CURRENT LITERATURE

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

General and Historical

Itakura, K. [Biwasaki Leprosarium—a century of dedication.] Jpn. J. Lepr. **61** (1992) 112–116. (in Japanese)

Biwasaki Leprosarium, or Biwasaki Tairo Hospital, was established by Father Jean Marie Corre, a French priest, in 1898. He was born in Brittany, France, in 1850. After being ordained to the priesthood, he came to Nagasaki, Kyushu, in 1876. He was just 26 years old. He was greatly moved by the sight of the Hansenites and other sick people around the Honmyoji Temple in Kumamoto, Kyushu, which is one of the well-known temples in Japan. They were barely making a living by the charitable contributions of the pilgrims who visited the Temple, in those days. Fr. Corre moved from Nagasaki to Kumamoto.

First, he built a church in Tetori, Kumamoto, and rented a house near the Honmyoji and began to look after the needs of the Hansenites. In 1896, he bought a large lot at Biwasaki in the Shimasaki area, Kumamoto, which is not far from Honmyoji. He remodeled some houses into the so-called sanatorium and started to accommodate those suffering from Hansen's disease. At the outset, he had the cooperation of nuns and church workers to look after the patients' needs. Furthermore, Fr. Corre appealed to Rome for the help and/or support in 1898 in order to expand this kind of project, and five nuns were dispatched in the following year from the Franciscan Missionaries of Mary in Rome. Since then the sisters have devoted themselves assiduously and faithfully to help the Hansenites as far as possible and they are still doing this.

In 1963, a fire broke out from one of the toilets and made a clear sweep of the whole building. The neighbors at first objected to the reconstruction since the leprosarium was located in the city and surrounded by many houses although no one was hurt in the fire. However, many people came and helped on the reconstruction of the leprosarium. Then, on 22 August in the following year, the new building as seen now was completed and an opening ceremony was held in the presence of Princess Chichibu. The Imperial Household has been very generous and well disposed to the Biwasaki Leprosarium.

In this Leprosarium there are only 16 patients now. They are fortunate at present, we believe. And yet, there are many unfortunate people in the world as the late Dr. M. Miyazake said in India. It is time now, indeed, for us to open our eyes to the people in distressful situations in developing countries. Sisters of the Franciscan Missionaries of Mary and many Japanese are working for or with the Hansenites in various places of the world. Let us give a helping hand to the afflicted and needy in the world, and also let us not forget the charity and relief works of the great pioneers.—Author's English Abstract

Saikawa, K. [The progress of National Leprosarium.] Jpn. J. Lepr. **61** (1992) 107–111. (in Japanese)

Leprosy Prevention Law was proclaimed in 1907 in Japan. According to the regulation, Leprosy Control Policy got under way by the government. In 1909, five leprosaria were established in the leprosy-endemic areas by local governments to admit vagrant leprosy patients who were estimated as 1200. The vagabonds had many troubles, especially they often escaped from leprosaria. Dr. Kensuke Mitsuda who was one of the directors of the leprosaria suggested that the Government establish the National Leprosarium on a small island to admit them. In 1930, the government had a ten-year program to eradicate leprosy and decided to set up 10,000 beds in existing leprosaria and the newly established National Leprosari-

um. The plan was almost completed by construction of five National Leprosaria by 1940. The number of inpatients was 9125, including 4389 in five national institutions. In 1941, five local leprosaria were transferred to the central government; after then, in 1943 and 1944, two National Leprosaria were established. The total number of National Leprosaria in Japan came to 13 in 1945.—Author's English Abstract

Vidya Sagar Reddy, J. Health and human resource mobilization: an assessment of staffing pattern in NLEP at operational level. Indian J. Lepr. **65** (1993) 81–93.

In this paper the staffing pattern, training and infrastructural facilities of the National Leprosy Eradication Program (NLEP) at operational level as well as the attendant problems in mobilizing human resources are discussed. The study shows that the major portion of the work of the NLEP is being shared by the para-medical workers (PMWs) (72%), followed by nonmedical supervisors (NMS) (14%) and medical officers (5%). The population served by the PMW in all the highly and moderately endemic regions is more than the prescribed limit except in Nagaland and Sikkim. In the same areas, the medical officer serves a population more than the norm in Andhra Pradesh, West Bengal, Maharashtra, Karnataka and Bihar. Regarding case load, in no state does the medical officer serve more than 2500 cases except in Bihar and Kerala, in moderately endemic and low-endemic regions, respectively. The PMW in Haryana and Punjab states attends more than 250 cases. In NLEP every 1 out of 4 sanctioned posts is vacant. There is also an urgent need to rationalize the training program so that there is optimal utilization of the training centers.—Author's Abstract

Chemotherapy

Crowle, A. J., Douvas, G. S. and May, M. H. Chlorpromazine—a drug potentially useful for treating mycobacterial infections. Chemotherapy **38** (1992) 410–419.

Chlorpromazine (CPZ) is one of several phenothiazines known to have antimicrobial properties. It can inhibit mycobacteria, and was reported in the early literature to improve tuberculosis clinically. CPZ was tested here for its ability to inhibit the replication of *Mycobacterium tuberculosis* and *M. avium* in cultured normal human macrophages, as determined by counts of viable bacteria at 0, 4, and 7 days after bacterial infection of the macrophages. CPZ inhibited the intracellular bacteria at a concentration range of 0.23–3.6 μg/ml, and was more effective intracellularly than extracellularly. It was further tested for its ability to cooperate with isoniazid, streptomycin, pyrazinamide, rifampin, rifabutin, penicillin and ethambutol (EMB) against intramacrophage *M. tuberculosis* and *M. avium*. CPZ enhanced the effectiveness of most of the drugs tested against intracellular mycobacteria. However, the combination of CPZ and EMB did not result in augmented antimycobacterial activity.—Authors' Abstract

Gidoh, M., Tsutsumi, S., Yamane, T., Yamashita, K., Hosoe, K. and Hidaka, T. Bactericidal action at low doses of a new rifamycin derivative, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl) benzoxazinorifamycin (KRM-1648) on *Mycobacterium leprae* inoculated into footpads of nude mice. Lepr. Rev. **63** (1992) 319–328.

Among a series of newly synthesized benzoxazinorifamycins, two of the 3′-hydroxy-5′-(4-alkyl-1-piperazinyl) derivatives, named KRM-1648 and KRM-2312, whose respective alkyl residues are isobutyl and isopropyl, were examined for efficacy against the nude mouse-model leprosy. KRM-1648 completely inhibited the growth of leprosy bacilli inoculated into nude mouse foot pads, even 6 months after the medication had been stopped, when given orally at a daily dose of 0.6 mg/kg, 5 or 6 times weekly, during 3–5 months' postinoculation. In comparison, the growth inhibition

by KRM-2312 was incomplete under the same conditions, although it was still stronger than that by rifampin. Complete growth inhibition by KRM-1648 was also observed when it was given orally at a dose of 1 or 3 mg/kg twice weekly during the same period. In contrast, the growth inhibition by rifampin was only slight at 1 mg/kg and partial at 3 mg/kg under the same condition.—Authors' Summary

Irshaid, Y. M., Al-Haddidi, H. F., Abuirjeie, M. A., Rawashdeh, N. M. and Gharaibeh, N. S. Acetylation of dapsone by human whole blood. Int. J. Clin. Pharmacol. Ther. Toxicol. **31** (1993) 18–22.

We studied the acetylation of dapsone (DDS) *in vitro* by whole blood taken from subjects with known acetylator phenotype. The acetylation of DDS by whole blood was both incubation time- and DDS concentration-dependent. Thus, it is highly recommended to separate plasma immediately after blood withdrawal during acetylation phenotyping using DDS. Para-aminobenzoic acid (PABA) substantially inhibited the acetylation of DDS by whole blood taken from both slow and rapid acetylators, while procainamide (PAD) significantly inhibited DDS acetylation by whole blood taken from slow acetylators. At the highest PAD concentration used (208 μM), DDS acetylation by whole blood taken from rapid acetylators was also inhibited. In contrast, sulfanilamide (SAD) failed to produce any significant inhibition of the acetylation of DDS by whole blood taken from either slow or rapid acetylators. Furthermore, there was no correlation between DDS acetylation by whole blood *in vitro* and the acetylator status of the subject. It is therefore not possible to predict the acetylator phenotype by studying DDS acetylation by human whole blood. These results indicate that the DDS N-acetyltransferase of human whole blood is most probably of the monomorphic type.—Authors' Abstract

Jadhav, V. H., Patki, A. H. and Mehta, J. M. Comparison of two multidrug regimens in multibacillary leprosy. Indian J. Lepr. **64** (1992) 501–504.

One of the technical problems relating to the multidrug therapy of leprosy is the slow decrease in the bacterial index (BI) in multibacillary (MB) patients. In this study we have compared a regimen containing rifampin given daily for 9 months with the standard WHO multidrug regimen for MB leprosy. We have found, at the end of 2 years, a significantly greater fall of BI in patients who had received the regimen containing daily rifampin as compared to those who had received pulsed doses of rifampin. The doses of dapsone and clofazimine were similar in these two groups. It appears that daily administration of rifampin may be useful in treating MB patients in whom reduction in the BI is slower than expected. However, in view of its high cost and the very much increased incidence of type-2 lepra reactions and hepatitis, daily rifampin therapy cannot be recommended for a control program.—Authors' Abstract

Li, W.-H., *et al.* [Effects of short-term pefloxacin on MB leprosy patients and follow up for them.] China Lepr. J. **8** (1992) 129–133. (in Chinese)

Fourteen newly detected MB cases of leprosy have been treated with pefloxacin made in China for 6 months and have shown significant improvement clinically, bacteriologically and pathologically. Among 9 cases out of these 14 patients, the vitality of the bacilli was measured before and after treatment. The bacilli in 2 cases still showed multiplication at the end of the first month of treatment, but no proliferation at the end of the second and third months. Two BT cases have been treated for 6 and 12 months, and when followed up 4 and 10 months after stopping of treatment, respectively, their conditions showed deterioration, suggesting that the treatment was not sufficient or that drug resistance has been induced.—Authors' English Abstract

Prabhakaran, K., Harris, E. B., Randhawa, B. and Hastings, R. C. Reversal of drug resistance in *Mycobacterium leprae* by ampicillin/sulbactam. Microbios **72** (1992) 137–142.

The multiplication of *Mycobacterium leprae* in foot pads of experimentally infected mice was suppressed by intramuscular administration of ampicillin combined with sulbactam or YTR-830H, two

potent inhibitors of beta-lactamase in the bacteria. The antibiotic or the inhibitors by themselves were inactive. Ampicillin/sulbactam also inhibited the growth of drug-resistant *M. leprae* which grew in the presence of rifampin or dapsone. The finding provides a new approach to treat leprosy and to overcome drug resistance of the mycobacteria.—Authors' Abstract

Progress in leprosy control through multidrug therapy. WHO Statis. Quart. **44** (1991) 1–46.

This issue comprises five papers: "Global review of multidrug therapy in leprosy" (S. K. Nordeen, L. Lopez Bravo and D. Daumerie, pp. 2–15); "Leprosy in the WHO African Region" (D. Daumerie, pp. 16–22); "The national leprosy eradication programme in India" (B. N. Mittal, pp. 23–29); "Leprosy control in Zambia" (G. J. Steenbergen, pp. 30–55); "Leprosy control activities of the International Federation of Anti-leprosy Associations" (D. Martineau-Needham and S. Lacey, pp. 36–46).

A useful overview of the contents and topic is provided. "In recent years leprosy control and the resulting world situation of the disease have been undergoing important changes for the better. This is due mainly to the introduction of the WHO-recommended multidrug therapy (MDT) in disease control and the increasing political commitment to and resources for leprosy control. Over the past 5 years, a reduction of 30% in the number of registered cases of leprosy has been observed with the figures for 1990 being about 3.7 million.

In spite of this progress, several problems remain. MDT coverage is still quite low in 40% of the endemic countries. There are considerable uncertainties about the proportion of undetected cases as those registered do not fully reflect the magnitude of the problem. Further MDT does not directly address the question of prevention of disabilities in leprosy which is a major concern in the disease.

It is important that this issue of the *Quarterly* highlights not only the major problem areas in leprosy control, such as those found in many parts of Africa, but also the success stories, in this case those of India and Zambia. The massive MDT operation in India aims to reach most leprosy patients within the shortest possible period of time, and the results so far are heartening thanks to the strong political commitment combined with the large mobilization of resources. The Zambian success demonstrates the effectiveness for leprosy work of integrated programs supported by specialized services as well as the relevance of community-based rehabilitation.

A major contributing factor to the current progress in recent years is the participation of nongovernmental organizations (NGOs), at both national and international levels. In some countries, national NGOs have provided critical inputs to ensure political commitment and increased priority for leprosy. However, international NGOs, such as the Member associations of the International Federation of Anti-leprosy Associations (ILEP), have played a key role in making available to countries substantial quantities of resources: in some instances, leprosy control could not have been possible without such support.

The future for leprosy control, and even the elimination of the disease as a public health problem, appears bright provided national commitments continue to be strengthened and sufficient resources are made available to ensure increasing coverage of MDT. WHO has played and continues to play a key role in technical collaboration with its Member states as well as in the mobilization and coordination of resources."

[Since this publication in 1991, WHO has produced new estimates for leprosy worldwide, giving a figure of 5.5 million for those requiring or receiving multiple drug therapy and a figure of between 2 and 3 million for those estimated to have (significant) deformities due to leprosy (see Bulletin of the World Health Organization, 1992, 70, 7).]—A. C. McDougall (Trop. Dis. Bull.)

Rao, P. S., Reddy, B. N., Krishnamurthy, P., Raja Rao, B., Sastrulus, M. V. and Dutta, A. Initial intensive therapy for multibacillary leprosy patients—in retrospect. Lepr. Rev. **63** (1992) 350–357.

We analyzed the results of 4845 multibacillary (MB) patients being treated with multidrug treatment (MDT) in the Srikak-

ulam District of Andhra Pradesh, India. Of these, 2309 (47.7%) patients were given an initial 14-day intensive therapy with rifampin, clofazimine and dapsone, followed by the WHO-recommended pulse therapy. The rest of the cases were given only pulse therapy. The improvement in terms of bacteriological clearance and the proportion of cases declared released from treatment (RFT) was found to be significantly higher among patients treated with only pulse therapy. Clinic attendance was found to be better and more regular in patients treated with intensive therapy, and no relapses were seen with either therapy. The implications of these findings on the operational aspects of program implementation are discussed.—Authors' Summary

Zhang, S., *et al.* [Effect of MDT on MB leprosy over 27 months and follow-up of 56 months thereafter.] China Lepr. J. **8** (1992) 133–136. (in Chinese)

Forty-seven leprosy patients have regularly taken WHO's regimen of MDT for 27 months. The treatment showed excellent curative effect, had few side effects, and was able to control the type 2 reaction. During 56 months' follow up after stopping treatment, their clinical conditions have been continuing to improve, the BI continuing to decline and pathological changes going on to subside in them. By the last follow up, 38 of 43 survival patients had been cured, and no relapse was found.—Authors' English Abstract

Clinical Sciences

Bhatki, W. S. and Ghulawala, R. G. Immunotherapeutic potential of ICRC vaccine: a case control study. Lepr. Rev. **63** (1993) 358–364.

A bacteriological follow up of 16 lepromatous patients with a high initial bacterial index (BI) showed that in 8 randomly selected patients who received single doses of ICRC vaccine (C44) at the onset of multidrug therapy (MDT), the average reduction of BI was from 4.4+ to 1+ in 2 years—3 of these patients became negative and 3 showed BI 1+ or less. Comparable bacteriological assessments in 8 nonvaccinated but otherwise similar patients showed an average reduction of BI from 4.7+ to 2.6+, i.e., consistent with the expected response to MDT in lepromatous patients. Here we discuss the role of immunotherapy and the selection of a desirable antileprosy vaccine in the context of fixed-duration MDT.—Authors' Summary

Brandsma, J. W., Heerkens, Y. F., Lakerveld-Heyl, K. and Mischner-Van Ravensberg, C. D. The international classification of impairments, disabilities and handicaps in leprosy-control projects. Lepr. Rev. **63** (1993) 337–344.

The use of a uniform language, which includes definitions of terms, is very important in the field of health care. It is important to have a common language for educational, research and communication purposes. Classifications can play a major role in the development of uniform reporting and registration systems. The purpose of this article is to familiarize leprosy workers with two classifications that are in common use in health care, a classification of diseases and a classification used to describe the overall health status of a person, and to relate the three terms that are used in the latter classification, impairments, disabilities and handicaps, to leprosy.—Authors' Summary

Cardoso Guillén, E., Ferrá Torres, T. M. Carranzana Hernández, G. B. [Ocular leprosy in the city of Camagüey, Cuba.] Rev. Leprol. Fontilles **18** (1992) 571–578. (in Spanish)

An ophthalmological study in 103 lepromatous leprosy patients (52.3% of the prevalence of this clinical form in 1989) was carried out in Camagüey city, Cuba; 46.7% of the cases presented ocular damage caused by leprosy. Madarosis of the eyelashes (40.8%) and chronic conjunctivitis (38.8%) were the most commonly seen lesions in the ocular annexal structures. Sequelae of keratitis and anterior uveitis were most fre-

quently observed in the anterior segment. Decrease of the visual acuity secondary to damage of the retina and optic nerve was present in 18.4% of the lepromatous patients.—Authors' English Summary

Chan, H., *et al.* [Observation on inhibitory activity of alpha$_1$ antipepsin in leprosy sera.] China Lepr. J. **8** (1992) 211–212. (in Chinese)

Inhibitory activity of α_1 antipepsin (α_1-AT) in the sera was determined with a modified Eriksson method in 55 leprosy patients (TT 15, B group 30 and LL 10) and 60 healthy persons as controls. The results showed that the α_1-AT level is 1.18 ± 0.15 mg/ml in TT without significant difference ($p > 0.05$), and 1.02 ± 0.28 mg/ml and 0.78 ± 0.42 mg/ml in B group and in LL with a highly significant difference ($p < 0.01$) respectively, as compared with controls. The mean of α_1-AT levels showed a tendency to decrease from TT to LL. The authors think that lack of α_1-AT might play some role in the pathogenesis of leprosy and purified α_1-AT or its preparation being able to promote the production of α_1-AT might be useful in the treatment of leprosy.—Authors' English Abstract

Courtright, P., Lewallen, S. and Howe, R. Cell-mediated immunity in trachomatous scarring—evidence from a leprosy population. Ophthalmology **100** (1993) 98–104.

There is limited understanding of the mechanisms that mediate immunity after infection by *Chlamydia trachomatis.* Since it is known that the clinical course of leprosy is related to cell-mediated immunity (CMI) and that such immunity contributes to the development of trachomatous conjunctival scarring, the authors examined patients to determine if there might be an association between leprosy status and trachomatous conjunctival scarring. Leprosy patients registered at Shashemane Hospital were interviewed, examined, and patients with siblings residing in the vicinity were asked to return for further clinical examination. A subsample of sibships was selected for laboratory evaluation of CMI measured by lymphocyte proliferative responses *in vitro* to stimulation by mycobacterial antigens. Conjunctival scarring was less severe in multibacillary leprosy patients (with suppressed CMI) than in their healthy siblings and more severe in paucibacillary leprosy patients (with enhanced CMI) than in their healthy siblings. The mean lymphocyte proliferative responses to mycobacterial antigens were greater in the sibling (whether leprous or healthy) with more severe conjunctival scarring, regardless of type of leprosy. The specific cellular immune responses to *Mycobacterium leprae* and p65 antigen in patients with increased conjunctival scarring provide evidence that early in the course of infection with *C. trachomatis* factors related to an individual's cellular response are crucial to the development of conjunctival scarring. A delayed-type hypersensitivity reaction ("reversal reaction") found in paucibacillary leprosy patients could contribute to the increased trachomatous conjunctival scarring in these patients.—Authors' Abstract

El Hassan, A. M., Hashim, F. A., Abdullah, M., Zijlstra, E. E. and Ghalib, H. W. Distinguishing post-kala-azar dermal leishmaniasis from leprosy: experience in the Sudan. Lepr. Rev. **64** (1993) 53–59.

In this study 4 patients with post-kala-azar dermal leishmaniasis (PKDL), whose lesions were similar to those of lepromatous and borderline leprosy, are described. In 2 patients there was no previous history of kala-azar, but they were residents of an area of known endemic kala-azar. Lack of proper clinical and laboratory assessment was behind the failure to diagnose PKDL. Consequently, the patients were treated with antileprosy drugs without proof of leprosy. The third and fourth patients, although suspected clinically of leprosy, were correctly diagnosed as PKDL with adequate history, clinical assessment and appropriate laboratory investigations. The salient points in distinguishing PKDL from leprosy are described and discussed.—Authors' Summary

Graf von Ballestrem, W., Alvarenga, A. and Namiki, M. Leprosy in a HIV-positive and syphilitic young Paraguayan man. Acta Leprol. (Genève) **8** (1992) 103–104.

In a borderline lepromatous patient a positive serology for syphilis and HIV-1 has

been detected. The patient also had a urinary tract infection with *Acinetobacter calcoaceticus*. In Paraguay until now no leprosy cases infected with HIV have been reported.—Authors' Summary

Hauhnar, C. Z., Kaur, S., Sharma, V. K. and Mann, S. B. S. A clinical and radiological study of maxillary antrum in lepromatous leprosy. Indian J. Lepr. **64** (1992) 487–494.

Seventy consecutive patients having multibacillary leprosy were questioned about symptoms of nasal involvement and sinusitis. Complete otorhinolaryngeal examination was carried out on all of these patients, and they were subjected to radiographic examination of paranasal sinuses. Radiological abnormality of maxillary antrum was found in 40 (57%) patients. Radiological changes were unilateral in 25 and bilateral in 15 patients. Localized or generalized mucosal thickening was the most common finding, followed by diffuse opacity. The development of radiological changes in the maxillary antrum correlated with high bacterial density (BI 3+ and above), nasal deformity, and disease duration of more than 2 years.—Authors' Abstract

Kannan, N. and Sivaram, M. Variables influencing regularity of leprosy patients in attending treatment clinics. Indian J. Lepr. **64** (1992) 505–511.

Regularity in attending clinics as well as taking drugs assumes a very significant place in a leprosy control program since irregularity of leprosy patients can lead to poor disease control, drug-resistant disease, and development of physical deformities and disabilities thus leading to program failure. Further, these complications also create socioeconomic and psychological problems to the victims as well as their families in myriad ways. This paper reports a study aimed at identifying the variables, among a set of 29 selected demographic, socioeconomic and disease-related variables, having significant association with the regularity of leprosy patients in attending treatment clinics. It was found that the age of the patients, type of family, duration of the disease, time lag between diagnosis of the disease and starting treatment, and knowledge of patients and their families about the disease were significantly associated with treatment regularity.—Authors' Abstract

Krotoski, W. A., Mroczkowski, T. F., Rea, T. H., Almodovar, P. I., Clements, B. C., Neimes, R. E., Kahkonen, M. K., Job, C. K. and Hastings, R. C. Lepromin skin testing in the classification of Hansen's disease in the United States. Am. J. Med. Sci. **305** (1993) 18–24.

Hansen's disease, or leprosy, although a relatively uncommon disease in the United States, continues to be important because of its implications—physical, psychological, and social—for the patient. Prognosis and treatment of the disease are based largely on clinical classification, which ranges from the multibacillary "lepromatous" to the paucibacillary "tuberculoid" forms, depending on the patient's specific immune capabilities. Traditionally, skin testing with lepromins—suspensions of the etiologic agent of Hansen's disease, *Mycobacterium leprae*—have been used as adjuncts to clinical parameters for classification in endemic areas. However, these have not been systematically studied in the United States. This report describes the results obtained from skin testing 38 volunteers (22 patients and 16 uninfected persons) with standard lepromin preparations. These results support the adjunctive value of lepromins for clinically classifying Hansen's disease in our "hypoendemic" population.—Authors' Abstract

Mishra, B., Malaviya, G. N., Girdhar, A., Hussain, S. and Girdhar, B. K. Paralysis of occipitofrontalis in a borderline case of leprosy. Lepr. Rev. **64** (1993) 60–63.

A patient with neuritic leprosy developed borderline skin lesions. Later, another skin lesion developed on the left side of the forehead with clinical involvement of the supraorbital branch of the ophthalmic division of the trigeminal nerve. Simultaneously, paralysis of the occipitofrontalis and mild paresis of orbicularis oculi occurred.—Authors' Summary

Myint, T., Htoon, M. T., Win, M. and Yin, C. Risk factors among defaulters in the urban leprosy control centre of Thaketa

Township in the City of Yangon, Myanmar, 1986. Lepr. Rev. **63** (1992) 345–349.

A total of 884 registered cases from the city of Yangon, Myanmar, were analyzed retrospectively. The defaulter proportion among cases registered for treatment at the Thaketa Health Center was 34.16%. It was established that patient sex and occupation are not factors in defaulting. Paucibacillary cases and cases with no disability are more likely to default.—Authors' Summary

Ohtaka, K. [Patients with calcinosis cutis in National Leprosarium Matsuoka Hoyo-En.] Jpn. J. Lepr. **61** (1992) 98–101. (in Japanese)

As of the year 1991 there were 358 leprosy patients in the National Leprosarium Matsuoka Hoyo-En, including 223 patients (62.3%) who had received injections of chaulmoogra oil before. Calcinosis cutis caused probably from the injections was noted on 73 patients (32.7%): 67 lepromatous and 6 tuberculoid cases. It has never been reported before that the T-type patient suffering from calcinosis cutis was observed in the cases of chaulmoogra oil injection in Japan. The detectable positions of calcinosis cutis were mostly at the injected sites, that is, outside the right brachium followed by bilateral-branchia and crura. In the group of patients with calcinosis cutis, the anti-PGL antibody was negative for the most part. Urinalyses, peripheral blood analyses, histopathological tissue examinations of calcium deposition, X-ray diffraction patterns, differential thermal and gravitational analyses, and chemical analyses were performed on all patients with this disease.

As the result of this study 6 patients with calcinosis cutis caused by sulpyrine was also found. The major component of the deposit by the drug was calcium phosphate. These calcinosis cutis were considered to be of trophopathic calcinosis based on the disorder of subcutaneous tissue due to the injections of respective drugs: chaulmoogra oil and sulpyrine.—Author's English Abstract

Pönnighaus, J. M., Fine, P. E. M., Sterne, J. A., Lucas, S. B. and McDougall, A. C. Long-term active surveillance of leprosy suspects—what are the likely returns? Lepr. Rev. **64** (1993) 25–36.

Data are presented from the Karonga District in northern Malaŵi on the long-term follow up of 277 leprosy suspects who were not given antileprosy treatment or kept on active surveillance. Individuals who were started on antileprosy treatment within a year after leprosy was first suspected, usually on the basis of histopathology results, are excluded from this analysis because their active surveillance would not usually cause an organizational or financial problem for leprosy control projects. After an average follow-up period of 4.5 years 35 of the 277 suspects included in the analysis (13%) were diagnosed with what we consider to be "unequivocal" leprosy, and 3 of the 35 had developed disabilities. In 211/277 (76%) all signs of leprosy had disappeared completely.

Comparing clinical certainties at first and last examinations and comparing clinical with histopathological certainties at last examinations, it is estimated that up to 50% of the 35 cases of unequivocal leprosy which "arose" in this group were attributable to misdiagnosis at the first or second examination rather than to genuine progression of the disease. This estimate is compatible with an overall sensitivity of 90% and an overall specificity of 95% at each examination. Leprosy suspects with one cardinal sign of leprosy, either a typical lesion without loss of sensation, or loss of sensation in an otherwise untypical lesion, should be considered a high-risk group in that approximately 25% of such suspects (19/78) were later found with unequivocal leprosy. Policies toward such suspects should be formulated by leprosy control projects.—Authors' Summary

Sanlorenzo, M., Ratrimoarivony, C., Caldera, D., Rakotondrajao, J. and Mounden, J. C. [Cutaneous neoplasia in two cases of leprosy.] Acta Leprol. (Genève) **8** (1992) 105–107. (in French)

The authors report on two cases of dermal neoplasia in foot trophic disorders observed in leprous subjects. The report includes the description of the clinical and histological data as well as the surgical technique used; finally, it underlines the importance of a

precocious diagnostic so as to obtain a good therapeutic result.—Authors' English Summary

Scheepers, A., Lemmer, J. and Lownie, J. F. Oral manifestations of leprosy. Lepr. Rev. **64** (1993) 37–43.

A total of 37 out of 187 patients with leprosy had oral lesions. All were biopsied. Oral lesions were found most frequently in patients with lepromatous leprosy. Prevalence of oral lesions was higher in males than in females (73%:27%). Oral lesions were recorded on the WHO topographical map, and in most cases (92%) several topographical locations were affected, including hard palate in all cases. Topographical locations affected increased with age; males were more extensively affected than females ($p = 0.001$); and patients with oral lesions who reported affected family members (11 out of 37) had more extensive oral lesions than those who did not. In 27 cases with oral lesions histopathological diagnosis was possible.—Authors' Summary

Shetty, V. P., Suchitra, K., Uplekar, M. W. and Antia, N. H. Persistence of *Mycobacterium leprae* in the peripheral nerve as compared to the skin of multidrug-treated leprosy patients. Lepr. Rev. **63** (1992) 329–336.

Skin and nerve biopsies obtained from 18 multibacillary (MB) and 16 paucibacillary (PB) cases of leprosy who had been fully treated by the WHO regimen were assessed for bacterial load using different staining techniques. In addition skin and nerve homogenates of 10 MB cases were tested for "persistor" *Mycobacterium leprae* using immunosuppressed mice. While significant amounts of integral bacilli and BCG cross-reactive antigen of *M. leprae* were detected both in skin and nerve tissues of all the MB cases (100%), 56% of skin and 62% of nerve biopsies of PB cases also showed the presence of BCG crossreactive antigen. Detection of "persistor" *M. leprae* in 2/10 skin biopsies (20%) and 3/10 nerve biopsies (30%) of MB cases was thought to be unexpectedly high after 2 years of MDT.—Authors' Summary

Shwe, T. Prevalence of color blindness among patients with leprosy. Indian J. Lepr. **64** (1992) 483–486.

Using Ishihara test plates the prevalence of color blindness was studied on 697 leprosy patients and 292 normal healthy controls; 7.88% of male patients with tuberculoid leprosy, 12.18% of male patients with lepromatous leprosy, and 0.67% of male controls were detected to be color blind (red-green deficiency or total color weakness). The differences between the different groups are significant. Among female patients and controls, only 1 lepromatous leprosy patient was detected to have red-green deficiency. This suggests the possibility of a genetic predisposition to *Mycobacterium leprae* infection in patients with leprosy.—Author's Abstract

Tanaka, M., Nishino, H., Gaku, K., Egawa, K. and Ozawa, T. [Survey of the antibody to HCV in National Leprosarium Suruga.] Jpn. J. Lepr. **61** (1992) 88–91.

Blood specimens from 210 leprosy patients (average age 67.4 years old) and 84 staff members (average age 43.5 years old) in National Leprosarium Suruga were tested for anti-hepatitis-C virus (HCV) antibody using Ortho's Ab ELISA system. Among the patients, 17 patients had chronic hepatic dysfunction as well as leprosy; 20 of the 210 patients (9.5%) had anti-HCV antibody in their blood; 11 of the 17 patients (65%) with chronic hepatic dysfunction were positive for anti-HCV antibody. Only one of the staff members was anti-HCV-antibody positive. This high positive ratio of anti-HCV antibody in the leprosy patients is similar to the results of other research reported from the National Leprosarium Oku Komyo-En. We, therefore, conclude that the prevalence of anti-HCV antibody in leprosy patients is higher than that of the general population and that anti-HCV antibody is related closely to chronic hepatic dysfunction.

Some investigators have recently reported that there was an increased incidence of hepatocellular carcinoma in leprosy patients. And so, it is speculated that this is due to the high prevalence of the hepatitis-C virus. However, the reason for this high prevalence of anti-HCV antibody in the

sample is obscure.—Authors' English Abstract

Tomioka, H., Saito, H., Fujii, K., Sato, K. and Hidaka, T. In vitro antimicrobial activity of benzoxazinorifamycin, KRM-1648, against *Mycobacterium avium* complex, determined by the radiometric method. Antimicrob. Agents Chemother. **37** (1993) 67–70.

MICs of a newly developed benzoxazinorifamycin derivative, KRM-1648, for *Mycobacterium avium* complex (MAC) were determined by the BACTEC 460 TB system and compared with those of other known antimicrobial agents. The radiometric method gave a fast, accurate, and reproducible MIC for each antimicrobial agent. MICs of KRM-1648 for 30 strains of MAC (10 strains each of *M. avium* isolated from AIDS and non-AIDS patients and of *M. intracellulare* isolated from non-AIDS patients) were measured. The MICs, ranging from 0.004 to 0.0625 μg/ml, were the lowest of all tested drugs, including rifampin, rifabutin, streptomycin, kanamycin, isoniazid, ethambutol, ofloxacin, ciprofloxacin, sparfloxacin, and clarithromycin. The MICs were 2 to 512 and 1 to 32 times lower than those of rifampin and rifabutin, respectively. With rifampin and ethambutol, there were some differences between the MICs for *M. avium* isolated from AIDS patients (American) and those for *M. avium* from non-AIDS patients (Japanese). Moreover, appreciable differences between the MICs of some drugs against *M. avium* and *M. intracellulare* isolated from non-AIDS patients were found. Many strains of *M. avium* were more susceptible to ofloxacin than *M. intracellulare,* but, conversely, *M. avium* was more resistant to rifampin, streptomycin, ethambutol, and clarithromycin than *M. intracellulare.*—Authors' Abstract

Yoshie, Y. [Memories on the investigation of leprosy of the upper respiratory tract.] Jpn. J. Lepr. **61** (1992) 102–103. (in Japanese)

The author has been engaged in medical consultant work with leprosy patients in the field of otorhino-laryngology at the National Leprosarium Tama Zensho-En for 5 years since 1938, and he has had many opportunities to conduct autopsies which helped him in pursuing clinical and pathological research on the upper respiratory tract, particularly from an otorhinolaryngological viewpoint.

The author wanted to conclude his 5 years research in a book under the title "Leprosy of the Upper Respiratory Tract," but the book was not published unfortunately, because it was burned in air raids during 1945.

The postwar appearance of Promin and DDS discouraged the author from republishing his work. However, in 1981, the author began to think that the clinical pictures of leprosy lesions in the nose, pharynx and larynx which were painted at the time of chaulmoogra oil had a rare value in literature on the subject, and this had led to a decision to republish the book "Leprosy of the Upper Respiratory Tract—Atlas of Clinical Picture and Notes on the Research (1938–'43)."—Author's English Abstract

Zaheer, S. A., Misra, R. S., Sharma, A. K., Beena, K. R., Kar, H. K., Mukherjee, A., Mukherjee, R., Walia, R. and Talwar, G. P. Immunotherapy with *Mycobacterium w* vaccine decreases the incidence and severity of type 2 (ENL) reactions. Lepr. Rev. **64** (1993) 7–14.

Immunotherapy with *Mycobacterium w* vaccine was given to 45 patients with multibacillary (MB) leprosy; 41 similarly classified patients served as controls. All patients received standard multidrug therapy (MDT). Incidence, severity and frequency of type 2 (ENL) reactional episodes were monitored in both groups in a follow up extending up to 4 years. Reactions were seen in fewer vaccinated (10/37) BL and LL patients than in the control group (12/34). A total of 20 episodes were recorded in the vaccine group as against 29 in the controls, 75% of reactions were mild in vaccinated and 51.72% were mild in the control group patients, and 3 patients in the control group had more than 3 reactional episodes. None of the vaccinated patients showed this. No additional incidences of neuritis were seen among vaccinated individuals during reactional episodes.—Authors' Summary

Immuno-Pathology

Abe, M., Ozawa, T., Minagawa, F. and Yoshino, Y. Immunoepidemiological studies on subclinical infection in leprosy. II. Geographical distribution of seropositive responders with special reference to their possible source of infection. Jpn. J. Lepr. **59** (1990) 162–168.

A percentage of positive fluorescent leprosy antibody absorption (FLA-ABS) tests showed significant differences among the inhabitants of different regions in Okinawa. Provided that this percentage indicates the frequency of subclinical leprosy infections, the numbers of inhabitants with subclinical infection per new case with leprosy in the same region ranged from 723 to 3039. The FLA-ABS test was positive in 16.2% of adults in Minami daito Island where no new case with leprosy was found during the survey for 7 years. The differences in the percentages could not be explained by the different prevalence and incidence rates of leprosy in each region or by the differences in age and sex of the individuals examined. A significant correlation between the FLA-ABS tests and neural signs or symptoms was found in three regions. None of the adults in Minami daito Island showed such signs or symptoms. The distribution of FLA-ABS-positive and -negative responders in two hamlets where the incidence of leprosy was relatively high suggested the localization of positive responders surrounding houses in which a leprosy case had recently been found and also the distribution of positive responders in the remote houses. These facts seem to indicate that a possible source of infection to the majority of positive responders might be from the environment rather than from direct contact with leprosy patients.—Authors' Summary

Abe, M., Ozawa, T., Minagawa, F. and Yoshino, Y. Immunoepidemiological studies on subclinal infection in leprosy. III. Yearly observations and follow-up studies of school children by using FLA-ABS and lepromin tests. Jpn. J. Lepr. **60** (1991) 72–84.

The schoolchildren in three regions of Miyako Islands, Okinawa Prefecture, were surveyed annually, from 1978 to 1984, by using the fluorescent leprosy antibody absorption (FLA-ABS) test and the lepromin reaction with the Dharmendra's antigen for detecting the individuals at high risk of leprosy and for evaluating the predictive value of these immunological tests. Constant potency of these tests was confirmed by the percentage of positive reactions among the children in the first grade of elementary schools (5 or 6 years old) surveyed from 1980 to 1984. A temporal rise or drop of the percentages during this period seemed to associate with the yearly reported number of new leprosy cases in each region. Among 1168 schoolchildren tested with the FLA-ABS test once or twice or more, the percentage of positive reactions was significantly higher in children with enlargement of peripheral nerve without sensory loss than in those without these signs and symptoms. The longer the duration of these signs and symptoms, the higher the percentage of positive FLA-ABS tests. A lepromin test did not show any significant correlation with these signs and symptoms. A concordant persistence or change between the FLA-ABS tests and neural signs or symptoms was observed in 133 out of 331 children examined twice or more. Discordant changes in the remaining were mainly attributed to a conversion to seronegativity before the disappearance of neural signs and symptoms. Changes of FLA-ABS and lepromin reactivities between the initial and final tests suggested a spontaneous cure of subclinical infection with *Mycobacterium leprae* among the children who were FLA-ABS positive but lepromin negative at the initial test and therefore considered to be a group at high risk of leprosy. Although neural signs and symptoms were found in the majority of these children, none of them so far developed overt symptoms of the disease. Based on these findings, the predictive value of FLA-ABS test together with lepromin are discussed.—Authors' Summary

Brandt, F., Shi, Z. R., Zhou, H. M., Lu, B., Wu, H. and Rai, N. A histological study of the eye lesions in 12 leprosy patients

with tuberculoid lesion in 4 eyes. Lepr. Rev. **64** (1993) 44–52.

The histological reactions in 12 eyes of 12 leprosy patients were studied (5 BT, 1 BB, 1 BL and 5 LL). Granuloma lesions composed of epithelioid cells, Langerhans' giant cells, macrophages and lymphocytes were found in various intraocular tissues, e.g., cornea, sclera, iris, ciliary body or retina in 4 patients (1 BT and 3 LL). Of the 3 LL patients, according to the records, 2 were cured and in the other patient the outcome of the treatment was not mentioned. In view of the finding of the granulomatous lesions in the clinically cured patients and tuberculoid granuloma in the intraocular tissues in the LL patients, could there be some peculiarities in the intraocular sites? Or perhaps the tuberculoid reaction is just a manifestation of an upgrading reaction? More examinations on human leprosy eye specimens will be needed to answer these questions.—Authors' Summary

Chanteau, S., Cartel, J. L. and Roux, J. [Serology of leprosy: current status and perspectives.] Acta Leprol. (Genève) **8** (1992) 65–70. (in French)

The different serological tests used for leprosy are firstly the methods for the detection of antibodies (anti-PGL-I, 35kDa, 36kDa, LAM), and secondly, the tests to detect the PGL-I antigen from the serum or urine. The antibody detection tests have a good but insufficient specificity for the diagnosis of leprosy patients and their sensitivity is generally high for multibacillary (MB) patients but low for paucibacillary patients. Their positive predictive value for the diagnosis of patients in a population is very low: 2.1% for the anti-PGL-I ELISA when the prevalence is 1/1000. For the early diagnosis of patients and the follow up of high-risk populations, these tests are not cost effective: the number of patients detected in these populations is 10-fold lower than in the general population and the relative risks for developing the disease are not different among seropositive and among seronegative groups. In treated MB patients, the IgM anti-PGL-I level decreases in correlation with the decrease of the bacterial index. For the diagnosis of *Mycobacterium leprae* infection in a population, there was no correlation between the anti-PGL-I seroprevalence and the prevalence of the disease. Concerning the PGI-I antigen detection tests, they are specific and sensitive for the diagnosis of MB patients but they cannot be used routinely for technical reasons. In conclusion and to date, the usefulness of serological tests in a leprosy control program is quite questionable.—Authors' English Abstract

Deshpande, R. G., Khan, M. B. and Navalkar, R. G. Immunoreactivity of mammalian liver component with leprosy sera. Int. Arch. Allergy Immunol. **97** (1992) 345–349.

Sera from 77 leprosy patients in various stages of infection—tuberculoid (TT), lepromatous (LL), borderline tuberculoid and borderline lepromatous—15 contacts and 21 normal healthy individuals, were assayed in an indirect enzyme-linked immunosorbent assay and dot enzyme immunoassay using ethanol-soluble and thermostable extract of liver as the antigen. The highest incidences of reaction were found in untreated LL patients (100%) and in TT patients (91%), while the sera from borderline patients showed a comparatively lower incidence (43%). Some of the sera from contacts of leprosy patients (6/15) also showed high reactivity. Assays using lecithin as an antigen did not exhibit any reaction.—AS (Trop. Dis. Bull.)

Friedland, J. S., Shattock, R., Remick, D. G. and Griffin, G. E. Mycobacterial 65-kD heat shock protein induces release of proinflammatory cytokines from human monocytic cells. Clin. Exp. Immunol. **91** (1993) 58–62.

Monocytes having phagocytosed mycobacteria are known to present the bacterial 65-kDa heat-shock protein (hsp) on their cell surface to alpha/beta and gamma/delta T lymphocytes. Cytotoxic CD4+ cells may then lyse monocytes expressing mycobacterial 65-kDa hsp. However, it is not known whether 65-kDa hsp directly stimulates monocyte functions other than antigen presentation. This study has demonstrated that following extraction of bacterial lipopolysaccharide, purified recombinant mycobacterial 65-kDa hsp may directly activate

THP-1 cells, a human monocytic line, to accumulate mRNA for and secrete tumor necrosis factor (TNF), a cytokine important in granuloma formation, the characteristic host immune response to mycobacterial infection. TNF gene expression and secretion following stimulation by hsp was dose-dependent and abolished by heat-induced proteolysis. Subsequently, THP-1 cells secreted IL-6 and IL-8, cytokines involved in recruitment and differentiation of T lymphocytes. The data indicate that secretion of proinflammatory cytokines from monocytes activated by mycobacterial 65-kDa hsp may be important in the host immune response and in the development of antigen-specific T-cell-mediated immunity.—Authors' Abstract

Fujita, M., Miyachi, Y., Nakata, N. and Imamura, S. Appearance of $\gamma\delta$ T cell receptor-positive cells following $\alpha\beta$ T cell receptor-positive cells in the lepromin reaction of human skin. Immunol. Lett. **35** (1993) 39–44.

To elucidate the involvement of human $\gamma\delta$ T cell receptor (TcR)$^+$ cells in mycobacterial infection, we examined the kinetics of these cells in skin lesions of human lepromin reaction. The majority of CD3$^+$ cells 2 days after induction of the lepromin reaction were $\alpha\beta$ TcR$^+$, while $\gamma\delta$ TcR$^+$ cells accounted for only 4.4 $\pm$ 1.4% of the CD3$^+$ cells. On day 21, the incidence of $\gamma\delta$ TcR$^+$ cells was greater (16.0 $\pm$ 2.1%), although $\alpha\beta$ TcR$^+$ cells remained the predominant population. These kinetics of $\alpha\beta$ TcR$^+$ cells and $\gamma\delta$ TcR$^+$ cells contradict the "early response, self-surveillance" hypothesis for $\gamma\delta$ TcR$^+$ cells in mice. Most of the $\gamma\delta$ TcR$^+$ cells in this study of the lepromin reaction were Vδ1$^-$ Vδ2$^+$ Vγ9$^+$, and some of them proliferated in the skin lesions, suggesting that $\gamma\delta$ TcR$^+$ cells in the lesions may respond to mycobacterial antigens and may play an active part in the lepromin reaction. However, these $\gamma\delta$ TcR$^+$ cells were not correlated with granuloma formation, the size of necrotic areas, mycobacterial content, or the incidence of CD4$^+$ cells and CD8$^+$ cells.—Authors' Summary

Fujiwara, T., Izumi, S. and Wu, Q. Does the difference of the properties of trisaccharide-BSA conjugate (NT-P-BSA) of *Mycobacterium leprae* phenolic glycolipid influence on its seroreactivity? Jpn. J. Lepr. **60** (1991) 132–138.

The sugar content of the trisaccharide-BSA conjugate of the phenolic glycolipid-I *Mycobacterium leprae* (NT-P-BSA) increased with the increase of the molar ratio of the trisaccharide to BSA used in the coupling reaction. The difference of the sugar content in NT-P-BSA did not give the influence on the seroreactivity and specificity in ELISA for both IgM and IgG class antibodies. During the course of the coupling reaction, about half of the amount of BSA was converted to dimeric form. However, there were no differences of the activity and specificity between monomeric form and dimeric form of NT-P-BSA. Based on these results, it was concluded that any lot of NT-P-BSA with a variety of sugar content can be used in ELISA without any difference of the seroreactivity.—Authors' Summary

Fukutomi, Y., Inui, S. and Onozaki, K. [Monokine production by mouse peritoneal macrophages after phagocytosis of mycobacteria.] Jpn. J. Lepr. **61** (1992) 92–97. (in Japanese)

It is well known that monkines IL-1 (interleukin-1) and TNF (tumor necrosis factor) are produced by macrophages after stimulation with various agents. These cytokines are involved in various aspects of the inflammatory process and immunological response in addition to their original activities to proliferate T lymphocytes and causing tumor necrosis, respectively. Recently, it has been reported that IL-1 and TNF also play an important role in mycobacterial infections such as granuloma formation. In the present study, IL-1 and TNF productions were observed by mouse peritoneal exudate and resident macrophages after incubation with heat-killed *Mycobacterium lepraemurium* and *M. avium in vitro*. The production was enhanced by phagocytosis of these mycobacteria in a dose-dependent manner, and the time course of the production was maximum within 24 hr after phagocytosis of these mycobacteria. Morphological changes were also shown and enhanced glucose consumption in media by these macrophages. These results suggest that phagocytosis of mycobacteria by mac-

rophages leads to monokine production, which would not only cause well known immunological reactions but also makes characteristic phenomena to be observed in mycobacterial infections.—Authors' English Abstract

Garbe, T., Harris, D., Vordermeier, M., Lathigra, R., Ivanyi, J. and Young, D. Expression of the *Mycobacterium tuberculosis* 19-kilodalton antigen in *Mycobacterium smegmatis*—immunological analysis and evidence of glycosylation. Infect. Immun. **61** (1993) 260–267.

The gene encoding a 19-kDa antigen from *Mycobacterium tuberculosis* was expressed as a recombinant protein in the rapid-growing species *M. smegmatis*. The recombinant antigen was expressed at a level approximately ninefold higher than in *M. tuberculosis* and, like the native antigen, was found in the pellet fraction after high-speed centrifugation of bacterial extracts. The 19-kDa antigen in crude bacterial extracts, and the purified recombinant antigen, bound strongly to concanavalin A, indicating the possibility of post-translational glycosylation. The recombinant antigen stimulated T-cell proliferation *in vitro* when added to assays either in the form of whole recombinant bacteria or as a purified protein. Homologous expression of mycobacterial antigens in a rapid-growing mycobacterial host may be particularly useful for the immunological characterization of proteins which are subject to post-translational modification.—Authors' Abstract

Kaplan, G. Recent advances in cytokine therapy in leprosy. J. Infect. Dis. **167** Suppl. 1 (1993) S18–S22.

Lepromatous leprosy is characterized by a selective anergy to *Mycobacterium leprae* and its antigens. The inadequate immune response and the resulting reduced interferon-gamma (IFN-gamma) production lead to a lack of macrophage activation and unrestricted bacterial growth. Purified protein derivative of tuberculin induced a normal local immune response in many lepromatous leprosy patients. Interleukin-2 induced an accelerated equivalent of an antigen response in the skin. In both, monocytes and T cells were recruited, and changes in keratinocytes, including expression of major histocompatibility complex class II antigens, were induced. Skin macrophages appeared to be activated and bacteria were eliminated. Similar effects were generated by IFN-gamma, a more distal molecule in the immune response. Cytokine treatment induced large amounts of tumor necrosis factor-alpha, which is toxic in this context but can be selectively down-regulated by thalidomide without interfering with other monocyte cytokines necessary for normal immune function.—Author's Abstract

Kar, H. K., Sharma, A. K., Misra, R. S., Zaheer, S. A., Mukherhjee, A., Mukherjee, R., Beena, K. R., Kaur, H., Nair, S. K. and Talwar, G. P. Induction of lepromin positivity by a candidate antileprosy vaccine, *Mycobacterium w*, in lepromin negative healthy contacts of multibacillary leprosy patients. Indian J. Lepr. **64** (1992) 495–500.

In a hospital-based study, 362 household contacts of multibacillary leprosy patients were screened for evidence of leprosy and 54 (14.9%) were found to have leprosy. The remaining 308 apparently healthy contacts were lepromin tested and 109 (35.4%) were observed to be negative to Mitsuda lepromin. *Mycobacterium w* vaccine was administered intradermally to 95 of these 109 lepromin-negative contacts; 68 of them could be retested for lepromin A reactivity, 56 (82.35%) manifested lepromin conversion. The 12 subjects who did not show lepromin conversion, received a second dose of the vaccine, and 11 subsequently became lepromin positive. The overall lepromin conversion rate was thus 98.5% (67 out of 68). Follow up of these contacts up to a period of 30 months did not demonstrate reversion of lepromin positivity back to negativity status. No untoward effects of vaccination were observed except for local ulceration at the site of vaccine administration.—Authors' Abstract

Krishnamurthy, P., Rao, P. S., Subramanian, M., Suresh Kumar, S. K. and Neelan, P. N. *Mycobacterium leprae* soluble antigen (Rees) skin test responses in an endemic population in India. Indian J. Lepr. **65** (1993) 49–57.

The response to intradermal administration of Rees' soluble skin-test antigen was studied in 12,142 randomly selected individuals living in a highly endemic area in South India. Taking a cut-off point of 12-mm induration as the criterion for "positivity," 73% of PB cases, 45% of MB cases and 63% of noncase population (67% in contacts and 63% in noncontacts) were found to be positive. Age-specific positivity rates were higher in males than in females and in adults than in children. The difference in age-adjusted positivity rates between cases, contacts and noncontacts in the female population was found to be significant. However, the differences in reaction response are not sufficient to identify the subpopulations of cases, contacts and noncontacts and, as such, this antigen is not likely to be useful in epidemiological studies of infection and evolution of clinical disease in high-endemic populations.—Authors' Abstract

Kumar, V., Katoch, K., Katoch, V. M. and Bharadwaj, W. P. A preliminary study of correlation of immuno-histological and ultrastructural characteristics of neural granuloma in leprosy patients. Acta Leprol. (Genève) **8** (1992) 87–94.

With an aim to better understand the pathogenesis of nerve damage in leprosy, peripheral nerve biopsies from 6 untreated leprosy cases (3 BT/TT and 3 BL/LL) were studied by electron-microscopy and immunohistology. In addition to routine histopathology for diagnosis, infiltrating cells of granuloma were characterized after preparation of single cell suspension. The lymphocytes in the lesion were characterized by E and EAC rosetting and macrophage phagocytic system (MPS) cells were studied using histochemical markers such as esterase and peroxidase. The results indicate that the lymphocyte content was significantly greater in tuberculoid neural granuloma compared to lepromatous nerves, and these formed rosettes with sheep erythrocytes (E) and expressed HLA-DR antigen suggesting that they are activated T cells. Infiltrating macrophages in both the tuberculoid and lepromatous neural granuloma were esterase positive, peroxidase negative and did not form rosettes with sheep erythrocytes or EAC. Ultrathin sections of tuberculoid granuloma showed lymphocytes clearly associated to epithelioid macrophages having well-developed golgi apparatus and rough endoplasmic reticulum. Correlation of these immunological and ultrastructural characters suggests that hypersensitivity mechanisms are possibly responsible for nerve damage in tuberculoid leprosy. Ultrastructural examination of lepromatous nerves, on the other hand, showed the predominance of macrophages with large nucleus, heavily bacillated Schwann cells, and a few lymphocytes. The correlation of immunohistological and ultrastructural characters indicates that the mechanism(s) of nerve damage in lepromatous leprosy are basically different, wherein hypersensitivity appears to play a very limited role.—Authors' Summary

Lal, H., Jain, V. K., Mittal, R. A., Chaudhary, S. D. and Saini, V. Detection of antibodies to phenolic glycolipid by ELISA in leprosy patients. Indian J. Lepr. **65** (1993) 95–99.

Antibody (IgM) response to PGL-I, a surface glycolipid unique to *Mycobacterium leprae,* has been studied in 25 cases each of lepromatous and tuberculoid leprosy and in 25 healthy controls. The absorbance value at 488 nm was expressed as antibody titer. Serum antibody titer was found to be significantly higher in patients than in controls. Results confirm that antibody response in leprosy patients depends upon bacterial load.—Authors' Abstract

Launois, P., N'Diaye, M., Sarthou, J.-L., Millan, J. and Bach, M.-A. Anti-peripheral nerve antibodies in leprosy patients recognize an epitope shared by the *M. leprae* 65 kDa heat shock protein. J. Autoimmun. **5** (1992) 745–757.

Binding of leprosy sera to peripheral nerve from different species (mouse, guinea pig and rabbit) was evaluated by ELISA. A majority of sera, whatever the clinical form of leprosy, bind to these antigens. Absorption with *Mycobacterium bovis* BCG demonstrated that these antibodies recognize crossreactive epitopes between peripheral nerve and mycobacteria. In immunoblot

analysis, both leprosy patient sera and a monoclonal antibody directed at the 65-kDa heat-shock protein of *M. leprae* were shown to react with a heat-shock 67–68-kDa sciatic nerve protein. Binding of the monoclonal antibody to this sciatic nerve antigen was prevented by incubation and lepromatous patient sera, showing that some peripheral nerve epitopes recognized by patient antibodies are shared by the 65-kDa heat-shock protein of *M. leprae.*—Authors' Abstract

Mahajan, P., Jogaikar, D. G., Jadhav, V. H. and Mehta, J. M. Gelatin particle agglutination assay to detect anti-PGL-I antibodies in leprosy patients and in household contacts: a preliminary study. Indian J. Lepr. **64** (1992) 461–467.

A preliminary study of anti-phenolic glycolipid-I (PGL-I) IgM antibody detection using a *Mycobacterium leprae* gelatin particle agglutination (MLPA) test kit is described. Antibodies were demonstrated in 70% of our leprosy patients taking antileprosy treatment. The percentage of positivity of multibacillary cases was 86.0; whereas that of paucibacillary cases was 30.0. Good correlation was found between the bacterial index and the presence of antibodies. Antibodies were detected in 28% of our patients released from treatment. Fourteen out of 27 household contacts were found to have antibodies but none of the normal controls were seropositive. These preliminary data demonstrate that the MLPA test is not applicable as a serodiagnostic test or as a test of cure, but may be useful for epidemiological studies and as a research tool.—Authors' Abstract

Mutis, T., Debueger, M., Bakker, A. and Ottenhoff, T. H. M. HLA class-II+ human keratinocytes present *Mycobacterium leprae* antigens to CD4+ Th1-like cells. Scand. J. Immunol. **37** (1993) 43–51.

In a variety of inflammatory skin diseases like leprosy, keratinocytes (KC) are induced to express MHC class-II molecules and may, therefore, serve as antigen-presenting cells (APC) for MHC class-II restricted T cells infiltrating the lesions. However, KC have been thought to be improper APC for MHC class-II restricted T cells and to drive T cells into an anergic rather than into an activation state. We evaluated this issue in relation to leprosy and tested whether HLA-DR+ KC could present *Mycobacterium leprae* antigens to well-defined, CD4+, cytotoxic as well as proliferative, Th1-like cell clones. Using a recently developed sensitive assay system which employs intact layers of basal KC as APC, we found that most T-cell clones (6/8) lysed HLA-DR+ KC pulsed with *M. leprae* antigens. KC were only recognized after the induction of HLA-DR expression by IFN-gamma, in an antigen-specific and HLA class-II restricted manner. All T-cell clones tested also showed significant proliferation and IFN-gamma production in response to *M. leprae* antigens presented by HLA-DR+ KC, arguing against a KC-dependent anergizing effect on T cells. Thus, HLA class-II+ KC can function as proper APC for HLA class-II restricted CD4+ TH1-like cells. It seems, therefore, possible that antigen presentation by KC contributes to the local cell-mediated immune responses in DTH lesions.—Authors' Abstract

Natarajan, M., Katoch, K., Bagga, A. K. and Katoch, V. M. Histological changes with combined chemotherapy and immunotherapy in highly bacillated lepromatous leprosy. Acta Leprol. (Genève) **8** (1992) 79–86.

Highly bacillated, untreated lepromatous cases with an initial bacterial index (BI) of 4+ to 6+ were treated with combined multidrug treatment (MDT) and immunotherapy with heat-killed *Mycobacterium w* or BCG. The vaccines were administered intradermally every 6 months. It was observed that a majority of cases on immunotherapy showed increased lymphocytic infiltration (both at local and distant sites) and some cases showed epithelioid cells as well. The lymphocytic infiltration was (slightly) more vigorous in those vaccinated with *Mycobacterium w.* Such changes were not seen in the patients on MDT alone. Also, the granuloma fraction reduced much faster in cases who were on additional immunotherapy as compared to those on MDT

alone. These changes along with evidence of clinical and bacteriological improvements suggest that immunotherapy may have an important supportive role, especially in the therapy of anergic lepromatous cases.—Authors' Summary

Peake, P. W., Britton, W. J., Davenport, M. P., Roche, P. W. and McKenzie, K. R. Analysis of B-cell epitopes in the variable C-terminal region of the *Mycobacterium leprae* 70-kilodalton heat shock protein. Infect. Immun. **61** (1993) 135–141.

The C-terminal region of the *Mycobacterium leprae* 70-kDa heat-shock protein is the major target for the humoral immune response to this protein and contains *M. leprae*-specific sequences. To examine the B-cell responses to this region more closely, we constructed and expressed a recombinant fragment of the *M. leprae* P70 gene that encodes the C-terminal 142 residues (C-142) and synthesized a series of 10 overlapping peptides to encompass this region. The affinities of three monoclonal antibodies (MAbs) reactive with this region of P70 were measured, and the binding site of the highest-affinity MAb was determined to lie between residues 498 and 515. This reactivity was confirmed by a fluid-phase inhibition enzyme-linked immunosorbent assay. By contrast, sera from leprosy patients which were strongly reactive with the C-142 fragment failed to bind directly to the conjugated or unconjugated peptides. To determine whether the *M. leprae*-specific C-terminal 70 residues could stimulate B-cell responses, the reactivity of hyperimmune anti-*M. leprae* P70 antisera with the peptides was examined. Rabbit polyclonal anti-*M. leprae* P70 antisera recognized epitopes between residues 498 and 515 and in the *M. leprae*-specific region between residues 567 and 591. The latter, in turn, when coupled to ovalbumin, was able to generate a strong anti-P70 response specific for mycobacterial, but not human, HSP70. Three strains of mice immunized with either C-142 or P70 recognized epitopes in the region between residues 487 and 532, but the response varied with the strain and immunogen. These data demonstrate that two regions in the C-terminal portion of *M. leprae* P70 contain linear B-cell epitopes recognized by MAbs or hyperimmune serum. Sera from leprosy patients, however, react predominantly with conformational determinants in the immunodominant C-terminal part of the protein.—Authors' Abstract

Reddy, B. S. N., Badrinath, S., Shantaraman, R., Harish, B. N., Sheriff, M. O., Rao, R. S. and Garg, B. R. Utility of gelatin particle agglutination test (MLPA) for rapid serodiagnosis of leprosy in a hyperendemic area. Indian J. Lepr. **64** (1992) 469–473.

The anti-PGL *Mycobacterium leprae*-specific antibodies were estimated by the MLPA test in 79 patients of leprosy, 8 contacts of lepromatous cases, and 10 healthy controls in a hyperendemic area. The results indicated an over-all seropositivity of 50.6% in leprosy patients. Three of the eight contacts and five of the controls also gave positive results. Higher seropositivity rates were noted in multibacillary patients (73% in lepromatous, 53.6% in borderline, 40% each in tuberculoid and indeterminate, and 10% in pure neuritic types). The practical application of the MLPA test in its present form as a serodiagnostic procedure for screening subclinical or clinical infections in leprosy patients appears to be of limited value in hyperendemic areas. Further studies involving large series of subjects are necessary for reaching definite conclusions.—Authors' Abstract

Said, G. and Hontebeyrie-Joskowicz, M. Nerve lesions induced by macrophage activation. Res. Immunol. **143** (1992) 589–599.

The neuropathies associated with infectious processes, including leprosy, retroviral infections, and Chagas' disease, represent the largest group of neuropathies in the world. Segmental demyelination and axonal degeneration of nerve fibers are associated with inflammatory infiltrates which contain a large number of mononuclear phagocytes. In order to learn more about the role played by macrophage activation in the nerve lesions observed in inflammatory neuropathies, [the authors] have performed a morphological study of [rodent] nerves injected with products of activation of macrophages

including proteolytic enzymes and cytokines (tumor necrosis factor and $\alpha\beta$-interferon). [The authors] have also studied the effects on nerve fibers of macrophages activated by ingestion of proteose-peptone, a foreign protein, and in the course of a delayed-type hypersensitivity (DTH) reaction. [The authors] have found that proteases and urokinase were potent demyelinating agents and that activated macrophages were also able to induce significant demyelination of neighboring fibers. In contrast, injection of TNFα induced more severe nerve lesions consisting of axonal degeneration of the majority of nerve fibers. [The authors] thus conclude that infected macrophages which penetrate the endoneurium and macrophages activated in a DTH reaction can both cause neuropathy.—AS (Trop. Dis. Bull.)

Sampaio, E. P., Moreira, A. L., Kaplan, G., *et al.* *Mycobacterium leprae*-induced interferon-γ production by household contacts of leprosy patients: association with the development of active disease. J. Infect. Dis. **164** (1991) 990–993.

"Identification of individuals at risk for developing leprosy and their early diagnosis are central to effective disease control. Lack of immunologic response to *Mycobacterium leprae* among persons exposed to the infectious agent may be predictive of susceptibility. *M. leprae*-induced interferon-γ (IFNγ) production by peripheral blood mononuclear cells was used as a measure of immune responsiveness. Household contacts of multibacillary patients likely to be at risk of developing active disease were identified [in Brazil], and a preliminary analysis after 2 years of follow up is presented. A persistent *in vitro* negative response to *M. leprae* was present in 34.6% of the contacts, and a decrease in IFN-γ production was noted in 52.5%. Five contacts (6.41%) developed leprosy during follow up and, as predicted, belonged to the group of individuals who were negative or showed reduced levels of IFN-γ in response to the antigen."

Of the 5 contacts who developed leprosy, 4 were classified as having indeterminate and 1 BT leprosy. They all had a negative bacterial index.—H. Dockrell (Trop. Dis. Bull.)

Saroyants, L. V., Polyanskaya, I. S., Alexeyev, L. P., Baranov, Y. N. and Yuschenko, A. A. [HLA antigens in lepromatous leprosy.] Vestn. Dermatol. Venerol. **4** (1992) 18–20. (in Russian)

HLA antigen distribution was studied in 120 patients with lepromatous leprosy; 122 Russian donors have made up the reference group. Eight HLA-1, 15 HLA-B, and 6 HLA-DR loci antigens were analyzed. A significantly higher incidence of HLA-B7 (relative risk RR = 2.51, $p_c = 0.049$) and HLA-DR3 (RR = 10.22, $p_c = 0.00014$) antigens was detected in leprosy patients.—AS (Trop. Dis. Bull.)

Sekar, B., Sharma, R. N., Leelabai, G., Anandan, D., Vasanthi, B., Yusuff, G., Subramanian, M. and Jayasheela, M. Serological response of leprosy patients to *Mycobacterium leprae* specific and mycobacteria specific antigens: possibility of using these assays in combinations. Lepr. Rev. **64** (1993) 15–24.

The serological response of 147 leprosy patients to three mycobacterial antigens, PGL-I, 35 kDa (*Mycobacterium leprae*-specific) and LAM (which is a common mycobacterial antigen), were analyzed. A stronger serological response was seen among the MB patients than the PB patients in all the assays. The three antibody levels correlated positively with each other in both MB and PB cases. An overlap of seropositivity was seen between anti-PGL-I and anti-LAM ($p > 0.05$). A progressive increase in seropositivity and a significant difference of absorbance or titer in antibody levels in all three assays over increasing grades of BI were seen in the MB patients ($p < 0.05$). A significant difference in seropositivity between untreated and treated groups of patients was observed for anti-PGL-I ($p < 0.05$) and anti-LAM ($p < 0.01$) antibodies. The sensitivity, specificity and efficiency of anti-PGL-I (50%; 99%; 70%), anti-LAM (43%; 95%; 64%) and anti-35 kDa (66%; 100%; 80%) assays taken individually were less than that of combinations of anti-PGL-I/anti-35 kDa (74%; 99%; 84%) or anti-PGL-I/anti-35 kDa/anti-LAM (80%; 94%; 86%). The difference in the efficiency of both sets of combination of assays were not statisti-

cally significant ($p > 0.05$).—Authors' Summary

Shi, Z., *et al.* [Histopathological study of six eye specimens of leprosy patients.] China Lepr. J. **8** (1992) 140–143. (in Chinese)

Histological changes in 6 eyes of 6 leprosy (1 BT and 5 LL) patients were studied. Tuberculoid granuloma composed of epithelioid cells, Langhans' giant cells, macrophages and lymphocytes were found in various intraocular tissues, e.g., cornea, sclera, iris, ciliary body or retina in 1 BT and 3 LL patients. According to the records, of the 3 LL patients 2 were cured, but the outcome of treatment was not mentioned in the third one. In view of the finding of granulomatous lesions in clinically cured patients and tuberculoid granuloma presenting in the intraocular tissues of LL patients, one could suspect that there might be some peculiarities in the intraocular sites or the tuberculoid reaction as a manifestation of upgrading reaction. It seems to need more eye specimens of leprosy patients to examine the reaction in them.—Authors' English Abstract

Silva, C. L., Lukacs, K. and Lowrie, D. B. Major histocompatibility complex non-restricted presentation to CD4+ lymphocytes-T of *Mycobacterium leprae* heat-shock protein-65 antigen by macrophages transfected with the mycobacterial gene. Immunology **78** (1993) 35–42.

When the immunodominant 65,000 MW heat-shock protein of *Mycobacterium leprae* (ML65hsp) was expressed from the transfected mycobacterial gene in the mouse macrophage cell line J774.G8, the antigen was recognized by specifically sensitized CD4+ splenocytes in association with major histocompatibility complex (MHC) class II and CD4. Inhibition by monensin, leupeptin and chloroquine but not brefeldin A indicated dependence of presentation upon endosomal antigen processing. Although direct access of the endogenously synthesized antigen to the endosomal pathway of presentation, without extracellular release followed by endocytosis, could not be discounted, antigen was present in supernatants of the transfected cells in a form that could be presented by fixed macrophages and a form that required further processing for presentation. Each of three monoclonal antibodies specific for widely separated linear amino-acid epitopes of the antigen strongly inhibited recognition, suggesting steric interference with antigen-presenting cell (APC)-T cell interaction. Tests with splenocytes from vaccinated congenic mice indicated that recognition was not restricted by MHC haplotype. The significance and mechanism of this apparent MHC context-independent interaction of the presented antigen with specific T-cell receptor (TcR) remain to be explored.—Authors' Abstract

Takahashi, D., Andrade, H. F., Jr., Wakamatsu, A., Manini, M. and De Brito, T. Treated indeterminate leprosy: a search for predictive histopathological and immunohistochemical parameters in skin biopsies taken from patients at admission and at clinical discharge. Acta Leprol. (Genève) **8** (1992) 95–102.

In a previous study an index ($\Sigma 3$) resulting from the summation of three parameters, i.e., presence of bacilli, even in small numbers, in various dermal structures, multiple positive antigen sites as detected by anti-BCG antiserum and dermal nerve involvement, identified 72.22% of cases of indeterminate leprosy which progressed to multibacillary leprosy.

The present study was undertaken to investigate possible parameters which might be indicative of indeterminate leprosy which would persist unchanged or be cured (treated cured patients). Thirty, treated, cured indeterminate leprosy patients were selected from the files of the São Paulo Health Institute [Brazil], and studied by histopathological, immunohistochemical and statistical methods similar to those employed in the previous study. The $\Sigma 3$ index was 4.10 ± 0.60, a finding that places this group of patients in a position close to that of patients changing to paucibacillary leprosy but statistically different from that of patients progressing to multibacillary leprosy. Moreover, it was found that patients belonging to this group have heterogeneous single parameters, some of them suggestive of multibacillary and others of paucibacillary leprosy.

Immunologically based techniques mainly employing rabbit anti-BCG serum as the primary antibody have proved to be valuable to detect antigen sites in biopsies from indeterminate leprosy patients and should be used together with the bacterial index during the follow up and clinical discharge control of such patients. In the present study, we show that clinical discharge of these patients did not mean a complete clearance of bacillary antigens.—Authors' Summary

Uyemura, K., Ho, C. T., Ohmen, J. D., Rea, T. H. and Modlin, R. L. Selective expansion of Vdelta1+T-cells from leprosy skin lesions. J. Invest. Dermatol. **99** (1992) 848–852.

T cells bearing gamma/delta T-cell receptors (TCRs) are prominent residents of murine epidermis and appear to be important participants in the immune response to infection in human skin. The Mitsuda reaction in leprosy, induced by intradermal challenge with *Mycobacterium leprae,* provides an opportunity to study the cellular events that mediate a form of delayed-type hypersensitivity (DTH) in skin. T cells bearing gamma/delta TCRs comprise a significant proportion of the T-cell population in these DTH reactions. Presently we have generated T-cell lines from Mitsuda reactions *in vitro* and compared their TCR repertoire to that found *in situ.* Gamma/delta T cells comprised 20%–40% of lines derived from these skin lesions, but < 10% of lines derived from the peripheral blood of the same individuals. Flow-cytometric analysis of variable (V) chain usage in T-cell lines derived from skin lesions indicated that Vdelta1 was predominant. Evaluation of the TCR repertoire using PCR indicated that Vdelta1-Jdelta1 and Vgamma2-JgammaP gene rearrangements were prevalent. In comparison, Vdelta2-Jdelta1 gene rearrangements predominated *in situ.* Furthermore, nucleotide sequence analysis of the V-J junction of one T-cell line revealed limited genetic diversity of the gamma/delta TCR. These findings suggest that the Vdelta1 subpopulation of gamma/delta T cells in Mitsuda skin reactions selectively outgrows from leprosy skin lesions *in vitro.* Such Vdelta1 + T-cell lines should be useful for determining the relevant antigens and restriction elements in this response to a pathogen in skin.—Authors' Abstract

Wallis, R. S., Paranjape, R. and Phillips, M. Identification by 2-dimensional gel electrophoresis of a 58-kilodalton tumor necrosis factor-inducing protein of *Mycobacterium tuberculosis.* Infect. Immun. **61** (1993) 627–632.

We have previously identified proteins in fractions of a culture filtrate of *Mycobacterium tuberculosis* with the capacity to induce cytokine production in monocytes, by using a technique we have defined as "monocyte Western blotting" (immunoblotting). In this series of experiments, we have extended this technique to two-dimensional gel electrophoresis and have identified a novel 58-kDa protein of *M. tuberculosis* which induces production of tumor necrosis factor by human monocytes. Nitrocellulose particles bearing this protein were used to develop murine monoclonal antibodies by the technique of intrasplenic immunization. The protein was purified by preparative isoelectric focusing and gel electrophoresis and subjected to N-terminal amino-acid sequence analysis. Since tumor necrosis factor is a mediator of both pulmonary necrosis and macrophage activation for intracellular killing, this 58-kDa protein may play an important role both in the immunopathogenesis of tuberculosis and in mycobacterial immunity.—Authors' Abstract

Wang, T., Butt, K. I., Maeda, Y., Kawatsu, K. and Izumi, S. Histo-bacteriological investigation of borderline tuberculoid leprosy. Jpn. J. Lepr. **60** (1991) 152–157.

The multi-sections, which were stained by Fite method, of skin biopsies taken from 12 active BT cases were examined under the guidance of special stains for demonstration of nerve components. All cases were AFB positive. Bacilli were found in infiltrated nerves in 11 cases, of which, in 7 cases, bacilli were detected in nerve fragments within epithelioid cell granuloma, and bacilli were seen in arrector pili muscles in 2 cases. No bacilli were detected in other sites. Since the survival of *Mycobacterium leprae* in nerves is one of the reasons causing relapse, this paper suggests that it would be

better to treat active BT cases with the multibacillary regimen recommended by WHO even though smear-negative.—Authors' Summary

Wang, T., Butt, K. I., Maeda, Y., Kawatsu, K. and Izumi, S. The use of improved silver impregnating staining method in the differential diagnosis of tuberculoid leprosy. Jpn. J. Lepr. **60** (1991) 146–151.

A comparative study on the usefulness of Kawatsu's silver impregnating staining method compared with S-100 protein ABC technique for the differential diagnosis of tuberculoid leprosy was carried out. The results of neurohistological examination obtained from both methods were almost the same. It can be said that Kawatsu's method is useful and cost-effective, and thus suitable for practical use in developing countries.—Authors' Summary

Wu, Q., *et al.* [Study on monoclonal antibody in leprosy. (II) Preliminary report on production of monoclonal antibody against supersonic products of *M. leprae.*] China Lepr. J. **8** (1992) 136–140. (in Chinese)

By fusion of the SP2/0 myeloma cells with the splenocytes of a BALB/c mouse immunized with MLSS as antigen, three strains of the fusion cells, which produce monoclonal antibody against MLSS protein, have been attained. A representative stain II $E_{10}H_8E_7$ can steadily secrete monoclonal antibody even through 59 generations of cultivation. The examination of subclasses of the monoclonal antibody showed that there are also monoclonal antibodies against whole leprosy bacilli and ND-O-BSA besides the antibody to MLSS protein.—Authors' English Abstract

Wu, Q., *et al.* [Study on monoclonal antibody in leprosy. (III) Production of monoclonal antibody to entire *M. leprae.*] China Lepr. J. **8** (1992) 204–206. (in Chinese)

After immunizing mice with *Mycobacterium leprae* and PGL-I as antigen, the splenocytes obtained from the mice have been added to SP2/0 mice myeloma cells in the proportion of 3 to 1 and then fused. In the test, positive results have been gained from 48 wells. Eight strains of the cells have been frozen after screening using both antigens, *M. leprae* and *M. tuberculosis,* and cloned of which 2 strains—(5) 24 A_3 and (5) 24D_b A_8—have been cultured in 53 serial generations over 4 months. It showed that the cells can produce sensitive and specific monoclonal antibody against entire leprosy bacilli, and its class is IgG. The prospects in the use of the antibody are discussed.—Authors' English Abstract

Wu, Q., *et al.* [Study on monoclonal antibody in leprosy. (IV) Basic conditions for the production of monoclonal antibody.] China Lepr. J. **8** (1992) 207–210. (in Chinese)

For preparation of monoclonal antibody to *Mycobacterium leprae* and its antigen component, schemes of immunizing mice, selections of feeding cells and relevant reagents and the factors influencing production of ascites have been systematically studied. The authors think that in the process of producing monoclonal antibody, for immunizing mice to add mycobacterial wall adjuvant and to make the determinant of immunogens expose itself are very important. For fusing and cloning, feeding cells may not be added and differences among some existing 1640 are not significant, but the quality of calf serum is extremely important. The nature of inducers and sex and source of animal have significant influence on the process of producing ascites, and the influences on it of other factors have to be further studied.—Authors' English Abstract

Yajima, M., Murata, J., Yamada, N. and Asano, G. Ultrastructural observations of small blood vessels in leprosy patients. Jpn. J. Lepr. **60** (1991) 121–127.

In lepromatous leprosy, blood vessels revealed the luminal protrusions of endothelial cells containing *Mycobacterium lepae* and thickening of the basement membrane of endothelial and smooth cells. Endothelial projections with increased pinocytotic vesicles were more often encountered in lepromatous leprosy than in the other types of leprosy. On the other hand, in tuberculoid leprosy, the extensive rough endoplasmic

reticulum suggesting protein synthesis was observed in the endothelial cells compared with the other types of leprosy and nonspecific lesions. It seems that the blood vessels associated with hyperfunction and proliferation in the endothelium could aggravate the degenerative changes in peripheral nerve fibers including the Schwann cells.—Authors' Summary

Zaheer, S. A., Mukherjee, R., Ramkumar, B., Misra, R. S., Sharma, A. K., Kar, H. K., Kaur, H., Nair, S., Mukherjee, A. and Talwar, G. P. Combined multidrug and *Mycobacterium w* vaccine therapy in patients with multibacillary leprosy. J. Infect. Dis. **167** (1993) 401–410.

Immunotherapy with *Mycobacterium w* vaccine was attempted in patients with borderline-borderline, borderline lepromatous (BL), or lepromatous leprosy (LL) to determine whether immunization can hasten recovery and reduce treatment time by invigorating cell-mediated immunity. *Mycobacterium w*, a nonpathogenic, rapidly growing, atypical mycobacterium, shares a number of common B- and T-cell determinants with *M. leprae* and *M. tuberculosis*. Patients receiving the vaccine had rapid clinical improvement and accelerated bacteriologic clearance. After treatment with vaccine for 2 years, 13 of 31 BL and LL patients were bacteriologically negative as were 5 of 25 controls. Vaccinated patients had one of two distinct histologic features, either an upgrading in the disease spectrum or complete clearance of granuloma. Some 80% of lepromin conversions were in BL and LL patients who received the vaccine versus none and 14.3% of BL and LL controls, respectively. Thirteen of 17 vaccinated LL patients were released from treatment after 2 years in contrast to 2 of 15 controls.—Authors' Abstract

Microbiology

Banerjee, D. K. and Patel, B. R. Evaluation of the activity of a number of antimicrobial agents against mycobacteria within mouse macrophages by a radiometric method. J. Antimicrob. Chemother. **31** (1993) 289–302.

[^{3}H]-uridine was incorporated by *Mycobacterium bovis* BCG with increasing intensity as the incubation period was increased. Rifampin and isoniazid inhibited incorporation of the label rapidly. Similar inhibition was seen with *M. tuberculosis* H37Rv and several clinical isolates of *M. tuberculosis* both in axenic medium and inside macrophages. Ofloxacin and ciprofloxacin were both inhibitory but clofazimine was not. The combination of rifampin with either isoniazid or ethambutol produced enhanced killing, but the combination of ethambutol and isoniazid was not synergic. *M. avium-intracellulare* isolates from AIDS patients were less susceptible to rifampin and were unaffected by isoniazid, ethambutol, clofazimine, ofloxacin and ciprofloxacin. The results obtained by inhibition of [^{3}H]-uridine incorporation by intracellular mycobacteria correlated with conventional *in vitro* MICs and was reproducible and rapid; a definitive result was obtainable within 7 days.—Authors' Abstract

Chakrabarty, A. N. and Dastidar, S. G. Leprosy-derived chemoautotrophic nocardioform (CAN) bacteria closely resemble, or are identical with, *Mycobacterium leprae* on mycolate and other lipid profiles. Indian J. Lepr. **64** (1992) 529–535.

On the basis of thin-layer chromatography and gas chromatography-mass spectrometric studies, the lipid profiles of all the chemoautotrophic nocardioform (CAN) bacteria derived from human and animal leprosy tissues appear to be identical with each other, and closest to or identical with the most probable profile of *Mycobacterium leprae*.—Authors' Abstract

Delforge, D., Depiereux, E., De Bolle, X., Feytmans, E. and Remacle, J. Similarities between alanine dehydrogenase and the N-terminal part of pyridine nucleotide transhydrogenase and their possible im-

plication in the virulence mechanism of *Mycobacterium tuberculosis*. Biochem. Biophys. Res. Comm. **190** (1993) 1073–1079.

Recent developments in simultaneous multiple alignment methods of protein sequences allow prediction of structural similarity in related proteins. Alanine dehydrogenase and the N-terminal sequence of pyridine nucleotide transhydrogenase were compared for their sequences. High similarities of sequences were observed especially in their NAD(H)-binding sites. These similarities suggest that antibodies which recognized the alanine dehydrogenase of *Mycobacterium tuberculosis* can also be directed against the membrane-bound pyridine nucleotide transhydrogenase. If this is the case, the virulent property of this pathogen could be linked to its higher synthesis of NADPH necessary for its anabolism.—Authors' Summary

Donelly-Wu, M. K., Jacobs, W. R. and Hatfull, G. F. Superinfection immunity of mycobacteriophage-L5—applications for genetic transformation of mycobacteria. Mol. Microbiol. 7 (1993) 407–417.

Mycobacteriophage L5 is a temperate phage of the mycobacteria that forms stable lysogens in *Mycobacterium smegmatis*. We show here that the 183-amino-acid product of L5 gene 71 confers immunity to L5 superinfection, is required for maintenance of the lysogenic state and contains a helix-turn-helix DNA-binding motif—properties associated with repressors of temperate phages. We have utilized these observations to demonstrate the use of L5 gene 71 as a selectable marker for genetic transformation of the mycobacteria. Significantly, the use of L5 gene 71 as a selectable gene avoids the requirement for antibiotic-resistance genes providing an important tool for the manipulation of the pathogens *M. tuberculosis* and *M. avium*, and for the construction of recombinant BCG vaccines.—Authors' Abstract

Eiglmeier, K., Honoré, N., Woods, S. A., Caudron, B. and Cole, S. T. Use of an ordered cosmid library to deduce the genomic organization of *Mycobacterium leprae*. Mol. Microbiol. 7 (1993) 197–206.

In an attempt to unify the genetic and biological research on *Mycobacterium leprae*, the etiological agent of leprosy, a cosmid library was constructed and then ordered by a combination of fingerprinting and hybridization techniques. The genome of *M. leprae* is represented by four contigs of overlapping clones which, together, account for nearly 2.8 Mb of DNA. Several arguments suggest that the gaps between the contigs are small in size, and that virtually complete coverage of the chromosome has been obtained. All of the cloned *M. leprae* genes have been positioned on the contig maps together with the 29 copies of the dispersed repetitive element, RLEP. These have been classified into four groups on the basis of differences in their organization. Several key housekeeping genes were identified and mapped by hybridization with heterologous probes, and the current genome map of this uncultivable pathogen comprises 72 loci.—Authors' Summary

Hatfull, G. F. and Sarkis, G. J. DNA sequence, structure and gene expression of mycobacteriophage-L5—a phage system for mycobacterial genetics. Mol. Microbiol. 7 (1993) 395–405.

Genetic studies of *Mycobacterium tuberculosis* and other mycobacterial pathogens have suffered from the lack of a sophisticated genetic system. To address this issue we have developed a viral system through a detailed characterization of mycobacteriophage L5, a temperate phage that infects both fast- and slow-growing mycobacteria. We describe here the complete DNA sequence of the L5 genome and initial characterization of L5 virion structure and gene expression. In addition to providing a genetic "tool-box" for the mycobacteria we find that L5 offers a new paradigm for dsDNA phages, being phenotypically temperate but employing genetic strategies for phage growth usually associated with lytic bacteriophages.—Authors' Abstract

Honore, N., Bergh, S., Chanteau, S., Doucet-Populaire, F., Eiglmeier, K., Garnier, T., Georges, C., Launois, P., Limpaiboon, T., Newton, S., Niang, K., del Portillo, P., Ramesh, G. R., Reddi, P., Ridel, P. R., Sittisombut, N., Wu-Hunter, S. and Cole,

S. T. Nucletoide sequence of the first cosmid from the *Mycobacterium leprae* genome project: structure and function of the Rif-Str regions. Mol. Microbiol. **7** (1993) 207–214.

The nucleotide sequence of cosmid B1790, carrying the Rif-Str regions of the *Mycobacterium leprae* chromosome, has been determined. Twelve open reading frames were identified in the 36716 bp sequence, representing 40% of the coding capacity. Five ribosomal proteins, two elongation factors and the β and β' subunits of RNA polymerase have been characterized and two novel genes were found. One of these encodes a member of the so-called ABC family of ATP-binding proteins, while the other appears to encode an enzyme involved in repairing genomic lesions caused by free radicals. This finding may well be significant since *M. leprae*, an intracellular pathogen, lives within macrophages.—Authors' Summary

Honore, N. and Cole, S. T. Molecular basis of rifampin resistance in *Mycobacterium leprae*. Antimicrob. Agents Chemother. **37** (1993) 414–418.

Rifampin is currently the most potent drug used in leprosy control programs. We show that the rifampin resistance which emerged in 9 patients with lepromatous leprosy, who had received rifampin monotherapy, stemmed from mutations in the *rpoB* gene, which encodes the beta subunit of RNA polymerase of *Mycobacterium leprae*. In 8 cases missense mutations were found to affect a serine residue, Ser-425, while in the remaining mutant a small insertion was found close to this site. These findings will be of use for the development of a rapid screening procedure, involving the polymerase chain reaction, for monitoring the emergence of rifampin-resistant *M. leprae* strains.—Authors' Abstract

Joshi, S., Nair, S. C. and Mahadevan, P. R. Antigenic similarity of a cultivable acid-fast bacterium to *Mycobacterium leprae*. Indian J. Med. Res. [A] **97** (1993) 18–24.

A cultivable acid-fast stainable bacterium obtained from a leprosy nodule showed similarity to *Mycobacterium leprae* in antigenicity to serum antibodies of lepromatous leprosy patients. The antigenic similarity has been seen more clearly in the delipidified cell components of both these bacteria. An antigen of 35–38 kDa has been seen as a common antigen between *M. leprae* and the cultivable bacilli with binding ability to sera from leprosy patients. This cultivable bacterial component could be used for serodiagnosis of lepromatous leprosy.—Authors' Abstract

Sharma, V. K., Kaur, S., Kaur, I., *et al.* Adenosine triphosphate content of *Mycobacterium leprae* by Percoll buoyant density centrifugation. Indian J. Exp. Biol. **30** (1992) 451–453.

This paper is confirmatory in nature: the authors show that the adenosine triphosphate (ATP) content of *Mycobacterium leprae* harvested from 39 untreated baciliferous patients is between 218 and 337 pg ATP/10^6 bacilli. Their results fit in with values obtained previously by others—all but one group found between 100 and 500 pg ATP/10^6 *M. leprae* organisms. The authors show that the ATP content is very slightly (10%) higher in bacilli harvested by density gradient centrifugation than by an enzyme treatment method; this difference is statistically significant. Correlation of ATP content with "morphological index" is not clear. ATP determinations represent a possible rapid method for determination of viability of *M. leprae*.—P. Wheeler (Trop. Dis. Bull.)

van der Vliet, G. M. E., Hermans, C. J. and Klatser, P. R. Simple colorimetric microtiter plate hybridization assay for detection of amplified *Mycobacterium leprae* DNA. J. Clin. Microbiol. **31** (1993) 554–670.

The detection of amplified products resulting from polymerase chain reactions (PCRs) remains a complicated process. To simplify the detection procedures, we developed a colorimetric microtiter plate hybridization assay for the specific detection of 5'-biotinylated PCR fragments of *Mycobacterium leprae* DNA. For this assay, an *M. leprae* DNA capture probe was made and immobilized on the wells of a microtiter plate. Hybridization of the biotin-labeled PCR fragments was detected through en-

zymatic color development. The resulting optical densities showed a logarithm-linear relationship with the amount of template DNA and corresponded to the intensity of the bands obtained through gel analysis and Southern blotting of the PCR products. The sensitivity of the assay was found to be 125 fg of genomic *M. leprae* DNA, or 20 lysed bacilli, revealing a dection limit similar to that of agarose gel analysis. The efficient coamplification of human DNA was used as a positive control for the presence of inhibitory substances in clinical material. For detection of human PCR products, a human DNA capture probe was also constructed for the colorimetric assay. This dual setup for hybridization, which thus detected both *M. leprae* and human DNA PCR products, was useful for ascertaining the presence of inhibiting substances in clinical specimens. All biopsy specimens (N = 10) from untreated patients with leprosy were positive. Apparently, this assay is more sensitive than microscopy, because biopsy specimens from half of the patients were negative upon histopathological examination. Biopsy specimens from three treated patients were negative, as were those from the three patients who did not have leprosy. We conclude that this colorimetric assay can replace agarose gel analysis and Southern hybridization, because it is as sensitive as those methods. Its advantages over conventional gel analysis and Southern hybridization are that it is less cumbersome and more rapid.—Authors' Abstract

Wilson, S. M., McNerney, R., Nye, P. M., Godfrey-Faussett, P. D., Stoker, N. G. and Voller, A. Progress toward a simplified polymerase chain reaction and its application to diagnosis of tuberculosis. J. Clin. Microbiol. **31** (1993) 776–782.

The complexity, expense, and susceptibility to contamination of the polymerase chain reaction (PCR) are all issues which need to be overcome if PCR is to be used outside of research laboratories. We addressed these problems with respect to the diagnosis of tuberculosis. First, we simplified the procedure for extracting *Mycobacterium tuberculosis* DNA from sputum samples. Two methods of sample preparation were compared: the chaotrope-silica method and a novel, more simple chloroform method. Second, we developed a colorimetric method for product detection. This method was as sensitive and specific as agarose gel electrophoresis for detection of PCR product. By using a one-tube nested protocol, 5 to 50 genome equivalents of *M. tuberculosis* DNA were detected. The simplified colorimetric PCR was compared with microscopy and culture for detection of *M. tuberculosis* in clinical specimens of sputum. A total of 171 sputum samples were investigated from 108 patients, 12 of whom were subsequently found to have tuberculosis by culture and/or microscopy. PCR of samples prepared by the chaotrope-silica method had a sensitivity of 75% and a specificity of 100%; whereas PCR of samples prepared by the chloroform method had a sensitivity of 92% and a specificity of 99% when compared with the sensitivities and specificities of the combined classical microbiological methods for the diagnosis of tuberculosis. The simplified colorimetric PCR in combination with the chloroform sample preparation method was at least as sensitive as microscopy but had a greater specificity because samples with atypical mycobacteria were not detected by PCR. The sensitivity of the method for detection of smear-negative and extrapulmonary tuberculosis remains to be investigated.—Authors' Abstract

Yoon, K.-H., Cho, S.-N., Lee, M.-K., Abalos, R. M., Cellona, R. V., Fajardo, T. T., Jr., Guido, L. S., dela Cruz, E. C., Walsh, G. P. and Kim, J.-D. Evaluation of polymerase chain reaction amplification of *Mycobacterium leprae*-specific repetitive sequence in biopsy specimens from leprosy patients. J. Clin. Microbiol. **31** (1993) 895–899.

Biopsy specimens were obtained from 102 leprosy patients before chemotherapy and examined by polymerase chain reaction (PCR) using the primers amplifying the 372-bp DNA of a repetitive sequence of *Mycobacterium leprae*. The PCR results were then compared with bacterial indices (BI) of slit-skin smears and biopsy specimens. The intensities of DNA bands were in general correlated with the numbers of acid-fast bacilli (AFB), and even a sample with only one organism gave a PCR positive result. Ten 5-μm sections from each frozen

tissue sample were pooled and processed for DNA preparation. PCR was positive for 11 (73.3%) of 15 biopsy specimens with BI of 0 determined for the paraffin sections from the same biopsy samples. PCR also gave positive results for 84 (96.6%) of 87 BI positive biopsy samples. Although the difference in overall results between the two methods was not statistically significant, PCR seemed to have an advantage over microscopic examination in detecting *M. leprae* in biopsy specimens negative for AFB. Further evaluation of PCR using more specimens from leprosy patients who are bacteriologically negative is warranted to ensure PCR's advantage over the conventional microscopic examination for the diagnosis of leprosy.—Authors' Abstract

Young, D. B. and Cole, S. T. Leprosy, tuberculosis, and the new genetics. J. Bacteriol. **175** (1993) 1–6.

While effective control of leprosy may be a realistic prospect for the year 2000, tuberculosis, considered a disease of poverty and of the past, has returned with a vengeance. The problems of understanding the complex interactions between mycobacteria and their mammalian hosts which occupied previous generations of researchers must now be faced once more. Can we exploit the progress in molecular genetics over the intervening decades to provide new perspectives on these problems? Perhaps with the new techniques at our disposal we will be able to discover the mechanisms which allow *Mycobacterium tuberculosis* to survive in the hostile environment of host macrophages and to uncover the molecular basis of the strong affinity between *M. leprae* and Schwann cells, which underlies the nerve damage characteristic of leprosy. Study of the genetics and physiology of mycobacterial pathogens represents a conceptually demanding and exciting challenge for modern bacteriology.—Authors' Concluding Remarks

Experimental Infections

Gangadharam, P. R. J. and Dhople, A. M. Utility of beige mouse in leprosy research. Indian J. Lepr. **64** (1993) 475–481.

Dissemination of *Mycobacterium leprae* to visceral organs is seen from 4 months onward only in beige (C57BL/6/bg^J/bg^J) but not BALB/c mice following intravenous or intraperitoneal infections. Inoculation of the beige mouse-derived *M. leprae* showed all the characteristics of *M. leprae,* including growth pattern in the foot pads of BALB/c mice. *M. leprae* inoculated into foot pads of beige mice multiplied faster than those in the foot pads of BALB/c mice. The possibility of using the beige mouse in chemotherapeutic studies in leprosy is discussed.—Authors' Abstract

Kohli, M., Vaishnavi, C., Jaswal, S., Kaur, S. and Ganguly, N. K. Respiratory burst metabolic status of macrophages in experimental leprosy. J. Hyg. Epidemiol. Microbiol. Immunol. **36** (1992) 201–206.

Peritoneal macrophages from uninfected controls and *Mycobacterium leprae*-infected Swiss albino mice were studied for their respiratory burst (RB) activity at different time intervals. The RB metabolic activity of macrophages declined significantly after 3 months infection using latex ($p < 0.001$) and *M. leprae* ($p < 0.01$) as stimuli. However, a significant rise ($p < 0.001$) in the oxidative metabolic activity was seen at 6 and 9 months' postinfection period on stimulation with both the stimuli. The sharp rise in the oxidative metabolic status at peak period of infection in the experimental animals suggests that the macrophages are functionally normal though *M. leprae* is unable to trigger the respiratory burst sufficiently.—AS (Trop. Dis. Bull.)

Vaishnavi, C., Ganguly, N. K., Kaur, S. and Kumar, B. Suppression of ADCC by immune complexes formed *in vitro* in *Mycobacterium leprae*-infected mice. Microbiol. Immunol. **37** (1993) 49–53.

Antibody-dependent cellular cytotoxicity (ADCC) was assessed in mice infected experimentally with *Mycobacterium leprae* and

injected simultaneously with *in vitro*-formed immune complexes (IC). Significant decrease in the ADCC function was observed in animals given IC at day zero (0dIC) and 3 months (3mIC) postinoculation with *M. leprae*, when ADCC activity was assessed at 3, 6 and 9 month periods. From the data obtained we believe that ADCC is suppressed by IC formed *in vitro*.—Authors' Abstract

Yogi, Y., Nakamura, K., Inoue, T., Kawatsu, K., Kashiwabara, Y., Sakamoto, Y., Izumi, S., Saito, M., Hioki, K. and Nomura, T. Susceptibility of severe combined immunodeficient (SCID) mice to *Mycobacterium leprae*: multiplication of the bacillus and dissemination of the infection at an early stage. Jpn. J. Lepr. **60** (1991) 139–145.

Inoculations of *Mycobacterium leprae* were made into both hindfeet at a dose of 4.8×10^6 bacilli per foot in order to determine the susceptibility to *M. leprae* of SCID mice which are severely deficient in both T- and B-cell immunity. SCID mice were found to have an extremely high susceptibility to *M. leprae*, and the progress of infection observed in the SCID mice showed a rapid systemic spread of infection in the overall tissues as well as the growth of the leprosy bacilli at the site of inoculation. Therefore, SCID mice can be used as a suitable multibacillary model for the study of leprosy.—Authors' Summary

Epidemiology and Prevention

Baquillon, G., Scandella, B., Testa, J., Desfontaines, M., André, J. and Limbassa, J. [Leprosy survey conducted in the Central African Republic from 1982 to 1985 among the Ba-Benzélé Pygmies.] Acta Leprol. (Genève) **8** (1992) 71–78. (in French)

A leprosy survey was conducted from 1982 to 1985 among 2650 semi-sedentarized Pygmies in two camp-villages in the Central African Republic. Leprosy is endemic there, with an estimated prevalence rate of 1.05% and an annual detection rate of 0.2%. In view of its close relations with other neighboring ethnic groups, this Pygmy community can be considered as a target population the study of which provides indications on the transmission and typical course of leprosy in the region and also as a potential focus of contamination. However, the concurrent presence of endemic tuberculosis made it necessary during the survey to look for clinical associations of leprosy and tuberculosis in patients so that the standard multidrug treatment schedules comprising rifampin could be adjusted accordingly.—Authors' English Summary

De Wit, M. Y. L., Douglas, J. T., McFadden, J. and Klatser, P. R. Polymerase chain reaction for detection of *Mycobacterium leprae* in nasal swab specimens. J. Clin. Microbiol. **31** (1993) 502–506.

The polymerase chain reaction (PCR) based on the selective amplification of a 531-bp fragment of the gene encoding the proline-rich antigen of *Mycobacterium leprae* was applied to nasal swab specimens from leprosy patients, occupational contacts, and endemic and nonendemic controls. To prevent false-positive amplification, we used dUTP and uracil-DNA-glycosylase in all PCRs. False-negative reactions were detected by using a 531-bp modified template as an internal control. Amplification products were found in 55% of untreated patients, in 19% of the occupational contacts, in 12% of endemic controls, and in none of the nonendemic controls. This study strongly suggests that not only leprosy patients but also healthy persons may carry *M. leprae*. We concluded that PCR is a reliable method to detect *M. leprae* in nasal specimens. The method holds promise for studying the spread and transmission of *M. leprae* within a population.—Authors' Abstract

Dockrell, H. M., Eastcott, H., Young, S., MacFarlane, A., Hussain, R. and Waters, M. F. R. Possible transmission of *My-*

cobacterium leprae in a group of UK leprosy contacts. Lancet **2** (1992) 739–743.

"A case of infectious leprosy in a residential accommodation in the U.K. prompted a study of the cellular and humoral response to *Mycobacterium leprae* in two groups of individuals who were in contact with the index case for almost a year. In the younger staff group (mean age 44 years) 23 of 30 individuals had positive Mitsuda skin tests, 25 showed lymphocyte transformation to a soluble sonicate of *M. leprae* and 2 had slightly raised IgM antibody concentrations to the terminal disaccharide of *M. leprae* phenolic glycolipid-I. In the older group of residents (mean age 83 years) 7 of 36 individuals were skin-test positive, 25 of 33 were positive by lymphocyte transformation, but none had raised antibody levels. When retested on two further occasions, the same 2 individuals in the younger group still had raised antibody concentrations, one of whom had a persistent lepromin skin-test response for over 8 months and showed a pronounced increase in lymphocyte transformation to mycobacterial antigens. The findings suggest that transmission of *M. leprae* may have occurred in these 2 contacts, who were therefore given 6 months' chemoprophylaxis with rifampin."—P. E. M. Fine (Trop. Dis. Bull)

Mora, N., Pérez, M., Bendiche, M., Gonzáles, A. B., González-Abreu, E. and Gil, R. E. [Determination of antiphenolic glycolipid antibodies in a general population of an area endemic for leprosy.] Rev. Leprol. Fontilles **18** (1992) 587–597. (in Spanish)

Anti-phenolic glycolipid-I antibody levels were studied in 26,806 individuals from the general population in the municipality of Guantanamo, Cuba, by the enzyme immunoassay in the Ultra Micro Analytic System (SUMA) developed in Cuba. Prevalence and case detection rates in this municipality were 5 and 4 times, respectively, higher than those in the country in the year of the study (1988). Of the total, 82.6% showed values below the cut-off level. Among the "seropositive" a decline in the frequency according to the increase of age and an increase in the feminine sex was observed. It was possible to find out that 828 "seropositive" individuals had known contact with leprosy cases and that, among them, in those showing the higher values the proportion of household contacts was significantly higher than that of the non-household ones. This might be associated with a greater exposure to *Mycobacterium leprae* and to the recognized greater risk of contracting the disease among the household contacts. The examination of the "seropositive" has led, to date, to the diagnosis of 12 new leprosy patients in whom it was possible to demonstrate the presence of acid-fast bacilli in 7 of them; therefore being sources of infection in the community.—Authors' English Summary

Smith, W. C. S. and Jesudasan, K. Elimination of leprosy and prospects for rehabilitation. (Letter) Lancet **1** (1993) 89–90.

The latest estimate of the number of leprosy cases world wide from the World Health Organization (WHO) is 5.5 million, which is about half the number estimated in the early 1980s. This striking change led the 44th World Health Assembly of the WHO in May 1991 to set the goal of eliminating leprosy as a public health problem by the year 2000.

What does it mean and is this target achievable? Treatment of leprosy was radically changed by the introduction of the WHO multidrug regimen in 1982. A prominent logistical impact of this policy has been the reduction in the duration of treatment to 6 months for paucibacillary disease, and to 2 years for multibacillary disease.

The prevalence rate of disease is mathematically the product of the incidence rate and the duration of disease, and is estimated from the number of cases registered for treatment. Consequently, when the treatment is shortened the prevalence rate falls. The new very short chemotherapy regimens (down to 28 days) now being developed could lead to further falls in disease prevalence without affecting incidence or disability rates. Should the definition of disease be determined by the treatment? Defining disease in this way means that untreated patients with a single skin lesion but no neurological impairment are regarded as cases;

whereas patients who have completed a course of chemotherapy but who are profoundly handicapped are not. For those concerned with control of transmission this approach may be appropriate (although incidence rates are still a better indicator) but for others it is very unsatisfactory. Rather than leprosy being eliminated it has undergone a transformation from a medical disorder requiring drug therapy to a rehabilitation and a social problem. Perhaps that is all to the good, but we must not neglect the continuing needs of patients with impairment and disability when chemotherapy for the mycobacterial infection has been completed.—From the Letter

Terencio de las Aguas, J. [Leprosy in the district of Marina (Spain).] Rev. Leprol. Fontilles **18** (1992) 599–636. (in Spanish)

The origin of leprosy in the district of the Marina of the Valencian Community that was an endemic focus at the end of the XIX century and first half of the present is studied. The history of the leprosy colony Sanatorio de Fontilles in the center of the District Marina Alta and its activities in teaching and investigation are explained and, finally, the present leprosy incidence in this district together with its decrease and possible erradication by the end of the century.—Author's English Summary

Rehabilitation

Birke, J. A., Novick, A., Patout, C. A. and Coleman, W. C. Healing rates of plantar ulcers in leprosy and diabetes. Lepr. Rev. **63** (1993) 365–374.

Comparison was made of wound-healing time in a consecutive series of leprosy and diabetic patients with plantar ulceration. In the leprosy group, 66 of 70 (94%) ulcers healed in a mean time of 42.7 (±36.1) days, and in the diabetic group, 75 of 80 (94%) ulcers healed in a mean time of 39.7 (±32.1) days. Analysis of all healed ulcers using a general linear model found wound depth ($p < 0.03$), and wound diameter ($p < 0.05$) significantly related to ulcer-healing time. Diagnosis, healing devices (cast, splint and cut-out sandal), age and sex were not significant. In diabetic subjects a regression model including depth, diameter and age explained 36% of the variation in healing time. A meaningful regression model was not found in leprosy patients.—Authors' Summary

Çakiner, T., Yüksel, A., Soydan, M., Saylan, T. and Bahçeci, E. Women and leprosy in Turkey. Indian J. Lepr. **65** (1993) 59–67.

Women in Turkey have many social, cultural and economic problems. Women with leprosy have problems in common with other women as well as those related to physical and social consequences of leprosy. There are 2414 patients with leprosy in Turkey, registered to Istanbul Leprosy Hospital and 829 of them are females. The mean age and duration of disease of our female leprosy patients are high. Most women with leprosy were born in the eastern part of Turkey where the prevalence of leprosy is higher, and most have moved to western regions. The proportion of women who have some kind of social security is very low. Their economic status is also not good and 79% of the patients had stigma about their disease. Three fourths of these cases have been hospitalized some time, for different reasons. Most of them (97.2%) have inactive disease at present. Disability degrees of patients are high. Patients with disability degrees over 1 constitute 54% of the total for eyes, 55% for hands and 51% for feet. High percentage of multibacillary form and long duration of disease, delayed diagnosis, insufficient self-care of patients due to low socioeconomic and cultural status and failure of health personnel to control patients periodically may be among the reasons for such high ratios of moderate and severe disabilities. In light of the data obtained in our study, some measures to alleviate the problems of patients resulting from their socioeconomic, cultural and social status have been suggested.—Authors' Abstract

Jiang, C., *et al.* [Sociomedical study of leprosy disability. (I) A survey of attitude to their disability in leprosy.] China Lepr. J. **8** (1992) 197–203. (in Chinese)

A questionnaire survey of attitudes toward their disability in 319 leprosy patients and 712 cures has been made in Yangzhou city, Jiangsu, and showed that 98.8% of them have had disabilities of I or II degrees. Their answers to the cause are "being affected with damp," "having a cold," "being inevitable outcome of leprosy" or "being a signal of leprosy" are in 48.2%, and being indifferent to their disability in 35.0%. Among the persons with disability 58.9% wanted to gloss over their disability, 37.3% decreased communication with others and 32.4% intended to commit suicide. These attitudes have nothing to do with the patients' sex, age and educational level, but have to do with their disability and its degree. The form of leprosy, patient's dwelling place and ability to work and live also have some impact on the attitude.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Al-Orainey, I. O., Gad El Rab, M. O., Al-Hajjaj, M. S. and Saeed, E. S. Detection of mycobacterial antigens in sputum by an enzyme immunoassay. Eur. J. Clin. Microbiol. Infect. Dis. **11** (1992) 58–61.

An enzyme immunoassay (EIA) for the detection of mycobacterial antigens in sputum was evaluated. The system utilizes commercially available anti-BCG immunoglobulin. BCG protein standard was used as positive control. Thirty-nine patients [in Saudi Arabia] with culture-proven pulmonary tuberculosis were tested. The EIA was positive in 24 of 29 patients with positive smears and cultures, giving a sensitivity of 86.2%. It was also positive in 6 of 10 patients with smear-negative culture-positive disease, resulting in a sensitivity of 60% in this group. In another 176 patients with different nontuberculous pulmonary infections, only 9 were positive by EIA, giving a specificity of 94.9%. The high sensitivity and specificity of the assay make it a useful tool for the early diagnosis of pulmonary tuberculosis.—AS (Trop. Dis. Bull.)

Anderson, P. and Heron, I. Specificity of a protective memory immune response against *Mycobacterium tuberculosis.* Infect. Immun. **61** (1993) 844–851.

We have investigated the memory T-cell immune response to *Mycobacterium tuberculosis* infection. C57BL/6J mice infected with *M. tuberculosis* were found to generate long-lived memory immunity which provided a heightened state of acquired resistance to a secondary infection. The T-cell response of memory-immune mice was directed to all parts of the bacilli, i.e., both secreted and somatic proteins. Major parts of the memory T-cell repertoire were maintained in a highly responsive state by cross-reactive restimulation with antigens present in the normal microbiological environment of the animals. A resting noncrossreactive part of the memory repertoire was restimulated early during a secondary infection to expand and produce large amounts of gamma interferon. The molecular target of these T cells was identified as a secreted mycobacterial protein with a molecular mass of 3 to 9 kDa.—Authors' Abstract

Ashtekar, D. R., Costaperira, R., Nagrajan, K., Vishvanathan, N., Bhatt, A. D. and Rittel, W. *In vitro* and *in vivo* activities of the nitroimidazole CGI-17341 against *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **37** (1993) 183–186.

CGI 17341 (2-ethyl-5-nitro-2,3-dihydro[2-1b]imidazo-oxazole) is a novel orally active representative of the 5-nitroimidazole series of antimicrobial agents. At concentrations ranging from 0.1 to 0.3 μg/ml, CGI 17341 inhibited the drug-susceptible and multidrug-resistant strains of *Mycobacterium tuberculosis.* CGI 17341 had no crossresistance with isoniazid, rifampin, streptomycin, or ethambutol. While the *in vitro* activity of CGI 17341 against *M. tu-*

berculosis was comparable to those of isoniazid and rifampin, it was superior to those of streptomycin, ciprofloxacin or norfloxacin, and oxazolidinone DuP 721. The MIC of CGI 17341 was not affected when the pH of the medium was decreased from 6.8 to 5.6, while four- to sixfold increases in the MICs of ciprofloxacin and isoniazid were observed. In mice infected with *M. tuberculosis,* the 50% effective dose for CGI 17341 was 7.7 mg/kg of body weight (95% confidence limits, 3.5 and 10.27) when administered on days 11 and 12 postinfection. CGI 17341 gave a dose-dependent (r = 0.995) and significant increase in the survival time. Our data indicate that the 5-nitroimidazole CGI 17341 is a promising and novel antituberculosis compound with potent *in vitro* and *in vivo* activities. Further investigations on this compound are warranted.—Authors' Abstract

Aspinall, G. O., Gammon, D. W., Sood, R. K., Chatterjee, D., Rivoire, B. and Brennan, P. J. Structures of the glycopeptidolipid antigens of serovar-25 and serovar-26 of the *Mycobacterium avium* serocomplex, synthesis of allyl glycosides of the outer disaccharide units and serology of the derived neoglycoproteins. Carbohydr. Res. **237** (1992) 57–77.

The pentasaccharide hapten released from the glycopeptidolipid (GPL) antigen of *Mycobacterium avium* serovar 26 has been characterized as *O*-(2,4-di-*O*-methyl-alpha-L-fucopyranosyl)-(1 → 4)-*O*-beta-D-glucopyranosyluronic acid-(1 → 4)-*O*-(2-*O*-methyl-alpha-L-fucopyranosyl)-(1 → 3)-alpha-L-rhamnopyranosyl-(1 → 2)-6-deoxy-L-talose. The allyl glycosides of the outer glycosyl and glycobiosyl units of this hapten have been synthesized, the latter by a route involving oxidation of the corresponding D-glucopyranose derivative. Conjugation of allyl glycosides to protein by ozonolysis and reductive coupling afforded neoantigens (neo 26-1 and 26-2), both of which interacted with antibodies to *M. avium* serovar 26. The terminal sugar residue of the pentasaccharide hapten of the serovar 25 GPL had been shown to have the galacto configuration on the basis of H-1-C-13 NMR correlation spectroscopy, but absolute configurational assignment for the sugar awaited the synthesis, as for neo 26, of two glycobiosyl NGPs bearing the terminal sugar in the D and l enantiomeric forms, respectively. Only the glycobiosyl NGP bearing the terminal sugar as the D-enantiomer interacted with antibodies to *M. avium* serovar 25, thus providing evidence for the absolute configuration of the sugar, and showing that the complete oligosaccharide hapten has the structure, *O*-(4-acetamido-4,6-dideoxy-2-*O*-methyl-alpha-D-galactopyranosyl)-(1→4)-*O*-beta-D-glucopyranosyluronic acid-(1 → 4)-*O*-(2-*O*-methyl-alpha-L-fucopyranosyl)-(1 → 3)-*O*-alpha-L-rhamnopyranosyl-(1 → 2)-6-deoxy-L-talose.—Authors' Abstract

Barnes, P. F., Abrams, J. S., Lu, S. Z., Sieling, P. A., Rea, T. H. and Modlin, R. L. Patterns of cytokine production by mycobacterium-reactive human T-cell clones. Infect. Immun. **61** (1993) 197–203.

To gain insight into the functional capacity of human T cells in the immune response against *Mycobacterium tuberculosis,* we evaluated the spectrum of cytokines produced by mycobacterium-reactive human T-cell clones. Nine of 11 T-cell clones bearing alpha/beta or gamma/delta T-cell receptors produced both Th1 and Th2 cytokines, a pattern resembling that of murine Th0 clones. The most frequent pattern was secretion of gamma interferon, tumor necrosis factor alpha (TNF), and interleukin-10 (IL-10), in combination with IL-2, IL-5, or both. Two clones produced only Th1 cytokines, and none produced exclusively Th2 cytokines. Although IL-4 was not detected in cell culture supernatants, IL-4 mRNA was detected by polymerase chain reaction amplification in 2 of 6 clones. There were no differences between the cytokine profiles of alpha/beta and gamma/delta T cells. A striking finding was the markedly elevated concentrations of TNF in done supernatants, independent of the other cytokines produced. Supernatants from mycobacterium-stimulated T-cell clones, in combination with granulocyte-macrophage colony-stimulating factor, induced aggregation of bone-marrow-derived macrophages, and this effect was abrogated by antibodies to TNF. The addition of recombinant TNF to

granulocyte-macrophage colony-stimulating factor markedly enhanced macrophage aggregation, indicating that TNF produced by T cells may be an important co-stimulus for the granulomatous host response to mycobacteria. The cytokines produced by T cells may exert immunoregulatory and immunopathologic effects and thus mediate some of the clinical manifestations of tuberculosis.—Authors' Abstract

Barrera, L. F., Skamene, E. and Radzioch, D. Assessment of mycobacterial infection and multiplication in macrophages of polymerase chain reaction. J. Immunolog. Meth. **157** (1993) 91–99.

The amplification of mycobacterium-specific DNA sequences from samples obtained from infected patients by the polymerase chain reaction (PCR) has been useful in the clinical diagnosis of mycobacterial diseases. Using 20-bp oligonucleotide primers that recognize a 123-bp repeated sequence present in *Mycobacterium bovis* and *M. tuberculosis* DNA, we describe in detail the conditions of the PCR reaction that allow an assessment of the mycobacterial content of infected macrophages. The results of the highly reproducible, time-efficient PCR technique show good correlation with the widely used colony forming unit (CFU) and [H-3]uracil incorporation methods for the detection of *Mycobacterium.* Our method allows an assessment of the level of *M. bovis* BCG infection from a variety of sources, including peritoneal macrophages and macrophage lines, within a few hours, making it the assay of choice for rapid determination of the level of mycobacterial growth in infected cells, in experimental models of mycobacterial infection.—Authors' Abstract

Bermudez, L. E. Differential mechanism of intracellular killing of *Mycobacterium avium* and *Listeria monocytogenes* by activated human and murine macrophages—the role of nitric oxide. Clin. Exp. Immunol. **91** (1993) 277–281.

Murine peritoneal macrophages activated with interferon-gamma (IFN-gamma) produce large quantities of nitric oxide and are efficient in the killing of certain intracellular pathogens. To examine the role of this mechanism in the killing of *Mycobacterium avium* by murine and human macrophages, we infected mouse peritoneal macrophages and human monocyte-derived macrophages with *M. avium* and *Listeria monocytogenes* and stimulated the cells with recombinant tumor necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor (GM-CSF) or IFN-gamma, in the presence or absence of N-monomethyl-L-arginine (NMA) or arginase. Neither competitive inhibition with NMA nor depletion of arginine by arginase had any effect on the inhibition of growth/intracellular killing of *M. avium* by activated human and murine macrophages. In contrast, activation of murine but not human macrophages infected with *L. monocytogenes* by IFN-gamma was significantly inhibited by the addition of NMA/arginase. Furthermore, murine macrophages produced large concentrations of nitric oxide following stimulation with recombinant cytokines, although no significant increase of nitric oxide production was observed with human monocyte-derived macrophages.—Author's Abstract

Brown, S. T., Edwards F. F., Bernard, E. M., Tong, W. and Armstrong, D. Azithromycin, rifabutin, and rifapentine for treatment and prophylaxis of *Mycobacterium avium* complex in rats treated with cyclosporine. Antimicrob. Agents Chemother. **37** (1993) 398–402.

Azithromycin, rifabutin, and rifapentine were used to treat or prevent disseminated *Mycobacterium avium* complex (MAC) infections produced in rats immunosuppressed with cyclosporine. Animals with bacteremic infections were treated 1 week after intravenous inoculation with 10^7 CFU of MAC with azithromycin, 100 mg/kg of body weight administered subcutaneously for 5 days and then 75 mg/kg on Monday, Wednesday, and Friday, or with rifabutin or rifapentine, 20 mg/kg administered intraperitoneally on Monday through Friday. All three drugs showed efficacy after 1 and 2 months. Rifabutin cleared the organisms from tissues more rapidly than azithromycin or rifapentine. To approximate prophylaxis, treatment was started 2 weeks before

intravenous inoculation with 10^4 organisms. MAC infections were undetectable in treated animals after 4 months, while control animals had disseminated infections. These findings support the rationale for clinical trials of treatment and prophylaxis with these agents. The cyclosporine-treated rat appears to be a useful model in which to evaluate compounds for the treatment and prophylaxis of disseminated MAC infections.—Authors' Abstract

Carlucci, S., Beschin, A., Tuosto, L., Ameglio, F., Gandolfo, G. M., Cocito, C., Fiorucci, F., Saltini, C. and Piccolella, E. Mycobacterial antigen complex A60-specific T-cell repertoire during the course of pulmonary tuberculosis. Infect. Immun. **61** (1993) 439–447.

The *Mycobacterium bovis* antigen complex A60 is known to be immunodominant in tuberculosis and to have a protective effect against experimental infection *in vitro* and *in vivo*. To identify immunodominant and possibly protective antigens in pulmonary tuberculosis, the T-cell repertoire directed to nitrocellulose-bound fractions of A60 antigen was analyzed in active tuberculosis patients during the course of the infection and after recovery. The results show that patients infected with *Mycobacterium tuberculosis* acquired reactivity only in the late phases of infection. At disease onset, patients with active tuberculosis were characterized by (i) T-cell unresponsiveness to most A60 fractions, (ii) high tumor necrosis factor-alpha production, and (iii) low gamma-interferon (IFN-gamma) release. Several weeks after chemotherapy, the unresponsive state disappeared and the following reverse situation was observed: (i) high blastogenic response to almost all A60 fractions, (ii) low tumor necrosis factor-alpha release, and (iii) high IFN-gamma production. In addition, 60% of these patients significantly responded against seven A60 fractions (61 to 58, 56 to 53, 49 to 46, 46 to 44, 35 to 33, 33 to 30, and 30 to 28 kDa), indicating that they included immunodominant antigens. Furthermore, only the fractions within the molecular-mass ranges of 56 to 44 and 35 to 28 kDa induced IFN-gamma synthesis. One year after complete recovery from infection, more than 60% of past-active tuberculosis subjects had memory T cells specific for the immunodominant fractions of 61 to 58, 56 to 53, 49 to 46, and 33 to 30 kDa. Since the same fractions induced the strongest IFN-gamma production, known to exhibit antimycobacterial effects, it is suggested that these may represent the inducers of a protective immune response.—Authors' Abstract

Costello, A. M. D., Kumar, A., Narayan, V., Akbar, M. S., Ahmed, S., Abou-Zeid, C., Rook, G. A. W., Stanford, J. and Moreno, C. Does antibody to mycobacterial antigens, including lipoarabinomannan, limit dissemination in childhood tuberculosis. Trans. R. Soc. Trop. Med. Hyg. **86** (1992) 686–692.

Serum immunoglobulin (Ig) G responses to a variety of mycobacterial antigens were measured in children from the U.K., in children with tuberculosis from Hyderabad, India, and Dhaka, Bangladesh, classified according to whether the disease was disseminated or localized, and in nontuberculous controls. Anti-lipoarabinomannan (LAM) IgG responses in U.K. children showed a marked trough between 6 months and 3 years coincident with the reported peak incidence of disseminated tuberculosis. Geometric mean IgG responses to sonicates of slow-growing mycobacteria (rich in LAM) in 36 children with disseminated tuberculosis were markedly lower than in 99 children with localized tuberculous lesions (for *Mycobacterium scrofulaceum* $p < 0.01$, for *M. tuberculosis* $p < 0.01$, and for *M. vaccae* $p < 0.01$). Responses to purified LAM were also lower in the disseminated tuberculosis group ($p < 0.05$) but there was no difference between the groups in their response to mycobacterial 65-kDa protein. Multiple regression analysis showed that the reduced response to sonicated mycobacterial antigens and to LAM in children with disseminated disease was independent of age, nutritional status, skin-test reactivity, duration of previous symptoms, and city of origin. There was no evidence for sequestration of antibody to immune complexes. These findings are compatible with the hypothesis that children with low levels of antibody to sonicated mycobacterial antigen

and to LAM, or those who cannot mount an antibody response, are predisposed to dissemination. A role for antibody in preventing disseminated forms of tuberculosis in childhood has implications for the development of improved vaccines and for the optimum timing of vaccination with bacille Calmette-Guérin.—Authors' Abstract

Dautzenberg, B., Saint Marc, T., Meyohas, M. C., Elias-Zewitch, M., Haniez, F., Rogues, A. M., Dewit, S., Cotte, L., Chauvin, J. P. and Grosset, J. Clarithromycin and other antimicrobial agents in the treatment of disseminated *Mycobacterium avium* infections in patients with acquired immunodeficiency syndrome. Arch. Intern. Med. **153** (1993) 368–372.

Disseminated infection with *Mycobacterium avium* is common with late-stage acquired immunodeficiency syndrome (AIDS), and no antimicrobial agent has been found to be clearly effective. A multicenter open trial was conducted to assess the antimicrobial activity and clinical efficacy of clarithromycin—a new macrolide antibiotic—against disseminated *M. avium* in 77 patients with late-stage AIDS. Blood cultures were taken at baseline and during treatment; side effects were also evaluated. *M. avium* was eradicated from blood cultures in 11 (63%) of 16 evaluable patients receiving daily doses or 500 or 1000 mg (N = 21) and in 45 of 46 (98%) of those receiving 1500 or 2000 mg (N = 56). Eradication after 2 months was influenced by continuity of drug treatment; 36 of 42 patients with no relapse had received continuous treatment vs 6 of 14 patients whose drug treatment had been stopped for 7 days or longer. After 2 to 7 months of treatment, acquired resistance associated with relapse was observed. Drug side effects were elevated liver enzyme levels (26%) and impaired hearing (4%). Concomitant AIDS drugs had no favorable effect on outcome and may have worsened liver toxicity. Clarithromycin has bacteriologic efficacy against *M. avium* infection in late-stage AIDS, although drug resistance eventually develops. Further studies are needed to investigate safe, effective concomitant drugs.—Authors' Abstract

De Kesel, M., Gilot, P., Misonne, M.-C., Coene, M. and Cocito, C. Cloning and expression of portions of the 34-kilodalton-protein gene to *Mycobacterium paratuberculosis*: its application to serological analysis of Johne's disease. J. Clin. Microbiol. **31** (1993) 947–954.

Paratuberculosis (Johne's disease), an endemic mycobacteriosis of cattle that is caused by *Mycobacterium paratuberculosis,* is characterized by incoercible diarrhea and fecal shedding of bacteria. The present work aimed at developing a specific serological test for this disease. We have recently shown that a 34-kDa protein belonging to the major antigen complex A36 of *M. paratuberculosis* is immunodominant and contains epitopes specific with respect to all mycobacteria tested, including *M. bovis* and the closely related species *M. avium.* From a λgt11 genomic library of *M. paratuberculosis,* three portions of the gene coding for this 34-kDa protein have been isolated. Two of them expressed crossreacting mycobacterial epitopes. One portion (in clone a362) expressed a polypeptide which crossreacted with all tested *M. paratuberculosis* strains but not with 20 other bacteria tested, including many strains of the *M. avium-M. intracellulare-M. scrofulaceum* group. The occurrence at the *M. paratuberculosis* surface of epitopes corresponding to the a362 polypeptide was shown by immune electron microscopy. The recombinant a362 polypeptide was used as reagent for an enzyme-linked immunoassay for paratuberculosis. This assay correctly diagnosed all of the tested blood samples from infected cattle at all stages of the disease.—Authors' Abstract

Dwyer, B., Jackson, K., Raios, K., Sievers, A., Wilshire, E. and Ross, B. DNA restriction fragment analysis to define an extended cluster of tuberculosis in homeless men and their associates. J. Infect. Dis. **167** (1993) 490–494.

A cluster of tuberculosis in men associated with shelters for the homeless in Melbourne, Australia, diagnosed between April 1984 and July 1991, was reviewed with respect to the genetic relatedness of the infecting strains. Relatedness was determined by examination of Southern blot analyses

of restriction enzyme-digested genomic DNA, using probes for repetitive sequences. From the initial 24 cases selected, isolates were available from 19, and 18 were identical. A total of 571 other Australian and Solomon Islands strains were examined for relatedness to these strains. Nine additional case strains were found to be identical; all were from recent Melbourne residents and at least 7 were epidemiologically related to the original case cluster. The identification of a single infecting strain of *Mycobacterium tuberculosis* in these 27 cases suggests that reactivation disease in this group may not be as important as infection or reinfection from another case or cases.—Authors' Abstract

Frieden, T. R., Sterling, T., Pablos Mendez, A., Kilburn, J. O., Cauthen, G. M. and Dooley, S. W. The emergence of drug-resistant tuberculosis in New York City. N. Engl. J. Med. **328** (1993) 521–526.

In the past decade the incidence of tuberculosis has increased nationwide and more than doubled in New York City, where there have been recent nosocomial outbreaks of multidrug-resistant tuberculosis. We collected information on every patient in New York City with a positive culture for *Mycobacterium tuberculosis* during April 1991. Drug-susceptibility testing was performed at the Centers for Disease Control and Prevention. Of the 518 patients with positive cultures, 466 (90%) had isolates available for testing. Overall, 33% of these patients had isolates resistant to one or more antituberculosis drugs, 26% had isolates resistant to at least isoniazid, and 19% had isolates resistant to both isoniazid and rifampin. Of the 239 patients who had received antituberculosis therapy, 44% had isolates resistant to one or more drugs and 30% had isolates resistant to both isoniazid and rifampin. Among the patients who had never been treated, the proportion with resistance to one or more drugs increased from 10% in 1982 through 1984 to 23% in 1991 ($p = 0.003$). Patients who had never been treated and who were infected with the human immunodeficiency virus (HIV) or reported injection-drug use were more likely to have resistant isolates. Among patients with the acquired immunodeficiency syndrome, those with resistant isolates were more likely to die during follow up through January 1992 (80% vs 47%; $p = 0.02$). A history of antituberculosis therapy was the strongest predictor of the presence of resistant organisms (odds ratio, 2.7; $p < 0.001$). There has been a marked increase in drug-resistant tuberculosis in New York City. Previously treated patients, those infected with HIV, and injection-drug users are at increased risk for drug resistance. Measures to control and prevent drug-resistant tuberculosis are urgently needed.—Authors' Abstract

Friedland, J. S., Shattock, R. J., Johnson, J. D., Remick, D. G., Holliman, R. E. and Griffin, G. E. Differential cytokine gene expression and secretion after phagocytosis by a human monocytic cell line of *Toxoplasma gondii* compared with *Mycobacterium tuberculosis*. Clin. Exper. Immunol. **91** (1993) 282–286.

Toxoplasma gondii infection may be clinically silent in immunocompetent individuals but may cause fatal disease in immunocompromised patients such as those with HIV infection. Proinflammatory cytokines are known to be important in murine resistance to *T. gondii* but there are no data from human models of infection. We have investigated whether phagocytosis of *T. gondii*, of *Mycobacterium tuberculosis* (a pathogen which elicits a granulomatous host immune response) and of inert latex particles by THP-I cells, a human monocytic line, caused gene expression and secretion of tumor necrosis factor (TNF), IL-6 and IL-8. These cytokines are important in recruitment and activation of T lymphocytes, and both TNF and IL-6 may have direct antitoxoplasmacidal and antimycobacterial activity. Phagocytosis of *T. gondii* by THP-1 cells resulted in minimal gene expression and secretion of TNF, IL-6 and IL-8 similar to that following phagocytosis of inert latex particles. In contrast, phagocytosis of *M. tuberculosis* resulted in increased gene expression of TNF and IL-8 as well as increased secretion of all three cytokines, particularly IL-8. These observations may partially explain the frequency of noninflammatory host responses to *T. gondii* in

immunocompetent individuals.—Authors' Abstract

Gangadharam, P. R. J. and Parikh, K. In-vivo activity of streptomycin and clofazimine against established infections of *Mycobacterium avium* complex in beige mice. J. Antimicrob. Chemother. **30** (1992) 833–838.

Beige mice were challenged with 10^6–10^7 colony forming units (cfu) of *Mycobacterium avium-intracellulare* strain 101 and 22 days later treated with streptomycin 150 mg/kg/day alone, clofazimine 20 mg/kg/day alone, streptomycin 150 mg/kg/day plus clofazimine 20 mg/kg/day, or no antimicrobial agent (untreated controls). Both single-drug therapies partially reduced the cfu counts in spleen, liver and lungs compared with the controls; however the combination was significantly more effective and completely eliminated the pathogen from the spleen and lungs of some animals after 8 weeks' treatment.—Authors' Abstract

Gascon, P., Sathe, S. S. and Rameshwar, P. Impaired erythropoiesis in the acquired immunodeficiency syndrome with disseminated *Mycobacterium avium* complex. Am. J. Med. **94** (1993) 41–48.

Anemia is an important negative predictor for survival with disseminated *Mycobacterium avium* complex (MAC) infection in the acquired immunodeficiency syndrome (AIDS). We analyzed the differences in AIDS patients with and without MAC infection with regard to anemia, severity of human immunodeficiency virus (HIV) infection, bone marrow morphology, and bone marrow erythroid progenitor colony growth (BFU-E and CFU-E). In addition, we determined the *in vitro* effect of sera obtained from these patients on normal BFU-E and CFU-E. A possible role of macrophages in the suppression of erythropoiesis was examined by studying *in vitro* the effect of supernatants from MAC-infected macrophages on cultured BFU-E and CFU-E. Hematocrit, serum levels of p24 antigen, erythropoietin, and CD4-positive cell count were determined in 14 AIDS patients with and 24 without MAC infection. Bone-marrow erythropoietic and granulocytic progenitor cells from 15 normal individuals, from 12 AIDS patients with MAC infection, and from 10 AIDS patients without MAC infection were cultured on methylcellulose. In addition, progenitor cells from normal individuals were cultured in the presence, and in the absence, of sera obtained from AIDS patients with (14), or without (24), MAC infection. Last, we studied the effect of supernatants (SNs) from MAC and *M. tuberculosis*-infected macrophages on erythropoietic progenitor cell growth.

The anemia in AIDS patients with MAC infection was associated with a selective suppression of erythropoietic progenitors despite bone-marrow morphology that was indistinguishable from that in patients without MAC infection. The degree of anemia could not be explained on the basis of severity of HIV infection or a deficiency of erythropoietin production. Bone-marrow mononuclear cells from AIDS patients with MAC generated significantly fewer erythroid progenitor colonies (BFU-E and CFU-E) than equivalent cells from AIDS patients without MAC infection ($p < 0.05$). Sera from MAC-infected AIDS patients were markedly inhibitory to the erythroid progenitors as compared with sera from patients without MAC infection ($p < 0.001$). SNs from MAC-infected macrophages were markedly inhibitory to the erythroid progenitors (BFU-E and CFU-E) as compared with the myeloid progenitors (CFU-GM). The profound anemia in MAC-infected AIDS patients is due to suppression of erythroid progenitors by a soluble factor(s) in the serum. The data suggest that the soluble factor(s) is probably elaborated by macrophages.—Authors' Abstract

Goble, M., Iseman, M. D., Madsen, L. A., Waite, D., Ackerson, L. and Horsburgh, C. R. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. N. Engl. J. Med. **328** (1993) 527–532.

The frequency of infection with multidrug-resistant *Mycobacterium tuberculosis* is increasing. We reviewed the clinical courses of 171 patients with pulmonary disease due to *M. tuberculosis* resistant to rifampin and isoniazid who were referred to our hospital between 1973 and 1983. The

patients' records were analyzed retrospectively. Their regimens were selected individually and preferably included three medications that they had not been given previously and to which the strain was fully susceptible. The 171 patients (median age, 46 years) had previously received a median of six drugs and shed bacilli that were resistant to a median of six drugs. Thus, their regimens were frequently not optimal. Of 134 patients with sufficient follow-up data, 87 (65%) responded to chemotherapy (as indicated by negative sputum cultures for at least 3 consecutive months); 47 patients (35%) had no response, as shown by continually positive cultures. The median stay in the hospital was more than 7 months. In a multivariate analysis, an unfavorable response was significantly associated with a greater number of drugs received before the current course of therapy (odds ratio, 4.0; 95% confidence interval, 1.6 to 9.9; $p < 0.001$) and with male sex (odds ratio, 2.5; 95% confidence interval, 1.1 to 6.2; $p < 0.03$). Twelve of the patients with responses subsequently had relapses. The overall response rate was 56% over a mean period of 51 months. Of the 171 patients, 63 (37%) died, and 37 of these deaths were attributed to tuberculosis. For patients with pulmonary tuberculosis that is resistant to rifampin and isoniazid, even the best available treatment is often unsuccessful. Only about half of such patients eventually have negative sputum cultures despite carefully selected regimens administered for extended periods. Failure to control this resistant infection is associated with high mortality and ominous implications for the public health.—Authors' Abstract

Gurumurthy, P., Rahman, F., Narayana, A. S. L. and Raghupati Sarma, G. Salivary levels of isoniazid and rifampicin in tuberculous patients. Tubercle **71** (1990) 29–33.

Concentrations of isoniazid and rifampin were determined in time-matched samples of saliva and serum from 30 tuberculous patients (18 with pulmonary tuberculosis and 12 with intestinal tuberculosis), comprising 18 slow and 12 rapid acetylators of isoniazid, following administration of isoniazid 300 mg and rifampin 12 mg/kg. The diffusion of isoniazid into saliva was quite rapid and the salivary concentrations were similar to those in serum, suggesting that saliva could be used in place of serum for all pharmacokinetic studies with isoniazid. The salivary concentrations of rifampin were much lower than those in serum, the mean peak concentrations being 0.9 and 8.5 μg/ml, respectively. Further, there was evidence of a significant delay in the diffusion of rifampin from serum to saliva.—AS (Trop. Dis. Bull.)

Haas, W. H., Kirschner, P., Ziesing, S., Bremer, H. J. and Bottger, E. C. Cervical lymphadenitis in a child caused by a previously unknown mycobacterium. J. Infect. Dis. **167** (1993) 237–240.

Acid-fast bacilli were isolated from lymph nodes of an immunocompetent child presenting with unilateral cervical lymphadenitis. The slowly growing mycobacterium could not be identified by traditional methods. Direct sequencing of the enzymatically amplified 16S rRNA gene revealed a unique sequence belonging to a previously unrecognized mycobacterium. Direct 16S rDNA sequencing enables definitive identification of mycobacterial isolates. The method is useful for rapid recognition of previously unrecognized pathogens.—Authors' Abstract

Haeseleer, F., Pollet, J. F., Haumont, M., Bollen, A. and Jacobs, P. Stable integration and expression of the *Plasmodium falciparum* circumsporozoite protein coding sequence in mycobacteria. Mol. Biochem. Parasitol. **57** (1993) 117–126.

The DNA coding for the circumsporozoite protein of *Plasmodium falciparum* (CSP; aa 1-412) has been placed under the control of the mycobacterial promoter derived from the gene encoding the 64-kDa antigen of *Mycobacterium bovis*-BCG. This expression cassette was cloned into pJRD184, an *Escherichia coli* multicloning, site vector, together with the kanamycin resistance gene from Tn903 and the attachment site and integrase gene from the temperate mycobacteriophage FRAT1. One of the resulting plasmids, pNIV2173, introduced by electroporation into both *M.*

smegmatis and *M. bovis*-BCG, integrated at a specific site in the genome of each recipient. Recombinants expressed immunoreactive polypeptides, ranging in size from 62 to 43 kDa, at a level of about 1% of total soluble proteins. Part of this material was present in the culture medium indicating that mycobacterial recombinants were able to secrete the CSP. The *M. smegmatis* and *M. bovis*-BCG recombinants, transformed with pNIV2173 and grown in absence of antibiotic, were followed for more than 400 and 50 generations, respectively. Over this time span, neither DNA rearrangement nor loss of expression was observed. Inoculation of the recombinant BCG to mice did not induce a humoral response to CSP nor a proliferative response to CSP Th2R CD4+ T lymphocyte epitope.—Authors' Abstract

Hoop, R. K., Böttger, E. C., Ossent, P. and Salfinger, M. Mycobacteriosis due to *Mycobacterium genavense* in six pet birds. J. Clin. Microbiol. **31** (1993) 990–993.

Six cases of mycobacteriosis due to *Mycobacterium genavense* in three budgerigars (*Melopsittacus undulatus*), one orange-winged amazon (*Amazona amazonica*), one flycatcher (*Cyanoptila cyanomelana*), and one zebra finch (*Taeniopygia guttata*) are discussed. Gross lesions associated with the infection included a high degree of muscular wasting (5 cases), hepatomegaly (4 cases), and thickening of the wall of the small intestine (4 cases). Granulomas were found in the lung (1 case) and the subcutis (1 case). Acid-fast bacilli were detected in the liver of all 6 birds. Only the use of acidic BACTEC mediums consistently led to growth; whereas the egg-based medium failed. These findings point to a possible role of the environment as a reservoir for *M. genavense*.—Authors' Abstract

Houssaini-Iraqui, M., Clavel-Seres, S., Rastogi, N. and David, H. L. Partial physical and functional map of a *Mycobacterium aurum* carotenogenesis operon. Curr. Microbiol. **26** (1993) 65–74.

The genes controlling the biosynthesis of the carotenes in *Mycobacterium aurum* were clustered in a 10.83-kb segment. Fragments generated by endonuclease digestions of the segment were cloned into a pHLD69 shuttle vector. The plasmids so constructed were used to transform a colorless (albino) *M. aurum* mutant (strain A11), a brick-red mutant accumulating large amounts of lycopene (strain NgR9), the buff-colored *M. smegmatis* MC2-155, and the buff-colored *M. tuberculosis* H37Ra. From the endonuclease digestion patterns and the phenotypes of the transformed strains, the partial physical and functional maps of a carotenogenesis operon were established. This investigation also showed that the genes controlling the conversion of lycopene into the xanthophylls were not located in the 10.83-kb segment.—Authors' Abstract

Hsieh, P.-C., Shenoy, B. C., Jentoft, J. E. and Phillips, N. F. B. Purification of polyphosphate and ATP glucose phosphotransferase from *Mycobacterium tuberculosis* H37Ra: evidence that poly(P) and ATP glucokinase activities are catalyzed by the same enzyme. Protein Express. Purif. **4** (1993) 76–84.

Polyphosphate [poly(P)$_n$]:D-(+)-glucose-6-phosphotransferase (EC 2.7.1.63) from *Mycobacterium tuberculosis* H37Ra was purified to homogeneity using an improved method which yielded a 634-fold purification with higher recovery. The purified enzyme migrated as a single band with M_r 33-kDa on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The native enzyme was shown to be a dimer by gel filtration using high-performance liquid chromatography (HPLC). The purified enzyme fractionated as a single peak on a C_8 reverse-phase HPLC column and was found to display both polyphosphate- and ATP-dependent glucokinase activities. Further evidence that a single protein was responsible for both activities was shown by nondenaturing PAGE, in which the two activities (as determined by an activity stain in dual experiments) were found to comigrate. The C-terminal analysis yielded a single sequence while the N-terminus which was blocked also yielded a single sequence after deblocking. The two activities were found to have the same temperature optimum of 50°C. The pH optima were 9.5 and 8.6–9.5 with poly(P)$_{32}$ and ATP as the phosphoryl donors, respectively. The ap-

parent K_m for poly(P)$_{32}$ was 18.4 μM while the K_m for ATP was 1.46 mM. In addition, the nucleotide analogue, reactive blue 4, was found to be a competitive inhibitor with ATP in the ATP-dependent glucokinase reaction, while it displayed noncompetitive inhibition patterns with poly(P) in the poly(P)-dependent glucokinase reaction. It is concluded that the poly(P) and ATP glucokinase activities are catalyzed by the same enzyme but that the two substrates may have different binding sites.—Authors' Abstract

Huebner, R. E., Good, R. C. and Tokars, J. I. Current practices in mycobacteriology: results of a survey of state public health laboratories. J. Clin. Microbiol. **31** (1993) 771–775.

Fifty-six state and territorial public health laboratories were surveyed to determine whether currently available rapid methods for the identification and drug susceptibility testing of *Mycobacterium tuberculosis* were being performed. Forty (71%) laboratories use fluorochrome rather than conventional basic fuchsin stains for screening clinical specimens for acid-fast bacilli. Of the 55 laboratories that routinely culture for mycobacteria, 16 (29%) use the more rapid radiometric methods. Species identification of isolates is done by biochemical tests in 13 (23%) laboratories; 40 (72%) use nucleic acid probes, high-performance liquid chromatography, or the BACTEC *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test (rapid tests); 3 laboratories do not perform species identification. Drug susceptibility testing is performed with solid media by 36 of 45 (80%) laboratories, while the more rapid radiometric methods are used by 9 (20%) laboratories. Compared with the laboratories that use conventional methods, laboratories that use rapid methods report results more quickly: for species identification, 43 days (conventional) versus 22 days (rapid); for drug susceptibility testing, 44 days (conventional) versus 31 days (rapid) from specimen processing. Rapid technologies for microscopy and species identification are being used by many, but not all, state and territorial public health laboratories; however, most laboratories do not use the more rapid radiometric methods for routine culture or drug susceptibility testing of mycobacteria. Implementation of such rapid technologies can shorten turnaround times for the laboratory diagnosis of tuberculosis and recognition of drug resistance.—Authors' Abstract

Lalande, V., Truffot-Pernot, C., Paccaly-Moulin, A., Grosset, J. and Ji, B. Powerful bactericidal activity of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* in mice. Antimicrob. Agents Chemother. **37** (1993) 407–413.

The bactericidal activities of various monotherapies and combined regimens were compared in mice at different stages after infection with *Mycobacterium tuberculosis*. These therapies mimicked the initial and continuation phases of chemotherapy for human tuberculosis. As monotherapy, the bactericidal activity of sparfloxacin (SPFX) was dose related; the activity of SPFX at 100 mg/kg of body weight was comparable to that of rifampin (RMP) and was significantly greater than those of isoniazid (INH), pyrazinamide (PZA), or ofloxacin (OFLO) during both the initial and continuation phases of chemotherapy. During the initial phase, the addition of SPFX did not enhance or diminish the activities of the combinations INH-RMP-PZA or RMP-PZA; the combinations SPFX-PZA-streptomycin (SM) and SPFX-PZA-kanamycin (KANA) displayed powerful bactericidal activity. Because the area under the plasma concentration-time curve of SPFX (100 mg/kg) in mice is similar to that of SPFX (400 mg) in humans, the promising bactericidal activity displayed by SPFX in mice might be achieved in humans by administration of the drug in a clinically tolerated dosage. In addition, the combinations SPFX-PZA-SM and SPFX-PZA-KANA may be useful for the treatment of multidrug-resistant tuberculosis.—Authors' Abstract

Mazurek, G. H., Hartman, S., Zhang, Y. S., Brown, B. A., Hector, J. S. R., Murphy, D. and Wallace, R. J., Jr. Large DNA restriction fragment polymorphism in the *Mycobacterium avium-M. intracellulare* complex—a potential epidemiologic tool. J. Clin. Microbiol. **31** (1993) 390–394.

Mycobacterium avium-M. intracellulare complex (MAI) isolates were studied by

comparing the large restriction fragment (LRF) patterns produced by digesting their DNAs with infrequently cutting restriction endonucleases and separating the resultant large fragments by pulsed-field gel electrophoresis. Four reference strains and 35 randomly selected clinical MAI isolates gave highly diverse LRF patterns when their DNAs were digested with *Xba*I or *Asn*I. The LRF patterns of random isolates identified to be the same species by DNA probe analysis were not similar. The LRF patterns of random isolates of the same serotype were also different. In contrast, all isolates recovered from the same patient gave identical patterns. This included 28 isolates from 9 patients. One isolate from sputum, one isolate from bone marrow, and two isolates from blood recovered over a 27-month period from a patient with AIDS were identical. Seven isolates recovered from the sputum of a second patient over 37 months also had identical patterns. The LRF patterns of unrelated MAI strains are highly polymorphic, appear to be strain specific, are relatively stable, and offer exciting promise as epidemiologic markers for the study of MAI infections.—Authors' Abstract

McFadden, J., Bacon, K. and Camp, R. Topically applied verapamil hydrochloride inhibits tuberculin-induced delayed-type hypersensitivity reactions in human skin. J. Invest. Dermatol. **99** (1992) 784–786.

Calcium channel antagonists have been reported to possess inhibitory effects on lymphocyte migration and activation *in vitro* and on cell-mediated immune reactions in the skin of experimental animals. We have therefore studied the effects of topically applied 8% (w/v) verapamil hydrochloride in propylene glycol on tuberculin-induced delayed-type hypersensitivity reactions in the skin of normal human volunteers. There was significant inhibition of the tuberculin reactions by the verapamil preparation compared to vehicle controls, as determined by forearm skin-fold thickness measurement and the assessment of the density of mononuclear cell infiltrates in skin biopsies. The precise mechanism of action of verapamil hydrochloride remains unclear, but could include effects on T-cell migration and activation, on antigen-presenting cells, and/or on other cells. The potential for the use of topical calcium channel antagonist preparations in inflammatory skin diseases warrants further study.—Authors' Abstract

Mor, N. and Heifets, L. MICs and MBCs of clarithromycin against *Mycobacterium avium* within human macrophages. Antimicrob. Agents Chemother. **37** (1993) 111–114.

The inhibitory and bactericidal activities of clarithromycin were determined quantitatively against the intracellular populations of five *Mycobacterium avium* strains growing in monocyte-derived human macrophages. The MICs were 1.0 μg/ml, and the MBCs ranged from 16.0 to 64.0 μg/ml; these values were similar to the MICs and MBCs found in broth cultures at pH 7.4 and were substantially lower than those found in broth cultures at pHs 6.8 and 5.0. Since the intracellular environment has a neutral or even an acidic pH, relatively low MICs and MBCs found in macrophage cultures can be associated with the fact that the drug concentrations in macrophages are substantially higher than those in the medium in which these cells are cultivated. Pretreatment of the macrophages 2 days prior to infection decreased the MICs twofold in comparison with results of experiments in which the drug was added to already infected macrophages.—Authors' Abstract

Orme, I. M., Furney, S. K., Skinner, P. S., Roberts, A. D., Brennan, P. J., Russell, D. G., Shiratsuchi, H., Ellner, J. J. and Weiser, W. Y. Inhibition of growth of *Mycobacterium avium* in murine and human mononuclear phagocytes by migration inhibitory factor. Infect. Immun. **61** (1993) 338–342.

Infections caused by *Mycobacterium avium*, the most common form of disseminated bacterial disease in AIDS patients, are difficult to treat because of their resistance to many antimycobacterial drugs. The results of the present study show that recombinant migration inhibitory factor, a 12-kDa molecule recently isolated by COS-1 cell expression screening of cDNA from a human T-cell hybridoma, has potent inhibitory activity on the growth of a panel of clinical

isolates of *M. avium* within both bone-marrow-derived murine macrophages and cultured human blood monocytes. These cells cultured in recombinant migration inhibitory factor exhibit various signs of activation, including cell division, morphological changes such as evidence of substantial phagolysosomal fusion, and enhanced secretion of tumor necrosis factor.—Authors' Abstract

Rao, S. P., Ogata, K. and Catanzaro, A. *Mycobacterium avium-M. intracellulare* binds to the integrin receptor alpha(V)beta3 on human monocytes and monocyte-derived macrophages. Infect. Immun. **61** (1993) 663–670.

Mycobacterium avium-M. intracellulare is an intracellular pathogen responsible for the highest incidence of disseminated bacterial infection in patients with AIDS. Treatment of the infection is difficult and has been of limited efficacy. Attachment of the organism to macrophages is a critical early step in the establishment of the disease. In the present study, we isolated and identified a receptor that mediates the attachment of *M. avium-M. intracellulare* to human peripheral blood monocytes and monocyte-derived macrophages. On Western blotting (immunoblotting), the receptor was found to crossreact with antibodies against a human vitronectin receptor (alpha(V)beta3). The receptor could be purified from monocyte extracts by using monoclonal antibodies (MAbs) against the alpha(V) subunit of vitronectin receptor coupled to CNBr-Sepharose 4B, as well as with the adhesive tripeptide sequence arginine-glycine-aspartic acid (RGD) coupled to CNBr-Sepharose 4B. Surface-bound MAbs directed against alpha(V)beta3 were found to inhibit the attachment of *M. avium-M. intracellulare* to monocyte-derived macrophages in an *in vitro* inhibition assay, while MAbs directed against CD14, CD18, alpha2beta1 and platelet glycoprotein gpIIb/IIIa receptors did not inhibit this attachment. These observations suggest that alpha(V)beta3 on the surface of human monocytes and monocyte-derived macrophages may function as a receptor for *M. avium-M. intracellulare.* Identification of a receptor for *M. avium-M. intracellulare* on macrophages may offer new approaches to the prevention and control of *M. avium-M. intracellulare* infection at the cellular level.—Authors' Abstract

Ross, B. C. and Dwyer, B. Rapid, simple method of typing isolates of *Mycobacterium tuberculosis* by using the polymerase chain reaction. J. Clin. Microbiol. **31** (1993) 329–334.

To develop a molecular typing method for *Mycobacterium tuberculosis* based on the polymerase chain reaction (PCR), oligonucleotide primers were designed to the ends of the insertion sequence IS6110 in an attempt to amplify DNA between clusters of this element on the genome. Although in many strains the copy number of this element is low and is distributed throughout the genome, most strains examined produced a banding pattern which varied between isolates including strains with one copy of IS6110. With strains isolated from patients in epidemiologic clusters of tuberculosis, the banding patterns were similar within each cluster but distinct from those in strains from different clusters. Similarly, multiple isolates from the same patient yielded a consistent banding pattern. Sequencing of four PCR products revealed that amplification was occurring between copies of IS6110 in two of the products and from a single copy of IS6110 to a nonspecific priming site in the other two. This technique provides a rapid and simple means of typing *M. tuberculosis* isolates for epidemiologic studies.—Authors' Abstract

Schroder, K. H., Kazda, J., Muller, K. and Muller, H. J. Isolation of *Mycobacterium simiae* from the environment. Zentralbl. Bakteriol. **277** (1992) 561–564.

An examination of 18 sphagnum samples collected in two different biotopes of the coastal region of southeastern Madagascar revealed an unexpectedly high positivity for mycobacteria (83.3%). The concentration of alcohol-acid-fast bacilli reached a high level of 10^5 and 10^6/g, respectively, compared with the sphagnum biotopes in moderate climates. Besides the habitat-specific mycobacterial species in sphagnum vegetation, such as *Mycobacterium sphagni, M. gor-*

donae and *M. madagascariense,* potentially pathogenic species, like *M. avium, M. scrofulaceum* and *M. xenopi* and *M. marinum,* were found. Furthermore, pathogenic *M. simiae* was found in sphagnum vegetation of Madagascar, first time isolated in the environment until now. It should be considered as a potential source of infection for human and animals.—Authors' Abstract

Serfling, U., Penneys, N. S. and Leonardi, C. L. Identification of *Mycobacterium tuberculosis* DNA in a case of lupus vulgaris. J. Am. Acad. Dermatol. **28** Suppl. 2 Part 2 (1993) 318–322.

A variety of cutaneous lesions are believed to result from the presence of *Mycobacterium tuberculosis.* Demonstration of *M. tuberculosis* directly or in culture in some of these eruptions can be difficult. We studied a typical case of lupus vulgaris that had been followed for several years with frequent unrewarding biopsies and cultures to see if *M. tuberculosis* DNA could be demonstrated in skin-biopsy specimens. We used the polymerase chain reaction (PCR) and a primer/probe set specific for a region in the gene for the 65-kDa antigen of *M. tuberculosis* to search for *M. tuberculosis* complex DNA. *M. tuberculosis* complex DNA was demonstrated in archival skin-biopsy specimens from the lesion of lupus vulgaris. The PCR and specific primer/probe sequences can be used to demonstrate *M. tuberculosis* complex DNA in skin lesions. A variety of skin lesions believed to be related to tuberculosis (tuberculids) can be revisited with these techniques and studied for the presence of an infectious agent.—Authors' Abstract

Takewaki, S., Okuzumi, K., Ishiko, H., Nakahara, K., Ohkubo, A. and Nagai, R. Genus-specific polymerase chain reaction for the mycobacterial dnaJ gene and species-specific oligonucleotide probes. J. Clin. Microbiol. **31** (1993) 446–450.

Identification of tuberculous and nontuberculous mycobacteria by biochemical methods is a long-term process that takes up to 8 weeks for completion and requires expertise to interpret the results. In order to detect and differentiate the major pathogenic mycobacterial species, we developed genus-specific primers that amplify the dnaJ gene from the broad spectrum of mycobacterial species and determined the nucleotide sequences within the dnaJ genes from 19 mycobacterial species (*Mycobacterium tuberculosis, M. bovis, M. bovis* BCG, *M. africanum, M. microti, M. kansasii, M. marinum, M. gastri, M. simiae, M. scrofulaceum, M. szulgai, M. gordonae, M. avium, M. intracellulare, M. xenopi, M. fortuitum, M. chelonei, M. haemophilum,* and *M. paratuberculosis*). On the basis of the dnaJ gene sequences, we developed dot-blot hybridization analysis with species-specific oligonucleotide probes for the *M. tuberculosis* complex, *M. avium, M. intracellulare,* and *M. kansasii,* allowing a rapid identification of these species following polymerase chain reaction (PCR) for the dnaJ gene. We conclude that PCR with the genus-specific primer that amplifies the dnaJ genes and subsequent dot-blot analysis with species-specific oligonucleotide probes are most useful for differential diagnosis of tuberculosis and nontuberculous mycobacterial infections.—Authors' Abstract

Telenti, A., Marchesi, F., Balz, M., Bally, F., Bottger, E. C. and Bodmer, T. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31** (1993) 175–178.

A method for the rapid identification of mycobacteria to the species level was developed on the basis of evaluation by the polymerase chain reaction (PCR) of the gene encoding for the 65-kDa protein. The method involves restriction enzyme analysis of PCR products obtained with primers common to all mycobacteria. Using two restriction enzymes, BstEII and HaeIII, medically relevant and other frequent laboratory isolates were differentiated to the species or subspecies level by PCR-restriction enzyme pattern analysis. PCR-restriction enzyme pattern analysis was performed on isolates (N = 330) from solid and fluid culture media, including BACTEC, or from frozen and lyophilized stocks. The procedure does not involve hybridization steps or the use of

radioactivity and can be completed within 1 working day.—Authors' Abstract

Thompson, P. J., Cousins, D. V., Gow, B. L., Collins, D. M., Williamson, B. H. and Dagnia, H. T. Seals, seal trainers, and mycobacterial infection. Am. Rev. Respir. Dis. **147** (1993) 164–167.

In 1986, three seals died in a marine park in Western Australia; culture of postmortem tissue suggested infection with *Mycobacterium bovis*. In 1988, a seal trainer who had been employed at the Western Australian marine park until 1985 developed pulmonary tuberculosis caused by *M. bovis* while working in a zoo 3000 km away on the east coast of Australia. Culture characteristics, biochemical behavior, sodium dodecyl sulfate polyacrylamide gel electrophoresis, and restriction endonuclease analysis suggested that the strains of *M. bovis* infecting the seals and trainer were identical but unique and differed from reference strains and local cattle strains of *M. bovis*. The infection in both the seals and the trainer had a destructive but indolent course. This is the first time that *M. bovis* has been observed in seals and the first time that tuberculous infection has been documented to be transmitted from seals to humans. Further investigation of the extent of tuberculous infection in seal populations elsewhere in the world seems warranted, and those working with seals and other marine animals should be monitored for infection.—Authors' Abstract

Tuberculosis control and research strategies for the 1990s: memorandum from a WHO meeting. Bull. WHO **70** (1992) 17–21.

Tuberculosis is the largest cause of death from a single infectious agent in the world, killing nearly 3 million people every year. This death toll represents 25% of avoidable adult deaths in developing countries. It imposes a heavy burden on the 8 million new individuals who contract the disease each year, and on their households; morbidity and mortality are concentrated in young adults. The association of tuberculosis and HIV infection will significantly exacerbate the situation in developed and developing countries, making the need for action all the more pressing. Effective control measures are available. Broad action is therefore warranted and should be aimed at introducing the effective strategies on as wide a scale as possible to reach the targets of 70% case detection and 85% cure of smear-positive patients by the year 2000. Research is needed to implement these strategies throughout the world and to ensure that effective tools will remain available for controlling tuberculosis despite emerging problems such as resistance to the major drugs currently available. To make a real impact on the tuberculosis problem, a focused global program must be created, under the leadership of WHO, to bring tuberculosis to the world's attention, to mobilize support on a major scale, and to provide direct guidance and support to national programs.—AS (Trop. Dis. Bull.)

van Embden, J. D., Cave, M. D., Crawford, J. T., Dale, J. W., Eisenach, K. D., Gicquel, B., Hermans, P., Martin, C., McAdam, R., Shinnick, T. M. and Small, P. M. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. **31** (1993) 406–409.

DNA fingerprinting of *Mycobacterium tuberculosis* has been shown to be a powerful epidemiologic tool. We propose a standardized technique which exploits variability in both the number and genomic position of IS*6110* to generate strain-specific patterns. General use of this technique will permit comparison of results between different laboratories. Such comparisons will facilitate investigations into the international transmission of tuberculosis and may identify specific strains with unique properties such as high infectivity, virulence, or drug resistance.—Authors' Abstract

Van Rensburg, C. E. J., Van Staden, A. M. and Anderson, R. The riminophenazine agents clofazimine and B669 inhibit the proliferation of cancer cell lines *in vitro* by phospholipase-A2-mediated oxidative and nonoxidative mechanisms. Cancer Res. **53** (1993) 318–323.

Clofazimine, a riminophenazine antimicrobial agent, and its analog B669 were investigated for their effects on FaDu cells, a

human squamous carcinoma cell line. These agents, at concentrations within the therapeutic range (0.25–2 μg/ml), caused a dose-dependent tumor cell cytotoxicosis which was greatly enhanced in the presence of human neutrophils. The neutrophil-mediated increment in tumoricidal activity, but not the direct antitumor effects of the drugs *per se,* was inhibited by catalase. The effects of these drugs on three more cell carcinoma lines as well as on two primary cultures and a noncarcinoma cell line were also investigated and compared with the activity of the standard antitumor chemotherapeutic agents bleomycin, cisplatin, and methotrexate. All 7 cultures were sensitive to clofazimine and B669 compared to 6 that were sensitive to cisplatin, 3 that were sensitive to bleomycin, and 1 that was sensitive to methotrexate. The treatment of FaDu cells with clofazimine and B669 was associated with enhanced activity of phospholipase A2, as evidenced by increased release of radiolabeled arachidonate and lysophosphatidylcholine from membrane phospholipids. Inhibitors of arachidonic acid metabolism, protein kinase C inhibitors, as well as water and lipid soluble antioxidants failed to protect the cells against the cytotoxic activity of clofazimine and B669. However, alpha-tocopherol, a lysophospholipid-complexing agent, completely blocked the antiproliferative effects of the riminophenazines and also protected the cells against the direct cytotoxic effect of lysophosphatidylcholine, while the lysophospholipid-neutralizing enzyme lysophospholipase protected against the riminophenazines. These observations demonstrate that the tumoricidal properties of clofazimine and B669 are probably due to increases in the lysophospholipid content of cell membranes.—Authors' Abstract