perineurial cells in leprosy. Acta Neuropathol. **81** (1991) 336–338.

In leprous neuropathy the perineurium very often has an abnormal multilayered appearance and is infiltrated by many different types of inflammatory cells. We report here 13 cases characterized by an abnormal endoneurial proliferation of fibroblasts which seems to differentiate in perineurial cells. In several instances there is formation of many intrafascicular microcompartments. Such aspects have been described in various, but infrequent, cases of experimental and human neuropathies. It seems that severe Wallerian degeneration, diffuse endoneurial macrophage infiltration and lesion of the perineurium might lead to such a process.—Authors' Summary

Venner, T. J. and Gupta, R. S. Nucleotide sequence of mouse HSP60 (chaperonin,

GroEl homolog) cDNA. Biochem. Biophys. Acta **1087** (1990) 336–338.

The cDNA sequence of the 60-kDa heat-shock protein from mouse 3T3 cells has been determined. The deduced amino acid sequence of mouse hsp60 protein differs from the corresponding proteins from Chinese hamster and human cells in 7 and 13 residues, respectively, most of which are conservative replacements.—Authors' Abstract

Wu, Q.-X., *et al.* [Study of blocking agents in ELISA for antibodies to leprosy.] China Lepr. J. **6** (1991) 208–213. (in Chinese)

The authors compared systematically the efficacies of different blocking agents in ELISA for detecting antibody in sera of leprosy patients using P/N and t test as evaluation indicators. The results indicated that: a) with P/N ratio, all the blocking agents used showed reliable blocking efficacy, i.e., P/N >10, with following order: 10% BSA (193.0), 10% SM (146.0), 20% EA (97.5), 5% EAC (26.1), 10% NGS (22.8), 10% EA (18.0) and 5% BSA (16.0); b) 10%, 15% and 20% EA, and 5% and 10% SM, both the blocking agents are inexpensive, and showed the best blocking results when the evaluations were conducted with combination of P/N ratio and t test. SM and EA are recommended for use as blocking agents because they are easier to prepare and do not require low temperature for shipping and storage.—Authors' English Abstract

Microbiology

Bhatia, V. N. Observations on nerve tissue infected with *M. leprae.* Indian J. Lepr. **62** (1990) 492–494.

Nerve tissue from leprosy patients showed a) small linear pinkish translucent crystalloid bodies, b) small round structures in relation to filamentous strands, c) short pieces of filaments with round spaces within them and d) miscellaneous structures, like pink granules, brown bodies and dark masses. These structures are being studied for their relationship to leprosy.—Author's Summary

de Wit, M. Y. L., Faber, W. R., Krieg, S. R., Douglas, J. T., Lucas, S. B., Montreewasuwat, N., Pattyn, S. R., Hussain, R., Ponnighaus, J. M., Hartskeerl, R. A. and Klatser, P. R. Application of a polymerase chain reaction for the detection of *Mycobacterium leprae* in skin tissues. J. Clin. Microbiol. **29** (1991) 906–910.

The polymerase chain reaction (PCR) based on the selective amplification of a 530-bp fragment of the gene encoding the proline-rich antigen of *Mycobacterium leprae* was applied on sections of fixed or frozen biopsy samples from leprosy patients. A simple procedure for the extraction of DNA from *M. leprae* in clinical specimens that provided suitable template DNA for amplification was developed. When PCR was applied on frozen sections, positive amplification in samples from all untreated acid-fast bacillus (AFB)-positive patients and in samples from 56% of the untreated AFB-negative patients could be detected, while biopsy samples from patients with skin diseases other than leprosy were all PCR negative. With neural Formalin-fixed biopsy samples, positive amplification in 92% of the samples from untreated AFB-positive patients and in 61% of the samples from untreated AFB-negative patients could be detected by PCR. Biopsy samples exposed to mercuric chloride or nonbuffered formaldehyde containing fixatives were not suitable for application of PCR. This PCR holds promise as a tool for studies on *M. leprae* infection.—Authors' Abstract

Lazraq, R., Clavel-Sérès, S. and David, H. L. Transformation of distinct mycobacterial species by shuttle vectors derived from the *Mycobacterium fortuitum* pAL5000 plasmid. Curr. Microbiol. **22** (1991) 9–13.

Mycobacterium tuberculosis H37Ra, *M. smegmatis* ATCC 607, *M. smegmatis* MC²155, *M. aurum* A+, *M. aurum* A11, and one representative strain of *M. flavescens* were transformed by electroporation with plasmid pMY10 and cosmid pDC100. Plasmid pMY 10 contained the origin of replication of pAL5000, the origin of replication of pBR322, a kanamycin resistance gene, and the origin of transfer of the Inc plasmid RK2; the cosmid pDC100 contained the pHC79 SS cosmid, the origin of replication of pAL5000, and a kanamycin resistance gene. The efficiency of transformation varied with the recipient cells used and was in decreasing order: 7×10^5 for *M. smegmatis* MC²155, 6×10^3 for *M. tuberculosis* H37Ra, 10^3 for *M. aurum,* 50 for *M. smegmatis* ATCC 607, and 5 for *M. flavescens.* A rapid protocol for plasmid extraction from mycobacteria was developed. The satisfactory transformation of the nonvirulent *M. tuberculosis* strain H37Ra was of interest for future studies on cloning of virulence genes, while the satisfactory transformation of *M. aurum* was of interest for future studies on the genetics of drug resistance because these bacteria are sensitive to drugs specifically used in the treatment of tuberculosis and leprosy. However, neither vector was stably maintained in *M. smegmatis,* indicating that further investigations are still necessary to resolve this difficulty.—Authors' Abstract

Luquin, M., Roussel, J., Lopez-Calahorra, F., Lanéelle, G., Ausina, V. and Lanéelle, M.-A. A novel mycolic acid in a *Mycobacterium* sp. from the environment. Eur. J. Biochem. **192** (1990) 753–759.

A fast-growing, non-photochromogenic mycobacterium isolated from the environment exhibited, on thin-layer chromatograms, a characteristic pattern of mycolates composed of unsaturated mycolates and also an unknown more polar component. Spectroscopic analysis and chemical degradation showed that this latter component was a novel mycolic acid containing a methoxy group at the ω-1 position (instead of ω-17 and ω-18 in known methoxymycolates), and two double bonds in the long mero aldehyde chain (instead of one as in known mycolates with additional oxygenated groups).—Authors' Abstract

Ridley, M. J. Reply: the use of xylene (xylol) in medical laboratories. (Letter) Lepr. Rev. **61** (1990) 80–81.

This letter follows previous correspondence (*Leprosy Review,* 1989, **60,** 67) and highlights the problems that can occur if acid-fast organisms are left in contact with immersion oil, xylene or mountant. The author describes a technique for re-staining smears which have faded. The problem appears to be more common with *Mycobacterium leprae* in material from patients than from animals, and rarely occurs with *M. tuberculosis* or BCG.—B. W. Allen (*Trop. Dis. Bull.*)

Wheeler, P. R. Recent research into the physiology of *Mycobacterium leprae.* Adv. Microb. Physiol. **31** (1990) 71–124.

Mycobacterium leprae has physiological features typical of many microbes (both saprophytic and pathogenic), in particular its carbon and energy metabolism. However, the overall view is of a well-adapted, obligate, intracellular parasite. Though it does not produce its own catalase, *M. leprae* inside host cells is surrounded by a thick (50-100 mm) zone of lipoidal capsular material which appears to protect it from the toxic environment that the host cell creates in its attempt to kill invading microbes. *M. leprae* has the capability of shutting down its TCA cycle, and thus oxidative metabolism, by proteolytic digestion of one of the cycle enzymes. Like dormant tubercle bacilli, it has high activities of the alterative glycoxylate bypass enzymes, though *M. leprae* are dividing with a mean generation time of 12 days. The physiology of *M. leprae* enables it to utilize important available nutrients in the host cell, such as nucleotides, which it can hydrolyze to nucleosides, some of which, particularly adenosine and uridine, are rapidly incorporated into its DNA and RNA. Lipids would also be available, and it is known that at least phospholipids can be used as nutrients, first being hydrolyzed with a phospholipase when the released fatty acids can be used as either energy sources, or elongated when their

metabolic fate is probably as structural lipids.

M. leprae acquires its nutrients without producing any toxins. There is no sign of the damage caused by phospholipase when it acts as a toxin, and it acquires iron without producing hemolysins. In these respects it is similar to mycobacterial pathogens in general.

An intriguing possibility is that there is a general mechanism at the level of the genome which slows down the physiology of *M. leprae* and enables it to be a successful pathogen. There are about 20 insertion sequences, apparently not transposable, which may block specific functions. Insertion sequences are also found in *M. paratuberculosis,* another chronic pathogen, and some persistent strains of *M. avium* from which *M. paratuberculosis* is virtually indistinguishable by taxonomic means. *M. leprae* is, however, a distinct species not just a slowed-down strain of another known mycobacterium.

M. leprae is so well adapted to intracellular growth that it has evaded axenic cultivation so far. Biochemical studies suggest that purines and a scavenger of peroxide are essential additions to media, while phospholipids (lecithin) and exochelin from *M. neoaurum* should promote growth and may be needed for isolation. However, these suggestions alone are not enough; more information is needed. In the meantime, inhibition of readily measurable activities in *M. leprae,* such as ATP content, palmitate oxidation, and hypoxanthine or (in macrophages) thymidine incorporation by a wide range of agents, should allow a drug-screening method to be set up without axenic culture, probably based on several of the activities being measured in combination.—Author's Conclusions

Experimental Infections

Gelber, R. H., Siu, P., Tsang, M. and Murray, L. P. Activities of various macrolide antibiotics against *Mycobacterium leprae* infection in mice. Antimicrob. Agents Chemother. **35** (1991) 760–763.

We evaluated the activities of several macrolide antibiotics against *Mycobacterium leprae* infections in mouse foot pads. Erythromycin and azithromycin were inactive, while both roxithromycin and clarithromycin were found to be consistently active and, in fact, bactericidal. By both methods, clarithromycin was found to be superior to roxithromycin, a finding which, at least in part, may be a consequence of the higher levels of clarithromycin at the site of infection.—Authors' Abstract

Epidemiology and Prevention

Abel, L. and Bonney, G. E. A time-dependent logistic hazard function for modeling variable age of onset in analysis of familial diseases. Genet. Epidemiol. **7** (1990) 391–407.

The paper presents an extension of the regressive logistic models proposed by Bonney to address the problems of variable age-of-onset and time-dependent covariates in analysis of familial diseases. This goal is achieved by using failure time data analysis methods, and partitioning the time of follow up in K mutually exclusive intervals. The conditional probability of being affected within the kth interval ($k = 1 \ldots K$) given not affected before represents the hazard function in this discrete formulation. A logistic model is used to specify a regression relationship between this hazard function and a set of explanatory variables including genotype, phenotypes of ancestors, and other covariates which can be time dependent. The probability that a given person either

becomes affected within the *k*th interval (i.e., interval *k* includes age of onset of the person) or remains unaffected by the end of the *k*th interval (i.e., interval *k* includes age at examination of the person) are derived from the general results of failure time data analysis and used for the likelihood formulation. This proposed approach can be used in any genetic segregation and linkage analysis in which a penetrance function needs to be defined. Application of the method to familial leprosy data leads to results consistent with our previous analysis performed using the unified mixed model, i.e., the presence of a recessive major gene controlling susceptibility to leprosy. Furthermore, a simulation study shows the capability of the new model to detect major gene effects and to provide accurate parameter estimates in a situation of complete ascertainment.—Authors' Abstract

Banerjee, R., Banerjee, B. D., Chaudhury, S. and Hati, A. K. Transmission of viable *Mycobacterium leprae* by *Aedes aegypti* from lepromatous leprosy patients to the skin of mice through interrupted feeding. Lepr. Rev. **62** (1991) 21–26.

Female *Aedes aegypti* which took partial blood meals from the skin lesions of untreated lepromatous leprosy (LL) patients were then allowed to continue feeding on 72–96-hr-old Swiss albino suckling mice (Rockefeller strain). The bitten portion of skin was removed, divided into two parts, and processed for the extraction of bacilli by two different methods using chloroform and petroleum ether. The proboscis of some of the fed mosquitoes was dissected out and examined for viable bacilli (stained by fluorescein diacetate and ethidium bromide) and acid-fast bacilli (AFB). Out of 50 probosces dissected, 45 were found positive for AFB, with bacillary counts ranging up to 246 (average 40.20 ± S.D. 41.80) per proboscis. The average percentage of viable bacilli (green solid) in the probosces immediately after feeding on LL patients was 43.90; thereafter it decreased gradually to 3 on the seventh day. In the petroleum ether extract of mouse skin, viable bacilli were observed in numbers up to 37 (average 15.25 ± S.D. 10.25) per smear. The number of fluorescing bacilli (green and red) correlated with the total number of AFB.—Authors' Summary

de Rojas y Lopez del Rincon, V., Garcia, S. and Diaz, J. M. [Some psychosocial and epidemiological characteristics of leprosy patients in the municipality of Artemisa.] Rev. Cubana Med. Trop. **42** (1990) 53–68. (in Spanish)

Psychosocial and hygienico-epidemiologic characteristics of prevalence of leprosy in Artemisa Municipality, Havana Province, Cuba, were studied and compared with a control group. In relation to psychical stability, they are normal individuals. The general personal profile shows a personality structure disposed to neuroticism. The hygienic conditions are good. The majority of patients do not feel marginated, and they have been incorporated into the social and labor life. There are no mutilating incapacities in these patients. All needing rehabilitation have it. The patients, in this municipality, know the main aspects of the disease.—Authors' English Summary

Dhandayuthapani, S., Anandan, D. and Bhatia, V. N. ELISA & lepromin skin tests in household contacts of leprosy patients. Indian J. Med. Res. [A] **91** (1990) 431–436.

A total of 438 household contacts of leprosy patients and 228 noncontacts were studied for anti-PGL-I antibodies and cell-mediated immune response to *Mycobacterium leprae* using ELISA and lepromin tests. The contacts showed relatively higher positivity to ELISA (29.4%) and lepromin (70.5%) as compared to noncontacts who showed 9.6% and 57% positivity. In contacts as well as noncontacts, the seropositivity was not found to be influenced by age and sex. However, seropositivity was higher (39.7%) in the contacts of patients with multibacillary leprosy as compared to contacts (27.7%) of patients with paucibacillary leprosy.—Authors' Abstract

Gupta, S. C., Humne, A. Y. and Ingole, D. L. A rapid survey technique for detection of leprosy. Indian J. Lepr. **62** (1990) 488–491.

Comparison of prevalence rates of leprosy as assessed by a rapid survey technique, in which only the exposed parts of the body were examined, with that assessed by a routine total body examination in a population of about 700 showed that most cases of leprosy were detected by the former.—Authors' Abstract

Louis, J. P., Trebucq, A., Hengy, C., Coddy-Zitsamele, R., Eozenou, P., Baya-Tsika, N., Obvala, D., Jannin, J., Gelas, H. and Cottenot, F. [Leprosy endemicity in Popular Republic of Congo. An epidemiologic and behavioral analysis.] Acta Leprol. (Genève) **7** (1990) 213–220. (in French)

The authors report the results of a national prevalence survey of leprosy made in 1989 in the Popular Republic of Congo. Leprosy is essentially found in rural areas and frequently causes disabilities. The prevalence rate is 5.8 ± 2.6‰ among people more than 15 years of age, and 10.5% of all forms are multibacillary. All patients are under dapsone monotherapy. One overwhelming risk factor is leprosy cases in the family history; active case-finding and surveillance of contact cases are recommended. Generally, leprosy is poorly understood by the general population; an educational effort is necessary.—Authors' English Summary

Mahmoud, S. F. and Azadeh, B. Leprosy in Qatar. Int. J. Dermatol. **30** (1991) 125–126.

Clinical and histopathologic features of 104 cases of leprosy diagnosed between 1982 and 1989 in the State of Qatar were studied. There were 88 male (84.6%) and 16 (15.4%) female patients aged 18 to 64 years. Eighty-four of the cases were diagnosed during the initial mandatory medical screening on arrival to Qatar. Almost all (103) were expatriates and only one 50-year-old woman was a Qatari. Indians formed the largest group (60.6%), follwed by Bangladeshi (6.7%), Pakistani (5.8%), and others. The majority of cases were classified as lepromatous (43.3%) or tuberculoid (43.3%). Although Qatar is a relatively leprosy-free country, the influx of a large number of workers from developing countries seeking employment forms a potential public health hazard.—Authors' Abstract

Ren, X.-W., *et al.* [Analysis of newly found cases of leprosy in Jiangsu Province from 1983 to 1987.] China Lepr. J. **6** (1990) 194–199. (in Chinese)

One-thousand-ninety-six cases of leprosy were found from 1983 to 1987 in Jiangsu Province, China, and on the average there were 219.2 new patients detected each year, which decreased by 64.8% as compared with that from 1976 to 1979. In these new cases, the proportion of male to female is 2.9 to 1 and MB are 376 (34.3%), being more than that in the 1970s. The cases with a disease duration of less than 2 years make up 68% of the patients. Children under 14 years account for 3.94%; 90.5% of the cases are passively found. Those with an evident source of infection were 27.1%, of which 69.9% were MB and 30.1% were PB. At the time of detection, there were skin lesions in 98.9% of the patients, nerve damage in 94.5%, lepra reaction in a 21%, positive smear in 49.6% with a mean BI of 1.39, specific histopathological change in 84.6%, and deformities of grades II and III in 35%. Most of the patients (99.6%) received regular treatment the eighth day after diagnosis, of which 88.6% had MDT and 88.5% were at their homes. The authors emphasize the training of medical workers at all levels for earlier detection of leprosy patients so as to treat them in time.—Authors' English Abstract

Tonglet, R., Pattyn, S. R., Nsansi, B. N., Eeckhout, E. and Deverchin, J. The reduction of the leprosy endemicity in northeastern Zaire 1975/1989. Eur. J. Epidemiol. **6** (1990) 404–406.

The leprosy endemy in Uele, northeastern region of Zaire, has declined considerably since the 1950s, particularly since 1975. The hypothesis is advanced that the most important causal factor has been the distribution of dapsone for many years, through a vertical program. The gradual increase of dapsone resistance has been taken care of by the introduction of combined, multidrug therapy in the 1980s. The cost per patient detected and treated rises in inverse pro-

portion to the decline in endemy; therefore, the time has come for the integration of leprosy control in the primary health care strategy. However, central specialized teams financed by voluntary organizations should be maintained to assure training of personnel, to give advice in diagnosis, prevention and treatment of complications, to provide drugs and to assist in rehabilitation.—Authors' Abstract

Weng, C.-Y., *et al.* [Effect of leprosy control in Yulin City of Guangxi Province over 32 years.] China Lepr. J. **6** (1990) 200–202. (in Chinese)

From 1956 to the present, prevention is always regarded as of first priority and leprosy patients are allowed to accept treatment at home in the city of Yulin, Guangxi Province, China. An accumulative total of 773 leprosy patients were found, of which 574 cases were cured (74.3%) and 165 died. Until July of 1987, there were 10 active cases of leprosy and the prevalence rate is 0.008%, decreased by 98.5% as compared with that in 1956. In the last 5 years the mean incidence rate is 0.15 per 100,000, decreased by 95% as compared with that in 1956 to 1960. The number of the villages with a combined population of about 140,000 where leprosy existed was 386, but at present only 12 such villages remain which is 31% of the former situation. In September of 1987, through examination at the prefectural level, it was proved that leprosy has already been basically eradicated in the city of Yulin.—Authors' English Abstract

Rehabilitation

Dandapat, M. C., Sahu, D. M., Mukherjee, L. M., Panda, C. and Baliarsing, A. Treatment of leprous neuritis by neurolysis combined with perineural corticosteroid injection. Lepr. Rev. **62** (1991) 27–34.

A study on leprous neuritis, involving the ulnar nerve, was carried out on 39 patients. The evaluation of nerve function was done before and after treatment by a score chart. Patients were divided into two groups. Group A (21 patients) was subjected to neurolysis only, and group B (18 patients) were given the combined treatment of neurolysis and perineural corticosteroid injection at the same time as neurolysis and, subsequently, at the end of the second and third weeks. In group B, 83.3% of patients showed a 10% or more increase in the posttreatment score in comparison with 57.1% in group A. Improvement was more marked in paucibacillary cases and when the duration of nerve involvement was less than 3 months. Patients with short segments of nerve involvement with minimal thickening had better recovery. This procedure was observed to be simple, easy, and well accepted by the patients, with a marked beneficial effect.—Authors' Summary

Kulkarni, V. N., Antia, N. H. and Mehta, J. M. Newer designs in foot-wear for leprosy patients. Indian J. Lepr. **62** (1990) 483–487.

Micro-cellular rubber (MCR) foot wear has been used widely over the past several years for the anesthetic feet of leprosy. Although MCR has got good shock absorbing and moulding qualities, many tend to reject the foot wear because of the stigma of the disease which it carries. Two newer models of foot wear which would meet the demands of anesthetic sole and avoid the stigma because of their resemblance to foot wear available in the market were tried. Model mark II fulfilled the needs and was acceptable to the patients. Such models must be tried and acceptable and effective foot wear need to be made available.—Authors' Abstract

Malaviya, G. N. Recent advances in restorative surgery of extremities in lepro-

sy. Acta Leprol. (Genève) 7 (1990) 239–245.

A better understanding of the pathology and mechanisms of nerve damage has changed the plan of treatment of neuritis. A concept of "preventive nerve surgery" has come up during the last few years. The genesis of plantar ulcers and its presentation is better understood now. The recent developments in the treatment of paralytic deformities of hand and foot have been outlined.—Author's Summary

Patil, K. M. and Srinath, M. S. New image-processing system for analysis, display and measurement of static and dynamic foot pressures. Med. Biol. Eng. Comput. **28** (1990) 416–422.

A new image-processing system, using a video digitizer with an IBM-compatible PC/AT, is developed for acquisition and processing of low-contrast, low-intensity barographic images of both feet for assessment of pressure distribution during standing and walking. Data displays, in the form of centers of pressures, isopressures contours, perspective views of pressures, grey scale image and walking pressure patterns, combined image of walking pressures, paths of centers of pressures and pressure variations with time, are developed. These have provided very useful and early information regarding the internal structural changes in the bones of the foot and sites at risk of ulcer development in leprosy subjects and enable suitable corrective orthopedic procedures to be adopted.—Authors' Abstract

Vulliet, F. and Tschibangu, P. [Treatment and rehabilitation of deformities in leprosy in a district hospital (Kapolowe, Zaire).] Acta Leprol. (Genève) 7 (1990) 225–228. (in French)

Since 1985, we continue to treat and rehabilitate the leprosy infirmities at the Kapolowe District Hospital (Shaba, Zaire). The setting up of a permanent autochtonous department (Tschibangu) has taken the place of the temporary expeditions. A first follow-up refers to 138 patients, on which 259 interventions have been done: 165 refer to foot ulcerations and their septic osteo-articular complications, 75 relate to neuritis, 8 only have been restorative interventions; 75% of our patients have had a good social reinsertion. The 25% relapses show the importance of a good limb prosthesis, of sanitary education of the patients and their supervision when they go back to brushwood. In the initial period, it is necessary to start by performing surgery in cleanliness, otherwise bright restorative interventions would be inefficient.—Authors' English Summary

Warren, G. Facial palsy–a leprosy surgeon's viewpoint. Aust. N.Z. J. Ophthalmol. **18** (1990) 257–266.

Facial palsy is cosmetically unacceptable, whether affecting eyes or mouth. It endangers the vision. Both upper and lower facial paralysis can be surgically dealt with, using the temporal muscle and fascia. This produces satisfactory results in a relatively short period of time.—Author's Abstract

Zhou, S.-N., *et al.* [Preliminary observation on 74 leprosy patients undergoing a rehabilitation plan for a year.] China Lepr. J. **6** (1990) 190–193. (in Chinese)

Seventy-four leprosy cases have received self-medication and physical therapy for a year on the basis of a rehabilitation program. Of 42 cases with hand deformity of Grade I 6 cases recovered the superficial sensation on their hands, accounting for 14.3%. There were 61 cases with foot deformity of Grade I, of which 9 cases, 17.4%, recovered the sensation on their feet. Of 21 plantar ulcers in 18 patients, 10 ulcers, 47.6%, healed. All of the deformities of Grades II and III had no exacerbation during this period of time.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Cline, J. M., Schlafer, D. W., Callihan, D. R., Vanderwall, D. and Drazek, F. J. Abortion and granulomatous colitis due to *Mycobacterium avium* complex infection in a horse. Vet. Pathol. **28** (1991) 89–91.

Although mycobacterial infections are uncommon in horses, infections with *Mycobacterium* spp. have been implicated as causative agents of granulomatous enteritis and colitis in a number of equine cases. Disseminated infections by members of the *M. avium-M. intracellulare* complex have been reported rarely, and a single case report documents equine abortion due to *M. terrae*. Experimental infections of horses with *M. paratuberculosis* have been established. We report a case of equine abortion associated with infection of the maternal gastrointestinal tract by an organism of the *M. avium* complex.—From the article

Crowle, A. J. and Elkins, N. Relative permissiveness of macrophages from black and white people for virulent tubercle bacilli. Infect. Immun. **58** (1990) 632–638.

Black people appear to be more susceptible to tuberculosis than white people, probably because of both genetic and environmental factors. These authors show that blood-derived macrophages from black donors killed more virulent tubercle bacilli (the Erdman strain of *Mycobacterium tuberculosis*) during phagocytosis *in vitro* than corresponding cells from white donors. However, in successfully infected cell cultures the bacteria grew significantly faster in macrophages from black donors than in macrophages from white donors, particularly in the presence of serum also from black donors. Macrophages from black donors were less well protected against the tubercle bacilli by 1,25-$(OH)_2$-vitamin D_3 (a hormonally active form of vitamin D) than cells from white donors. "These results demonstrate some inherent and environmental liabilities in the monocytic phagocytes and serum of black people compared with white people, which may contribute to their greater susceptibility to tuberculosis."—C. A. Brown (*Trop. Dis. Bull.*)

David, H. L. Probability distribution of drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis*. Appl. Microbiol. **20** (1970) 810–814.

The fluctuation test shows that *Mycobacterium tuberculosis* mutates to resistance to isoniazid, streptomycin, ethambutol and rifampin spontaneously and at random. The average mutation rates for the drugs, in the same order, were calculated to be 2.56×10^{-8}, 2.95×10^{-8}, 10^{-7}, and 2.25×10^{-10} mutation per bacterium per generation. The relatively high mutation rate to ethambutol resistance and the low mutation rate to rifampin resistance were confirmed by analyzing the increase in the proportion of mutants with time in a growing population of the tubercle bacilli. The highest proportions of mutants to be expected in unselected populations of the tubercle bacilli were calculated from the results of fluctuation tests.—Author's Abstract

Dorozhkova, I. R., Krudu, V. N. and Popesku, T. T. [Clinical value of *M. tuberculosis* L-form detection in subjects with residual tuberculous changes in the lungs.] Probl. Tuberk. **12** (1990) 5–8. (in Russian)

To clarify the role of *Mycobacterium tuberculosis* L-form in a recurring tuberculous process in subjects with residual tuberculous changes in the lungs, microbiological, clinicoroentgenologic and laboratory examinations of 1651 persons recorded as having VIIA, VIIB, III and 0 groups were undertaken. The causative agent of tuberculosis was isolated in a typical bacillary and L-transformed forms in 3.1% and 5.0% of the screened persons, respectively. Active respiratory tuberculosis was diagnosed in 64.7% of sputum-positive patients, including 86.3% of them as bacillary excreters and 42.7% of those excreting L-forms of the tuberculosis agent. The detection of biologically altered forms of the causative agent

indicates a potential activity of the process and requires an intensified medical examination control and differential prevention activities.—Authors' English Abstract

Emre, S., Sumrani, N. and Hong, J. Beneficial effect of thalidomide and ciclosporin combination in heterotopic cardiac transplantation in rats. Eur. Surg. Res. **22** (1990) 336–339.

The effect of thalidomide on the prevention of early rejection was studied in heterotopic cardiac transplants between AC1 (donors) and Lewis (recipients) rats, in combination with subtherapeutic doses of cyclosporin. Although allografts treated solely with thalidomide (5 mg/kg/day intraperitoneally) survived longer than controls (9.4 $\pm$ 2.7 and 6.3 $\pm$ 0.6 days, respectively, $p < 0.001$), the survival rates of animals treated with low dose cyclosporin (1.25 mg/kg/day intraperitoneally) plus thalidomide (1.25, 2.5 and 5 mg/kg/day) were significantly better at 21 days (70, 88.9 and 88.9%, respectively), compared to 55.6% in those treated with cyclosporin (1.25 mg/kg/day) alone. Graft survival rates at 90 days were not significantly different in the thalidomide-cyclosporin combination groups (60, 77.8 and 55.6%, respectively) compared to the cyclosporin group alone (55.6%). We conclude that thalidomide is effective in preventing early rejection of rat cardiac allograft when combined with subtherapeutic doses of cyclosporin, thus avoiding the dose-dependent side effects of cyclosporin in the early posttransplant period.—Authors' Abstract

Fourche, J., Capdepuy, M., Maugein, J. and Le Moigne, F. Analysis of cellular fatty acids and proteins by capillary gas chromatography and sodium dodecyl sulphate polyacrylamide gel electrophoresis to differentiate *Mycobacterium avium, Mycobacterium intracellulare* and *Mycobacterium scrofulaceum* (MAIS) complex species. J. Chromatogr. **532** (1990) 209–216.

Infections due to atypical mycobacteria have increased during the past 30 years. Species of *Mycobacterium avium, M. intracellulare* and *M. scrofulaceum* are among the most common nontuberculous mycobacteria isolated from patients with AIDS or immunosuppressed. These three organisms are taxonomically closely related and identification, according to cultural characteristics and biochemical tests, is not always evident, so some of these related strains are grouped in a "MAIS" complex. Analysis of cellular constituents is an aid to identification. Gas chromatography was used to study mycolic acids and a secondary alcohol was found which is a discriminating constituent between *M. scrofulaceum* and the other two species. The lipidic analysis was not able to separate *M. avium* and *M. intracellulare,* so cell proteins were considered. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins reflects genetic relatedness between strains; the different patterns obtained from these three species are described and it is shown that this method is very useful in classification and epidemiology.—Authors' Abstract

Frelier, P. F., Templeton, J. W., Estes, M., Whitford, H. W. and Kienle, R. D. Genetic regulation of *Mycobacterium paratuberculosis* infection in recombinant inbred mice. Vet. Pathol. **27** (1990) 362–364.

Genetic mechanisms involved in host resistance to viruses, bacteria, and protozoa are well documented in mice. Natural resistance of mice to the intracellular organisms *Salmonella typhimurium, Leishmania donovani, Mycobacterium bovis, M. intracellulare, M. tuberculosis,* and *M. lepraemurium* is thought to be regulated by genes on chromosome 1. These genes are either identical or are closely linked on the chromosome 1 and exist in two allelic forms, i.e., resistant (Bcg^r) and susceptible (Bcg^s). It has been suggested that the macrophage, under the control of the *Bcg* alleles, inhibits the proliferation of the infecting organism. This study demonstrated intraspecies variability between C57BL/6J and C3H/HeJ parental strains to infection with *M. paratuberculosis.* The study also demonstrated complete concordance of phenotypic assignment between *Para* and *Bcg* in the recombinant inbred strains examined. This indicates that either the same locus regulates resistance/susceptibility to *M. para-*

tuberculosis and *M. bovis,* or the phenomena are regulated by linked loci. These findings suggest that resistance to *M. paratuberculosis* infection may be regulated by the *Bcg* gene or a gene linked to the *Bcg* gene on mouse chromosome 1.—From the article

Larsson, H. Treatment of severe colitis in Behcet's syndrome with thalidomide (CG-217). J. Intern. Med. **228** (1990) 405–407.

A 35-year-old male patient, known in our department since 1979 because of a severe and complete Behcet's syndrome, was treated with thalidomide (CG-217) as a final pharmacological measure to avoid colectomy during a severe attack of Behcet colitis. Prior to the administration of thalidomide, the patient had been treated for 7 weeks with full parenteral nutrition and high doses of steroids intravenously without a satisfactory effect on the colitis. Treatment with sulfasalazine was unsuccessful because of a decreasing number of platelets on this drug. After a few days on thalidomide, 300 mg given once daily at bedtime, the patient's stools were normalized and without reaction for blood, his oral ulcers and pleural effusion disappeared, and his steroid doses could be reduced. Gradually he was put on oral nutrition again, and his rectal mucosa became normalized. The dose of thalidomide was reduced to 200 mg, and then to 100 mg daily when the patient was discharged from hospital, less than 3 weeks after institution of the drug treatment. After 5 months as an outpatient his condition is still satisfactory and without symptoms of his former disease. Thalidomide has previously been reported to be of value in treatment of Behcet's syndrome, but to my knowledge never with such a dramatic effect on a severe colitis as reported in this case.—Author's Abstract

Larsson, L. O., Skoogh, B.-E., Bentzon, M. W., Magnusson, M., Olofson, J., Taranger, J. and Lind, A. Sensitivity to sensitins and tuberculin in Swedish children. II. A study of preschool children. Tubercle **72** (1991) 37–42.

Non-BCG-vaccinated preschool children (4 or 5 years of age) were simultaneously tested on separate arms with a 2 IU PPD RT23 and 0.1 μg *Mycobacterium avium* sensitin RS10 or 0.1 μg *M. scrofulaceum* sensitin RS95. None of the 762 children had any known exposure to tuberculosis. A total of 8.8% reacted with an induration (≥ 3 mm to PPD RT23 while 2% reacted with ≥ 6 mm. Half the children were tested with *M. avium* sensitin: 18.9 and 7.8% reacted when 3 and 6 mm cut-off points, respectively, were taken. The remaining children were tested with *M. scrofulaceum* sensitin: 18.4 and 6.3%, respectively, reacted. In a previous study of schoolchildren aged 8 or 9 years, reactions to sensitins were considerably more frequent. Thus, sensitisation by atypical mycobacteria seems to increase from the preschool to the early school age. This finding probably reflects a continuous exposure of the children to atypical mycobacteria from various sources. The preschool children with a reaction to PPD RT23 ≥ 6 mm were examined and chest X-rays were performed. All children were healthy but one child had enlarged lymph nodes in the mediastinum and abdomen. It cannot be excluded that these pathological findings were caused by atypical mycobacteria.—Authors' Summary

Larsson, S., Shrestha, M. P., Pokhrel, B. M., Upadhyay, M. P., and Shrestha, K. B. The glutaraldehyde test as a rapid screening method for pulmonary tuberculosis; a preliminary report. Ann. Trop. Med. Parasitol. **84** (1990) 111–117.

A test is described involving the monitoring of gelling of blood samples after mixing with EDTA and glutaraldehyde. The authors report that the gelling time for blood from 267 tuberculosis patients in Nepal was consistently lower than that with blood from 272 control individuals. The test is simple to perform and levels of 89% and 95% are reported for sensitivity and specificity, respectively. It is thought that the phenomenon is due to elevated levels of gammaglobulin and fibrinogen in the blood of tuberculosis patients [although the simplicity of the test is much to be admired, it will be important to clarify the extent to which such nonspecific changes are shared by other clinical conditions unrelated to tuberculosis]. The authors point out that 2 pa-

tients with visceral leishmaniasis scored positive in the test, but were readily distinguished from tuberculosis patients on clinical grounds. The authors consider the test as a substitute for smear examination [but the results are not presented in terms of sensitivity in smear-positive versus smear-negative].—D. B. Young (*Trop. Dis. Bull.*)

Lind, A., Larsson, L. O., Bentzon, M. W., Magnusson, M., Olofson, J., Sjögren, I., Strannegard, I.-L. and Skoogh, B.-E. Sensitivity to sensitins and tuberculin in Swedish children. I. A study of schoolchildren in an urban area. Tubercle **72** (1991) 29–36.

Non-BCG-vaccinated schoolchildren (8 or 9 years of age) were simultaneously tested on separate arms with 2 IU PPD RT23 and 0.1 μg *Mycobacterium avium* sensitin RS10 or 0.1 μg *Mycobacterium scrofulaceum* sensitin RS95. None of the 2819 analyzed children had any known exposure to tuberculosis. A total of 3.4% reacted with an induration ≥ 6 mm to PPD RT23. Half the number of children were tested with *M. avium* sensitin and 25.4% reacted, while the remaining were tested with *M. scrofulaceum* sensitin and 32.4% reacted when the cut-off was 6 mm. For about 90% of the children the sensitin reaction was larger than or equal to the tuberculin (PPD RT23) reaction. Correlation analyses showed that moderate and high PPD RT23 values were combined with still higher sensitin values, indicating that the tuberculin reactions were mainly cross-reactions due to the antigenic similarity between tuberculin and sensitins. The presence of birds, dogs, and cats in the homes was combined with an increased frequency of children reacting to the sensitins used. The children with reactions to PPD RT23 ≥ 6 mm were examined and chest X-rays were performed. None of them showed any signs or symptoms of mycobacterial disease. In non-BCG-vaccinated Swedish schoolchildren without clinical signs of tuberculosis and without known contact with a contagious tuberculous person, indurations less than 12 to 14 mm on tuberculin testing are probably caused by atypical mycobacteria. In such cases sensitin tests should be performed to verify the suspicion.—Authors' Summary

Manjunath, N., Shankar, P., Rajan, L., Bhargava, A., Saluja, S. and Shriniwas. Evaluation of a polymerase chain reaction for the diagnosis of tuberculosis. Tubercle **72** (1991) 21–27.

A polymerase chain reaction for the specific detection of *Mycobacterium tuberculosis* has been developed and evaluated for clinical applicability. Primers were designed to amplify a 240 base pair region in the MPB 64 protein coding gene (nts 460–700). From among 15 different DNA templates tested (including 10 species of mycobacteria) PCR amplified the DNA from *M. tuberculosis* complex only, demonstrating its exquisite specificity. Sensitivity studies using serial ten-fold dilutions of *M. tuberculosis* bacilli determined the limit of detectability to be 10 organisms. A total of 143 clinical specimens were analyzed. This consisted of 26 known nontuberculous specimens (control group) and 117 specimens received at the Tuberculosis Diagnostic Service of AIIMS (test group). None of the specimens in the control group was positive by PCR. Out of 117 specimens in the test group, 19 were culture positive for mycobacteria and 17 of these isolates were identified as *M. tuberculosis.* All the specimens from which *M. tuberculosis* was grown were also PCR positive. The remaining two isolates were identified as mycobacteria other than *M. tuberculosis,* and these two specimens were PCR negative. An additional 14 culture negative specimens were PCR positive, yielding an overall *M. tuberculosis* positivity rate of 26.5% (31/117) compared to 14.5% (17/117) by culture. The superior sensitivity of PCR over culture was more evident in nonpulmonary cases where PCR picked up 10 cases in addition to 3 culture positives out of 69 specimens. On the other hand, out of 48 pulmonary specimens only 4 cases in addition to 14 culture positives were picked up by PCR.—Authors' Summary

McAdam, R. A., Hermans, P. W. M., van Soolingen, D., Zainuddin, Z. F., Catty, D., van Embden, J. D. A. and Dale, J. W. Characterization of a *Mycobacterium tuberculosis* insertion sequence belonging to the IS*3* family. Mol. Microbiol. **4** (1990) 1607–1613.

A repetitive element (IS*986*), previously isolated from *Mycobacterium tuberculosis* and shown to detect multiple restriction fragment-length polymorphisms (RFLPs), has been sequenced. It consists of a potential insertion sequence of 1358bp, with 30-bp inverted repeat ends. IS*986* has four potentially significant open reading frames (ORFs): ORFa1, ORFa2 and ORFb on one strand and ORFc on the complementary strand. The sequences of the potential translated products identify IS*986* as a member of the IS*3* family, with an apparent frameshift between ORFa1 and ORFa2. IS*986* has potential as a highly specific probe for detection and typing of *M. tuberculosis,* as well as for transposon mutagenesis of mycobacteria. The sequence of IS*986* is virtually identical to that of another recently described element, IS*6110*. —Authors' Summary

Nakamura, M., Harano, Y. and Koga, T. [Isolation of a strain of *M. tuberculosis* which is considered to be rifampicin-dependent, from a patient with long-lasted smear positive and culture difficult (SPCD) mycobacteria.] Kekkaku **65** (1990) 569–574. (in Japanese)

During the course of clinical examination of drug sensitivity tests for *Mycobacterium tuberculosis,* a strain of *M. tuberculosis* which is considered to be rifampin-dependent was isolated from a patient with persisting smear-positive, culture-negative (SPCN) or culture-difficult (SPCD) mycobacteria status. The strain isolated produced a few tiny colonies on the control Ogawa-egg yolk medium; whereas it showed abundant growth like a bacteria plaque on the medium containing rifampin 50 μg/ml. Furthermore, the growth of the strain on Ogawa medium containing rifampin 50 μg/ml is much better than that on the medium containing rifampin 10 μg/ml.—Authors' English Summary

Rodrigues, C. J., de Campos, F. P. F., Furtado-Mendonça, L. L., Pereira, R. M. R., Langer, B., Diament, J., de Oliveira, R. M. and Cossermelli, W. Mycobacterial subcutaneous arteritis. Rev. Inst. Med. Trop. São Paulo **32** (1990) 346–350.

The authors report three patients with subcutaneous erythematous nodules in different phases of development, nonspecific systemic symptoms, positive PPD test, and normal chest X-rays. The histopathological study of the older nodules showed a granulomatous arteritis with a few acid-fast bacillin (AFB) in the vascular wall. The nodules at an early phase showed a nonspecific panniculitis with some AFB in apparently normal cutaneous vessels. These findings suggest that the mycobacterium has a vascular tropism and may cause a primary granulomatous arteritis.—Authors' Summary

Rook, G. A. W. and Al Atiyah, R. Cytokines and the Koch phenomenon. Tubercle **72** (1991) 13–20.

We outline the mechanisms contributing to the human form of the Koch phenomenon, which we define as necrosis occurring within 24–48 hr of injection of mycobacterial antigen into the skin of past or present tuberculosis patients. It is probable that tissue damage mediated in the same way occurs in the lesions themselves. We suggest that the necrosis is mediated in part by cytokines, particularly tumour necrosis factor (TNF), and that this occurs for three reasons. First, *Mycobacterium tuberculosis* evokes an immunoregulatory abnormality characterized by raised agalactosyl IgG. This abnormality, also found in rheumatoid arthritis, Crohn's disease, and erythema nodosum leprosum, seems to be associated with dysregulation of cytokine release. Secondly, *M. tuberculosis* itself triggers further cytokine release. Thirdly, the normally protective role of TNF is distorted by several interacting properties of components of *M. tuberculosis,* which render the cytokine toxic to the host tissues. The immunoregulatory abnormality may be susceptible to correction by immunotherapy.—Authors' Summary

Safrin, S., Sattler, F. R., Lee, B. L., Young, T., Bill, R., Boylan, C. T. and Mills, J. Dapsone as a single agent is suboptimal therapy for *Pneumoncystis carinii* pneumonia. J. Acquir. Immune Defic. Syndr. **4** (1991) 244–249.

In a prospective, noncomparative study, seven patients with mild *Pneumocystis carinii* pneumonia, characterized by room air arterial Po_2 greater than 60 mm Hg at the time of presentation, were treated with dapsone alone at a dose of 200 mg daily. Two of the seven patients required mechanical ventilation for respiratory failure on day 5 of dapsone therapy; both died. Four patients experienced major side effects during dapsone therapy. None of the seven patients successfully completed a full course of therapy with dapsone. We conclude that high-dose, single-agent dapsone is not suitable for further study as therapy for *Pneumocystis carinii* pneumonia.—Authors' Summary

Singh, I. G., Mukherjee, R. and Talwar, G. P. Resistance to intravenous inoculation of *Mycobacterium tuberculosis* H37Rv in mice of different inbred strains following immunization with a leprosy vaccine based on *Mycobacterium w.* Vaccine **9** (1991) 10–14.

Four strains of mice, namely BALB/c, C57BL/6 NCrl (Bcg^s), C3H He NCrl, and CBA'N (Bcg^r) were experimentally infected with *Mycobacterium tuberculosis* H37Rv (Trudeau Institute, Saranac Lake, N.Y.) to induce sublethal infection. The level of infection was assessed by screening tuberculin reaction, pulmonary lesions, and viable units of mycobacteria recovered from the lung, spleen and liver. On prior immunization with 10^7 heat-killed suspension of *Mycobacterium w,* an antileprosy vaccine currently under large-scale human trials in India, protection was observed against tuberculosis in all the four strains of mice used in the study as assessed by significant reduction of both pulmonary lesions and viable units of mycobacteria recovered from different organs. In parallel experiments, live BCG was able to confer protection to mice of Bcg^s strains but not to mice of the Bcg^r strains. Results of these experiments suggest that a vaccine based on heat-killed *Mycobacterium w* has the potential also to confer protection against tuberculosis in mice of genetic strains whose immune system is less triggered by intravenous injection of viable BCG.—Authors' Abstract

Stoner, G. L. Implications of progressive multifocal leukoencephalopathy and JC virus for the etiology of MS. Acta Neurol. Scand. **83** (1991) 20–33.

JC virus (JCV) infects oligodendrocytes and, to a lesser extent, astrocytes in the brain and spinal cord and causes the demyelinating disease known as progressive multifocal leukoencephalopathy (PML) in immunocompromised individuals. The possibility exists that this opportunistic infection reactivates from a latent state in the brain. It is proposed that the pathogenetic immune response in a multiple sclerosis (MS) brain may be directed predominantly toward antigens of a DNA virus, such as JCV, which is latent in glial cells. The target antigens could be synthesized only during transient viral reactivation or could persist, thus explaining the two basic patterns of neurological symptoms in MS. It is further proposed that the viral genome as a minichromosome becomes focally distributed in glial cells following vertical passage in dividing progenitor cells after infection early in life. The concept that the host response to a single agent can evoke two distinct pathologies (PML and MS) derives from a chronic mycobacterial infection of peripheral nerves—leprosy.—Author's Abstract

Street, M. L., Umbert-Miller, I. J., Roberts, G. D. and Su, W. P. D. Nontuberculous mycobacterial infections of the skin. J. Am. Acad. Dermatol. **24** (1991) 208–215.

This study comprised 14 patients from whose skin nontuberculous mycobacteria were recovered. Most clinical manifestations were relatively nonspecific. Various histopathologic patterns were observed in 22 biopsy specimens. Recurrences were common, and prolonged treatment was often necessary. Culture of tissue remains the definitive diagnostic procedure. Cutaneous lesions can be the first or only site of nontuberculous mycobacteriosis.—Authors' Abstract

Tanaka, K., Wilks, M., Coates, P. J., Farthing, M. J. G., Walker-Smith, J. A. and Tabaqchali, S. *Mycobacterium paratuberculosis* and Chrohn's disease. Gut **31** (1991) 43–45.

The possible etiological role of *Mycobacterium paratuberculosis* in Crohn's disease was investigated. The immunological response was studied using an enzyme-linked immunosorbent assay (ELISA), Western blotting, and immunocytochemistry. The antibody response to two protoplasmic antigen preparations of *M. paratuberculosis* in the sera of patients with inflammatory bowel disease was measured by ELISA. IgG and IgM antibodies to these antigens were measured in serum samples from 52 patients with Crohn's disease, 15 patients with ulcerative colitis, and 41 control patients without inflammatory bowel disease. Although there was wide variation in the concentrations of antibody detected, patients with Crohn's disease had concentrations that were not significantly different from those of the other two groups. In addition, mycobacterial antigens were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the immune response to each antigen was then examined separately and assayed for IgG and IgM in 10 patients from each of the three groups. An indirect peroxidase test was also used to detect *M. paratuberculosis* in sections of tissue from 18 patients with Crohn's disease and 10 with ulcerative colitis. The results were negative in all cases. This study does not support a role for *M. paratuberculosis* in Crohn's disease.—Authors' Abstract

Truffot-Pernot, C., Ji, B. and Grosset, J. Activities of pefloxacin and ofloxacin against mycobacteria: in vitro and mouse experiments. Tubercle **72** (1991) 57–64.

The minimal inhibitory concentrations for 90% of strains (MIC90) of ofloxacin against *Mycobacterium tuberculosis* and *M. xenopi* was 2 mg/l. This was three dilutions lower than that of pefloxacin and was well within the range of drug concentrations achievable in man. The antituberculosis activities of both quinolones were independent of resistance of the strains to other antimycobacterial agents. *M. avium-intracellulare* was resistant to both compounds with MIC90s greater than 16 mg/l. The maximum serum levels (C_{max}) of both compounds increased proportionally with increasing dose size. The terminal elimination half-life ($T_{½}$) of pefloxacin was longer than that of ofloxacin, but the $T_{½}$ of both compounds in mice were much shorter than in man. The area under the concentration curve (AUC) of pefloxacin was double than that of ofloxacin. In the mouse, pefloxacin at doses up to 150 mg/kg daily was inactive against *M. tuberculosis* infection: in terms of survival rate, the minimal effective dose of ofloxacin against *M. tuberculosis* infection was 150 mg/kg daily when given by gavage or by incorporation into the mouse diet at a concentration of 0.1%, but in terms of cfu counts, ofloxacin 150 mg/kg daily only displayed a moderate degree of activity similar to ethambutol 100 mg/kg daily. The therapeutic effects of ofloxacin against *M. tuberculosis* infection were dose-related: 300 mg/kg daily by gavage or 0.4% in mouse diet displayed much better therapeutic effects than lower dosages. Since the AUC in mice treated with ofloxacin 150 mg/kg daily is close to that in man treated with a clinically tolerated dose—600 mg daily—such a dosage may be only moderately effective against human tuberculosis.—Authors' Summary

Wallace, R. J., Jr., Brown, B. A. and Onyi, G. O. Susceptibilities of *Mycobacterium fortuitum* biovar. *fortuitum* and the two subgroups of *Mycobacterium chelonae* to imipenem, cefmetazole, cefoxitin, and amoxicillin-clavulanic acid. Antimicrob. Agents Chemother. **35** (1991) 773–775.

Minimum inhibitory concentrations of imipenem, cefoxitin, cefmetazole, and amoxicillin-clavulanic acid were determined against 100 strains of *Mycobacterium fortuitum* and 200 strains of *M. chelonae.* Imipenem and cefmetazole were more active against *M. fortuitum* than cefoxitin was, and imipenem (which inhibited 39% of strains at 8 μg/ml) was the only beta-lactam active against *M. chelonae* subsp. *chelonae.*—Authors' Abstract

Yamaguchi, R., Matsuo, K., Yamazaki, A., Kagawa, H., Nagai, S. and Yamada, T. Fusion protein based epitope mapping of the MPB57 protein from *Mycobacterium bovis* BCG and its epitope insertion into

the native protein. Can. J. Microbiol. **37** (1991) 7–13.

The gene coding for the 12-kDa protein (MPB57) of *Mycobacterium bovis* BCG has recently been cloned and sequenced. To map linear B-cell epitopes by β-galactosidase fusion proteins, we have constructed convenient vectors (pUR278S, pUR288S, and pUR289S) with the *Sma*I site. Based on recognition by polyclonal antibodies, two epitope regions on the MPB57 protein were identified, both of which corresponded to the amino acid sequence Glu^{20} to Val^{45} (26 residues, epitope I region) and Ile^{78} to Leu^{86} (9 residues, epitope II). Complementary oligonucleotides encoding epitope II were synthesized, polymerized by a ligase reaction, inserted into the native MPB57 protein gene, and expressed in *Escherichia coli,* giving rise to epitope-inserted proteins. Their stability and potential uses are described.—Authors' Abstract

Zhang, Y.-X. and Neu, H. C. Fleroxacin combined with rifampin. Diagn. Microbiol. Infect. Dis. **14** (1991) 23–27.

We determined the effect of the combination of rifampin and fleroxacin against *Enterobacteriaceae* and streptococcal species. None of the 65 isolates tested by checkerboard assay demonstrated synergy, 12% of isolates showed an additive effect; 86.7% were indifferent, and only 1 isolate showed antagonism. The mean FIC was 1.2. When using 2 and 8 μg/ml of rifampin, fleroxacin MICs of 285 isolates of *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus influenzae,* staphylococci, streptococci, *Bacteroides,* and *Clostridium* were not increased, but synergy was not demonstrated. Time-kill studies against *Escherichia coli, P. aeruginosa, Enterobacter cloacae, Staphylococcus aureus,* and *Enterococcus faecalis* failed to show increased killing when the two agents were present at one-half the MBC. The fleroxacin-rifampin interaction is one of indifference but provides coverage for species not adequately inhibited by fleroxacin.—Authors' Abstract