# CURRENT LITERATURE

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

## General and Historical

**Bainson, K. A.** Integrating leprosy control into primary health care: the experience in Ghana. Lepr. Rev. **65** (1994) 376–384.

Integration of leprosy control into primary health care is the most comprehensive and permanent system of delivering care to leprosy patients. But so far only a few countries have adopted this approach, largely on account of a fear of failure.

Over the past decade Ghana has developed a model approach toward the transition from a vertical to an integrated program. The highlights of our approach included the development of the leprosy service as part of the overall development of the health service, increasing capacity building for leprosy control at the district and subdistrict levels as well as the establishment of regular and effective monitoring to identify and correct operational problems early.

This paper describes the principles behind the integration, the strategies adopted and how they were implemented. It also includes the achievements made as well as the problems that were encountered and how they were solved.—Author's Summary

**Feenstra, P.** Will there be a need for leprosy control services in the 21st century? (Editorial) Lepr. Rev. **65** (1994) 297–299.

"With the reducing number of patients the cost per patient cured will increase. WHO has roughly estimated the direct cost for the health services to diagnose and cure a leprosy patient under different prevalence situations. With a prevalence of more than 10 per 10,000 the average costs for a PB patient are US$30 and for an MB patient $150; at a prevalence rate of about 5 per 10,000 the costs are $70 and $280, respectively, and at a prevalence below 1 per 10,000 $100 for a PB patient and $400 for an MB patient. It may be expected that with the declining incidence and prevalence of leprosy and, thus, the decreasing relative importance of leprosy as a public health problem, governments in the endemic countries will make less funds available for leprosy control. In order to achieve eradication there will be a continuing need during the next decades for technical and financial resources from international donor agencies. Here we face the danger that the recent success of leprosy control may have negative effects on fund raising by the NGOs. It is usually not a problem to sell a success story, but this may become the case in leprosy control. Leprosy control should not become a victim of its own success, just as we are getting close to our goal to eradicate the disease. Therefore, whenever the elimination goal is presented it should be made clear that even when this goal is attained, there will continue to be significant numbers of (new) cases of leprosy and people with severe psychological, economical and social problems caused by leprosy who need assistance. Leprosy will not be under control when the 'elimination' goal has been achieved."—From the Editorial

**Kaur, H. and Ramesh, V.** Social problems of women leprosy patients—a study conducted at 2 urban leprosy centres in Delhi. Lepr. Rev. **65** (1994) 361–375.

Leprosy seems to afflict women less commonly than men, but for cultural reasons this difference may be more apparent than real. Unfortunately, the effects are equally as devastating, if not more so, in women than in men. This study, carried out at the Urban Leprosy Centres of Safdarjung Hospital and Dr. Ram Manohar Lohia Hospital in Delhi, showed that the impact of stigma attached to leprosy had more effect on educated women belonging to a higher socio-

economic group than on less-fortunate women. Discriminative attitudes were more common in joint than nuclear families. Although many got support from their families, the disease had definite psychological effects. Because of the fear of infecting the family members, women sufferers kept themselves aloof and were constantly worried about divorce. Fear of social ostracism prevented the disclosure of disease to the community. Deformities and disabilities led to a deterioration in their functional capabilities and their psychological state of mind. Pregnancy did not affect regularity of treatment. Many women needed an escort to attend the clinic. Solutions to minimize some problems have been suggested.—Authors' Summary

**Sommerfeld, P.** Voluntary donor agencies in antileprosy work; present contribution and probable future. (Editorial) Lepr. Rev. **65** (1994) 1–8.

This 8-page editorial reviews, in considerable detail, the activities and contribution of the International Federation of Anti-Leprosy Associations (ILEP) over the past 27 years. The opening paragraphs emphasize that the account deals with voluntary donor agencies only and does not include the extremely important contributions made by many local organizations in leprosy-endemic countries. The author is General Secretary of ILEP, but a footnote makes it clear that he is writing as an individual, recording personal views which do not necessarily represent those of ILEP members. The main headings include: (1) the financial contribution, (2) coordination of support, (3) public health, targets and the humanitarian imperative, (4) when is a case not a case?, (5) progress toward the ILEP target of multiple drug therapy (MDT) for all, (6) the continuing load, (7) action on disability: are the targets possible?, (8) supporting systems or projects: the dilemma of associations.

The final section, "A lasting commitment to leprosy," refers to a recent consultation with ILEP members on the possible need for changes in the Federation as such and to their overwhelming decision against making any changes at this stage. The section also refers to the continuing commitment of most agencies mainly to leprosy work, although a few have now embraced tuberculosis, dermatology or general disability/rehabilitation. ILEP is committed to the provision of multiple drug therapy (MDT), essentially as advised by WHO in 1982, for all cases in need by the year 2000, and to do all possible to prevent disability (or to manage it as best possible, once it is established), while at the same time giving considerable attention to humanitarian aspects. The Federation spent over US$70 million on various projects in 1993 [and has budgeted to spend $67 million in 1994, thus maintaining leprosy in a remarkably high position in the "external aid flow list" compared with tropical diseases, diarrhoea, malaria and tuberculosis].—A. C. McDougall (Trop. Dis. Bull.)

## Chemotherapy

**Bharti, R.** Pefloxacin in leprosy. Indian J. Lepr. **66** (1994) 443–448.

Fluoroquinolones, a new class of compounds characterized by a broad antimicrobial spectrum, including mycobacteria together with limited toxicity, recently have been introduced in the chemotherapy of various human infectious diseases. Pefloxacin, one of the members of this class, was recently demonstrated to be bactericidal against *Mycobacterium leprae* in the mouse foot pad model and clinically beneficial in lepromatous leprosy patients. Clinical response to standard MDT with added pefloxacin in 10 previously untreated patients (both PB and MB) was compared with that in 10 similar patients on MDT alone in the present trial. The results of chemotherapy were quantified by a method of clinical scoring. This pilot study showed that the addition of pefloxacin led to significant and

rapid clinical improvement. There were no side effects attributable to pefloxacin.—Author's Abstract

**Costa, H. C., Opromolla, D. V. A., Madeira, S., Marques, F. B., Martelli, A. C. C. and Ura, S.** [Prevalence of sulfone resistance in patients with hanseniasis in the city of Bauru, state of Sao Paulo (Brazil)]. Hansen. Int. **18** (1993) 5–10. (in Portuguese)

The aim of this study was to determine the prevalence of dapsone (DDS) resistance in lepromatous leprosy patients in Bauru, São Paulo. In a population of 349 such patients (205 with more than 5 years of treatment and thus at high risk of developing resistance) 30 (14.63%) showed clinical signs of possible resistance (BI of 3+ or higher). Ten patients presented bacilli resistant to DDS (2.86%). For this reason, the authors conclude that nationwide implementation of MDT is of the utmost importance.—Authors' English Summary

**de Rijk, A. J., Gabre, S., Byass, P. and Berhanu, T.** Field evaluation of WHO-MDT of fixed duration, at ALERT, Ethiopia: the AMFES project—I. MDT course completion, case-holding and another score for disability grading. Lepr. Rev. **65** (1994) 305–319.

We report on 286 new leprosy patients (128 PB, 158 MB) enrolled in the AMFES project, a field study in which patients are monitored during WHO-MDT, and during 5 years thereafter, by active surveillance. The first paper describes the purposes, organization and methods of the study, patient enrollment and preliminary results of MDT completion and case-holding.

Of 128 PB patients 102 (79.7%) completed MDT and of 91 on surveillance for more than 1 year, coverage with reviews had been good or very good for 31, fair or poor for 36, and very poor or nil for 21 PB patients. Of 158 MB patients 64 had completed MDT, and 26/128 (20.3%) PB and 18/158 (11.4%) MB patients were lost to follow up during treatment, with 76 MB patients still on treatment.

At first diagnosis, 159/286 (55.6%) had nerve function impairment, with no significant differences in disability grade by gender or between PB and MB patients. The proportion of disability grade 0 among new cases decreased very significantly with age, from 28/41 (68.3%) for age 0–14 years to 13/57 (22.8%) for 50 years and above. In view of the limitations of patient disability grades, a score per patient of the sum of disability grades for the four extremities, named "HF-impairment score," is shown to be more informative.

Incidence of leprosy reactions and neuritis in these patients, during treatment and during surveillance, is reported upon in Part II.—Authors' Summary

**de Rijk, A. J., Gabre, S., Byass, P. and Berhanu, T.** Field evaluation of WHO-MDT of fixed duration, at ALERT, Ethiopia: the AMFES project—II. Reaction and neuritis during and after MDT in PB and MB leprosy patients. Lepr. Rev. **65** (1994) 320–332.

For a cohort of 286 leprosy patients the incidence rates and clinical manifestations of leprosy reactions during treatment and surveillance are described. Currently, individual patients had been observed for up to 4 years. It is intended that surveillance within this project should continue for up to 5 years after treatment. Of 128 PB patients observed for 267 person-years (mean 2.1), 27 had 35 episodes of reaction, corresponding to an overall incidence rate of 131 events per 1000 person-years-at-risk (pyar). Of 158 MB patients observed for 402 person years (mean 2.5), 64 had 114 reactions, with an overall incidence of 284 events per 1000 pyar. For both PB and MB patients, incidence rates during treatment and post-MDT surveillance were similar. For PB patients, pre-existing physical impairment at the start of MDT was a significant risk factor for the occurrence of subsequent events, but this was not found in MB patients.—Authors' Summary

**Dhople, A. M., Ibanez, M. A. and Dhople, A. A.** *In vitro* activities of 2,2′-bipyridyl analogs against *Mycobacterium leprae.* Antimicrob. Agents Chemother. **38** (1994) 2908–2909.

*In vitro* susceptibility of *Mycobacterium leprae* to two bipyridyl analogs was studied by using two biochemical parameters to measure the metabolic activity of the or-

ganism. VUF-8514 at 0.16 μg/ml, but not VUF-8842, completely inhibited the metabolic activity of *M. leprae,* and the action was bactericidal. When compared to rifampin (MIC 0.3 μg/ml), VUF-8514 was equally bactericidal against *M. leprae.*—Authors' Abstract

**dos Santos, S. N. M. B., Araujo, M. G., Patrus, O. A. and Aguilar, C. R.** [Thrombocytopenia associated with the use of sulfones?] An. Bras. Dermatol. **69** (1994) 513–515. (in Portuguese)

Thrombocytopenia rarely has been observed in association with sulfones. We report a case of an adult male who developed thrombocytopenia during treatment of hanseniasis with dapsone. His platelet counts returned near to normal values after sulfone was substituted by clofazimine.—Authors' English Summary

**Gelber, R. H.** Chemotherapy of lepromatous leprosy: recent developments and prospects for the future. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 942–952.

Leprosy is a major debilitating infectious disease, primarily of the developing world. In this paper the current status and future prospects of antimicrobial therapy of the severe anergic lepromatous form of the disease are reviewed. Until the last few years only dapsone, rifampin, clofazimine and ethionamide have had practical application in its therapy and only rifampin was bactericidal. Recently, antibiotics from three different classes have been found to be bactericidal in lepromatous patients: a tetracycline (minocycline), a macrolide (clarithromycin), and several fluoroquinolones (including pefloxacin, ofloxacin and sparfloxacin). Against a background of drug resistance and bacterial persistence, recommendations for multidrug therapy and the means to devise rationally based therapy for the future are discussed.—Author's Abstract

**Guo, Q., *et al.*** [Effect of MDT on endemicity of leprosy.] China Lepr. J. **10** (1994) 159–160. (in Chinese)

Ten years after implementation of MDT in Guangxi Autonomous Region, the finding rate of leprosy decreased from 1.8 to 0.65/100,000; the incidence from 1.39 to 0.04/100,000 and the prevalence from 0.06 to 0.005‰. The counties without leprosy patients have increased from 0 to 80. The authors believe that decline of leprosy there was due to the action of MDT and other factors in concert.—Authors' English Abstract

**Huang, W.-J., *et al.*** [Leprosy control in Xinjuang (China) since 1986.] China Lepr. J. **10** (1994) 158–159. (in Chinese)

An overall leprosy control program with the use of MDT has been launched in Xinjiang Autonomous Region since 1986. Up to 1992, 1360 persons cured of leprosy with DDS monotherapy were retreated with MDT, of which no relapse was seen after follow up of 2 to 6 years. The number of active patients decreased from 345 to 71, the prevalence from 0.024% to 0.005%, and those who had disability Grade II and III made up 12.8% of the new cases.—Authors' English Abstract

**Jakeman, P. and Smith, W. C. S.** Evaluation of a multidrug therapy programme of leprosy control. (Editorial) Lepr. Rev. **65** (1994) 289–296.

MDT programs for leprosy control have two objectives, controling leprosy in populations and controling leprosy in individuals. Evaluation of such programs needs to address both objectives and this can be done by a review of the trends in key indicators and by site visits. Site visits are more expensive and should be done less frequently, but they can reveal issues not apparent in routinely produced statistics. Evaluation on an annual basis is the responsibility of program managers and program funders. Evaluation by program staff themselves should be encouraged and supported.

Evaluation of an MDT program's effectiveness in controling leprosy in a population should be by analysis of case detection as a proxy for incidence. Prevalence rates will continue to be monitored because of the WHO elimination goal, but these do not reflect disease transmission. Case detection is a proxy measure of incidence and depends on consistency in case detection activities. Case detection data by age, gender, mode

of detection, disability ratio and lepromatous (MB) rate need to be analyzed over at least 5 years and preferably 10 years to give an indication of trends in incidence. Caution is needed, however, as the pattern seen when case detection deteriorates may resemble the pattern expected when transmission is reduced. The site visit is important in this situation in allowing examination of the case detection activities, as well as in looking for new, undetected cases in the population.

Evaluation of the MDT program's effectiveness in controling leprosy in patients should be by analysis of both the effectiveness of MDT delivery and the changes in disability. For drug delivery, MDT coverage of new and registered patients is used, but this only reflects the basic minimum of treatment, that each patient has started MDT. MDT completion rates are the best indicators, with PB rates reflecting the situation in the previous year, and MB rates the longer-term position. In monitoring disability, WHO gradings are of limited use in assessing change, and are not always recorded. If they were available for the start and end of each patient's treatment it would give a crude indicator of the program's effectiveness in preventing disabilities. Better methods have not yet been proved to be either adequately reproducible or simple enough for PHC-based programs. More research is urgently needed in this field. It may be that simple counts of sole wounds will prove to be the most suitable indicator of effectiveness in the prevention and treatment of disabilities. A site visit will help to reveal what is actually going on in the area of prevention of disabilities as well as in treatment delivery. Remember that it is always worthwhile speaking to the patients and not only to the staff!—Authors' Summary

**Lowe, N. J., Fakouhi, T. D., Stern, R. S., Bourget, T., Roniker, B. and Swabb, E. A.** Photoreactions with a fluoroquinolone antimicrobial: evening versus morning dosing. Clin. Pharmacol. Ther. **56** (1994) 587–591.

Quinolone antimicrobials absorb ultraviolet radiation and, with appropriate drug concentrations, may cause photoreactions. Photoreactions have been reported for several quinolones, including lomefloxacin, a difluorinated quinolone antimicrobial. This study was designed to determine whether the interval between administration of lomefloxacin and exposure to ultraviolet A (UVA) light would affect skin responses. The minimal erythema dose (MED) and severity of local reactions were the main parameters of evaluation. Exposure to UVA radiation 2 hr after morning dosing caused an increase in skin sensitivity as assessed by changes in MED ($p < 0.05$). No changes were observed with exposure 16 hr after evening dosing ($p = 1.00$). Edema and blisters at the radiation sites were observed in only the morning dosing group. A significant negative correlation was observed between lomefloxacin plasma concentrations and change MEDs ($r = -0.72$; $p < 0.05$). An evening dosing strategy may minimize the risk of phototoxic effects.—Authors' Summary

**Naik, S. S., Vartak, R. R. and Sequiera, E. B.** Improving patient compliance—a multicentre evaluation of the DDS tile test. Indian J. Lepr. **66** (1994) 473–475.

The feasibility and utility of the "DDS tile test" under field conditions was assessed in 112 leprosy centers in Maharashtra. About 10% of the 2952 urine samples tested negative for dapsone. Feed-back information from 54 centers 1 year later showed that the test could be performed easily under field conditions and also that counseling of patients showing poor compliance helped to improve drug compliance in over 80% of the cases.—Authors' Abstract

**Opromolla, D. V. A., Costa, H. C. and de Oliveira, P. R. D.** [Secondary multiple drug resistance in hanseniasis.] Hansen. Int. **18** (1993) 11–16. (in Portuguese)

Four patients with leprosy bacilli secondarily resistant to two drugs of WHO-MDT (rifampin and dapsone) are reported. One patient's bacilli were also resistant to ethionamide. The authors discuss a development of this multidrug resistance and the possibility of its primary occurrence. They remind what is happening in the U.S. regarding tuberculosis, where cases with initial multidrug resistance have been shown, probably in most cases due to AIDS, and

admit the risk of the appearance of this same situation in leprosy. Finally, they warn of the undue use of new drugs, easily available in pharmacies, which are yet under experiment for leprosy treatment.—Authors' English Abstract

**Poenca, N. G.** [Use of thalidomide in dermatology.] An. Bras. Dermatol. **70** (1995) 61–67. (in Portuguese)

A review of the use of thalidomide in dermatology. Between 1965 and 1982 thalidomide had already been accepted and employed in the treatment of the following diseases: erythema nodosum leprosum, actinic prurigo, relapsing aphthosis, Behçet's disease, discoid lupus erythematosis. More recently (1988–1993), thalidomide has been indicated for the treatment of two more conditions: recurrent painful ulcers in patients with acquired immunodeficiency syndrome (HIV-positive) and chronic graft-versus-host disease. Some proven mechanisms of the activity of thalidomide have been highlighted: (a) for inhibiting the production of tumor necrosis factor-alpha (TNF-alpha); (b) for changes in surface receptors, such as integrin and other adhesion receptors, in CD4 lymphocytes and in leukocytes; (c) for decreasing the CD4 lymphocytes in the peripheric circulating blood.—Authors' English Summary

**Ramu, G.** The past, present and future of chemotherapy of leprosy. NLO Bull. **23** (1994) 37–49.

1. MDT and the fixed duration of treatment have resulted in a fast decline of the prevalence of leprosy all over the world. Relapses in multibacillary leprosy would only be occurring now in many of the centers since the median incubation period for LL/BL relapses is 9 years. Unfortunately the surveillance for M.B. leprosy has been fixed as 5 years. Therefore most of these relapses will go unnoticed. Similarly the surveillance period for paucibacillary leprosy has been fixed as 2 years whereas most relapses occur at 3 years.

2. There has been an over-emphasis on reversal reactions. Relapses can manifest as BT lesions. There may be even some response to steroids. But the subsidence is never complete in relapses. The disease in the nerve along with the skin lesion progress, since steroids do not stem the disease process, and patients are seen to develop deformities because of this misunderstanding. Denying patient proper treatment and allowing him to get deformed is not in keeping with good medical practice.

3. Stopping treatment in BL/LL patients at 2 years when the BI is 3 or above would be inviting the persisters to multiply and produce relapses; they would also act as reservoirs of infection. Patients with a BI of 3 and over would be only a few in number in any one given center and the treatment can be continued till the BI comes to 2.5. If it does not come down multidrug resistance should be suspected and investigated.

4. Paucibacillary patients should be examined at 6 months before releasing from treatment for signs of regression. If the lesions have not regressed or show extension or new lesions are observed skin smears should be taken. There might have been a mis-categorization of M.B. cases as P.B.

5. Poor compliance for self-administered drugs is observed in 30% of cases even in the best centers. For paucibacillary cases this is not serious. In multibacillary patients this is serious. Some improvement can be achieved by an injection of 1 g streptomycin along with the supervised pulse of rifampicin.

6. In patients whose BI is high and who get repeated reactions immunotherapy with the available vaccine might be of help.

7. For future therapy ofloxacin and minocyclin are not suitable for women and children. Clarithromycin and sulbactam ampicillin, dapsone + brodimoprim need to be studied carefully using both mouse footpad and ATP measurement in selected centers before multi-centric studies are undertaken without such monitoring. The criterion of cure is the absence of living or multiplying bacilli in the tissues of a person so as to ensure freedom from relapse.—Author's Conclusions

**Rao, P. S., Subramanian, M. and Subramanian, G.** Deformity incidence in leprosy patients treated with multidrug therapy. Indian J. Lepr. **66** (1994) 449–454.

The records of 2285 [2007 paucibacillary (PB) and 278 multibacillary (MB)] cases of

leprosy which were declared as released from treatment (RFT) after multidrug therapy (MDT) and under surveillance, as per the National Leprosy Eradication Programme (NLEP) guidelines in the rural field practice area of Central Leprosy Teaching & Research Institute (CLTRI), Chengalpattu, India, between September 1986 and September 1993, were analyzed for collecting data on the incidence of deformity. Of the 2285 cases 2053 (1947 PB and 106 MB) did not have deformity at the commencement of treatment. Three MB cases and one PB case out of the 2053 developed deformity (all grade II) during the course of treatment. No patient developed deformity during surveillance. Thus, the deformity incidence in the population of patients was 0.681 per 1000 person-years of observation. Age, sex, type of disease, prior dapsone monotherapy and nerve involvement at the commencement of treatment appear to influence the deformity incidence. The risk of development of deformity in patients treated with MDT appears to be very low and analysis of larger data sets is suggested to corroborate the above findings as the information would be useful for planning prevention and management of deformity services.—Authors' Abstract

**Rhodes, L. E., Tingle, M. D., Park, B. K., Chu, P., Verbov, J. L. and Friedmann, P. S.** Cimetidine improves the therapeutic/toxic ratio of dapsone in patients on chronic dapsone therapy. Br. J. Dermatol. **132** (1995) 257–262.

We have previously shown that cimetidine, given concurrently for 2 weeks to patients on chronic dapsone therapy, reduced methemoglobinemia by inhibiting the formation of the toxic hydroxylamine metabolite of dapsone. The aim of the present study was to examine the effect of this combination on the benefit/toxic ratio of dapsone over a longer period.

Eight patients (six dermatitis herpetiformis, one linear IgA disease, one folliculitis decalvans) on long-term dapsone 50–100 mg daily, took cimetidine 1.6 g daily concurrently for 3 months. At 3-weekly intervals, a clinical assessment was made, plasma dapsone and methemoglobin were measured, and parameters of oxidative hemolysis were monitored. The dapsone level rose from 2298 ± 849 ng/ml (mean ± S.D.) at baseline to 3006 ± 1131 ng/ml at week 3 of cimetidine ($p < 0.01$). This rise in plasma dapsone was sustained during cimetidine administration, falling to 2446 ± 954 ng/ml when cimetidine was stopped ($p < 0.02$). The methemoglobin fell from 5.5 ± 2.2% (mean ± S.D.) at baseline to 3.9 ± 1.1% at week 3 ($p < 0.01$), and remained low until week 12, when there was a return to baseline values ($p < 0.01$). The hemoglobin did not change from the baseline of 12.7 ± 0.3 g/dl (mean ± S.D.), and other parameters of hemolysis were unaltered. There was a fall in the visual analog score for headache ($p < 0.05$), but this was not associated with any deterioration in control of the skin disorders.

Hence, long-term concurrent cimetidine results in increased plasma dapsone levels without increased hemolysis, and is accompanied by reduced methemoglobinaemia for more than 2 months. Cimetidine thus improves the therapeutic/toxic ratio of dapsone. Such a therapeutic strategy may be appropriate for patients who require high-dose dapsone, or those who are particularly susceptible to dapsone-induced hemotoxicity.—Authors' Abstract

**Ronn, A. M., Lemange, M. M., Angelo, H. R. and Bygbjerg, I. C.** High-performance liquid chromatography determination of dapsone, monoacetyldapsone, and pyrimethamine in filter paper blood spots. Therapeut. Drug Monitor. **17** (1995) 79–83.

A high-performance liquid chromatography method for the simultaneous analysis of dapsone (DDS), the major metabolite of DDS, monoacetyldapsone (MADDS), and pyrimethamine (PYR) was modified for capillary blood samples obtained by finger prick and dried on filter paper. Limit of quantitation using 150 μl whole blood dried on filter paper was found to be 20 ng/ml for DDS and PYR and 15 ng/ml for MADDS (precision < 15%). The clinically relevant concentrations of DDS are 50–2000 ng/ml and for PYR 25–150 ng/ml. No interference from several drugs was observed. The accuracy of the filter paper method and the original whole-blood method was almost

comparable. Standardization could, therefore, be obtained by the more simple whole-blood method. Dried filter paper samples stored at 19–22°C were stable for months and for 2 weeks stored at 35°C. The concentrations of simultaneously collected capillary blood and conventional venous blood samples correlated well. The present method using capillary blood dried on filter paper is reliable, simple, sensitive, and applicable in the field with limited technical facilities.—Authors' Abstract

**Rubenstein, E., Dautrey, S., Farinoti, R., St. Julien, L., Ramon, J. and Carbon, C.** Intestinal elimination of sparfloxacin, fleroxacin, and ciprofloxacin in rats. Antimicrob. Agents Chemother. **39** (1995) 99–102.

The intestinal transepithelial elimination of sparfloxacin and fleroxacin was compared with that of ciprofloxacin in a rat model following a single parenteral administration of 25 mg of each of the antibiotics per kg of body weight. All three fluoroquinolones were eliminated through the small intestine. Ciprofloxacin was eliminated in the proximal jejunum at a rate of 1.97 ± 0.70 $\mu g/cm^2$, while the elimination rates of fleroxacin and sparfloxacin were 0.64 ± 0.26 and 0.21 ± 0.10 $\mu g/cm^2$, respectively, over a 90-min collection period. In the ileum, the elimination rates of ciprofloxacin, fleroxacin, and sparfloxacin over the same period were 1.44 ± 0.77, 1.00 ± 0.33, and 0.41 ± 0.26 $\mu g/cm^2$, respectively. These data suggest that these fluoroquinolones undergo a transepithelial elimination process in the small intestine. This route of elimination may be important in the therapy of bacterial diarrhea.—Authors' Abstract

**Sahu, K. and Das, R. K.** Reducation of clastogenic effect of clofazimine, an antileprosy drug, by vitamin A and vitamin C in bone marrow cells of mice. Food Chem. Toxicol. **32** (1994) 911–915.

Clofazimine (CLF), an antileprosy drug, has earlier been proved to be clastogenic in mice *in vivo*. It is an important constituent of the triple-drug regimen recommended by WHO for the treatment of leprosy. In this study the protective role of vitamins A and C (vit A and vit C) against the clastogenic effect of CLF in mouse bone marrow cells has been evaluated. Two doses (20 and 40 mg/kg) of vit C and two doses (2500 and 5000 IU/kg) of vit A were tested against a dose of 40 mg CLF/kg. The drug alone induced chromosomal aberrations of about 8 times the control value. Neither of the doses of vit C exhibited any clastogenic effect and, when administered simultaneously with CLF, both reduced the effect of CLF very significantly, the higher dose reducing chromosomal aberrations almost to the control value. Conversely, both doses of vit A, when administered alone, brought about significant increases in chromosome aberrations over the control value; the higher, but not the lower dose, given simultaneously with CLF, minimized the effect of CLF significantly but not as greatly as vit C. A scavenging effect of the vitamins, removing free radicals produced by CLF, is assumed to be responsible for modulation of the clastogenic effect of CLF.—Authors' Abstract

**Sylla, P. M., Blanc, L., Sow, S. and Diallo, A. S.** [Factors determining the irregularity of multidrug therapy patients in Bamako district (Mali).] Acta Leprol. (Genève) **9** (1994) 69–75. (in French)

In order to examine the factors determining irregularity among patients undergoing multidrug therapy in Bamako district, we conducted a nonexperimental study based, in the first instance, on medical records and later on a questionnaire; 1175 treatment cards were reviewed in this way. The results of our study show that 3.1% of the patients fail to attend treatment sessions regularly and that multibacillary patients have more irregular attendance than paucibacillary patients. We have not observed any statistically meaningful difference between old and new patients as far as irregularity in attending multidrug therapy sessions is concerned.

The second part of our research based on a questionnaire targeting a group of cases (36 patients who did not attend regularly) and a random control group (50 patients who attended treatment regularly but had missed at least one treatment) has shown that it is only for Item VI ("Have you ever missed your appointment because you perhaps considered yourself cured?") that a sta-

tistically meaningful difference emerges between cases and controls regarding the rates of affirmative responses ($p < 0.05$).—Authors' English Abstract

**Vage, C., Saab, N., Woster, P. M. and Svenson, C. K.** Dapsone-induced hematologic toxicity; comparison of the methemoglobin-forming ability of hydroxylamine metabolites of dapsone in rat and human blood. Toxicol. Appl. Pharmacol. **129** (1994) 309–316.

The relative methemoglobin (MetHgb) forming ability of two metabolites of dapsone, dapsone hydroxylamine (DDS-NOH) and monoacetyldapsone hydroxylamine (MADDS-NOH), were compared in rat and human whole blood. Concentration-response curves for the two metabolites were generated *in vitro* in whole blood. Data were fit to both the E(max) and Sigmoid E(max) models. The E(max) values for MetHgb formation in rat blood for MADDS-NOH and DDS-NOH fitted to the E(max) model were 83 (8) and 84 (2)%; while the EC(50) values were 1087 (283) and 828 (104) $\mu$M, respectively (mean $\pm$ S.D.). Neither these values nor those generated for the Sigmoid E(max) model differed significantly between the two metabolites. Similarly, the E(max) values in human blood for MADDS-NOH and DDS-NOH fitted to the E(max) model were 79 (5) and 80 (2)%; while the EC(50) values were 90 (17) and 95 (19) $\mu$M, respectively. These values also did not differ between the two metabolites using either pharmacodynamic model. MetHgb was produced at the same rate, reached similar peak concentrations, and exhibited the same rate of decline with both metabolites. The area under the MetHgb content versus time curve did not differ between the two metabolites. These data demonstrate that MADDS-NOH and DDS-NOH are equipotent and equally efficacious in their MetHgb-forming ability. Investigation of the disposition of these metabolites is necessary to assess their relative role in dapsone-induced toxicity *in vivo*.—Authors' Abstract

## Clinical Sciences

**Abbot, N. C., Beck, J. S., Rao, B. B., Feval, F., Stanford, J. L., Weiss, F. and Mobayen, M. H.** Circulation and sensation at the fingertips of claw hands. Lepr. Rev. **65** (1994) 341–349.

Measurements of skin blood flow (by laser Doppler flowmetry) and temperature were made under environmental conditions promoting peripheral vasodilatation at the fingertips of a disfigured "clawed" hand in 12 leprosy patients long-resident at Baba Baghi Leprosy Hospital, Tabriz, Iran. Sensory function was assessed by measuring the responses to light touch, pain and temperature of each finger, and peripheral autonomic function was gauged by estimating palmar sweating and by measuring skin vasomotor reflexes in response to inspiratory gasp.

In 2 patients all measured fingers had laser Doppler flux (LDFlux) values and skin temperatures lower than the 95% confidence limits for the mean of 20 healthy controls, i.e., were impaired; in 2 patients all fingers had normal values for LDFlux and temperature; and in 8 patients there was a combination of impairment with most fingers normal for these parameters but with the small finger most commonly impaired. There were 10 (67%) fingers with impaired LDFlux and temperature values who had significant sensory impairment; whereas only 5 (18%) of the fingers with normal LDFlux values and temperatures had a similar sensory deficit. Overall, the fingers with the most impaired sensation had significantly ($p < 0.05$) lower LDFlux and temperature values than those with no sensory deficit. Microcirculatory impairment was not related to disordered skin vasometer reflexes or dysfunction of sweating.

We concluded that the relationship between motor (skeletal muscle) nerve paralysis and any subsequent sensory neuropathy and/or microcirculatory impairment is more

complex than might be expected from previous understanding of the disease.—Authors' Summary

**de Rijk, A. J. and Byass, P.** Field comparison of 10-g and 1-g filaments for the sensory testing of hands in Ethiopian leprosy patients. Lepr. Rev. **65** (1994) 333–340.

In ALERT's leprosy control program sensory testing of hands and feet is done with a nylon filament giving a 10-g stimulus, but doubts arose that early, partial sensory loss in hands would not thus be discovered. In order to evaluate the relative performance of 1-g and 10-g filaments for sensory testing on the palms of hands, both filaments were used separately in a series of 1021 examinations on several consecutive occasions in 159 leprosy patients and 97 nonleprosy controls. The 1-g filament was always felt on normal hands and does not lead to false-positive findings of nerve dysfunction. If the 1-g filament were used routinely, almost twice as many instances of "neuritis" would be discovered and treated, if the criterion for diagnosis and treatment of new nerve dysfunction remained as it is for nerves tested with the 10-g filament. It appears desirable to distinguish between testing for early sensory loss and for loss of protective sensation. The two tests may each need their own instrument and separate recording of the results.—Authors' Summary

**Jayakumar, J., Aschhoff, M. and Job, C. K.** Pathogenesis of generalized nodular type I reaction in a borderline leprosy patient. Indian J. Lepr. **66** (1994) 477–482.

In an earlier communication we reported a case of borderline tuberculoid leprosy presenting with multiple subcutaneous nodules during a type I reactive phase (Jayakumar, *et al.* 1993). The histopathological examination showed that the nodules were composed of a granulomatous inflammation and edema with areas of necrosis. It was hypothesized that the eruption of nodules was due to the release of *M. leprae* antigens into the circulation resulting in the formation of generalized lesions throughout the body. We report here another such case which seems to unfold the true pathogenesis of such lesions.—From the article

**Jayasheela, M., Sharma, R. N., Sekar, B. and Thyagarajan, S. P.** HIV infection amongst leprosy patients in South India. Indian J. Lepr. **66** (1994) 429–433.

In a pilot study, 463 leprosy patients (374 males and 89 females) were investigated for HIV-1 and HIV-2 antibodies by screening tests. Sera positive by the screening tests were subjected to confirmatory tests. Three cases were confirmed to be positive for HIV, two for HIV-1 and one for HIV-2. All three positive cases were young males who had visited commercial sex workers. No correlation was found between the type of leprosy and HIV infection. This is the first report of HIV infection among leprosy patients from South India.—Authors' Abstract

**Jing, Z.** [Nerve damage on the face of 89 leprosy patients.] China Lepr. J. **10** (1994) 162–163. (in Chinese)

Damage to the trigeminal and facial nerves was analyzed in 89 leprosy patients. The results showed that facial palsy in PB was more and later than in MB and it almost always followed damage to the trigeminal nerve. Some 43.5% of these facial palsies were caused by type 1 reaction. The authors think that timely use of corticosteroids could prevent the palsy.—Authors' English Abstract

**John, S., Roul, R. K. and Anderson, G. A.** Cancer associated with leprosy. Indian J. Lepr. **66** (1994) 321–325.

Eighty-seven leprosy patients with cancer, seen between 1960 to 1984, were studied. Cancer in patients with leprosy occurred in a younger age group compared to the general population. The most common type of malignancy seen among males was squamous cell carcinoma of the lower extremity while in a hospital patient population it was cancer of the head and neck. Among the females, carcinoma cervix was the most common as in the hospital patients. The types of malignancy occurring among leprosy patients were similar to that of the hospital patient population with the exception of an increase in incidence of squamous cell carcinoma of extremities.—Authors' Abstract

**Kar, P. K., Arora, P. N., Ramasastry, C. V., Sayal, S. K. and Dhaka, R. S.** A clinicopathological study of macular lesions in leprosy. Indian J. Lepr. **66** (1994) 435–442.

One-hundred-twenty histologically confirmed cases of leprosy having macular lesions were evaluated clinically and histopathologically according to Ridley-Jopling classification. Of these 120 cases, the majority (91 or 75.8%) were young adults. The main clinical findings were: a single macule in 42 patients (35%), multiple macules 2–5 in number in 35 patients (29.1%), 6–10 macules in 17 patients (14.1%) and more than 10 macules in 26 patients (21.6%). Impairment of sensation over the macular lesions was present in 62 cases (51.6%), total loss of sensation was noticed in 31 patients (25.8%) and sensation was intact in 27 patients (22.5%). Acid-fast bacilli were detected in 11 cases (9.1%) by slit-skin smear examination.

Clinical examination of the 120 cases revealed features of TT in 16 (13.3%), BT in 41 (34.1%), BB in 11 (9.1%), BL in 13 (10.8%), LL in 7 (5.8%) and indeterminate leprosy (IL) in 32 patients (26.6%). On the contrary, histologically there were 22 cases of TT (18.3%), 38 cases of BT (31.6%), 8 (6.5%) BB, 10 BL (8.3%), 7 LL (5.8%) and 35 cases of IL (29.1%). Histopathological features were consistent with the clinical picture in 84 patients (70%).—Authors' Abstract

**Koyuncu, M., Celik, O., Ozturk, A. and Saunders, M.** Audiovestibular system, fifth and seventh cranial nerve involvement in leprosy. Indian J. Lepr. **66** (1994) 421–428.

Thirty-nine patients with leprosy and 15 sex- and age-matched controls were investigated for disorders of the fifth and seventh cranial nerves and that of the audiovestibular system. Sensorineural hearing loss found to be of cochlear origin was detected in eight (22%) of the patients with leprosy compared to none in the control group ($p > 0.05$). Vestibular dysfunction was noted in four patients (11.1%) compared to none in the control group ($p \leq 0.05$). Two cases were found to have fifth nerve involvement and one (2.8%) had seventh nerve involvement. None in the control group had fifth or seventh nerve deficit.—Authors' Abstract

**Liao, J., *et al.*** [Changes of mood and mentality among leprosy patients.] China Lepr. J. **10** (1994) 217–221. (in Chinese)

The mood and mentality of persons with leprosy were analyzed with SAS and SDS table. The result showed that there generally are strain sensations in them, the mean score by the SAS and SDS criteria was very significant of the difference between them and healthy persons, and anxiety and depression are common among them. The authors discuss suicide attempts among them.—Authors' English Abstract

**Nagaraju, B. and Gupta, M. D.** Diagnostic problems of early leprosy in field studies. Indian J. Lepr. **66** (1994) 463–472.

A series of exercises were undertaken in order to develop methodology for consistency and reliability of clinical diagnosis of leprosy under field conditions in longitudinal studies. It was observed in initial studies that the field investigators could miss about 35% of leprosy cases, mostly those with early manifestations. After training and experience, the proportion of missed cases came down to about 20%. In about 14% of females with patches suggestive of leprosy the patches were present in the covered areas of the body and so are likely to be missed during examination in field situations. One-hundred-forty-two individuals with suspicious and definite leprosy lesions detected by paramedical workers were examined by a senior medical officer experienced in leprosy on two different occasions at an interval of 3 months for leprosy diagnosis. The concordance rates for diagnosis and classification of leprosy were about 80% and 70%, respectively; and corresponding values for kappa were 0.59 and 0.62, similar to earlier experiences in inter-observer variation studies.—Authors' Abstract

**Qiu, W.-P.** [On stomatologic disorder and its treatment in persons with leprosy.] China Lepr. J. **10** (1994) 93–97. (in Chinese)

Stomatologic disorders in 193 patients and persons cured of leprosy had been treated, including 132 MB and 61 PB, 21 active cases and 164 cures, and 138 men and 55 women with the mean age of 55.8 years. Among the examinees, 91 cases have dental caries (47.1%), 181 have residual roots (93.8%) and average 5.44 dental caries, being more than those in healthy persons. Those who have both hands disabled and seem to have lost the ability to clean their teeth account for 25.4%, of which 51% are still maintaining their habit of teeth cleaning with a brush in their disabled palms every day; 80.8% of them are dirty in their oral cavity, 89.6% have dental calculus, and 77.7% suffer from periodontal disorders, being similar to the situation in the healthy population. As the treatment, 342 teeth were pulled in 186 persons, 45 teeth repaired in 20 persons, 42 artificial teeth inserted in 4 persons and 8 cases of periodontal disorders and 11 cases of stomatologic disease were treated, accounting only for 5% to 47% of those who should be treated. The author points out that almost no treatment is given to stomatologic disorders in leprosy patients, including active and cured, in our country up to now. Society and government should pay special attention to them and bring them into the national leprosy rehabilitation program.—Authors' English Abstract

**Qiu, W.-P.** [Analysis of the nerve damage in maxillofacial area among 607 leprosy patients.] China Lepr. J. **10** (1994) 80–84. (in Chinese)

The nerves of the dental pulp were examined by microamp current in 507 cases of leprosy with a mean age of 53 years, including 383 MB, 124 PB, 418 men and 89 women, of which 211 showed slow response (41.6%) and 296 no response (58.4%), including 237 MB (61.9%) and 59 PB (47.6%) ($p < 0.01$). Among these patients, 217 showed dysesthesia in the face (42.8%), of which 24.5% might be attributed to the supraorbital nerve, 24.3% to the zygomatic, 20.3% to the buccal, 17.2% to the infraorbital, 13.2% to the supratrochlear and 3.9% to the mandibular nerve according to the area supplied by the nerves, and 124 cases showed thickened supraorbital nerves, of which 55 showed dysesthesia at the same time.—Authors' English Abstract

**Tan., W.-P.** [Effect of a modified temporal muscle transposition on leprous lagophthalmos.] China Lepr. J. **10** (1994) 147–149. (in Chinese)

Forty-two leprosy patients with lagophthalmos (68 eyes) have been corrected by using Johnson's modified suspension with musculus temporalis and fascia lata in the period April 1992 to February 1993. The width of the palpebral fissure on closing the eyes was 2–10 mm before the operation. The follow up in November 1993, 8 months to 1.5 years after the operations, has found those who have the fissure less than 1 mm and equaling to 1–3 mm were 16 and 36 persons, respectively, and lacrimation was improved for 56 eyes. The author suggests that lagophthalmos should be corrected as early as possible for protection of the cornea.—Authors' English Abstract

**Van Brakel, W. H. and Khawas, I. B.** Silent neuropathy in leprosy: an epidemiological description. Lepr. Rev. **65** (1994) 350–360.

This paper presents epidemiological data on silent nerve function impairment in leprosy based on a retrospective study of 536 patients registered at Green Pastures Hospital, Pokhara, West Nepal. Because of the multiple possible etiologies it is proposed that the clinical phenomenon should be named "Silent Neuropathy" (SN). We defined this as sensory or motor impairment without skin signs of reversal reaction or erythema nodosum leprosum (ENL), without evident nerve tenderness and without spontaneous complaints of nerve pain (burning or shooting pain), paresthesia or numbness. The functioning of the main peripheral nerve trunks known to be affected in leprosy was assessed using a nylon filament to test touch thresholds and a manual voluntary muscle test to quantify muscle strength.

Almost 7% of new patients had SN at first examination. The incidence rate of SN among the 336 new patients who were available for follow up was 4.1 per 100 person years at risk. In total, 75% of all SN episodes diagnosed after the start of chemotherapy

occurred during the first year of treatment. During steroid treatment the sensory and motor function in nerves affected by SN improved significantly ($p = 0.012$, Wilcoxon matched-pairs signed ranks test) over a period of 3 months. The patients with more extensive clinical disease (3/9 or more body areas involved, more than 3 enlarged nerves or a positive skin smear) were found to be at increased risk of developing SN.

We discuss four different possible etiologies of SN: 1) Schwann cell pathology; 2) nerve fibrosis; 3) cell-mediated immune reaction; and 4) intra-neural ENL. Some epidemiological evidence is presented that suggests that SN cannot be equated with a "reversal reaction expressing itself in the nerves."

It is recommended that all patients should have a nerve function assessment at every visit to the clinic at least during their first year of treatment. Regular nerve function assessment is essential to detect SN at an early stage and to prevent permanent impairment of nerve function.—Authors' Summary

## Immuno-Pathology

**Beckman, E. M., Porcelli, S. A., Morita, C. T., Behar, S. M., Furlong, S. T. and Brenner, M. B.** Recognition of a lipid antigen by CD1-restricted $\alpha\beta^+$ T cells. Nature **372** (1994) 691–694.

Major histocompatibility complex (MHC) class I and class II molecules bind immunogenic peptides and present them to lymphocytes bearing the $\alpha\beta$ T-cell antigen receptor (TCR). An analogous antigen-presenting function also has been proposed for the non-MHC-encoded CD1 molecules, a family of non-polymorphic, $\beta_2$-microglobulin-associated glycoproteins, expressed on most professional antigen-presenting cells. In support of this hypothesis, CD1 molecules are recognized by selected $CD4^-CD8^-$ $\alpha\beta$ or $\gamma\delta TCR^+$ T-cell clones, and we have recently shown that CD1 molecules restrict the recognition of foreign microbial antigens by $\alpha\beta TCR^+$ T cells. But the substantial structural divergence of CD1 from MHC class I and class II molecules raises the possibility that the antigens presented by the CD1 system may differ fundamentally from those presented by MHC-encoded molecules. Here we report that a purified CD1b-restricted antigen of *Mycobacterium tuberculosis* presented to $\alpha\beta TCR^+$ T cells is mycolic acid, a family of $\alpha$-branched, $\beta$-hydroxy, long-chain fatty acids found in mycobacteria. This example of nonprotein microbial antigen recognition suggests that $\alpha\beta TCR^+$ T cells recognize a broader range of antigens than previously appreciated and that at least one member of the CD1 family has evolved the ability to present lipid antigens.—Authors' Abstract

**Bottasso, O. A., Ingledew, N., Keni, M., Morini, J., Pividori, J. F., Rook, G. A. W. and Stanford, J. L.** Cellular immune response to common mycobacterial antigens in subjects seropositive for *Trypanosoma cruzi*. Lancet **344** (1994) 1540–1541.

The immune response is impaired in the silent stage of Chagas' disease. We used quadruple skin testing with new tuberculins in 37 adults who were symptom free but seropositive for *Trypanosoma cruzi* and in 37 matched seronegative controls. Whereas 19% of controls responded to common mycobacterial antigens, none of the Chagas'-seropositive group responded to them ($p < 0.006$), demonstrating specificity in their unresponsiveness. The enhanced tuberculin reactivity after BCG vaccination in the control group was suppressed in seropositive subjects ($p < 0.002$). Selective loss of response to common mycobacterial antigens may have implications for the autoimmune pathology of Chagas' disease, and for susceptibility to tuberculosis, leprosy, and HIV disease.—Authors' Abstract

**Champsi, J. H., Bermudez, L. E. and Young, L. S.** The role of cytokines in mycobacterial infection. Biotherapy **7** (1994) 187–193.

Mycobacterial infections are a major cause of morbidity and mortality worldwide. The pathogenesis of infection and the mechanisms for the development of protective immunity are poorly known, but cytokines appear to play an important role in the modulation of the immune response. Evidence exists for the role of tumor necrosis factor (TNF-alpha), granulocyte macrophage colony stimulating factor (GM-CSF), and interferon-gamma (IFN-γ) in the host defense against mycobacteria. In this article we discuss recent findings about the role of cytokines in leprosy, tuberculosis and *Mycobacterium avium* infection, using *in vitro* and *in vivo* human and murine data.—Authors' Abstract

**Chensue, S. W., Warmington, K., Ruth, J., Lincoln, P., Kuo, M. C. and Kunkel, S. L.** Cytokine responses during mycobacterial and schistosomal antigen-induced pulmonary granuloma formation—production of Th1 and Th2 cytokines and relative contribution of tumor necrosis factor. Am. J. Pathol. **145** (1994) 1105–1113.

Synchronized pulmonary granulomas (GRs) were induced in presensitized mice by intravenous embolization of polymer beads bound with purified protein derivative (PPD) of *Mycobacteria tuberculosis* or soluble antigens derived from *Schistosoma mansoni* eggs (SEA). Uncoated beads served as a foreign body control (CON). Antigen-coated beads elicited GRs with characteristic epithelioid macrophages and multinucleate giant cells by 4 days after embolization. Unlike PPD GR, SEA bead lesions contained eosinophils; whereas CON beads elicited only a limited mononuclear infiltrate. GRs and draining lymph nodes (LN) were assessed on days 2, 4, and 8 for Th1- (interleukin-2 [IL-2], interferon-gamma [IFN-γ] and Th2-type (IL-4, IL-5, and IL-10) cytokines. CON GR produced only a small amount of IFN-γ on day 2 and failed to induce a significant response in draining LN. In contrast, both PPD and SEA antigen-coated beads induced reactive lymphoid hyperplasia but differed greatly in local and regional cytokine profiles. PPD GR produced IFN-γ on day 2 and the draining LN produced predominantly Th1 cytokines on days 2 and 4. In contrast, SEA bead GRs were dominated by Th2 cytokines. The corresponding LN produced IL-2 and IL-4 on day 2; IL-2, IL-4, IFN-γ, and IL-10 on day 4; then IL-2, IFN-γ, and IL-4 on day 8, probably reflecting maturational changes of T cells. Macrophages (MP) from bead GR also showed different patterns of IL-6 and tumor necrosis factor (TNF) production. Compared with CON GR, MPs from PPD GR were weak sources of IL-6; whereas those of SEA GR showed enhanced and accelerated production. In contrast, MP of PPD GR had augmented TNF-producing capacity; whereas those of SEA GR showed delayed TNF production. *In vivo* depletion of TNF, respectively, caused 40% and 10% decreases in PPD GR and SEA GR but had no effect on CON GR area, indicating that TNF contributed to a greater degree to the PPD response. These data show that, depending on the inciting agent, GR can be mediated by different cytokines. Characterization of inflammatory lesions by cytokine profiles should allow design of more rational therapeutic interventions.—Authors' Abstract

**Clemens, D. L. and Horwitz, M. A.** Characterization of the *Mycobacterium tuberculosis* phagosome and evidence that phagosomal maturation is inhibited. J. Exp. Med. **181** (1995) 257–270.

We have used the cryosection immunogold technique to study the composition of the *Mycobacterium tuberculosis* phagosome. We have used quantitative immunogold staining to determine the distribution of several known markers of the endosomal-lysosomal pathway in human monocytes after ingestion of either *M. tuberculosis, Legionella pneumophila,* or polystyrene beads. Compared with the other phagocytic particles studied, the *M. tuberculosis* phagosome exhibits delayed clearance of major histocompatibility complex (MHC) class I molecules, relatively intense staining for MHC class II molecules and the endosomal marker transferrin receptor, and relatively weak staining for the lysosomal membrane glycoproteins, CD63, LAMP-1, and LAMP-2 and the lysosomal acid protease, cathepsin D. In contrast to

*M. tuberculosis*, the *L. pneumophila* phagosome rapidly clears MHC class I molecules and excludes all endosomal-lysosomal markers studied. In contrast to both live *M. tuberculosis* and *L. pneumophila* phagosomes, phagosomes containing either polystyrene beads or heat-killed *M. tuberculosis* stain intensely for lysosomal membrane glycoproteins and cathepsin D. These findings suggest that a) *M. tuberculosis* retards the maturation of its phagosome along the endosomal-lysosomal pathway and resides in a compartment with endosomal, as opposed to lysosomal, characteristics; and b) the intraphagosomal pathway, i.e., the pathway followed by several intracellular parasites that inhibit phagosome-lysosome fusion, is heterogeneous.—Authors' Abstract

**Drowart, A., Huygen, K., Launois, P., Jann, E., Nyabenda, J. and van Vooren, J. P.** IgG humoral response against the antigen 85 complex homologues in leprosy. Scand. J. Immunol. **40** (1994) 643–647.

Antigen 85 complex is the major protein component present in *Mycobacterium bovis* BCG culture filtrate (CF). It consists of a family of three proteins: 85A, 85B and 85C. Combining isoelectric focusing and Western blot analysis, we have previously identified different antigenically related proteins present in the CF of other mycobacteria (*M. tuberculosis, M. kansasii, M. avium, M. gordonae, M. fortuitum* and *M. phlei*) using monoclonal antibodies (MoAbs) directed against the antigen 85 complex of *M. bovis* BCG. Humoral immune response directed against these crossreactive homologues was analyzed in sera from 20 patients with multibacillary leprosy (BL/LL), from 20 patients with paucibacillary leprosy (BT/TT) and from 15 healthy leprosy contacts. All the antigen 85 homologues identified in the seven CFs by MoAbs were also recognized by IgG present in sera from multibacillary leprosy patients, but not or very faintly in sera from paucibacillary leprosy patients or from healthy subjects. These results suggest that some of the *M. leprae* epitopes inducing a significant humoral response in multibacillary leprosy are common to the various 85 antigenically related proteins present in all mycobacterial species.—Authors' Abstract

**Fine, P. E. M., Sterne, J. A. C., Ponnighaus, J. M. and Rees, R. J. W.** Delayed-type hypersensitivity, mycobacterial vaccines and protective immunity. Lancet **344** (1994) 1245–1249.

There is a longstanding debate over the implications of natural and vaccine-induced delayed-type hypertensivity (DTH) for protective immunity to mycobacterial infections. The identification of correlates of vaccine-induced protective immunity should help explain the inconsistent behavior of BCG vaccines in different populations and assist in efforts to devise improved vaccines.

More than 70,000 subjects in Karonga District, northern Malawi, were skin tested with soluble antigens of the tubercle and leprosy bacilli, and then followed up for 5 years for tuberculosis and leprosy incidence. Incidence rate ratios were calculated to compare subjects with different levels of prior skin test sensitivity, after controlling for the effects of age, sex and previous BCG vaccination. BCG vaccination protected against leprosy without persistent DTH to tuberculin or to soluble antigens of the leprosy bacillus. In subjects who had not received BCG, hypersensitivity to tuberculin or to antigens of the leprosy bacillus was associated with strong protection against leprosy. In BCG-vaccinated and unvaccinated subjects, there was a J-shaped relation between hypersensitivity to tuberculin and subsequent rates of tuberculosis, with lowest rates associated with low-grade sensitivity (induration 1–10 mm).

This study shows that DTH to antigens has different implications for tuberculosis and leprosy: low-level hypersensitivity (probably attributable to environmental mycobacteria) is associated with protection, but persistent vaccine-associated hypersensitivity to mycobacterial antigens is not a correlate of vaccine-derived protection against mycobacterial diseases.—Authors' Abstract

**Flad, H. D., Richter, E., Schluter, C., Duchrow, M., Arnoldi, J., Hahn, M., von Ballestrem, W. C., Alvarenga, A. E. and Gerdes, J.** *Mycobacterium leprae* DNA content, cellular and cytokine patterns in skin lesions of leprosy patients undergo-

ing multidrug therapy (MDT). Immunobiology **191** (1994) 388–394.

Skin biopsies from untreated and MDT-treated patients were examined for infiltrating cells and cells producing the cytokines TNF-alpha, IFN-gamma, and IL-1 beta using immunohistochemistry. Biopsy specimens item untreated tuberculoid leprosy patients were characterized by the presence of cells producing TNF-alpha, IFN-gamma, and IL-1 beta and of subepidermal Langerhans' cells. These cells were rarely found or completely absent in biopsies of untreated lepromatous leprosy patients, but tended to increase under MDT.

In a short-term therapy trial for 3 months with brodimoprim, dapsone, and rifampin, 12 patients were monitored by follow-up biopsies. Semiquantitative PCR for mycobacterial DNA revealed two groups of patients: one group in which mycobacterial DNA in follow-up biopsies remained constant and a second group in which a decrease of mycobacterial DNA during therapy was noted. Immunophenotyping in these follow-up biopsies revealed that in the latter group IFN-gamma-positive cells and Langerhans' cells were present and gamma delta T cell receptor-positive cells tended to decrease during therapy. In contrast, in patients whose mycobacterial DNA did not change during therapy, these phenotypical manifestations were not observed.

We, therefore, conclude that assessment of mycobacterial DNA in combination with phenotyping of infiltrating cells and determination of cytokine patterns may be useful tools in establishing criteria for the effectiveness and duration of MDT in patients with leprosy.—Authors' Abstract

**Hussain, R., Dockrell, H. M. and Chiang, T. J.** IgG subclass antibody to *Mycobacterium leprae* 18,000 MW antigen is restricted to $IgG_1$ and $IgG_3$ in leprosy. Immunology **83** (1994) 495–500.

IgG subclass responses to *Mycobacterium leprae* 18,000 MW recombinant antigen (18K) were determined in sera from untreated leprosy patients using an ELISA-based assay with specific monoclonal antibodies. Antibodies to *M. leprae* 18K were restricted to $IgG_1$ and $IgG_3$ antibodies with higher seropositivity in lepromatous patients (25.5% for $IgG_1$ and 12.8% for $IgG_3$) compared to patients with tuberculoid disease (11.5% for $IgG_1$ and 5% for $IgG_3$). No significant antibody response was detectable in $IgG_2$ and $IgG_4$ in patients with either lepromatous or tuberculoid leprosy. The selective production of antibodies in $IgG_1$ and $IgG_3$ subclasses could not be related to polyclonal activation in these subclasses as all IgG subclasses showed similar elevated levels at the polyclonal level. The major difference noted between lepromatous and tuberculoid leprosy patients with the IgG subclass antibody response was a strong linear correlation between $IgG_1$ and $IgG_3$ responses to *M. leprae* 18K in lepromatous patients ($r = 0.703$, $p < 0.001$) but not in tuberculoid leprosy patients ($r = 0.007$, $p > 0.10$) which may be related to immunoglobulin class switching of $IgG_3$ to $IgG_1$ rather than selective shifts in T-helper subsets. Our results, therefore, do not support the hypothesis that activation of Th2 cells occurs in lepromatous leprosy; this issue needs further examination.—Authors' Abstract

**Hussain, R., Kifayet, A. and Chiang, T. J.** Immunoglobulin $G_1$ ($IgG_1$) and $IgG_3$ antibodies are markers of progressive disease in leprosy. Infect. Immun. **63** (1995) 410–415.

*Mycobacterium leprae*-specific and polyclonal immunoglobulin G (IgG) subclass and IgE antibodies in leprosy patients across the histopathological spectrum were determined by using a quantitative enzyme-linked immunosorbent assay. Antibody responses to *M. leprae* sonicates were detected only in $IgG_1$, $_2$, and $_3$ subclasses. Even at 100-times-lower dilutions, very little $IgG_1$ and IgE antibody activity against *M. leprae* was detected in any group of leprosy patients. Quantitatively, antibody responses were highest at the lepromatous pole and decreased toward the tuberculoid pole. The greatest quantitative difference in antibodies between the lepromatous and tuberculoid poles was observed with $IgG_1$ (140-fold), this was followed by the difference with $IgG_3$ antibodies (32-fold). Polyclonal antibodies, on the other hand, were elevated for all four IgG subclasses as well as IgE in both lepromatous and tuberculoid leprosy patients

compared with healthy controls from a leprosy-endemic area. Selective elevation of *M. leprae*-specific antibody responses in $IgG_1$ and $IgG_3$ subclasses, therefore, could not be attributed to selective polyclonal activation in these particular subclasses. Furthermore, polyclonal activation for IgE was observed in both lepromatous and tuberculoid leprosy patients, with higher levels in the tuberculoid group, which does not support selective TH2 activation in lepromatous leprosy patients. $IgG_1$ and $IgG_3$ antibodies also showed the highest Spearman rank correlation with the bacterial index in these patients ($r = 0.748$ and $p < 0.001$ for $IgG_1$; $r = 0.721$ and $p < 0.001$ for $IgG_3$). Thus, disease progression in leprosy showed a significant correlation with selective increases in $IgG_1$ and $IgG_3$ responses.—Authors' Abstract

**Hussain, R., Menz, B., Dockrell, H. M. and Chiang, T. J.** Recognition of *Mycobacterium leprae* recombinant 18,000 MW epitopes by IgG subclasses in leprosy. Immunology **84** (1995) 290–297.

IgG subclasses are known to be differentially regulated by cytokines (elaborated by activated T cells), which act as growth factors and immunoglobulin switch factors on B cells. In leprosy, we have previously shown that IgG subclass antibodies to a purified recombinant antigen of *Mycobacterium leprae* (18,000 MW) are restricted to $IgG_1$ and $IgG_3$ across the disease spectrum. The only significant difference observed was that lepromatous patients with low to undetectable T-cell responses showed a strong correlation between $IgG_1$ and $IgG_3$ ($p < 0.001$) antibodies, while tuberculoid patients who showed strong T-cell responses did not show such a correlation. To examine if these differences were related to T-cell-mediated class switching in tuberculoid leprosy patients, we have studied epitope recognition by $IgG_1$ and $IgG_3$ using a panel of synthetic peptides spanning the 18,000 MW molecule in an enzyme-linked immunosorbent assay (ELISA). In lepromatous patients there was little similarity in peptide recognition by $IgG_1$ and $IgG_3$, with $IgG_1$ recognition being restricted to a single dominant carboxy-terminal peptide, while the $IgG_3$ antibodies recognized a diverse set of peptides in the *N*-terminal half of the 18,000 MW molecule. In tuberculoid patients both $IgG_1$ and $IgG_3$ antibody showed recognition of similar peptides in the *N*-terminal half of the 18,000 MW molecule. Our results therefore support the hypothesis that immunoglobulin class switching is occurring in tuberculoid but not in lepromatous patients.—Authors' Abstract

**Kaplan, G.** Cytokine regulation of disease progression in leprosy and tuberculosis. Immunobiology **191** (1994) 564–568.

Studies in our laboratory have focused on the role of cytokines in the regulation of the cellular immune response and disease progression in two important mycobacterial infections of man, namely, leprosy and tuberculosis. Our studies in leprosy have involved the use of key regulatory cytokines such as IFN-gamma in the modulation of the cellular response of infected patients. We have investigated the effect of intradermal administration of low dose IFN-gamma on the lesions of anergic lepromatous patients and have reported an accelerated bacillary clearance from the skin. This was associated with the local accumulation of mononuclear cells and killing of infected macrophages. However, IFN-gamma administration also resulted in the induction of erythema nodosum leprosum, a toxic syndrome associated with excess TNF-alpha production. Both the toxic symptoms and the high levels of TNF-alpha production could be inhibited by thalidomide treatment, a drug we have shown reduces the half-life of TNF-alpha mRNA. In preliminary clinical trials in tuberculosis patients we have attempted to use thalidomide to reduce TNF-alpha production and toxicities. These results are discussed.—Author's Abstract

**Kaufmann, S. H. E. and Ladel, C. H.** Role of T cell subsets in immunity against intracellular bacteria: experimental infections of knock-out mice with *Listeria monocytogenes* and *Mycobacterium bovis* BCG. Immunobiology **191** (1994) 509–519.

The generation of knock-out mice with targeted gene deletions has already proven its enormous value for our understanding

of the antimicrobial immune response. Here we describe studies with knock-out mice deficient in the TCR-beta gene, lacking alpha/beta T cells; in the TCR-delta gene, lacking gamma/delta T cells; in the beta 2m gene, lacking beta 2-microglobulin, and hence cell surface expressed MHC class I and functional CD8 T cells; and in the H-21-A beta gene, lacking cell surface expressed MHC class II and, hence, functional CD4 T cells. These mice were infected with *Listeria monocytogenes* or *Mycobacterium bovis* BCG as representative microbes which primarily activate CD8 T cells or CD4 T cells, respectively. Data described in this treatise demonstrate that the different gene deletions had an impact of varying degree on antibacterial defense and on the formation of granulomatous lesions. At the same time, the data point to a compensatory potential of the incomplete immune system. We assume that deletions in the major immune effector cells promote the emergence of a second line of defenders which frequently remain silent in the normal immune system. Thus, our data illustrate an enormous redundancy of the immune system, which, however, is not abundant since it takes over essential functions in the immunodeficient situation.—Authors' Abstract

**Khanolkar Young, S., Rayment, N., Brickell, P. M., Katz, D. R., Vinayakumar, S., Colston, M. J. and Lockwood, D. N. J.** Tumour necrosis factor-alpha (TNF-$\alpha$) synthesis is associated with the skin and peripheral nerve pathology of leprosy reversal reactions. Clin. Exp. Immunol. **99** (1995) 196–202.

Leprosy may be complicated by episodes of increased cell-mediated immunity toward *Mycobacterium leprae* (reversal reactions) which result in severe local immunopathology in skin lesions and peripheral nerves. Using *in situ* hybridization and monoclonal techniques we have demonstrated TNF-alpha mRNA and TNF-alpha protein in macrophages infiltrating leprosy skin and peripheral nerve. Levels of TNF-alpha mRNA are significantly increased in reactional skin and nerve, particularly in borderline tuberculoid patients. TNF-alpha mRNA and TNF-alpha protein levels are higher in reactional nerves then reactional skin. In both reactional skin and nerve TNF-alpha mRNA is more abundant than TNF-alpha protein; this may reflect the rapid turnover of TNF-alpha protein in an immunologically dynamic situation, such as is seen in reversal reaction. Our findings emphasize the importance of documenting both mRNA and protein production when assessing the role of cytokines in pathology. The leprosy reversal reaction may be regarded as a useful model of tissue immunopathology in which TNF-alpha is generated as part of the host response to infection, but also produces local tissue damage.—Authors' Abstract

**Kode, J. A., Chiplunkar, S. V., Samson, P. D., Deo, M. G. and Gangal, S. G.** Immunoprecipitation of mycobacterial antigens with sera from patients with leprosy. Acta Leprol. (Genève) **9** (1994) 89–94. (in French)

Pooled sera from leprosy patients across the clinical spectrum, tuberculosis patients and healthy individuals were tested for their reactivity with antigens of *Mycobacterium leprae* and a panel of cultivable mycobacteria by immunoprecipitation technique. Sera from lepromatous leprosy patients demonstrated exclusive reactivity with the 26-kDa protein of *M. tuberculosis* H37Ra, 28-kDa protein of *M. kansasii*, 45-kDa protein of *M. smegmatis*, and 158, 40 and 14 kDa proteins of *M. phlei*. Sera from patients with borderline tuberculoid leprosy, tuberculoid leprosy, tuberculosis and healthy individuals failed to identify these antigens. Our studies indicate that analysis and characterization of immunodominant antigenic epitopes present on proteins of cultivable mycobacteria, sharing crossreactive epitopes with *M. leprae* may prove to be important in the serodiagnosis of multibacillary leprosy as well as for developing vaccines for immunotherapy of leprosy.—Authors' English Summary

**McHugh, S. M., Rifkin, I. R., Deighton, J., Wilson, A. B., Lackmann, P. J., Lockwood, C. M. and Ewan, P. W.** The immunosuppressive drug thalidomide induces T helper cell type 2 (Th2) and concomitantly inhibits TH1 cytokine pro-

duction in mitogen- and antigen-stimulated human peripheral blood mononuclear cell cultures. Clin. Exp. Immunol. **99** (1995) 160–167.

Thalidomide is an effective immunomodulatory drug in man, but its mechanism of action remains unclear. We hypothesized that, in addition to its reported inhibitory effects on production of monocyte-derived tumor necrosis factor-alpha (TNF-alpha), thalidomide might be effective at the level of Th immunoregulation. In a comparative study with the immunosuppressant cyclosporin A, we have demonstrated a potent and specific effect of thalidomide on cytokine production relating to the distinct Th1 and Th2 subsets. It induced and enhanced the production of IL-4 and IL-5 and, at the same dose (1000 ng/ml), significantly inhibited interferon-gamma (IFN-gamma) production in phytohemagglutinin (PHA)-stimulated human peripheral blood mononuclear cell (PBMC) cultures. Stimulation of PBMC with recall antigen [streptokinase: streptodornase (SKSD)] at 144 hr in the absence of thalidomide resulted in a predominantly Th1 response, with the production of IFN-gamma and IL-2. Thalidomide switched this response from a Th1 to a Th2 type. The effect was most pronounced at 1000 ng/ml thalidomide, where inhibition of IFN-gamma and enhancement of IL-4 production was maximal. In unstimulated cultures thalidomide alone induced IL-4 production. Cyclosporin A, in contrast, inhibited both Th1 and Th2 cytokine production by PHA-stimulated PBMC. Time course data from thalidomide-treated cultures revealed that the augmented IL-4 production diminished as the culture time increased; whereas IFN-gamma production was significantly increased. This response might be due to activation-induced apoptosis of Th2 cells or the induction of Th2 cell anergy, in the continued presence of stimulating agents, with the emergence of IFN-gamma-secreting Th1 cells when Th2 antagonism declines. The effects of thalidomide and related compounds may enhance our understanding of the mechanisms of T-helper cell selection, offer the possibility of controlled therapeutic switching between Th1 and Th2 responses, and may lead to a rational approach for the treatment of some T-cell-mediated immunological disorders.—Authors' Abstract

**Modlin, R. L. and Nutman, T. B.** Type 2 cytokines and negative immune regulation in human infections. Curr. Opin. Immunol. **5** (1993) 511–517.

"Recent studies indicate that the human immune response to infection is regulated by the balance between the T-helper type 1 cytokines, interleukin-2 and interferon-$\gamma$, and the T-helper type 2 cytokines, interleukin-4, interleukin-5 and interleukin-10. Interleukin-4 and interleukin-10 can facilitate antibody production but can also suppress cell-mediated immune responses. The net effect of these negative immunoregulatory cytokines is to favor progression of infection." The reviewers discuss the role of type 2 cytokines in the regulation of the human immune response in leprosy, tuberculosis, leishmaniasis, malaria, and helminth infections. References of special and of outstanding interest are highlighted.—C. A. Brown (Trop. Dis. Bull.)

**Mustafa, A. S., Deggerdal, A., Lundin, K. E. A., Meloen, R. M., Shinnick, T. M. and Oftung, F.** An HLA-DRw53-restricted T-cell epitope from a novel *Mycobacterium leprae* protein antigen important to the human memory T-cell repertoire against *M. leprae.* Infect. Immun. **62** (1994) 5595–5602.

Cellular immunity mediated by T cells plays a major role in protection against intracellular infections including leprosy, a chronic disease caused by *Mycobacterium leprae.* In this work, we describe CD4+ T-cell clones, isolated from healthy humans immunized with *M. leprae,* which recognize a novel *M. leprae* protein antigen previously isolated from a lambda gt11 DNA expression library. On the basis of the deduced primary structure of the carboxyl-terminal part of the antigen, we have used a synthetic-peptide approach to exactly define the T-cell epitope recognized. Importantly, major histocompatibility complex restriction studies showed that the epitope is presented by an HLA-DRw53 molecule which is frequently expressed in many populations. In addition, we have demonstrated that a long-term, cell-mediated immunity response

against the peptide epitope is present after immunization with *M. leprae*. In conclusion, the *M. leprae* T-cell epitope described here fulfills the primary criteria for subunit vaccine candidates against leprosy.—Authors' Abstract

**Launois, P., Ndiaye, M. N., Cartel, J.-L., Mane, I., Drowart, A., Van Vooren, J. P., Sarthou, J. L. and Huygen, K.** Fibronectin-binding antigen 85 and the 10-kDa GroES-related heat shock protein are the predominant Th-1 response inducers in leprosy contacts. Infect. Immun. **63** (1995) 88–93.

Peripheral blood mononuclear cells from 27 healthy leprosy contacts were analyzed for lymphoproliferation and TH-1 cytokine secretion (interleukin-2 and gamma interferon) in response to heat shock proteins with molecular masses of 65, 18, and 10 kDa from *Mycobacterium leprae* and the 30–32-kDa antigen 85 (Ag 85) from *M. bovis* BCG. Cells from 18 and 19 of 19 lepromin-positive contacts proliferated or produced TH-1 cytokines in response to the *M. leprae* 10-kDa protein and to Ag 85, respectively. Limiting-dilution analysis for two lepromin-positive contacts indicated that about one-third of *M. leprae*-reactive T cells displayed specificity to the *M. leprae* 10-kDa protein and Ag 85. The *M. leprae* 65- and 18-kDa proteins were less potent TH-1 response inducers: gamma interferon and interleukin-2 could be measured in 14 and 9 of 19 lepromin-positive contacts, respectively. In contrast, very low or undetectable proliferative and cytokine responses were found for 8 lepromin-negative contacts. Our data demonstrate that the fibronectin-binding Ag 85 and the 10-kDa GroES homolog are powerful mycobacterial TH-1 response inducers in the vast majority of lepromin-positive contacts and suggest that they might be valuable candidates for a future subunit vaccine.—Authors' Abstract

**Lombardi, C., Cohen, S., Leiker, D. L., Souza, J. M. P., Cunha, P. R., Martelli, C. M. T., Andrade, A. L. S. S. and Zicker, F.** Agreement between histopathological results in clinically diagnosed cases of indeterminate leprosy in São Paulo, Brazil. Acta Leprol. (Genève) **9** (1994) 83–88.

Histopathological slides from skin biopsies of 57 self-reporting patients diagnosed as indeterminate leprosy by the Leprosy Control Program in São Paulo, were sent to three independent histopathologists. Agreement between the reports was based on the following diagnosis: "indeterminate leprosy," "suggestive leprosy" or "no leprosy." A great variation was observed in the interpretation of the histopathological examination. The three pathologists reported "indeterminate leprosy," respectively, in 7.0%, 54.4% and 84.2% of the cases studied. A kappa index of agreement between any two pathologists ranged from 0.08 to 0.32, showing poor agreement between observers. Agreement improved by pooling together the reports "suggestive leprosy" and "indeterminate leprosy." The three pathologists agreed in the results of 24 biopsies of the 27 classified as leprosy by any one of the three observers. Eight cases were considered as "no leprosy" by all pathologists. Higher agreement indices were obtained for positive and negative proportionate concordance between any two examiners. The implications of the variation in the diagnosis of indeterminate leprosy and early leprosy are discussed in the context of public health and case management.—Authors' Summary

**Oftung, F., Geluk, A., Lundin, K. E. A., Meloen, R. H., Thole, J. E. R., Mustafa, A. and Ottenhoff, T. H. M.** Mapping of multiple HLA class II-restricted T-cell epitopes of the mycobacterial 70-kilodalton heat shock protein. Infect. Immun. **62** (1994) 5411–5418.

By combining a DNA subclone and synthetic-peptide approach, we mapped epitopes of the immunogenic mycobacterial 70-kDa heat shock protein (HSP70) recognized by human CD4(+) T-cell clones and lines. In addition, we identified the respective HLA-DR molecules used in antigen presentation. The donor groups used were healthy persons immunized with killed *Mycobacterium leprae* and tuberculoid leprosy patients. The results show that the N-terminal part of the HSP70 molecule contains three different T-cell epitopes, of which two were

presented by DR7 (amino acids [aa] 66 to 82 and 210 to 226) and one was presented by DR3 (aa 262 to 274). The C-terminal part contains one epitope (aa 413 to 424) presented by HLA-DR2. The C-terminal epitope shows extensive homology to the corresponding region of the human HSP70 sequence. All of the T-cell epitopes identified were presented by only one particular HLA-DR molecule. We also found that HLA-DR5 and DRw53 can present HSP70 to T cells, demonstrating the presence of additional epitopes not yet defined at the peptide level. On the basis of the donors used in this study, recognition of HSP 70 at the epitope level seems to be ruled by the restriction elements expressed by the donor rather than by any difference in reactivity between healthy individuals and patients. In conclusion, mycobacterial HSP70 is relevant to subunit vaccine design since it contains a variety of T-cell epitopes presented in the context of multiple HLA-DR molecules.—Authors' Abstract

**Roche, P. W., Triccas, J. A., Avery, D. T., Fifis, T., Billman Jacobe, H. and Britton, W. J.** Differential T cell responses to mycobacteria-secreted proteins distinguish vaccination with bacille Calmette-Guerin from infection with *Mycobacterium tuberculosis*. J. Infect. Dis. **170** (1994) 1326–1330.

The immune responses of healthy recipients of *Mycobacterium bovis* bacille Calmette-Guerin (BCG) vaccine, tuberculosis (TB) patients, and contacts of TB patients were examined to three major secretory proteins *M. tuberculosis*, MPB59, MPB64, and MPB70. MPB59 evoked a T-cell response in 78% of BCG vaccinees, 62% of TB patients, and 60% of contacts. MPB64 and MPB70 were recognized by < 15% of BCG vaccinees, half of TB patients, and three-quarters of contacts. TB and leprosy patients had antibody responses to MPB59, but few had antibodies to MPB64 or MPB70. Hybridization of mycobacterial DNA with specific gene probes demonstrated the absence of a gene for MPB64 in the vaccine strain of BCG, but the MPB70 gene was found in all virulent and vaccine BCG strains tested. Since MPB64 and MPB70 can induce delayed-type hypersensitivity reactions in infected animals, either of these proteins may have potential as skin test reagents for detecting infection with *M. tuberculosis*.—Authors' Abstract

**Shannon, E. J., Howe, R. C., McLean, K. and Hastings, R. C.** Thalidomide does not perturb CD2, CD4, CD5 CD8, HLA-DR, or HLA-A, B, C molecules *in vitro* on the membranes of cells with immune potential. Immunopharmacol. Immunotoxicol. **16** (1994) 717–729.

Thalidomide dramatically relieves the signs and symptoms of erythema nodosum leprosum (ENL). ENL is an acute inflammatory complication of lepromatous leprosy. The cause(s) of ENL as well as the mechanism of action of thalidomide in arresting ENL are unknowns. It has been suggested that ENL is the consequence of a transient activation of a cell-mediated-immune (CMI) response to *Mycobacterium leprae*. To initiate a CMI response, an interaction between adhesion and/or signal transducing molecules on T cells and molecules on antigen presenting cells would occur. An alteration, induced by thalidomide, of one or more of the molecules on T cells or antigen presenting cells that are essential to maintaining the reactive state of ENL, could explain thalidomide's ability to attenuate ENL. Thalidomide did not modify: (a) adhesion and/or signal transducing molecules such as CD2, CD4, CD5 and CD8, or (b) molecules that facilitate antigen presentation such as HLA-DR, HLA-A, HLA-B, or HLA-C.—Authors' Abstract

**Shen, J.-P., *et al.*** [The use of S-100 protein to diagnosis of leprosy.] China Lepr. J. **10** (1994) 152–155. (in Chinese)

Twenty-three cases of PB leprosy were examined with immunoperoxidase staining technique for S-100 protein, of which 18 revealed destroyed nerves in granulomas of epithelioid cells but only 11 had AFB in their sections. Thirty-seven cases of MB leprosy were examined with the same method, of which 22 showed swelling and perineural proliferation of cutaneous nerves and inflammatory infiltration in the nerves, and strongly positive staining of the mycobacterial colloid particles was found in their sections. In addition, 25 normal controls

showed intact skin nerves. The authors think the technique to be useful for the diagnosis of leprosy, especially PB leprosy.—Authors' English Abstract

**Sieling, P. A. and Modlin, R. L.** Cytokine patterns at the site of mycobacterial infection. Immunobiology **191** (1994) 378–387.

Distinct patterns of T-cell cytokine production have been shown to influence the outcome of infection in mouse models and humans. Th1 or Type 1 cytokines, interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) are generally associated with resistance to infection; whereas Th2 or Type 2 cytokines, IL-4 and IL-10 are associated with progressive disease. Leprosy is a useful model for studying the role of cytokines in modulating T-cell responses in human infectious disease. Infection by *Mycobacterium leprae* results in disease manifestations that encompass an immunological spectrum. Tuberculoid patients are able to restrict the growth of the pathogen and mount strong T-cell responses to *M. leprae*. In contrast, lepromatous patients manifest disseminated infection and their T cells weakly respond to *M. leprae*. We have found that tuberculoid leprosy lesions have a predominance of CD4+ T cells producing the Type 1 cytokine pattern. Secondly, IL-12 mRNA was expressed at 10-fold higher levels in tuberculoid lesions as compared to lepromatous lesions and that IL-12 promotes the selective expansion of the Type 1 cytokine-producing cells. In contrast, lepromatous lesions contain CD8+ IL-4-producing cells that suppress antigen-specific T-cell responses and promote the outgrowth of additional suppressor-T cells. IL-10, also expressed at higher levels in lepromatous as compared to tuberculoid lesions, was found to be produced by macrophages, effectively inhibiting cytokine production and macrophage activity.—Authors' Abstract

**Silva, C. L., Silva, M. F., Pietro, R. C. L. R. and Lowrie, D. B.** Protection against tuberculosis by passive transfer with T-cell clones recognizing mycobacterial heat shock protein 65. Immunology **83** (1994) 341–346.

We have previously shown that mice vaccinated by injection with J774 macrophage-like tumor cells that expressed *Mycobacterium leprae* heat-shock protein (hsp) 65 as a transgene had acquired a remarkably high degree of protection against subsequent challenge with virulent *M. tuberculosis*. We show here that antigen-specific T cells cloned from spleens of such vaccinated animals can transfer a high level of protection to non-vaccinated recipients. The most efficient cells were of T-cell receptor (TCR) alpha beta+ and CD4− CD8+ type and specifically lysed mycobacteria-infected macrophages. These findings are consistent with the importance for protective immunity of engaging the endogenous antigen-presenting pathway to bias the immune response towards a cytolytic action against a mycobacterial antigen that is expressed at the surface of infected macrophages. TCR gamma delta+ and TCR alpha beta+ cells interacted synergistically.—Authors' Abstract

**Singh, S., Jenner, P. J., Narayan, N. P. S., Ramu, G., Colston, M. J., Prasad, H. K. and Nath, I.** Critical residues of the *Mycobacterium leprae* LSR recombinant protein discriminate clinical activity in erythema nodosum leprosum reactions. Infect. Immun. **62** (1994) 5702–5705.

We reported earlier (S. Singh, N. P. Shanker Narayan, P. J. Jenner, G. Ramu, M. J. Colston, H. K. Prasad, and I. Nath, Infect. Immun. 62:86–90, 1994) that polyclonal antibodies directed against selective sequences in the *Mycobacterium leprae* recombinant protein designated LSR were present in lepromatous leprosy patients undergoing erythema nodosum leprosum (ENL) reactions (type 2 reactions). In this study using peptides with single-residue deletions from positions 6 to 24, we define three distinct regions, GVTY, NAA, and RGD, which were important for antibody recognition and for the discrimination of clinically silent and active ENL reactions. Antibodies against NAA were found only in patients undergoing active reactions. This is in contrast to the results for the RGD motif, which was recognized in all ENL patients, irrespective of the clinical status. Though GVTY was recognized in both groups of patients, its recognition was

masked by the flanking glutamic acid. These findings point towards a specific molecular recognition pattern that emerges when a lepromatous leprosy patient undergoes immune perturbations leading to ENL reactions. Moreover, the fine specificity of immunological recognition changes during the natural evolution of the host-parasite interaction.—Authors' Abstract

**Soares, D. J., Failbus, S., Chalise, Y. and Kathet, B.** The role of IgM antiphenolic glycolipid-I antibodies in assessing household contacts of leprosy patients in a low endemic area. Lepr. Rev. **65** (1994) 300–304.

This study was carried out to assess the role PGL-I antibodies may have to play in assisting with early diagnosis in close contacts of leprosy patients. Blood samples were collected from patients and contacts. It was found that 6.9% of index cases and 1% of healthy contacts were positive for PGL-I antibody. None of the healthy contacts developed clinical leprosy and all had become seronegative at follow up. We conclude that screening for PGL-I antibodies has a limited role in the screening of healthy contacts and may not be of use in low endemic areas.—Authors' Summary

**Thawani, G., Bhatia, V. N. and Mukherjee, A.** Anticardiolipin antibodies in leprosy. Indian J. Lepr. **66** (1994) 307–314.

Eighty-four leprosy patients were studied clinically and for IgG and IgM anticardiolipin (aCL) antibodies. Following WHO criteria, 41 patients could be classified as multibacillary (MB) and 43 as paucibacillary (PB). Baseline levels of IgG and IgM antibodies were 27 ± 4.8 GPL and 20 ± 3.4 MPL per ml, respectively. Comparing with these, 60.9% of MB and 39.5% of PB cases showed rise in IgG and IgM anticardiolipin antibodies; 19.5% of MB and 4.6% of PB sera showed rise in only IgG antibodies, while 4.8% of MB and 13.9% of PB cases showed rise only in IgM antibodies. Rise in aCL antibodies had no correlation with cardiovascular involvement, bacteriological index, reactive state and duration or regularity of treatment.—Authors' Abstract

**Thawani, G., Mukherjee, A. and Bhatia, V. N.** Evaluation of modified lepro-agglutination as screening test for leprosy. Indian J. Lepr. **66** (1994) 315–320.

One-hundred thirty-three leprosy sera [83 multibacillary (MB) and 50 paucibacillary (PB) cases] were screened by lepro-agglutination (LA) and *M. leprae* particle agglutination (MLPA) tests. Larger number of MB sera were positive by LA (86.75%) than by MLPA (45.12%) test. Thirty-seven of the 45 MB sera negative by MLPA test were positive by LA test. The reverse was true in 3 out of 11 MB sera. PB sera showed positivity of 16% in LA test and 24% in MLPA test. All the 55 sera from normal healthy individuals and 18 VDRL positive sera from syphilis patients were found to be negative by the LA test.—Authors' Abstract

**Weir, R. E., Morgan, A. R., Britton, W. J., Butlin, C. R. and Dockrell, H. M.** Development of a whole blood assay to measure T cell responses to leprosy: a new tool for immuno-epidemiological field studies of leprosy immunity. J. Immunol. Methods **176** (1994) 93–101.

A whole blood assay is described to measure T-cell-mediated immune responses to leprosy and provide an alternative to the conventional lymphocyte transformation test. Optimal conditions were defined for the whole blood assay, and interferon-gamma measurement was found to be a more sensitive way of measuring responses than tritiated thymidine incorporation. The assay was shown to be useful for investigating responses to a range of leprosy antigens. A whole blood assay has the advantages of being quick, simple and requiring only a small volume of blood, making it more appropriate as an immuno-epidemiological field test in leprosy endemic areas.—Authors' Abstract

**Xia, L.-P., *et al.*** [A preliminary study of SDS-PAGE standardization and serum spectrum of patients with leprosy.] China Lepr. J. **10** (1994) 214–217. (in Chinese)

A method of SDS-PAGE for determination of antigen in the sera in leprosy, tuberculosis and rheumatoid arthritis, etc., has

been established. With this method, it was found that the optimal concentration of the gel is 12.5%, and in examined sera there were basically the same bands among various individuals with the same disease but in different diseases the bands were weakened or absent, compared with that of the normal control, especially without the bands C, E and I in MB leprosy and without the bands C and E in PB leprosy, suggesting that the method could be useful in the diagnosis and classification of leprosy.—Authors' English Abstract

**Xie, Y.-L., *et al.*** [Determination of distribution and density of *M. leprae* in the skin of leprosy patients.] China Lepr. J. **10** (1994) 90–93. (in Chinese)

Histological sections of the skin of 57 patients with various forms of leprosy have been re-examined for observing the distribution and density of *M. leprae.* The authors thought that there is some shortcoming with the current method of calculating the BIG in leprosy pathology, i.e., it does not take the bacilli outside the granuloma into account, but there also is a lot of the bacilli inside nonspecific infiltrations and in the areas without infiltration, specifically in the small blood vessels there. This also involves the HI. For the sake of reflecting the fact, it is suggested that the score of the BIG should be recorded on the basis of the mean number of the bacilli in high power fields, which can be obtained if the sum total of the bacilli in all the inspected fields is divided by the number of the fields inspected. For an early case of leprosy, the authors believe it better to set up a bacterial index in nonspecific infiltration (BINSI) so as to record the number of the bacilli in the section of the skin. It is emphasized that the changes and the bacilli in the small blood vessels should be taken into account for pathologic diagnosis of early leprosy.—Authors' English Abstract

**Zhang, M., Gong, J.-H., Iyer, D. V., Jones, B. E., Modlin, R. L. and Barnes, P. F.** T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. J. Clin. Invest. **94** (1994) 2435–2442.

Tuberculosis causes more extensive and life-threatening disease in patients with HIV infection than in immunocompetent persons. To investigate the hypothesis that these severe manifestations of tuberculosis may be due to alterations in cytokine production, we evaluated cytokine patterns in HIV-infected tuberculosis patients. Upon stimulation with *Mycobacterium tuberculosis in vitro,* PBMC from HIV-infected tuberculosis patients had reduced proliferative and type 1 responses, compared with HIV-seronegative tuberculosis patients. The reduction in proliferative responses was independent of the CD4 cell count, but the reduced type 1 response was a direct result of CD4 cell depletion. There was no enhancement of type 2 cytokine production in HIV-infected patients, although production of IL-10 was prominent in all tuberculosis patients. In HIV-infected tuberculosis patients, *M. tuberculosis*-induced proliferative responses were significantly enhanced by neutralizing antibodies to IL-10 but not by antibodies to IL-4 or by recombinant IL-12. The *M. tuberculosis*-induced type 1 response was augmented both by antibodies to IL-10 and by recombinant IL-12. Tuberculosis in the context of HIV infection is characterized by diminished type 1 responses, probably induced by immunosuppressive cytokines produced by macrophages/monocytes, rather than by type 2 cells.—Authors' Abstract

**Zhou, X.-T., *et al.*** [Histopathology of the skin in LL patients after completion of treatment.] China Lepr. J. **10** (1994) 223–224. (in Chinese)

Pathological examination of the skin was made in 100 cases of lepromatous leprosy after completion of the course of treatment. Acid-fast bacilli were seen only in the section of one case. There still were foamy cells seen in 35 cases. The cutaneous appendages showed atrophy in 58 and regeneration to a certain extent in 42. The free zone disappeared in 81, pigment in the epidermis increased in 69, and evident proliferation of the epidermis like pseudopapilloma was found in two cases. In the dermis, there was significant fibrosis in 85 and small foci of lymphocyte infiltration in 66 cases.—Authors' English Abstract

# Microbiology

**Bhatia, V. N.** Morphology of cystic structures seen in leprosy biopsy suspensions kept at cooler temperatures. Indian J. Lepr. **66** (1994) 293–298.

Cystic structures were seen in good numbers in biopsy suspensions obtained from leprosy patients and kept at a cooler temperature. The structures were found arranged in singles, clusters or straight lines. In clusters, small round structures were seen surrounding a large spherical body. The small cystic bodies appeared empty, the medium-sized bodies showed fine particles, while the large ones showed spherules in and around them. It appears that the seed structure of the cycle emerges from the large spherical bodies.—Author's Abstract

**Fiss, E. H., Yu, S. W. and Jacobs, W. R.** Identification of genes involved in the sequestration of iron in mycobacteria: the ferric exochelin biosynthetic and uptake pathways. Mol. Microbiol. **14** (1994) 557–569.

Mycobacteria produce two siderophores, mycobactin and exochelin. *Mycobacterium smegmatis* mutants defective in the production of exochelin were isolated using agar medium containing chrome azural S for the sensitive detection of siderophores. Cosmids of genomic libraries from *M. smegmatis* and *M. bovis* BCG were screened for complementation of the mutation. Subcloning of the complementing *M. smegmatis* cosmid identified a 4.3-kb fragment required for restoring exochelin biosynthesis. Sequencing of the DNA revealed four open reading frames whose genes were named fxuA, fxuB, fxuC, and fxbA. fxuA, fxuB, and fxuC share amino acid sequence homology with the iron permeases fepG, fepC, and fepD from *Escherichia coli*, respectively. Deletion analysis identified fxbA as the gene required to restore exochelin biosynthesis in our mutant. Although fxbA does not share amino acid sequence homology with any of the published sequences for siderophore biosynthetic genes, it does show limited homology with the phosphoribosyl-glycineamide formyltransferases (GAR enzymes) and methionyl-tRNA formyltransferase over a limited region of the sequence, suggesting that fxbA may code for an enzyme which adds a formyl group in the synthesis of exochelin. A fusion of fxbA with the *E. coli* lacZ gene demonstrated regulation of gene expression by iron. The ability of *M. smegmatis* mutants to produce mycobactin in the absence of exochelin supports the hypothesis that exochelin is not a precursor of mycobactin, and suggests that the siderophores have independent biosynthetic pathways. In addition, complementation of the *M. smegmatis* mutant with the BCG cosmid restored the synthesis of the *M. smegmatis* exochelin, demonstrating the use of *M. smegmatis* as a surrogate host for analysis of exochelins from slow-growing mycobacteria.—Authors' Abstract

**Goswami, S., Sarkar, S., Basu, J., Kundu, M. and Chakrabarti, P.** Mycotin: a lectin involved in the adherence of mycobacteria to macrophages. FEBS Lett. **388** (1994) 183–186.

Pathogenic mycobacteria colonize host macrophages. Attachment of these organisms to macrophages is the preliminary step prior to invasion of the macrophages by the bacteria. Western blot confirmed that walls of *Mycobacterium avium* and *M. tuberculosis* contain molecules which are immunologically related to mycotin, a lectin found in *M. smegmatis*. We have demonstrated that the adherence of mycobacteria to macrophages is significantly inhibited by anti-mycotin antibody or the mycotin-specific sugar, mannan. These observations suggest that prevention of the interaction of mycotin-related molecules on the surfaces of mycobacteria with mannose-specific receptors on macrophages offers an important approach for blocking attachment of pathogenic mycobacteria to macrophages, thereby preventing infection.—Authors' Abstract

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

Rifampin is a key component of multiple-drug therapy regimens for the treatment of leprosy. To date, rifampin resistance is a rare phenomenon which results from inappropriate treatment; however, the widespread emergence of rifampin resistance would greatly threaten the success of leprosy control. Because *Mycobacterium leprae* cannot be grown *in vitro* and when grown *in vivo* multiplies very slowly, the identification of drug resistance is a difficult and slow procedure. The current paper describes the identification of mutations in the *rpoB* gene which result in rifampin resistance. Drawing on information from work on *Escherichia coli*, the authors focused on the parts of the *Mycobacterium leprae rpoB* gene in which mutations are known to affect rifampin sensitivity. Sequencing of these parts of the gene from 9 rifampin-resistant strains revealed the mutations responsible; in 8 cases missense mutations were found to affect a serine residue, Ser-425, while the ninth mutant resulted from an insertion of 2 additional amino acids, lysine and phenylalanine, probably as a result of a replication error.—Authors' Abstract

**Imai, T., Ohta, K., Kigawa, H., Kanoh, H., Taniguchi, T. and Tobari, J.** Preparation of high-molecular-weight DNA: application to mycobacterial cells. Analyt. Biochem. **222** (1994) 479–482.

A method for isolating high-molecular-weight DNA from bacteria is described. A special feature of the method is the treatment of whole bacterial cells with an organic solvent (chloroform-methanol (2:1, v/v) or ethanol-ether (1:1, v/v)) prior to DNA extraction from the cells. The DNA preparations obtained from organic solvent-pretreated bacterial cells such as *Mycobacterium smegmatis, M. phlei,* and *Escherichia coli* contained highly polymerized DNA, as revealed by pulse-field gel electrophoresis. The size and yield of the DNA obtained from *E. coli* pretreated with the organic solvent were quite similar to that of the DNA obtained from protoplasts. The results strongly suggest that the organic solvent pretreatment is effective for extracting very large DNA from bacterial cells and especially from bacteria whose protoplasts cannot be easily formed.—Authors' Abstract

**Inglis, N. F., Stevenson, K., Hosie, A. H. F. and Sharp, J. M.** Complete sequence of the gene encoding the bacterioferritin subunit of *Mycobacterium avium* subspecies *silvaticum*. Gene **150** (1994) 205–206.

A gene encoding the bacterioferritin subunit (Bfr) of *Mycobacterium avium* (Ma) subspecies *silvaticum* has been cloned, sequenced and expressed. The 477-bp open reading frame codes for 159 amino acids, which were shown to share up to 92% identity with the Bfr of five bacterial genera. The recombinant Bfr exhibits serological cross-reactivity with *M. paratuberculosis* antigen D, a protein of approx. 20 kDa in cell lysates of *M. paratuberculosis* and *Ma silvaticum* and a protein of 20–22 kDa in sonicates of *M. leprae*.—Authors' Abstract

**Klegerman, M. E., Oner, F., Morris, P., Son, K. and Groves, M. J.** Isolation of a fibronectin-binding tryptic peptide from the antigen 85A protein of *Mycobacterium bovis* BCG. Microbios **80** (1994) 173–180.

Antigen 85A, a major secreted mycobacterial fibronectin-binding protein, was isolated from culture fluids of the Tice® substrain BCG vaccine. Tryptic digestion of this protein and passage of the digest through a fibronectin affinity chromatographic column resulted in the identification of a polypeptide fragment of molecular weight $\leq 6.5$ kD, which had marked fibronectin-binding activity. The identity of a 62-residue polypeptide was deduced from the amino-terminal sequence, indicating that the primary structure may define the integrin-binding capability.—Authors' Abstract

**Madhusudan, K., Ramesh, V. and Nagaraja, V.** Molecular cloning of gyrA and gyrB genes of *Mycobacterium tuberculosis*: analysis of nucleotide sequence. Biochem. Mol. Biol. Int. **33** (1994) 651–660.

We have recently reported the cloning of gyrA and gyrB genes from *Mycobacterium tuberculosis* H37Ra [Curr. Science (1994) 66, 664–667)]. Here, we present the complete nucleotide sequence of gyrB gene from *M. tuberculosis* H37Ra along with the flanking regions. The gyrA gene has been located on 34 nucleotides downstream of gyrB and has been partially sequenced; both of the

genes seem to be transcribed from the promoter elements located upstream of gyrB coding sequence. The gyrB gene encodes a polypeptide of 714 amino acids. The deduced amino acid sequences of gyrB and a part of gyrA show extensive homology to the corresponding genes from other bacterial species. The DNA gyrase of *M. tuberculosis* could be utilized to develop new line of antitubercular drugs.—Authors' Summary

**Pinho, J. R. R., Barr, P. J., Vicente, E. J. and Schenberg, A. C.** Expression of the 18-kDa protein of *Mycobacterium leprae* in *Saccharomyces cerevisiae*. Biotechnol. Lett. **16** (1994) 1241–1246.

*Mycobacterium leprae*, the etiologic agent of leprosy, until now has not been grown *in vitro*, resulting exceedingly in obstacles for the production of purified antigens. It is therefore of interest to clone the relevant *M. leprae* antigens in other easy to handle microbial hosts. Here we describe two different systems for expressing the 18kDa antigen of *M. leprae* in *S. cerevisiae*. Each system was shown to be effective in antigen expression, but the secretion system provided easier purification. Working with different host strains under different growth conditions, large quantities of biologically active proteins were obtained.—Authors' Abstract

**Prabhakar, M. C.** [Comparative evaluation of AAFB from the nose and skin of LL patients.] China Lepr. J. **10** (1994) 84–86. (in Chinese)

A large number of AAFB (acid alcohol-fast bacilli) could be extracted from the nasal flushings obtained by "Jalaneti." A quantitative comparative study was made, with respect to the length and morphological index, between the AAFB of the skin and those of nose. AAFB from the nose had significantly larger length and higher MI when compared to those of the skin, and the significance of which has been discussed.—Author's English Abstract

**Richter, E., Duchrow, M., Schluter, C., Hahn, M., Flad, H. D. and Gerdes, J.** Detection of *Mycobacterium leprae* by three-primer PCR. Immunobiology **191** (1994) 351–353.

Recently, polymerase chain reaction (PCR) has been introduced for the species-specific assessment of *Mycobacterium leprae*. To avoid Southern blotting techniques using radioactively labelled oligonucleotide probes, the aim of this study was to establish a three primer-based single-step PCR technique. Using primers designed for this purpose we amplified a part of the gene encoding for the 16S ribosomal RNA of slowly growing mycobacteria. Due to the species-specific antisense primer a second, smaller fragment specific for *M. leprae* was amplified. Our results show that the employment of a second antisense primer in the PCR may be a substitution for Southern blot hybridization.—Authors' Abstract

**Richter, E., Schluter, C., Duchrow, M., Hahn, M., Rusch-Gerdes, S., Galle, J., Flad, H. D. and Gerdes, J.** An improved method for the species-specific assessment of mycobacteria in routinely formalin-fixed and paraffin-embedded tissues. J. Pathol. **175** (1995) 85–92.

A polymerase chain reaction (PCR) assay for the rapid and species-specific diagnosis of mycobacterial infections in paraffin-embedded clinical specimens was developed using oligonucleotide primers to amplify a fragment of the DNA coding for the ribosomal 16S RNA of mycobacteria. The oligonucleotide primers amplified DNA from all 14 species of mycobacteria tested. By means of a reamplification protocol, as few as one to two mycobacteria could be detected in the presence of human DNA. The method of DNA isolation and amplification was applied on sections of routinely formalin-fixed and paraffin-embedded tissues. PCR for the beta-actin gene served as a control for successful DNA isolation. Mycobacterial DNA could be detected in cases of mycobacterial infections. The mycobacterial species was determined by additional sequencing of the PCR fragment. This PCR method may be a powerful tool for the diagnosis of mycobacterial infections from histopathological material and for the assessment of those mycobacteria that cannot readily be cultured, such as *Mycobacterium leprae*.—Authors' Abstract

**Shinnick, T. M. and Good, R. C.** Mycobacterial taxonomy. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 884–901.

The minimal standards for including a species in the genus *Mycobacterium* are: i) acid-alcohol fastness, ii) the presence of mycolic acids containing 60–90 carbon atoms which are cleaved to C22 to C26 fatty acid methyl esters by pyrolysis, and iii) a guanine + cytosine content of the DNA of 61 to 71 mol%. Currently, there are 71 recognized or proposed species of *Mycobacterium* which can be divided into two main groups based on growth rate. The slowly growing species require > 7 days to form visible colonies on solid media while the rapidly growing species require < 7 days. Slowly growing species are often pathogenic for humans or animals while rapidly growing species are usually considered nonpathogenic for humans, although important exceptions exist. The taxonomic and diagnostic characteristics of medically important species and of newly described species of the *Mycobacterium* genus are reviewed.—Authors' Abstract

**Shinnick, T. M., King, C. H. and Quinn, F. S.** Molecular biology, virulence, and pathogenicity of mycobacteria. Am. J. Med. Sci. **309** (1995) 92–98.

The diseases resulting from infections with *Mycobacterium* species are important sources of morbidity and mortality throughout the world today, with particularly devastating effects in tropical and developing countries. Almost 2 billion people have been infected with *Mycobacterium tuberculosis,* the causative agent of tuberculosis, and approximately 3 million people die each year from this disease. Tuberculosis also has re-emerged as an important public health problem in the United States, and this resurgence has been accompanied by an increased incidence of tuberculosis resistant to the standardly used antituberculosis drugs. Researchers' ability to investigate the molecular basis of the pathogenicity and drug resistance of the mycobacteria has been hampered by a lack of appropriate experimental tools. However, during the past 5 years, tremendous progress has been made in the development of the molecular biology of mycobacteria, and molecular tools are now available for detailed analysis of their genetics and for elucidation of the molecular mechanisms of their pathogenicity. The development of these tools is briefly reviewed, and the uses of the tools to investigate drug resistance in *M. tuberculosis,* to identify mycobacterial virulence factors, and to explore intracellular survival strategies are described.—Authors' Abstract

**Sreevatsa and Katoch, V. M.** Comparative assessment of viability of *M. leprae* by mouse foot pad and fluorescent staining techniques. Indian J. Lepr. **66** (1994) 455–462.

Morphological characteristics have been used as a parameter to assess the viability of *Mycobacterium leprae* in leprosy patients. However, with the advent of the mouse foot-pad technique, viability of *M. leprae* is determined by growing the bacilli in the mouse foot pad. In recent years, a fluorescent staining technique using fluorescent diacetate-ethidium bromide (FDA-EB) has been used to assess the viability of cultivable mycobacteria as well as *M. leprae.* The purpose of this study was to compare the viability of *M. leprae* by both mouse foot pad and fluorescent staining techniques. *M. leprae* strains from both untreated and treated patients as well as mouse passaged strains of *M. leprae* were used for the comparison. Percentage of green-stained bacilli in the inoculum was compared with that of multiplication of *M. leprae* in the mouse foot pad. It was observed that there was no correlation between the estimates of viable *M. leprae* by fluorescent staining and by mouse foot pad inoculation. FDA-EB staining appears to reflect only trends as absence of green-staining cells had overall general correlation with loss of infectivity to mouse foot pad, but the converse was not found to be true.—Authors' Abstract

**Wayne, L. G.** Dormancy of *Mycobacterium tuberculosis* and latency of disease. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 908–914.

There is ample circumstantial evidence from observation of the natural history of tuberculosis in humans and experimental animals that *Mycobacterium tuberculosis* is

capable of adapting to prolonged periods of dormancy in tissues, and that these dormant bacilli are responsible for latency of the disease itself. Furthermore, the dormant bacilli are resistant to killing by antimycobacterial agents. A systematic evaluation of the mechanism of dormancy, and of attempts to abrogate latency will require a better understanding of the physiologic events that attend the shiftdown into dormancy. There are probably two or more stages in the shift down of *M. tuberculosis* from active replication to dormancy as bacilli in unagitated cultures settle through a self-generated $O_2$ gradient into a sediment where $O_2$ is severely limited. One step involves a shift from rapid to slow replication. The other involves complete shutdown of replication, but not death. Presumably this last step includes completion of a round of DNA synthesis. The shiftup on resumption of aeration includes at least three discrete sequential steps, the production of RNA, the ensuing synchronized cell division and, finally, the initiation of a new round of synthesis of DNA. Three markers of the process of shiftdown of *M. tuberculosis* to dormancy have been described, namely, the changes in tolerance to anaerobiosis, the production of a unique antigen and the tenfold increase in glycine dehydrogenase production. Additional markers represented in the shiftup and shiftdown process may yet be discovered, and determination of their specific functions should provide insights into the mechanisms of dormancy and latency in tuberculosis, and into strategies for preventing reactivation of the bacilli and development of disease.—Authors' Abstract

**Wichitwechkarn, J., Karnjan, S., Shuntawuttisettee, S., Sornprasit, C., Kampirapap, K. and Peerapakorn, S.** Detection of *Mycobacterium leprae* infection by PCR. J. Clin. Microbiol. **33** (1995) 45–49.

PCR amplification of the 531-bp fragment of the *Mycobacterium leprae* pra gene in fresh biopsy and slit-skin smear samples was evaluated for its usefulness in the detection of leprosy bacilli in patients in Thailand. In multibacillary patients, 87.1% (27 of 31) of biopsy specimens and 41.9% (13 of 31) of slit-skin smear specimens were positive by PCR; whereas in paucibacillary patients, 36.4% (8 of 22) of biopsy specimens and 18.2% (4 of 22) of slit-skin smear specimens yielded detectable PCR amplification. Compared with other diagnostic procedures, PCR showed a clear advantage over both microscopic examination of slit-skin smears and serologic detection of antiphenolic glycolipid I antibody, especially in paucibacillary patients when bacterial indexes were 0 and seropositivity was only 6.25%. PCR was also evaluated for its potential to help monitor bacterial clearance in some of these patients during chemotherapeutic treatment. The PCR results on slit-skin smear samples at 1, 3, and 6 months of chemotherapy showed that the number of PCR-positive cases of both multibacillary and paucibacillary types decreased sequentially. The results of this study are encouraging. However, investigation of a larger number of clinical specimens with an improvement in PCR methods, especially on slit-skin smears, needs to be done before PCR can be established as a diagnostic procedure for leprosy patients and subclinical cases or as a tool for drug assessment.—Authors' Abstract

**Wiese, M., Lindner, B. and Seydel, U.** Development of an *in vitro* drug screening system for *Mycobacterium leprae* based on the determination of the intrabacterial sodium-to-potassium ratio of individual bacterial organisms. Int. J. Antimicrob. Agents **4** (1994) 271–279.

*In vitro* drug effects on *Mycobacterium leprae* in a cell-free system have been monitored by mass spectrometric determination of the ratio of the intrabacterial concentrations of the sodium and potassium ions (Na+, K+ ratio) of a limited number of individual bacteria per sample. From the drug-induced increase of the median values of the distributions of the Na+, K+ ratio, information on the concentration and time dependence of drug effects as well as on antagonistic or synergistic interactions of drugs has been obtained. Moreover, absolute values for the percentage of killed bacteria (% kill) have been derived from the distribution of the Na+, K+ ratios within a bacterial population. For this, the limiting value of the Na+, K+ ratio (up to which bacteria are viable)—which had been deter-

mined as 0.45 for cultivable bacteria—has been presumed to be valid also for *M. leprae*. Highest killing rates have been observed for fusidic acid and clarithromycin, followed by rifabutin, rifampin, and clofazimine. Minocycline and dapsone have shown only moderate killing effects and isoniazid and—probably due to the restricted metabolism of *M. leprae* in a cell-free medium—ofloxacin have been completely inactive. Strong ofloxacin effects, however, have been observed for cultivable mycobacteria and intracellular *M. leprae* phagocytized by a murine macrophage cell line.—Authors' Abstract

**Wolucka, B. A., McNeil, M. R., De Hoffmann, E., Chojnacki, T. and Brennan, P. J.** Recognition of the lipid intermediate for arabinogalactan/arabinomannan biosynthesis and its relation to the mode of action of ethambutol on mycobacteria. J. Biol. Chem. **269** (1994) 23328–23335.

Despite major advances in our understanding of the structure of mycobacterial cell walls, little is known of their biogenesis, and yet they are the site of action of many antituberculosis drugs and implicated in much of the pathology of tuberculosis and leprosy. A family of monoglycosyl polyprenylphosphates was isolated from *Mycobacterium smegmatis*, containing arabinose, ribose, and mannose. The isoprenoid nature of the lipid components was established by H-1 NMR, and fast atom bombardment mass spectroscopy (FAB-MS) demonstrated the presence of C-50 decaprenyl-P derivatives and smaller amounts of the C-35 octahydroheptaprenyl-P products. The configuration of the mycobacterial decaprenol was established as mono-trans, octa-cis, pointing to carriers of unusual structure. Combined gas chromatography (GC)/MS, FAB-MS/MS, and H-1 MMR allowed characterization of one of the primary components as beta-D-arabinofuranosyl-1-monophosphodecaprenol. Pulse chase metabolic labeling of cells with D-[C-14]glucose indicated that the decaprenyl-P-arabinose is an active intermediate in the biosynthesis of the arabinan of cell wall arabinogalactan and arabinomannan. The identification of poly-prenyl-P-ribose suggests the existence of ribose containing polysaccharides in the cell walls of *M. smegmatis* or/and of a novel epimerase in the D-arabinose biosynthetic pathway. Ethambutol, a powerful antituberculosis drug known to inhibit arabinogalactan and arabinomannan biosynthesis, results in the rapid accumulation of decaprenyl-P-arabinose, indicating that the drug interferes with either the transfer of arabinose from the donor or, alternatively, the synthesis of the arabinose acceptor itself.—Authors' Abstract

**Wu, Q.-X., *et al.*** [A preliminary study on screening antileprosy Chinese herbs with CAMS-A as an indicator.] China Lepr. J. **10** (1994) 155–157. (in Chinese)

Preliminary results of studying activities of Chinese herbs against M. CAMS-A as an indicator with diphasic medium assay designed by the authors themselves for screening antileprosy drugs were reported. The results indicated that of 200 Chinese herbs tested, seven have high activity against M. CAMS-A, 15 have moderate, and 26 have low activity. The authors consider the diphasic medium assay as a useful technique for preliminary screening of antileprosy drugs because it is simple, rapid, inexpensive and especially combining characteristics of liquid and solid media. In addition, streaking inoculation on surface of solid medium at different times may display pharmacokinetic properties of the herbs.—Authors' English Abstract

**Wu, X.-Q., *et al.*** [Evaluation of leprosy control program with strata analysis.] China Lepr. J. **10** (1994) 87–90. (in Chinese)

To evaluate the quality of leprosy control work is an important item in the management. The selection and use of the indexes and method for the evaluation have great influence on the results. The authors have used the stratified analysis to evaluate the leprosy work for the last 5 years in Enshi County, Hubei Province. The result obtained is in accord with the actual situation. The stratified analysis regards leprosy control as a complex system with a lot of aims and strata, and divides the factors which have impact on the system into different strata. Then, the items or indices in the strata are compared with others and given a

score respectively, so as to set up the matrix for judgment, to calculate the proportion of every index in the total aim, after all to multiply combinate weighted coefficient by the actual value and through accumulating to acquire comprehensive indices for evaluation. According to the size of every index it might be judged to be good or bad.—Authors' English Abstract

## Experimental Infections

**Shen, J.-P., *et al.*** [On antileprosy activity of China-made ofloxacin in nude mice.] China Lepr. J. **10** (1994) 212–214. (in Chinese)

Four groups of nude mice were being fed with the diet containing 0.05% ofloxacin (OFLO), made in China and imported, and 0.1% China-made OFLO and 0.01% RMP, respectively, for 88 days since the day 92 after $1 \times 10^4$ *M. leprae* had been inoculated into the subcutis of their hind foot pads. The China-made OFLO showed high activity against *M. leprae* in the early stage of stopping treatment, being similar to those of imported OFLO and RMP. *M. leprae* was not found in the foot pads of the mice taking 0.1% China-made OFLO for the period of the experiment, showing that it has strong bactericidal activity and might be used for treatment of leprosy clinically.—Authors' English Abstract

**Zhou, H.-M., *et al.*** [Histologic study of the eyes of armadillos experimentally infected with *M. leprae* before and after chemotherapy.] China Lepr. J. **10** (1994) 73–76. (in Chinese)

Twenty-four armadillos were inoculated with *M. leprae*. Eleven months after the inoculation, the right eyes of the animals were extracted. The animals then were divided into two groups for treatment, one group with MDT and the other with DDS. Two months later the animals were sacrificed. The histological changes of their eyes, the lepromas at the sites of the inoculation, and the viscera were studied. It was found that the contents of the bacilli in the liver or spleen of the animals in both groups were similar. It seems that the sizes of the lepromas before and after treatment were of no statistically significant difference. The inflammatory reaction of the nictating membranes and eyelids in eight animals were quite severe, and lepra cell infiltration and acid-fast bacilli were found in these tissues of four animals. Intraocular inflammatory cell infiltrations in these animals all were mild, and no acid-fast bacilli was found within the eyes, except in one. In view of these findings, it might be suggestive that some intraocular leprosy could be the result of spreading of infection from the lesions of the periocular tissues. If so, local drug treatment in the eye in addition to the general treatment in the patients would be useful in preventing or minimizing the intraocular lesions.—Authors' English Abstract

## Epidemiology and Prevention

**He, R., *et al.*** [Analysis of 407 new cases of leprosy in Hubei Province (China).] China Lepr. J. **10** (1994) 99–100. (in Chinese)

Four-hundred-seven new cases of leprosy had been registered for the period of 1986 to 1990 in Hubei Province, with older mean age, higher L/T ratio, uneven distribution relevant to the economic situation and more of early cases. The detection of most cases was passive.—Authors' English Abstract

**Huang, Y.-X., *et al.*** [Leprosy control in Quanzhou City, Fujian, (China).] China Lepr. J. **10** (1994) 222–223. (in Chinese)

Up to the end of 1992, 5565 cases of leprosy were accumulatively detected in Quanzhou City, Fujian; most of them had been cured and there yet were 90 active cases with the prevalence of 0.016‰. The incidence was decreased by 99.6%. The proportion of children among the patients decreased from

8.4% to 0.15%. The area with leprosy had significantly lessened.—Authors' English Abstract

**Kumaresan, J. A., Khulumani, P. and Maganu, E. T.** Case finding survey for leprosy in Botswana. E. Afr. Med. J. **70** (1993) 635–638.

"A baseline survey to establish the point prevalence of leprosy was carried out in July and August, 1991 in northern Botswana, where cases of leprosy have existed over the years. A total of 799 contacts of 127 index cases and 8235 schoolchildren from 18 schools were clinically screened for leprosy. In all, 44 active cases of leprosy were registered and started on multidrug therapy recommended by WHO. Of these cases, 32% were newly identified during the survey. Due to the moderate outcome, surveillance and control of leprosy have been integrated with the existing TB control program. This is the first time that a systematic attempt was made to establish a program for control of leprosy in Botswana."

This is an excellent account of a survey leading to new control policies in Botswana, including the integration of leprosy into the existing tuberculosis program (which has been well established for many years). The authors consider that the relatively high lepromatous rate and age of recorded active cases (84% of cases found were in patients older than 25 years) suggest that leprosy may be dying out in Botswana. Although the survey revealed that there are only a few active cases requiring multiple drug therapy, there are three times as many with disability and the authors comment that "this total number is a more accurate indicator of the present magnitude of leprosy in Botswana."—A. C. McDougall (Trop. Dis. Bull.)

**Lai, Z., *et al.*** [Analysis of 1493 new cases of leprosy.] China Lepr. J. **10** (1994) 160–162. (in Chinese)

In the last 9 years, 1493 cases of leprosy have been found in Liangshan Prefecture, Sichuan. Among them male patients were 2.91 times the number of female ones, 97.65% were rural residents, and the type rate was 60%. In 1985, the new case-detection rate was 9.77/100,000, the prevalence was 1.03‰, and children made up 7.48% of the patients; in 1993 the figures were 2.35/100,000, 0.27‰ and 3.44%, respectively, but the disability rate was still 20.68%. The authors think that the stress of leprosy control there should continue being on early case finding.—Authors' English Abstract

**Li, W.-Z., *et al.*** [A seroepidemiological study of leprosy in the household contacts with ELISA using ND-O-BSA and PGL as antigens.] China Lepr. J. **10** (1994) 207–212. (in Chinese)

A seroepidemiological study of leprosy was carried out in 723 household contacts (HCP), taking 1632 healthy persons in an endemic area of Yunnan Province (EHP) and 131 healthy persons in nonendemic area of Gansu Province (NHP) as controls for detecting antibodies to ND-O-BSA (A-ND) and to PGL-I (A-PGL) with ELISA. The criteria of positivity were decided on the basis of the antibody levels in EHP and NHP, namely, EHPC and NHPC which are 0.23 and 0.25 in EHP, and 0.14 and 0.17 in NHP, respectively. By the NHPC, the positive rates for A-ND and A-PGL were 20.19% and 15.21% in HCP vs. 15.13% and 9.38% in EHP, respectively ($p < 0.01$). By EHPC, both the rates were 6.38% and 8.44% in HCP. The differences were significant ($p < 0.01$) among the three groups of examinees according to the mean levels of both the antibodies except to PGL between NHP and EHP. With NHPC, the relative risk was 4.04 for HCP and 3.02 for EHP times that of NHP by A-ND level vs. 3.04 and 1.88 by A-PGL level, respectively, but with EHPC, the relative risk for HCP was 1.27 times that of EHP by A-ND and 1.88 by A-PGL. The authors think that the antibody examination could be used in epidemiological study but not yet in early diagnosis of leprosy.—Authors' English Abstract

**Pan, H.-Y., *et al.*** [Endemicity and control of leprosy in Gansu Province (China).] China Lepr. J. **10** (1994) 203–206. (in Chinese)

Since 1949, 4669 cases of leprosy were registered accumulatively in Gansu Province, including 3273 men and 1396 women; 3596 lepromatous, 36 borderline, 966 tuberculoid and 71 indeterminate ones; 3331 cases had been cured, of which 246 relapsed.

There still were 130 active cases of leprosy at the end of 1990. The incidence decreased from 2.28/100,000 (1950) to 0.077/100,000 (1986); 75% of the patients live in Longnan, Gannan and Linxia Prefectures. Besides the action of leprosy control, by the authors' opinion, development of the local economy might also be one of the important factors causing the decrease in the number of leprosy patients.—Authors' English Abstract

**Wu, Q.-X., *et al.*** [Seroepidemiology of leprosy.] China Lepr. J. **10** (1994) 149–152. (in Chinese)

The result of a sampling survey on seroepidemiology of leprosy in different populations, including in Jiangsu, Shaanxi, Hubei, Hunan, Liaoning Provinces and in Chicago was reported. The sum of 5861 samples were examined, including 1083 household contacts (HC), 452 matched random persons (MRP), 3171 random persons (RP) and 380 normal controls (NC) from endemic and nonendemic areas of leprosy (ENC 95 and NNC 285). Except in Liaoning, where Ms-ELISA was used, all the samples were detected by PGL-ELISA for antibody against PGL-I. The results are as follows: 1) The order of the positive rates is HC $\approx$ MRP > RP > ENC > NNC. 2) The levels of Ig increase from HC to LL gradually, but in BT/TT IgG > IgM and in LL-BL IgM > IgG. 3) The PR is 29.1% in the group aged 15–25, being more than those in the other groups, and higher in HC of MB than in those of PB. The PR has something to do with the age and the blood relationship of the contacts with patients, form of index cases, the degree of contacting, individual resistance and so on.—Authors' English Abstract

**Yang, Z.-L., *et al.*** [Feasibility of basic eradication of leprosy in Xinjiang (China).] China Lepr. J. **10** (1994) 142–143. (in Chinese)

On the basis of epidemiological materials in 1987 to 1992, the feasibility of basically eradicating leprosy by the end of the century in Xinjiang Autonomous Region was evaluated with mathematic models. The result showed that both the incidence and new case detection rate of leprosy were gradually decreasing ($p < 0.05$), and the covering rate and compliance of MDT were steadily increasing. By inference of the authors, the aim of basic eradication of leprosy would be reached on time.—Authors' English Abstract

**Yang, Z.-M., *et al.*** [Seasons of occurrences of leprosy and the disability.] China Lepr. J. **10** (1994) 144–146. (in Chinese)

The data of a survey for leprosy in Yangzhou, Jiangsu, were analyzed. The results showed that the peak times of onset and detection of leprosy and occurrence of the deformity were March, June and April, respectively, when about 15% of the events occurred. The peak period of the occurrence of the events were March–June, May–August and March–June, respectively, when some 50% of them occurred. So, the authors suggest that leprosy control managers at the operational level should gather more resources in the peak time or period to deal with case finding as early as possible for reducing the deformity to get better cost benefit.—Authors' English Abstract

**Zheng, D.-Y., *et al.*** [Trend facet analysis of geographic distribution of leprosy.] China Lepr. J. **10** (1994) 97–99. (in Chinese)

The trend facet analysis is a statistical method based on polynary regression theory and can be used to analyze systematic and partial variance in geographic distribution of some disease as a whole. The authors have used the method to analyze the accumulative number of leprosy patients for the period of 1955 to 1990 in Weifang City, Shandong Province, taking the county as a unit. The result showed that the trend function is of significance, i.e., $F = 20.2757$, $R = 0.8028$ and $q = 1.9419$, coinciding basically with the actual distribution of leprosy in the area under jurisdiction of the city that is more in the north and less in the south. But the fitting goodness of the actual value with the estimate is lower, $C = u/s \times 100\% = 46.13\%$, being due to the fact that the grouping of the materials was too small and the population density and the distribution of leprosy as clusters were not taken into account. The use and significance of the method for research on the distribution of leprosy were discussed.—Authors' English Abstract

## Rehabilitation

**Chen, Q., *et al.*** [Effect of footwear on insensitive feet in leprosy.] China Lepr. J. **10** (1994) 163–164. (in Chinese)

The follow up for 12 to 21 months in 200 leprosy patients with insensitive feet, who have been wearing protective footwear, showed that the footwear have good protective effect on the fissures and wounds of the sole. The authors pointed out that the supply of the footwear and teaching the patients to use them would be able to prevent foot deformity and its deterioration.—Authors' English Abstract

**Gokhale, S. D.** Changing horizon of rehabilitation. Indian J. Lepr. **66** (1994) 327–337.

Since rehabilitation was started as a method to restore the lost function or limb, we have come a long way. The roles of the state and society have been well-defined. Recently, the government of India has created the Rehabilitation Council of India. It is unfortunate that leprosy has not been mentioned as an important cause of disability. It is true that leprosy itself is not a disability but it is a major cause resulting in stigma and, therefore, I very strongly appeal to the Ministry of Welfare to consider leprosy disability as a special type so that attention could be focussed on the social and economic rehabilitation of those with leprosy-related disabilities and handicaps. The requirements for rehabilitation, such as assessment, counselling, vocational training, job adjustment and follow-up, ought to be taken up while planning for rehabilitation services in India. I hope the new Rehabilitation Council can undertake the planning of integrated rehabilitation of all disabilities on CBR foundation. While leprosy as a public health problem will hopefully be eliminated by 2000 A.D., prevention of disability and the need for rehabilitation of cured leprosy patients is going to be an issue for the next 25 years to come. Therefore, while leprosy work in India is being reoriented, it is necessary for us to start planning for rehabilitation services on sound footing.

We have a very long way to go. I still remember the board outside Disneyland, which reads: "This work is incomplete and will remain incomplete as long as human imagination is there." I would say, "the planning for rehabilitation is incomplete and shall remain incomplete as long as leprosy and human suffering is there."—Author's Conclusion

**Malaviya, G. N. and Husain, S.** Surgical correction of saddle nose deformity in leprosy—one stage procedure. Acta Leprol. (Genève) **9** (1994) 76–82.

In 24 leprosy patients having mild to moderate nasal deformity, a corticoperiosteal bone graft obtained from second metatarsal bone was placed in a pocket created between the lining and cover of the nose. The cases were evaluated later, mean follow-up period being 4½ years. The results were satisfactory in 13 patients. Improved shape was reported in 7 and poor in 3. The bone graft was incorporated and consolidated at the recipient site in 16 cases. The main donor site problem was overriding of the donor toe. The operated cases need health education for after-care of nose since nasal mucosa is atrophic.—Authors' Summary

**Lu, W., *et al.*** [Endemic situation of leprosy recently in Sichuan Province (China).] China Lepr. Jr. **10** (1994) 101–102. (in Chinese)

Since 1988, Sichuan Provincial Institute of Dermatology has organized training courses in leprosy workers for early case finding and timely and proper treatment of the reaction. In 1989 a survey and registration of disability and deformity in leprosy began. Since 1990 focal point of leprosy control in Liangshan Prefecture and Panzhihua City has been on dealing with silent neuritis, self-care of the patients, and prevention and treatment of plantar ulcers with surgery, protective footwear, amputation and artificial limbs. The authors consider that the self-care should be widely popularized because of its efficiency and economy.—Authors' English Abstract

**Tan, W.-P.** [Effects of overall therapy on leprous plantar ulcers.] China Lepr. J. **10** (1994) 164–166. (in Chinese)

From May 1992 to December 1993, 46 cases of plantar ulcer in Taihe Leprosarium, Guangzhou, China, have been treated with an overall therapy including to soak the feet in 5% $MgSO_4$ warm solution for 20 to 30 minutes every day, then to scrape the hyperkeratotic skin and necrotic tissue, to apply vaseline, and bind it up. It must teach the patients themselves to do it and to wear the protective footwear. So far, 34 of 78 ulcers have been cured.—Authors' English Abstract

**Thappa, D. M.** Disability grading in leprosy: current status. Indian J. Lepr. **66** (1994) 299–306.

The systems of classification and grading of disabilities in leprosy patients, suggested by WHO and others, are reviewed. Taking into account the drawbacks observed in these classifications, a new system of grading of disabilities of hands and feet of leprosy patients based on the 1960 WHO classification is proposed for use in institutions.—Author's Abstract

**Theuvenet, W. J., Ruchal, S. P., Soares, D. J. and Roche, P.** Advantages, indications and the manufacturing of melted PVC waterpipe splints. Lepr. Rev. **65** (1994) 385–395.

There are several indications when to use splints in the treatment of leprosy. PVC waterpipe is a cheap and easily available material in developing countries. Its advantages, indications, and the manufacturing of splints are described.—Authors' Summary

**Verma, K. K. and Gautam, S.** Psychiatric morbidity in displaced leprosy patients. Indian J. Lepr. **66** (1994) 339–343.

One-hundred confirmed leprosy patients, all of them migrated from elsewhere, were examined for psychiatric co-morbidity. Forty-six of them were from an "ashram" and the others were from a slum area; 76% of the patients were found to be having psychiatric illness. Of these, 55% were having neurotic depression and 21% had anxiety neurosis. Single, unemployed, socioeconomically backward and patients with physical deformities were suffering significantly more often with psychiatric symptoms. Psychiatric morbidity was found to be more frequent in the patients staying in slum than in those in the "ashram" where they had some security.—Authors' Abstract

**Yan, L.-B., *et al.*** [Analysis of 1973 cases of plantar ulcer in leprosy.] China Lepr. J. **10** (1994) 78–80. (in Chinese)

There were 1973 cases with plantar ulcers (13.84%) in 11 counties of Yangzhou and Dongtai Cities, Jiangsu Province, of which 1421 have ulcer on one sole and 552 on both, accounting for 30.4% of their foot disorders. Among the patients, 689 cases are MB and 1284 PB. Complications on the feet with the ulcer are significantly more than in those without the ulcer and the ability to live and labor in the patients with the ulcer is lower than that in those without it; 247 persons (43.7%) out of 565 amputees have been using artificial limbs. The estimated number of existing leprosy patients, including cured, with plantar ulcers would be at least about 35,000, and if so, annual expenditure for dressing and drugs in them would amount to some 6.3 million yuan RMB; 44.2% of the first ulcers occurred in the patients with duration of leprosy less than 2 years, and it is more in males (14.75%) than in females (11.43%). The ulceration rates in those who had leprosy over 25 years with the age of over 50 years and in farmers and workers are 10%–34%. The ulcers located on the front of foot make up 58.6% and complicated ulcers 53.88%. The cause and inducing factors of plantar ulcer and the attitude of the patients to the ulcer are discussed.—Authors' English Abstract

**Yin, Y., *et al.*** [Surgical correction of lagophthalmos in 49 persons.] China Lepr. J. **10** (1994) 166–167. (in Chinese)

Forty-nine cases of leprosy with lagophthalmos, including 30 cases complicated by ectropion, have been corrected with the resection of levator palpebrae superioris for simple lagophthalmos, the partial tarsectomy for those with ectropion and suture or syndesmopexy of the inner canthus or a combination of some of the operations. The results of the operations showed that 61 out

of 69 eyes with lagophthalmos and 38 of 41 ones complicated by ectropion have basically recovered.—Authors' English Abstract

## Other Mycobacterial Diseases and Related Entities

**Afghani, B. and Lieberman, J. M.** Paradoxical enlargement or development of intracranial tuberculomas during therapy: case report and review. Clin. Infect. Dis. **19** (1994) 1092–1099.

Intracranial tuberculomas can sometimes develop or increase in size despite administration of appropriate therapy. We report the case of a child whose intracranial tuberculomas paradoxically enlarged while therapy was being administered, and we review 23 other cases in which tuberculomas increased in size or number and 17 cases in which tuberculomas appeared during therapy. These phenomena generally occurred within 3 months of the start of therapy. All but four patients had neurological deterioration that prompted obtaining a repeated computed tomographic scan. One patient died, about one fourth of the patients had residual neurological symptoms, and less than one third of the patients required surgical intervention. Most patients received a 12–18 month course of antituberculous therapy. Adjunctive therapy with steroids appears to diminish neurological symptoms and may improve outcome. Paradoxical enlargement or development of tuberculomas usually does not represent failure of antituberculous therapy; the most likely explanation for these phenomena is an interaction between the host's immune response and the direct effects of mycobacterial products.—Authors' Abstract

**Anderson, P.** The T cell response to secreted antigens of *Mycobacterium tuberculosis.* Immunobiology **191** (1994) 4–5.

Recent information from several laboratories points to proteins secreted from live *Mycobacterium tuberculosis* as being involved in protective immunity. We have studied protein release from *M. tuberculosis* during growth and have defined three different groups of proteins: excreted proteins, secreted proteins of the outer cell wall and cytoplasmic proteins released at late culture timepoints. These findings have led to the definition of a short-term culture filtrate (ST-CF) enriched in excreted/secreted proteins and with a minimal content of autolytic products.

ST-CF was tested as antigen in experimental vaccines against tuberculosis. A vaccine based on the adjuvant dimethyldioctadecylammonium chloride (DDA) was constructed and demonstrated to induce a potent cell-mediated immune response of the Th-1 type. The vaccine was tested in parallel with a BCG standard vaccine and both vaccines induced a highly significant protection of the same magnitude.

Molecules within the Ag85 complex and a 6-kDa secreted protein were mapped as the major antigenic targets for long-lived T cells involved in protective immunity against *M. tuberculosis.*—Author's Abstract

**Aralachaves, M., Vilanova, M., Ribeiro, A. and Pinto, J.** Immunostimulatory effect of thalidomide in normal C57BL/6 mice is compatible with stimulation of a highly connected central immune system. 1. Scand. J. Immunol. **40** (1994) 535–542.

Although thalidomide has been used with success in the treatment of increasing numbers of autoimmune diseases, the therapeutic effects have not been satisfactorily explained so far. We describe here some findings that may contribute to a better understanding of the immunomodulatory effects of this drug. Several immunological changes were observed after treating C57BL/6 mice with 3 mg of thalidomide. The numbers of natural IgM PFC against sheep red blood cells were increased in the spleen, and occasionally a dramatic oscillatory increase in the numbers of nonspecific splenic IgM and IgG PFC was observed in these mice. How-

ever, these oscillatory increases were progressively lower, after two and three treatments with thalidomide at 20-day intervals. Furthermore, the absolute numbers of splenic CD5+ B and CD5− B lymphocytes were increased; whereas depletion of CD4+ CD8+ cells in the thymus and of lymphoid cells in the bone marrow was seen after a single treatment with 3 mg of thalidomide. Taken together, these results suggest that thalidomide stimulates both peripheral and central immune systems and, consequently, enhances the connectivity of the central immune system.—Authors' Abstract

**Audit, C. O.** Thalidomide-induced polyamine acylation: a new insight into the acylation mechanism. Biogen. Amines **10** (1994) 543–554.

The deprivation of biological polyamines resulting from an acylation reaction of polyamines by thalidomide has been postulated to explain the teratogenic activity of this drug (Fabro, *et al.*, 1965). However, the acylation mechanism has been questioned later on (Brode, 1968; Jonsson, 1972). In this study, the acylation reaction has been re-examined. In ethanol, the interaction of thalidomide with putrescine, spermidine or spermine results in the cleavage of the phthalimide ring of thalidomide and in the formation of an acylation product. At physiological pH, the yield of the acylation reaction is lowered owing to the partial hydrolysis of thalidomide. In comparison to phthalimide itself or related phthalimide derivatives, thalidomide displays a markedly enhanced acylating activity toward polyamines. The binding of a glutarimide ring to the phthalimide moiety in the thalidomide molecule intensifies the acylation reaction. For thalidomide and related phthalimide derivatives, the teratogenic activity appears to be correlated with a high acylating power toward polyamines.—Author's Abstract

**Bermudez, L. E., Inderlied, C. B., Kolonoski, P., Petrofsky, M. and Young, L. S.** Clarithromycin, dapsone, and a combination of both used to treat or prevent disseminated *Mycobacterium avium* infection in beige mice. Antimicrob. Agents Chemother. **38** (1994) 2717–2721.

Bacteremic infection caused by organisms of the *Mycobacterium avium* complex (MAC) is common in patients with AIDS. We evaluated both clarithromycin and dapsone alone and in combination for the treatment and prevention of disseminated MAC disease in beige mice. In the therapeutic model, C57BL/6 beige mice were infected intravenously with strain 101 of MAC (serovar 1). After 1 week postinfection, mice were given clarithromycin (200 mg/kg of body weight per day) and dapsone (15 mg/kg of body weight per day) alone or in combination by gavage. Treatment with clarithromycin resulted in a significant reduction in bacteremia and the numbers of CFU of MAC in the liver and spleen. Treatment with dapsone had no effect on the mycobacterial counts in blood, liver, or spleen, and the combination of dapsone with clarithromycin was no better than clarithromycin as a single agent. Clarithromycin and dapsone were used to prevent systemic disease in beige mice infected orally with MAC 101. Clarithromycin prophylaxis was associated with a significant reduction in the numbers of bacteria in the liver, spleen, and appendix compared with those in controls. Prophylaxis with dapsone resulted in a mild reduction in the numbers of MAC in the spleen but not in the other tissues. Clarithromycin both treats and prevents MAC disease in beige mice. Dapsone has no therapeutic effect, but it does have a slight prophylactic effect, and in combination with clarithromycin it does not abrogate the effect of clarithromycin.—Authors' Abstract

**Boom, W. H., Balaji, K. N., Nayak, R., Tsukaguchi, K. and Chervenak, K. A.** Characterization of a 10- to 14-kilodalton protease-sensitive *Mycobacterium tuberculosis* H37Ra antigen that stimulates human gamma delta T cells. Infect. Immun. **62** (1994) 5511–5518.

γδ T-cell receptor-bearing T cells (γδ T cells) are readily activated by intracellular bacterial pathogens such as *Mycobacterium tuberculosis*. The bacterial antigens responsible for γδ T-cell activation remain poorly characterized. We have found that heat treatment of live *M. tuberculosis* bacilli released into the supernatant an antigen which stimulated human γδ T cells; γδ T-cell ac-

tivation was measured by determining the increase in percentage of γδ T cells by flow cytometry in peripheral blood mononuclear cells stimulated with antigen and by proliferation of γδ T-cell lines with monocytes as antigen-presenting cells. Supernatant from heat-treated *M. tuberculosis* was fractionated by fast-performance liquid chromatography (FPLC) on a Superose 12 column. Maximal γδ T-cell activation was measured for a fraction of 10 to 14 kDa. Separation of the supernatant by preparative isoelectric focusing demonstrated peak activity at a pI of < 4.0. On two-dimensional gel electrophoresis, the 10- to 14-kDa FPLC fraction contained at least seven distinct molecules, of which two had a pI of < 4.5. Protease treatment reduced the bioactivity of the 10- to 14-kDa FPLC fraction for both resting and activated γδ T cells. Murine antibodies raised to the 10- to 14-kDa fraction reacted by enzyme-linked immunosorbent assay with antigens of 10 to 14 kDa in lysate of *M. tuberculosis*. In addition, γδ T cells proliferated in response to an antigen of 10 to 14 kDa present in *M. tuberculosis* lysate. γδ T-cell-stimulating antigen was not found in culture filtrate of *M. tuberculosis* but was associated with the bacterial pellet and lysate of *M. tuberculosis*. These results provide a preliminary characterization of a 10- to 14-kDa, cell-associated, heat-stable, low-pI protein antigen of *M. tuberculosis* which is a major stimulus for human γδ T cells.—Authors' Abstract

**Bottger, E. C.** *Mycobacterium genavense*: an emerging pathogen. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 932–936.

Disseminated mycobacterial infection, often due to *Mycobacterium avium* complex, occurs frequently in patients with AIDS. More recently, a newly identified mycobacterium, *M. genavense*, has been repeatedly isolated from AIDS patients. *M. genavense* has unusual fastidious growth requirements and shows poor and variable growth *in vitro*. Molecular biology techniques are necessary for accurate diagnosis of infection and have established *M. genavense* to be a definite cause of disseminated mycobacterial infection in immunosuppressed patients. The clinical manifestations of infection caused by *M. genavense* are similar to those of infection caused by *M. avium* complex organisms. This similarity in clinical presentation and inherent difficulties in applying standard, culture-based techniques for detection and identification of mycobacteria have probably led to underestimation of the prevalence of disseminated *M. genavense* infection in patients with AIDS.—Author's Abstract

**Chapuis, L., Ji, B. H., Truffot-Pernot, C., O'Brien, R. J., Raviglione, M. C. and Grosset, J.-H.** Preventive therapy of tuberculosis with rifapentine in immunocompetent and nude mice. Am. J. Respir. Crit. Care Med. **150** (1994) 1355–1362.

The effectiveness of intermittent administration of rifapentine (RPT), with or without isoniazid (INH), for preventive therapy of tuberculosis was evaluated in immunocompetent (normal) and nude mice. After infection with a small inoculum of *Mycobacterium tuberculosis* H37Rv, normal mice developed a chronic and nonfatal infection, and the bacterial population became relatively stable after an initial period of limited multiplication. On the other hand, nude mice developed an acute and fatal infection, and all untreated mice died within 5 wk, with very high colony-forming-unit (CFU) counts in their organs. Various degrees of bactericidal activity were shown in normal mice after daily treatment with rifampin (RMP) plus pyrazinamide (PZA) for 13 wk, INH daily for 26 wk, or RPT once weekly for 13 wk or 26 wk or once fortnightly for 26 wk. The activity of RPT was significantly enhanced when INH was added at the same dosing frequency. In nude mice the response of *M. tuberculosis* infection to certain regimens was less favorable than that in normal mice, suggesting that preventive therapy may be less effective in severely immunodeficient hosts even during treatment. After chemotherapy was stopped, virtually all nude mice relapsed within 12 wk regardless of the regimen administered; whereas no or very few relapses were observed in normal mice that had been treated with RMP + PZA daily for 13 wk, or RPT alone or RPT + INH once weekly for 26 wk. The latter three regimens and RPT + INH once weekly for 13 wk may be applied for fixed-duration preventive therapy in human im-

munodeficiency virus (HIV)-negative subjects. Because relapse of tuberculosis was almost unavoidable in nude mice after stopping chemotherapy, immunodeficient hosts, such as HIV-positive subjects, may require lifelong preventive therapy for tuberculosis. Because RPT alone or RPT + INH once weekly displayed significant bactericidal activity against *M. tuberculosis,* and RPT + INH once fortnightly prevented the bacterial population from increasing in nude mice, the three regimens may be considered for trials of lifelong preventive therapy in HIV-positive subjects.—Authors' Abstract

**Colston, A., McConnell, I. and Bujdoso, R.** Cloning and expression in *Escherichia coli* of DNA encoding a 60 kDa stress protein of *Mycobacterium paratuberculosis,* the causative agent of Johne's disease. Microbiology **140** (1994) 3329–3336.

Polymerase chain reaction (PCR) was used to generate DNA encoding a 60-kDa stress protein of *Mycobacterium paratuberculosis* using primers complementary to sequences at the 5′ and 3′ ends of 60-kDa stress protein genes (encoding the "65-kDa antigens") of *M. leprae* and *M. tuberculosis.* The predicted PCR product of 1.8 kb contained the entire coding sequence of an *M. paratuberculosis* 60-kDa stress protein, with noncoding regions of 124 bp and 1 bp at the 5′ and 3′ ends, respectively. DNA encoding the entire ORF for the 60-kDa stress protein, as well as thrombin and Factor Xa proteolytic cleavage sites, was ligated into the bacterial expression vector pGEX-2T and used to transform *Escherichia coli* strain JM83. Transformed bacteria, induced by IPTG, expressed an 85-kDa fusion protein comprising glutathione S-transferase (GST) and *M. paratuberculosis* 60-kDa stress protein. This fusion protein was purified by adsorption to glutathione-agarose beads and shown to crossreact in Western blot analysis with an anti-mycobacterial 60-kDa stress protein monoclonal antibody. Recombinant *M. paratuberculosis* 60-kDa stress protein was liberated from GST by proteolytic cleavage with either thrombin or Factor Xa enzyme. Authenticity of liberated recombinant stress protein was confirmed by N-terminal amino acid sequencing.—Authors' Abstract

**Comstock, G. W.** Tuberculosis: is the past once again prologue? Am. J. Public Health **84** (1994) 1729–1731.

Tuberculosis has been considered the result of hereditary susceptibility, miasmas in the environment, and contact with contagious patients. During most of the latter half of this century, tuberculosis control efforts have concentrated almost exclusively on contagion by treating patients to make them noninfectious, treating latent tuberculosis to prevent reactivation, and in some countries, vaccinating uninfected persons to protect them from the consequences of infection. With the resurgence of tuberculosis in 1985, interest in all methods of tuberculosis control has been rekindled. Much remains to be discovered and much needs to be done. If renewed efforts succeed in again forcing tuberculosis rates downward, will we have the wisdom to eliminate tuberculosis in the United States, or will we relax and bring about another resurgence?—Author's Abstract

**Dannenberg, A. M.** Roles of cytotoxic delayed-type hypersensitivity and macrophage-activating cell-mediated immunity in the pathogenesis of tuberculosis. Immunobiology **191** (1994) 461–473.

The tubercle bacillus is a facultative intracellular parasite that grows well in nonactivated macrophages. When large numbers of these bacilli have grown intracellularly within such macrophages, a cytotoxic immune response, herein called tissue-damaging (or necrotizing) delayed-type hypersensitivity (DTH), kills the macrophages (and usually some of the surrounding tissue), forming the caseous center of the developing tubercle. In solid caseum, tubercle bacilli may survive, but do not multiply. When bacilli escape from the edge of the caseum, they are rapidly ingested by nearby viable macrophages. If these macrophages have not been activated, the bacilli again multiply intracellularly, and the cytotoxic immune response kills the bacilli-laden macrophages (and surrounding tissue), thus enlarging the caseous center. In hosts that develop poor activation of macrophages, this process is repeated until much of the lung is destroyed. In hosts that can develop good activation of macrophages (by cytokines

from antigen-specific T cells), herein called cell-mediated immunity (CMI), the caseous centers become surrounded by these activated macrophages, which ingest and destroy the bacilli escaping from the caseum. This process can arrest the disease.

Unfortunately, the caseous center may liquefy in such resistant hosts. In the liquefied menstruum, the bacilli may grow extracellularly (for the first time during the course of the disease), reaching tremendous numbers. The cytotoxic immune response to these numerous bacilli and their tuberculin-like products causes much tissue necrosis, including erosion of the walls of small bronchi, which results in cavity formation. From such cavities, the bacilli spread to other parts of the lung and to the environment. The extracellular multiplication of tubercle bacilli in the liquefied caseum is the main reason why tuberculosis perpetuates itself in mankind. It is also the reason why antimicrobial, drug-resistant bacillary strains develop. To elucidate the various mechanisms involved in macrophage activation, caseation, and liquefaction is a major challenge for tuberculosis researchers today.—Author's Abstract

**Donnabella, V., Martiniuk, F., Kinney, D., Bacerdo, M., Bonk, S., Hanna, B. and Rom, W. N.** Isolation of the gene for the beta subunit of RNA polymerase from rifampicin-resistant *Mycobacterium tuberculosis* and identification of new mutations. Am. J. Respir. Cell Mol. Biol. **11** (1994) 639–643.

Tuberculosis (TB) is one of the most important infections worldwide, with an estimated incidence of 10 million active cases per year. Rifampin is a key component of the first-line therapy used in the treatment of tuberculosis. In *Escherichia coli* and *Mycobacterium leprae,* rifampin has been shown to inhibit the beta subunit of RNA polymerase. The gene (rpoB) encoding this enzyme has been described in both species. We report the isolation of the homologous functional rifampin resistance gene from *M. tuberculosis.* A library was constructed with 15- to 25-kb BamHI-digested DNA fragments from a rifampin-resistant *M. tuberculosis* clinical isolate that was ligated into an *E. coli*-mycobacterial shuttle plasmid. Southern analysis of BamHI-digested DNA from 200 recombinant plasmids was performed and filters were hybridized to a 411-bp fragment of the beta subunit of RNA polymerase from *M. tuberculosis.* Only DNA from one plasmid (#86) hybridized, which suggested that the gene is found as a single copy per genome. This plasmid was able to transfer rifampin resistance to sensitive *M. smegmatis* and thus codes for a functional genetic unit. Sequence analysis in the expected "hotspot" region in eight rifampin-resistant *M. tuberculosis* strains (including one multidrug-resistant strain) revealed two novel mutations as well as others previously described.—Authors' Abstract

**Doran, T. J., Davies, J. K., Radford, A. J. and Hodgson, A. L. M.** Putative functional domain within ORF2 on the mycobacterium insertion sequences. Immunol. Cell Biol. **72** (1994) 427–434.

Repeated DNA sequences have been identified in a range of mycobacterial species and have been implicated in the increased virulence of some of these species, namely, *Mycobacterium paratuberculosis* and *M. avium* subsp. *silvaticum.* Here we present a case to suggest that the insertion sequences IS900 and IS902 encode a protein from a putative gene positioned on the complementary strand to their transposase genes. Based on amino acid homology analyses, this open reading frame (ORF2) could encode a transport protein. The ORF2 protein, thus IS900 and IS902, may have a role in the increased pathogenicity of *M. paratuberculosis* and *M. avium* subsp. *silvaticum* from an *M. avium* background.—Authors' Abstract

**Douvas, G. S., May, M. H., Pearson, J. R., Lam, E., Miller, L. and Tsuchida, N.** Hypertriglyceridemic serum, very low density lipoprotein, and iron enhance *Mycobacterium avium* replication in human macrophages. J. Infect. Dis. **170** (1994) 1248–1255.

The growth of *Mycobacterium avium* 7497, serovar 4, in cultured human macrophages is enhanced by $Fe^{3+}$ and serum lipids over 7 days. Iron (1–80 μg/ml) added to macrophages cultured in normal serum resulted in 10-fold increases in growth. If

iron-supplemented macrophages were cultured in serum from hypertriglyceridemic donors after infection, *M. avium* growth increased $10^3$- to $10^4$-fold. Without macrophages, differences in bacterial growth between sera were not seen. Removal of very low-density lipoprotein (VLDL) eliminated the differences between sera. Isolated VLDL from hyperlipidemic serum resulted in $10^5$-fold increases in growth over that seen with VLDL from normal sera. Accelerated *M. avium* growth in macrophages cultured with hyperlipidemic serum was partly inhibited by the addition of superoxide dismutase (1000 IU/ml). Results suggest that iron stimulates $O_2$-induced oxidation of VLDL and its subsequent accumulation in macrophages. The resultant iron- and lipid-laden cells become excellent hosts for mycobacterial growth.—Authors' Abstract

**Gardner-Medwin, J. M. M., Smith, N. J. and Powell, R. J.** Clinical experience with thalidomide in the management of severe oral and genital ulceration in conditions such as Behcet's disease: use of neurophysiological studies to detect thalidomide neuropathy. Ann. Rheum. Dis. **53** (1994) 828–832.

Objective—To examine the efficacy, dose, and safety profile, including neurophysiological testing, of thalidomide used in 59 patients (including 23 with Behcet's disease) to treat severe oral or genital ulceration (OGU).

Methods—We identified prospectively subjects (including women of childbearing potential) who had persistent OGU over periods lasting 1 to 40 years and whose active ulceration was not controlled by other therapies. They were treated with thalidomide. Retrospectively, we identified the number of subjects with complete resolution of the ulcers at 1 and 2 months of thalidomide therapy, and the dose required to maintain that improvement in those individuals who relapsed after stopping thalidomide. The decrease from the baseline sensory nerve action potential (baseline SNAP) amplitude value (derived from median, radial and sural nerve SNAPs) at which the development of paraesthesiae was likely to occur was also determined.

Results—Complete resolution of the ulcers occurred in 81% of the patients within 1 month of thalidomide therapy at doses of 200 mg/day. No further thalidomide was required by 20% of the patients responding, and in the remainder improvement was maintained with smaller doses (7–200 mg/day). Using an approximate 50% decrease from baseline SNAP as an indication to discontinue thalidomide, the incidence of symptomatic neuropathy was 13.5%. No patients with a decrease of $< 42\%$ developed neuropathy, and a further 13.5% were asymptomatic with a decrease in SNAP between 42% and 69%. Other side effects were seen in 44% of patients. There were no pregnancies and no requirement for urgent pregnancy testing.

Conclusions—Thalidomide provided a useful therapeutic option in severe oral and genital ulceration which had not responded to other therapies. The physician must remain vigilant to the continuing danger of axonal neuropathy and teratogenesis at all times during thalidomide therapy.—Authors' Abstract

**Havlir, D. V.** *Mycobacterium avium* complex: advances in therapy. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 915–924.

Disseminated *Mycobacterium avium* complex (MAC) is one of the most common opportunistic infections in AIDS patients and is increasingly recognized as a significant pathogen in chronic pulmonary disease in nonimmunocompromised patients. Important progress in therapy has occurred over the last several years. In AIDS patients, multidrug therapy has been shown to be beneficial in terms of reducing circulating bacteremia and improving clinical symptoms. Clarithromycin and azithromycin, two broad-spectrum antimicrobials with minimal activity against *M. tuberculosis,* have emerged as potent, well tolerated agents pivotal to treatment regimens. In AIDS patients, rifabutin prophylaxis reduced the frequency of MAC bacteremia by 50% in two placebo controlled trials. Despite these advances, there remains a need for determining the optimal combination regimens for therapy, and more effective drugs for prophylaxis which are beneficial both in

terms of survival and functional capacity of patients.—Author's Abstract

**Hawes, G. E., Struyk, L., Godthelp, B. C. and Vandenelsen, P. J.** Limited restriction in the TCR-alpha beta V region usage of antigen-specific clones—recognition of myelin basic protein (amino acids 84–102) and *Mycobacterium bovis* 65-kDa heat shock protein (amino acids 3–13) by T cell clones established from peripheral blood mononuclear cells of monozygotic twins and HLA-identical individuals. J. Immunol. **154** (1995) 555–566.

We have analyzed the TCR-alpha beta repertoire specific for a given peptide/MHC complex by using pairs of HLA-identical individuals, ranging from monozygotic twins to unrelated individuals, to examine the contribution of genetic background and HLA expression in shaping the potential response to a single antigenic epitope. This panel has been previously defined, demonstrating that the concordance of the peripheral TCR-alpha beta repertoires directly correlates to the level of relation and HLA identity. We have analyzed peptide-specific T-cell clones derived from T-cell lines from these individuals specific for MHC class II-restricted peptides: *Mycobacterium bovis* 65-kDa heat shock protein (65-kDa hsp) amino acids (aa) 3-13 (DR3-restricted), and myelin basic protein aa 84-102 (DR2-restricted). DNA sequence analysis was used to determine the composition of the TCR-alpha beta V regions. Although the overall TCR-alpha beta repertoires between individuals were diverse, an intra-individual limited restriction was evident. There was also a limited conservation in the response to the different peptides: high frequencies of V beta 2, 4, 7, 19, V alpha 21, and J alpha 17 responded to the MBP aa84-102, whereas these V/J regions were limited or absent in the 65-kDa hsp aa3-13 repertoire. Similarly, V beta 5.1 and J alpha 9 were increased in the 65-kDa hsp aa3-13 repertoire. Within the CDR3s, motifs could be identified that were similar between twins. Furthermore, one of these motifs resembled CDR3s previously found in corresponding animal models. Similarities could also be seen in the CDR3s of T-cell clones sharing V gene usage and peptide specificity. Thus, the *in vitro* response to antigenic peptides seems to be quite heterogeneous overall and individual specific.—Authors' Abstract

**Heym, B., Alzari, P. M., Honore, N. and Cole, S. T.** Missense mutations in the catalase-peroxidase gene, *katG*, are associated with isoniazid resistance in *Mycobacterium tuberculosis*. Mol. Microbiol. **15** (1995) 235–245.

The toxicity of the powerful antituberculosis drug isoniazid (INH) is believed to be mediated by the heme containing enzyme catalase-peroxidase, encoded by the *katG* gene of *Mycobacterium tuberculosis*. Compelling evidence for this was obtained by studying a panel of INH-resistant clinical isolates using a novel strategy based on the polymerase chain reaction and single-strand-conformation polymorphism analysis (PCR-SSCP) to detect mutations in *katG*. In most cases INH resistance was associated with missense mutations while in a small number of strains the gene had been completely, or partially, deleted. The missense mutations fell into two groups, the larger of which contained several independent mutations that affected the *N*-terminal peroxidase domain of the protein, resulting in the production of a catalase peroxidase with strongly reduced enzyme activity and increased heat lability. The effects of these substitutions could be interpreted by means of molecular modelling using the crystal structure of the related enzyme cytochrome *c* peroxidase from yeast as a template. The second group comprises a frequently occurring amino acid substitution and a single mutation that are both located in the *C*-terminal domain but do not noticeably alter either enzyme activity or heat stability.—Authors' Summary

**Hirsch, C. S., Yoneda, T., Averill, L., Ellner, J. J. and Toossi, Z.** Enhancement of intracellular growth of *Mycobacterium tuberculosis* in human monocytes by transforming growth factor-beta 1. J. Infect. Dis. **170** (1994) 1229–1237.

The production of transforming growth factor-beta (TGF beta 1) by human monocytes (MN) infected with *Mycobacterium tuberculosis* and its effects on the intracellular fate of the organism were studied. *M.*

*tuberculosis* infection of MN induced both expression of mRNA and secretion of tumor necrosis factor-alpha (TNF alpha) and TGF beta 1 protein. Neutralizing antibody to TGF beta 1 reduced the intracellular growth of *M. tuberculosis*. The growth-enhancing effects of TGF beta 1 could not be explained by increased initial bacterial load. Preculture with TGF beta 1 decreased uptake of *M. tuberculosis*. Exposure of MN to increasing concentrations of TGF beta 1 before or after infection with *M. tuberculosis* accelerated intracellular bacterial replication. Both TNF alpha and interferon-gamma (IFN-gamma) limited mycobacterial replication. TGF beta 1 (10 ng/mL) abrogated the bacteriostatic effects of TNF alpha and IFN-gamma. Within the infected focus, TGF beta 1 produced by mononuclear phagocytes may play an important role in the pathogenesis of tuberculosis, in part by modulating the response to potentially protective cytokines such as TNF alpha and IFN-gamma.—Authors' Abstract.

**Hoffner, S. E.** Pulmonary infections caused by less frequently encountered slow-growing environmental mycobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 937–941.

Pulmonary mycobacteriosis is usually caused by *Mycobacterium tuberculosis* or *M. avium* complex. There are, however, other slow-growing mycobacteria that can cause pulmonary infection. *M. kansasii, M. malmoense, M. xenopi, M. szulgai* and *M. simiae* typically infect middle-aged to elderly persons with preexisting lung disease. Differentiation of infection with these five mycobacteria from infection with *M. tuberculosis,* by culture and determination of the antimicrobial susceptibility pattern of the organism are important for several reasons. All five organisms are found in water and soil. They probably infect humans from environmental habitats; human-to-human spread of infection is thought not to occur. Furthermore, isolation of the organisms in culture may represent contamination of the specimen or colonization of the patient, and not necessarily an infection. Finally, although the antituberculosis drugs—isoniazid, ethambutol, rifampin and streptomycin—have been used for treatment of infection with these five organisms, there are often differences between the antimycobacterial susceptibility patterns of *M. tuberculosis* and those of the nontuberculous mycobacteria. Thus, the optimal choice of drug therapy may differ from that used for tuberculosis.—Author's Abstract

**Honore, N., Marchal, G. and Cole, S. T.** Novel mutation in 16S rRNA associated with streptomycin dependence in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **39** (1995) 769–770.

Molecular characterization of a streptomycin-dependent mutant of *Mycobacterium tuberculosis* revealed the presence of a novel mutation in the *rrs* gene encoding 16S rRNA. Insertion of an additional cytosine in the 530 loop of 16S rRNA, a region known to be involved in streptomycin susceptibility and resistance, was associated with streptomycin dependence.—Authors' Abstract

**Kapur, V., Li, L. L., Hamrick, M. R., Plikaytis, B. B., Shinnick, T. M., Telenti, A., Jacobs, W. R., Banerjee, A., Cole, S., Yuen, K. Y., Clarridge, J. E., Kreiswirth, B. N. and Musser, J. M.** Rapid *Mycobacterium* species assignment and unambiguous identification of mutations associated with antimicrobial resistance in *Mycobacterium tuberculosis* by automated DNA sequencing. Arch. Pathol. Lab. Med. **119** (1995) 131–138.

*Objective.* To develop and demonstrate the utility of automated DNA sequencing strategies for rapid and unambiguous identification of *Mycobacterium* species and mutations associated with antimicrobial resistance in *Mycobacterium tuberculosis.*

A 360-base pair segment of the gene (hsp65) encoding a 65-kd heat shock protein was characterized from 91 isolates assigned to 24 *Mycobacterium* species by traditional biochemical techniques. Areas of seven genes recently shown to contain mutations associated with antimicrobial resistance in *M. tuberculosis* strains were also sequenced in a sample of 128 resistant organisms. Early positive BACTEC 460 cultures and acid-fast, bacterium-positive sputum specimens from patients with tuberculosis were also studied. Automated DNA sequencing iden-

tified species-specific polymorphism in the target segment of hsp65, successfully identified organisms to the species level in smear-positive sputum samples, and unambiguously characterized seven genes associated with antimicrobial resistance in *M. tuberculosis.*

Rapid identification of *M. tuberculosis* and other *Mycobacterium* species is possible by automated DNA sequencing of a portion of hsp65. The technique is also feasible for analysis of some smear-positive sputum specimens. Unambiguous characterization of target segments of genes harboring mutations associated with antimicrobial resistance in *M. tuberculosis* is possible from primary patient specimens. Taken together, the data demonstrate the feasibility of mycobacterial species identification and potential to identify mutations associated with antimicrobial resistance in less than 48 hr.—Authors' Abstract

**Kiehn, T. E. and White, M.** *Mycobacterium haemophilum*: an emerging pathogen. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 925–931.

*Mycobacterium haemophilum* is emerging as a pathogen of immunocompromised patients, particularly those with AIDS and organ transplants. Infection has also occurred in healthy children. Adults usually present with cutaneous manifestations, septic arthritis or occasionally pneumonia. Children have perihilar, cervical or submandibular adenitis. The organism grows on mycobacterial media supplemented with ferric ammonium citrate or hemin, incubated at 30°C to 32°C, 2 to 3 weeks after inoculation. The most active antimicrobial agents *in vitro* are amikacin, ciprofloxacin, clarithromycin, rifabutin and rifampin. Development of resistance to the rifamycins has been demonstrated after patients were treated for several months with several antimycobacterial agents, including the rifamycins. Treatment for several months with at least two agents demonstrated to have low MICs for the organism has been shown to be effective.—Authors' Abstract

**Mor, N., Vanderkolk, J., Mezo, N. and Heifets, L.** Effects of clarithromycin and rifabutin alone and in combination on intracellular and extracellular replication of *Mycobacterium avium.* Antimicrob. Agents Chemother. **38** (1994) 2738–2742.

The combined effect of clarithromycin and rifabutin against *Mycobacterium avium* multiplying either within human monocyte-derived macrophages or extracellularly in a liquid medium was additive: both MICs and MBCs were twofold lower for the combination than they were for each drug alone. Prolonged exposure for 4 weeks of *M. avium*-infected macrophages to combined concentrations that were only twofold greater than the MICs resulted in a 100-fold decrease in the number of viable bacteria, while in the drug-free controls a 100-fold or greater increase in comparison with the initial viable counts took place. Comparison of this effect with the results of the prolonged exposure to each drug alone suggested that under these experimental conditions rifabutin enhanced the antimicrobial activity of clarithromycin against intracellular bacteria. At the same time, inhibition of intracellular growth by a 2-hr pulsed exposure of the infected macrophages to the combination of the two drugs was not different from the effect induced by clarithromycin alone. In conclusion, clarithromycin played the major role in the antimicrobial activity of the tested combination, while rifabutin may have enhanced this effect during a prolonged exposure of the intracellular bacteria to these two agents.—Authors' Abstract

**Levin, M., Newport, M. J., D'Souza, S., Kalabalikis, P., Brown, I. N., Lenicker, H. M., Agius, P. V., Davies, E. G., Thrasher, A., Klein, N. and Blackwell, J. M.** Familial disseminated atypical mycobacterial infection in childhood: a human mycobacterial susceptibility gene? Lancet **345** (1995) 79–83.

Inherited defects in specific components of the immune system have provided many clues to the immunological mechanisms underlying resistance to microbial infection. We report a familial immune defect predisposing to disseminated atypical mycobacterial infection in childhood.

Six children with disseminated atypical mycobacterial infection and no recognized form of immunodeficiency were identified. Four, including two brothers, come from a

village in Malta, and two are brothers of Creek Cypriot origin. They presented with fever, weight loss, lymphadenopathy, and hepatosplenomegaly. They had anemia and an acute-phase response. A range of different mycobacteria (*Mycobacterium fortuitum, M. chelonei,* and four strains of *M. avium intracellulare* complex) were isolated. Treatment with multiple antibiotics failed to eradicate the infection, although treatment with gamma interferon was associated with improvement. Three have died and the surviving children have chronic infection. Tumor necrosis factor-$\alpha$ production in response to endotoxin and gamma-interferon was found to be defective in affected patients and their parents. T-cell proliferative responses to mycobacterial and recall antigens were reduced in parents of affected children and gamma-interferon production was diminished in the affected patients and their parents. Clinical and immunological features suggest that these patients are phenotypically similar to Lsh/Ity/Bcg susceptible mice. Understanding of this defect may provide insights into the mechanisms responsible for susceptibility to mycobacteria.—Authors' Abstract

**Lim, E. M., Rauzier, J., Timm, J., Torrea, G., Murray, A., Gicquel, B. and Portnoi, D.** Identification of *Mycobacterium tuberculosis* DNA sequences encoding exported proteins by using *phoA* gene fusions. J. Bacteriol. **177** (1995) 59–65.

The activity of bacterial alkaline phosphatase (PhoA) is dependent on it being exported across the plasma membrane. A plasmid vector (pJEM11) allowing fusions between *phoA* and genes encoding exported proteins was constructed to study protein export in mycobacteria. Introduction of the *Mycobacterium fortuitum* $\beta$-lactamase gene (*blaF**) into this vector led to the production in *M. smegmatis* of protein fusions with PhoA activity. A genomic library from *M. tuberculosis* was constructed in pJEM11 and screened in *M. smegmatis* for clones with PhoA activity. Sequences of the *M. tuberculosis* inserts directing the production of protein fusions in these PhoA-positive clones were determined. They include part of the already known exported 19-kDa lipoprotein, a sequence with similarities to the exported 28-kDa antigen from *M. leprae,* a sequence encoding a protein sharing conserved amino acid motifs with stearoyl-acyl-carrier-protein desaturases, and unknown sequences. This approach thus appears to identify sequences directing protein export, and we expect that more extensive screening of such libraries will lead to a better understanding of protein export in *M. tuberculosis.*—Authors' Abstract

**Lounis, N., Ji, B.-H., Truffot-Pernot, C. and Grosset, J.** Selection of clarithromycin-resistant *Mycobacterium avium* complex during combined therapy using the beige mouse model. Antimicrob. Agents Chemother. **39** (1995) 608–612.

Sixteen weeks of treatment with clarithromycin (CLARI) alone displayed significant bactericidal activity against *Mycobacterium avium* complex infection in beige mice. Only two combined regimens, CLARI combined with an initial 4 or 8 weeks of amikacin (AMIKA), displayed activity greater than that displayed by CLARI alone. Four other combined regimens, CLARI combined with ethambutol (EMB), rifabutin (RBT), or both EMB and RBT during the entire 16 weeks of treatment or with AMIKA administered in an initial 2-week course showed bactericidal activity not significantly greater than that of CLARI alone. After 16 weeks of treatment, CLARI-resistant mutants were isolated from the majority of mice that had been treated with CLARI alone, CLARI-RBT, CLARI-EMB, or CLARI-EMB-RBT, as was the case for untreated controls, but the frequencies of occurrence of mutants were significantly greater in the groups treated with these combinations or CLARI alone. On the other hand, no CLARI-resistant mutants were isolated from the mice that had been treated with the combination of CLARI plus an initial 4 or 8 weeks of AMIKA and were isolated from only a tiny proportion of mice that had been treated with CLARI plus an initial 2 weeks of AMIKA. Therefore, only treatment with CLARI combined with an initial 4 or 8 weeks of AMIKA but not combined with RBT or EMB or both, could enhance the activity of the drug treatment and prevent the selection of CLARI-resistant mutants.—Authors' Summary

**Nabeshima, S., Hiromatsu, K., Matsuzaki, G., Mukasa A., Takada, H., Yoshida, S. and Nomoto, K.** Infection of *Mycobacterium bovis* bacillus Calmette-Guerin in antibody-mediated gamma delta T-cell-depleted mice. Immunology **84** (1995) 317–321.

To evaluate the hypothesis that gamma delta T cells participate in protective immunity against mycobacterial infection, we depleted gamma delta T cells from mice by administration of anti-T-cell receptor (TCR) gamma delta monoclonal antibody (mAb) and analyzed protection against *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). The gamma delta T-cell-depleted mice did not show any exaggerated bacterial multiplication compared with control mice. In contrast, alpha beta T-cell-depleted mice, which were administrated anti-TCR alpha beta mAb before BCG infection, showed a depressed protective immunity. These results suggest that gamma delta T cells are not essential for coping with a primary BCG infection.—Authors' Abstract

**Nettleman, M. D., Fredrickson, M., Good, N. L. and Hunter, S. A.** Tuberculosis control strategies: the cost of particulate respirators. Ann. Intern. Med. **121** (1994) 37–40.

Objective: To assess the cost of the mandatory use of high-efficiency particulate respirators to treat patients with known or suspected tuberculosis.

A questionnaire was used to determine the number of high-efficiency particulate respirators required and the number of cases of tuberculosis in employees that could potentially be prevented. Indirect costs included the training and fitness testing of employees. The clinical efficacy of respirators is not known. To provide a best-case scenario, it was assumed that the respirators could prevent as many as 25% of tuberculosis cases in health care workers.

Setting: 159 acute care facilities administered by the Department of Veterans Affairs. Participants: Quality improvement, infection control, and employee health specialists. Measurements: Cost of the respirators compared with their maximum predicted efficacy. Results: The use of the respirators would cost $7 million per case of tuberculosis prevented and $100 million per life saved.

Conclusions: High-efficiency particulate respirators are a costly means of trying to prevent tuberculosis. Costs could be reduced by reusing masks or by restricting the number of health care workers allowed to have contact with potentially infectious patients. As the health care budget undergoes further restrictions, specific means of accommodating the cost of new regulations must be found.—Authors' Abstract

**Neubert, R., Helge, H. and Neubert, D.** Thalidomide and the immune system 4. Down-regulation of the CD26 receptor, probably involved in the binding of HIV components to T cells in primates. Life Sci. **56** (1994) 407–420.

Thalidomide (Thd) is capable of down-regulating the CD26 receptor on CD4+ lymphocytes after treatment of healthy volunteers. Similar effects are observed when marmosets (*Callithrix jacchus*) are treated with Thd. The Ta-1 epitope of the CD26 receptor has recently been shown to bind the HIV-1 Tat trans-activating protein, and CD26 has also been suggested to be a co-receptor for the binding of the V3 loop of the gp120 HIV envelope protein. This might provide a hint for possible therapeutic interventions.—Authors' Abstract

**O'Brien, L., Carmichael, J., Lowrie, D. B. and Andrew, P. W.** Strains of *Mycobacterium tuberculosis* differ in susceptibility to reactive nitrogen intermediates *in vitro*. Infect. Immun. **62** (1994) 5187–5190.

The effects on the viability of *Mycobacterium tuberculosis* strains and one *M. bovis* strain from exposure to sodium nitrite for 24 hr, in both neutral and acidic media, were tested. The *in vitro* resistance of mycobacteria to reactive nitrogen intermediates, generated at an acidic pH, was found to have a significant ($p < 0.05$) positive correlation to the virulence of strains in guinea pigs.—Authors' Abstract

**Ordway, D. J., Sonnenberg, M. G., Donahue, S. A., Belisle, J. T. and Orme, I. M.** Drug-resistant strains of *Mycobacterium tuberculosis* exhibit a range of virulence

for mice. Infect. Immun. **63** (1995) 741–743.

A panel of clinical isolates of *Mycobacterium tuberculosis,* several of which were resistant to one or more antimycobacterial drugs, were tested for their capacity to give rise to active disease following aerogenic infection of normal immunocompetent mice. The panel exhibited a range of virulence in this model, which followed no clear trend in terms of geographical source, degree of drug resistance, or rate of growth *in vitro.* Several isolates grew very quickly over the first 20 days in mouse lungs before being contained by emerging immunity. In view of this latter observation, we hypothesize that it is possible that such so-called fast growers may be responsible for the rapid fatality sometimes seen in immunocompromised patients with tuberculosis. Moreover, the results of the study do not support the belief that increased drug resistance usually associates with loss of virulence of the isolate.—Authors' Abstract

**Orme, I. M., Roberts, A. D., Furney, S. K. and Skinner, P. S.** Animal and cell-culture models for the study of mycobacterial infections and treatment. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 994–999.

Emerging problems with the treatment of infections caused by *Mycobacterium avium* and *M. tuberculosis* require the development of new models, both *in vitro* and *in vivo,* in which new chemotherapeutic and immunotherapeutic approaches can be tested. In this brief review, the use of cell culture models, in which drugs can be tested for their capacity to inhibit mycobacterial growth within the infected host macrophage, and new models *in vivo* in which drugs and/or cytokines can be tested in infected mice are discussed. In this latter case, new emerging mouse models include animals with engineered gene disruptions, in which severely disseminated infections can be produced, thus mimicking events in severely immunocompromised human patients.—Authors' Abstract

**Paterson, D. L., Georghiou, P. R., Allworth, A. M. and Kemp, R. J.** Thalidomide as treatment of refractory aphthous ulceration related to human immunodeficiency virus infection. Clin. Infect. Dis. **20** (1995) 250–254.

In recent years, thalidomide has been used for the treatment of a variety of ulcerative and immunologic conditions. Several previous reports have suggested that thalidomide therapy is beneficial for patients with aphthous ulceration related to human immunodeficiency virus (HIV) infection. We describe the use of thalidomide in 20 HIV-infected patients with oropharyngeal, esophageal, and rectal ulceration. Nineteen patients had a dramatic response to thalidomide therapy, with both subjective and objective abatement in the signs and symptoms of their ulcerative disease. The standard treatment course was 200 mg of thalidomide for 14 days (the drug was administered at night). Four patients required additional courses of treatment because symptoms recurred after thalidomide therapy was stopped. Side effects due to thalidomide included rash (5 patients), peripheral neuropathy (1 patient), and excessive fatigue (1 patient). There did not appear to be any adverse immunologic effects in thalidomide-treated patients. The mechanism of the effect of thalidomide is uncertain, although recent studies have suggested that thalidomide selectively inhibits the production of tumor necrosis factor-alpha.—Authors' Abstract

**Pietrzak, J., Frei, R., Senn, H. P. and Moroni, C.** Comparison of polymerase chain reaction with standard methods in the diagnosis of *Mycobacterium tuberculosis* infection. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 1079–1083.

The polymerase chain reaction (PCR) method was evaluated in the detection of *Mycobacterium tuberculosis* in comparison with direct microscopy and culture procedures including the standard radiometric system (BACTEC). Amplified DNA fragments from clinical samples were analyzed by dot-blot and nonradioactive oligonucleotide hybridization techniques or by agarose gel electrophoresis. The results revealed nested PCR to be the method of choice. The combination of three culture methods could detect *M. tuberculosis* in only 79% of PCR-positive cases. The mean time

required to achieve a positive culture result was 17 days in contrast to the PCR method requiring only 2 to 3 days. The nested PCR assay provided rapid and reliable results allowing a definitive diagnosis, particularly in a number of samples which were negative on culture.—Authors' Abstract

**Ran, Y. P., Zhou, G.-P., Guo, Z.-P., *et al.*** [A case of tuberculosis verrucosa cutis diagnosed by polymerase chain reaction.] Chin. J. Clin. Dermatol. **23** (1994) 250–251. (in Chinese)

A case of 45-year-old female patient with tuberculosis verrucosa cutis for 13 years is reported. Granulomatous skin eruptions were present on her right buttock, right thigh, right ear, right lateral chest. Tuberculosis verrucosa cutis was suspected by histopathological examination, but the acid-fast staining yielded a negative result. The *Mycobacterium tuberculosis*-specific DNA fragment was successfully amplified by polymerase chain reaction (PCR) from paraffin-embedded tissues. The skin lesions were cleared by antituberculosis treatment.—Authors' English Abstract

**Raszka, W. V., Skillman, L. P., McEvoy, P. L. and Robb, M. L.** Isolation of nontuberculous, non-avium mycobacteria from patients infected with human immunodeficiency virus. Clin. Infect. Dis. **20** (1995) 73–76.

*Mycobacterium avium* serovars account for 97% of typeable *M. avium* complex (MAC) organisms causing infection in patients with AIDS. We reviewed 216 consecutive cultures that yielded nontuberculous mycobacteria (NTM) from 212 patients. Only the first isolate of each species of NTM recovered from each patient was analyzed in the study. Among the 92 patients infected with the human immunodeficiency virus, 96 NTM organisms were identified; *M. avium* was recovered from 50 (77%) of the 65 NTM-positive cultures of blood or bone marrow, while *M. intracellulare* and other non-avium NTM accounted for 18% and 5% of the isolates, respectively. Little difference in the susceptibility of isolates to antibiotics was noted between HIV-positive and HIV-negative patients or between *M. avium* and *M. intracellulare*. These data demonstrate that HIV-positive patients develop disseminated disease with NTM other than *M. avium* more frequently than has been previously reported and that these patients do not appear to be infected with NTM that are more resistant to antimicrobial agents than are NTM isolated from HIV-negative patients.—Authors' Abstract

**Raviglione, M. C., Snider, D. E. and Kochi, A.** Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. JAMA **273** (1995) 220–226.

This article describes the global epidemiology of tuberculosis and reviews recent estimates of tuberculosis incidence and mortality in the world. The highest prevalence of tuberculosis infection and estimated annual risk of tuberculosis infection are in sub-saharan Africa and Southeast Asia. Overall, almost 3.8 million cases of tuberculosis were reported in the world in 1990, of which 49% were in Southeast Asia. From the period 1984 through 1986 to the period 1989 through 1991, notification rates increased in all World Health Organization regions, except the American and the European regions. In 1990, there were an estimated 7.5 million cases of tuberculosis and 2.5 million deaths worldwide. The human immunodeficiency virus epidemic is causing increases in the number of tuberculosis cases, particularly in Africa, although increases are also expected in Southeast Asia. In many industrialized countries, tuberculosis has recently failed to decline, and in eastern Europe and the former Soviet Union, cases and deaths are increasing. Drug resistance is a serious problem, especially in the United States. If worldwide control of tuberculosis does not improve, 90 million new cases and 30 million deaths are expected in the decade 1990 through 1999.—Authors' Abstract

**Ravn, P. and Pedersen, B. K.** Non-major histocompatibility complex-restricted cytotoxic activity of blood mononuclear cells stimulated with secreted mycobacterial proteins and other mycobacterial antigens. Infect. Immun. **62** (1994) 5305–5311.

Several observations indicate that non-major histocompatibility complex (MHC)-restricted cytotoxicity, mediated, for example, by natural killer cells and lymphokine-activated killer cells, may serve as an important antimicrobial defense mechanism. The purpose of the present study was to investigate the influences of different mycobacterial antigens on non-MHC-restricted cytotoxicity and, further, to investigate the ways by which various lymphocyte subpopulations contribute to the development of this cytotoxicity. Non-MHC-restricted cytotoxicity was induced following stimulation of mononuclear cells with tuberculin purified protein derivative, *Mycobacterium bovis* bacillus Calmette-Guerin (BCG), short- and long-term culture filtrates of virulent *M. tuberculosis* H37Rv, and 30–31-kDa secreted mycobacterial protein. These antigens also induced proliferation and production of gamma-interferon. The CD4+ cells proliferated and expressed interleukin-2 (IL-2) receptors following stimulation with mycobacterial antigens. Depletion studies after antigen stimulation showed that the cytotoxic effector cells were CD16+, CD56+, and CD4−; the CD4+ cells alone did not mediate non-MHC restricted cytotoxicity. To evaluate the influence of CD4+ cells on the development of non-MHC-restricted cytotoxicity, blood mononuclear cells were depleted of CD4+ cells before antigen stimulation. When mononuclear cells were incubated with purified protein derivative or short-term culture filtrate in the absence of CD4+ cells, cytotoxic activity was reduced. This reduction was abolished by IL-2 but not by gamma-interferon. We conclude that several mycobacterial antigens are able to induce non-MHC-restricted cytotoxicity. This study indicates that non-MHC-restricted cytotoxicity following stimulation with mycobacterial antigens is induced by cytokines released by antigen-specific activated CD4+ cells.—Authors' Abstract

**Reddy, P. S., Raghavan, A. and Chatterji, D.** Evidence for a ppGpp-binding site on *Escherichia coli* RNA polymerase: proximity relationship with the rifampicin-binding domain. Mol. Microbiol. **15** (1995) 255–265.

On amino acid starvation, *Escherichia coli* cells exhibit an adaptive facility termed the stringent response. This is characterized by the production of high levels of a regulatory nucleotide, ppGpp, and concomitant curtailment in rRNA synthesis. Various studies reported earlier indicated that RNA polymerase is the site of action of ppGpp although a direct demonstration of the interaction of ppGpp with *E. coli* RNA polymerase is still lacking. Here we report the labelling of ppGpp with a fluorescent probe, 1-aminonapthalene-5-sulphonate (AmNS), at the terminal phosphates. AmNS-ppGpp responded much like a ppGpp molecule in an *in vitro* total transcription assay at selective promoters. Fluorescence titration of the tryptophan emission of RNA polymerase by AmNS-ppGpp indicated a unique binding site in the absence of template DNA. Competition experiments showed that unlabelled ppGpp binds to the enzyme at the same site. Sigma factor seems to have no effect on this binding. The titration profile is also characterized by a single slope in the Scatchard analysis. The presence of GTP or GDP does not influence the binding of AmNS-ppGpp with RNA polymerase. Forster's distance measurement was carried out which placed AmNS-ppGpp 27 Angstrom away from the rifampin-binding domain of RNA polymerase.—Authors' Abstract

**Roche, P. W., Peake, P. W., Billman Jacobe, H., Doran, T. and Britton, W. J.** T-cell determinants and antibody binding sites on the major mycobacterial secretory protein MPB59 of *Mycobacterium bovis*. Infect. Immun. **62** (1994) 5319–5326.

Among the first proteins encountered by the host immune system upon infection or vaccination with mycobacteria are those secreted by the bacillus during growth. The antigen 85 complex of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) is composed of three closely related members. The mature 85B protein of *M. bovis* (MPB59) has a high degree of amino acid identity with the *M. bovis* 85A protein (76%) and the *M. tuberculosis* 85B (99%) and 85A (76%) proteins. We have examined the regions of MPB59 which stimulate human

T- and B-cell responses by use of a set of 28 synthetic peptides, 20 amino acids (aa) in length and overlapping by 10 aa. Initial proliferative assays with recombinant MPB59 demonstrated that peripheral blood mononuclear cells from 95% of BCG vaccinees and 52% of tuberculosis patients responded to the whole mature protein. Peripheral blood mononuclear cells from MPB59 responders, but not nonresponders, were stimulated by peptides in a dose-dependent fashion. Five peptides were reactive in more than half of the MPB59 responders. The T-cell-reactive regions were essentially identical in the *M. bovis* and *M. tuberculosis* 85B proteins. Subjects with a variety of HLA-DR phenotypes responded to a number of these peptides. The dominant T-cell-reactive regions were distinct from the peptides recognized by sera from tuberculosis patients (aa 71 to 100) and the murine monoclonal antibody HYT27 (aa 61 to 90). The region reactive with antibodies overlapped part of the MPB59 sequence recently shown to participate in the binding of MPB59 to fibronectin.—Authors' Abstract

**Saha, B., Das, G., Vohra, H., Ganguly, N. K. and Mishra, G. C.** Macrophage-T cell interaction in experimental mycobacterial infection; selective regulation of costimulatory molecules on *Mycobacterium*-infected macrophages and its implication in the suppression of cell-mediated immune response. Eur. J. Immunol. **24** (1994) 2618–2624.

The most important immunopathological consequence of experimental mycobacterial infection is the suppression of T-cell-mediated immune response to both mitogens and mycobacterial antigens. We registered that there was decreased concanavalin A induced spleen cell proliferation in infected susceptible BALB/c mice as compared to normal mice. In resistant (C3H/HeJ) mice, infection with the bacteria did not induce any suppression in the mitogen-induced lymphoproliferation. Likewise, delayed-type hypersensitivity (DTH) responses to keyhole limpet hemocyanin and mycobacterial crude soluble antigen were suppressed in infected BALB/c mice but not in C3H/HeJ mice. This depressed T-helper cell function may either be due to defective T-cell-receptor occupancy by antigen-Ia complex or altered co-stimulatory signals provided by antigen-presenting cells. In the present study, we have investigated the status of certain co-stimulatory molecules on the infected macrophages from both susceptible and resistant mice. Our results demonstrate that upon mycobacterial infection, the macrophages are rendered incapable of delivering the co-stimulatory signals to T-helper cells, possibly due to the involvement of prostaglandin, as inhibition of its biosynthesis by indomethacin reversed the defect. Furthermore, the selective regulation was bacteria-induced as killing of the bacteria by rifampin abrogated the derangements in the expression of co-stimulatory molecules on the mycobacterium-infected macrophages. Our observations revealed that upon infection with *Mycobacterium tuberculosis,* B7 was down-regulated while ICAM-1 was increased only in BALB/c but not in C3H/HeJ mice. Expression of VCAM-1 did not change during the infection in either strain of mice. We found that these changes in ICAM-1 and B7 expression on the surface of infected macrophages resulted in inhibition of DTH-mediating functions of T-helper cells from BALB/c mice. The results obtained in this study describe not only a novel immune evasion strategy adopted by mycobacteria, but also open up the possibility of immunotherapy of mycobacterial infection by selective manipulation of costimulatory molecules.—Authors' Abstract

**Sedlaczek, L., Gorminski, B. M. and Lisowska, K.** Effect of inhibitors of cell envelope synthesis on beta-sitosterol side chain degradation by *Mycobacterium* sp. NRRL MB 3683. J. Basic Microbiol. **34** (1994) 387–399.

The role of the lipid bilayer and the peptidoglycan of the mycobacterial cell wall in the permeation of p-sitosterol into the cell and its transformation to androst-1-ene-3, 17-dione (AD) and androsta-1, 4-diene-3, 17-dione (ADD) was studied. Specific inhibitors were used at concentrations affecting the biosynthesis of the assumed target structures, but causing only partial cell

growth inhibition or exerting no effect on growth.

m-Fluorophenylalanine and DL-norleucine which are known to disorganize the biosynthesis of amphipatic components of the outer layer of the lipid bilayer, used at concentrations 250 μg/ml and 400 μg/ml, respectively, increased the formation rate of AD+ADD from 0.3 (control) to 0.7 and 0.8 mg products/g dry weight/hr.

The disorganization of the underlying mycolyl-arabinogalactan structure by the action of ethambutol at the concentration 40 μg/ml, at which the cell growth was apparently not affected, caused the decrease of the product formation from 135 mg/l to 70 mg/l. In the presence of isoniazid (350 μg/ml) only trace amounts of AD accumulated during 48 hr of transformation indicating much lower activity than that of the intact cells.

The most effective among the tested inhibitors of peptidoglycan synthesis were glycine (15 mg/ml) and vancomycin (150 μg/ml) which enhanced the transformation activity of the treated cells nearly three times. Increased transformation rate was also obtained by the action of colistin at concentrations ranging from 10 μg/ml to 15 μg/ml.—Authors' Abstract

**Shafer, R. W., Small, P. M., Larkin, C., Singh, S. P., Kelly, P., Sierra, M. F., Schoolnik, G. and Chirgwin, K. D.** Temporal trends and transmission patterns during the emergence of multidrug-resistant tuberculosis in New York City: a molecular epidemiologic assessment. J. Infect. Dis. **171** (1995) 170–176.

To ascertain the role of human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* transmission on multidrug-resistant (MDR) tuberculosis (TB) emergence in New York City, medical records, drug susceptibilities, and restriction fragment length polymorphisms (RFLPs) of TB cases at a city hospital between two 9-month periods (1987–1988 and 1990–1991) were reviewed. The proportion of TB patients with MDRTB increased from 10% (27/267) to 17% (38/222; p = 0.03). Among MDRTB patients of known HIV status, the proportion with HIV increased from 16% (3/19) to 58% (22/38; p = 0.006). HIV-infected MDRTB patients were more likely than the seronegative ones to have initial MDRTB (88% vs. 56%; p = 0.03). Among 56 MDR cases with RFLP results, 12 had unique patterns; 44 belonged to one of six clusters. During 1990–1991, 27 (75%) of 36 MDRTB patients were infected with strains isolated from HIV-seronegative patients during 1987–1988. The increase in MDRTB caused by transmission from immunocompetent to immunocompromised persons underscores the urgency of TB control in populations with increasing HIV prevalence.—Authors' Abstract

**Shah, J. S., Liu, J., Buston, D., Stone, B., Nietupski, R., Olive, D. M., King, W. and Klinger, J. D.** Detection of *Mycobacterium tuberculosis* directly from spiked human sputum by Q-beta replicase-amplified assay. J. Clin. Microbiol. **33** (1995) 322–328.

We report on a rapid, sensitive, Q-Beta replicase-amplified nucleic acid hybridization assay for the detection of *Mycobacterium tuberculosis* directly from spiked human sputum. Specimens were processed by either an *N*-acetyl-L-cysteine-NaOH or a 2% NaOH digestion-decontamination method and then washed to neutralize the pH of the cell pellet. The washed sputum pellets were heated at 100°C to inactivate the *M. tuberculosis* organisms. The heat-inactivated samples were mechanically lysed at 5000 rpm for 6 min in the GENE-TRAK Sample Processing Instrument in the presence of zirconium oxide beads and a buffer containing guanidine thiocyanate. The released nucleic acid was subjected to the GENE-TRAK Q-Beta replicase-amplified, dual-capture assay. The assay sensitivity was $10^3$ purified rRNA targets or 1 CFU of *M. tuberculosis* spiked into *M. tuberculosis*-negative human sputum. There was a low level of noise because of the limitations of performing a signal amplification assay in an open system. High levels of other mycobacterial rRNA (approximately $10^7$ organisms), including rRNAs of *M. avium* and *M. gordonae*, did not interfere with the sensitivity of the assay.—Authors' Abstract

**Sharples, C. E., Shaw, M. A., Castes, M., Convit, J. and Blackwell, J. M.** Immune

response in healthy volunteers vaccinated with BCG plus killed leishmanial promastigotes; antibody responses to mycobacterial and leishmanial antigens. Vaccine **12** (1994) 1402–1412.

Antibody (IgG) responses to mycobacterial (BCG, PPD; *Mycobacterium leprae* soluble antigen, MLSA) and leishmanial (*Leishmania mexicana* LV4) antigens were measured in 208 initially PPD and leishmanin skin-test negative volunteers divided into four vaccine groups as follows: 68 received BCG plus killed promastigotes (group A), 47 received BCG alone (group B), 47 received killed promastigotes alone (group C), and 46 formed the diluent control (placebo, group D). Three vaccine doses were administered at 8–12 week intervals. Vaccinees were bled immediately prior to each vaccination, and again at 3- and 12-month follow up. Skin tests were performed prevaccination, and again at the 3- and 12-month follow up. Anti-BCG, anti-PPD and anti-MLSA IgG levels increased significantly in groups A and B receiving BCG. The presence of leishmanial antigen (with BCG) in the inoculum suppressed the IgG response to *M. tuberculosis/M. bovis*-related (PPD and BCG), but not *M. leprae*-related (MLSA)-related antigens. A small but significant increase (relative to prevaccination level) in response to MLSA, but not to BCG or PPD was observed in the non-BCG-vaccinated groups. The background level of response to mycobacterial and leishmanial antigens was higher in the Venezuelan vaccinees than in nonendemic (British) volunteers. Responses to leishmanial antigen were not enhanced in the two vaccine groups receiving killed promastigotes (with/without BCG) compared with the BCG alone and placebo groups. Instead, all vaccine groups showed a pattern of response consistent with either (i) a response to the skin-test antigen or, more likely, (ii) seasonal endemic exposure to leishmanial antigen. Interestingly, this endemic response to leishmanial antigen was enhanced in the vaccine groups receiving BCG, despite the fact that group B received no leishmanial antigen in the vaccine inoculum. When prevaccination IgG levels (mean + 3 standard deviations) were used to determine a negative cut-off, a low percentage (< 38%) of vaccinees converted to responder status for either antimycobacterial or antileishmanial responses, and those who did would be classified as "low-responder" status compared with titers observed in severe forms of disease. Hence, although there was evidence for a background endemic response to both leishmanial and mycobacterial antigens, there was no evidence that vaccination *per se* led to a potentially disease exacerbatory level of TH2-associated antibody response especially with respect to the antileishmanial response. Taken together with our earlier report that a high proportion of vaccinees, particularly in the BCG plus killed promastigotes group (> 90%), converted for T-cell responder phenotypes (skin test; proliferative response; interferon-gamma production) to leishmanial antigens, these results indicate that this vaccine is potentially protective for the majority of vaccinees. The results of this small trial thus provide a level of confidence in extending the protocol to test the efficacy of BCG plus whole killed parasite vaccines in preventing disease.—Authors' Abstract

**Singh, A. P. and Khuller, G. K.** Induction of immunity against experimental tuberculosis with mycobacterial mannophosphoinositides encapsulated in liposomes containing lipid A. FEMS Immunol. Med. Microbiol. **8** (1994) 119–126.

Mycobacterial mannophosphoinositides (PIMs) encapsulated in liposomes made of egg phosphatidylcholine (EPC) and cholesterol (CH) (2:15 molar ratio) were able to induce humoral and delayed-type hypersensitivity (DTH; foot-pad swelling reaction) responses in mice without the help of any carrier protein. Animals immunized with this glycophospholipid antigen demonstrated enhanced percent survival on intravenous challenge with virulent *Mycobacterium tuberculosis*. On fractionation of PIMs, pentamannophosphoinositide ($PIM_5$) was found to induce higher antibody and DTH reaction and proved to be more immunoprotective than other fractions. Inclusion of lipid A as immunomodulator in liposomes containing PIMs or $PIM_5$ resulted in a significantly increased immune response. Further, mice immunized with PIMs or $PIM_5$

in lipid A-containing liposomes exhibited decreased mortality on challenge with *M. tuberculosis* $H_{37}Rv$, which was comparable to BCG vaccinated animals.—Authors' Abstract

**Strachan, D. P., Powell, K. J., Thaker, A., Millard, F. J. C. and Maxwell, J. D.** Vegetarian diet as a risk factor for tuberculosis in immigrant south London Asians. Thorax **50** (1995) 175–180.

In a previous retrospective study of tuberculosis in south London among Asian immigrants from the Indian subcontinent, Hindu Asians were found to have a significantly increased risk for tuberculosis compared with Muslims. This finding has been further investigated by examining the role of socioeconomic and lifestyle variables, including diet, as risk factors for tuberculosis in Asian immigrants from the Indian subcontinent resident in south London.

Using a case-control study technique Asian immigrants from the Indian subcontinent diagnosed with tuberculosis during the past 10 years and two Asian control groups (community and outpatient clinic controls) from the Indian subcontinent were investigated. Cases and community controls were approached by letter. A structured questionnaire concerning a range of demographic, migration, socioeconomic, dietary, and health topics was administered by a single trained interviewer to subjects (56 cases and 100 controls) who agreed to participate.

The results confirmed earlier findings that Hindu Asians had an increased risk of tuberculosis compared with Muslims. However, further analysis revealed that religion had no independent influence after adjustment for vegetarianism (common among Hindu Asians). Unadjusted odds ratios for tuberculosis among vegetarians were 2.7 (95% CI 1.1 to 6.4) using community controls, and 4.3 (95% CI 1.8 to 10.4) using clinic controls. There was a trend of increasing risk of tuberculosis with decreasing frequency of meat or fish consumption. Lactovegetarians had an 8.5-fold risk (95% CI 1.6 to 45.4) compared with daily meat/fish eaters. Adjustment for a range of other socioeconomic, migration, and lifestyle variables made little difference to the relative risks derived using either community or clinic controls.

These results indicate that a vegetarian diet is an independent risk factor for tuberculosis in immigrant Asians. The mechanism is unexplained. However, vitamin D deficiency, common among vegetarian Asians in south London, is known to affect immunological competence. Decreased immunocompetence associated with a vegetarian diet might result in increased mycobacterial reactivation among Asians from the Indian subcontinent.—Authors' Abstract

**Steingrube, V. A., Gibson, J. L., Brown, B. A., Zhang, Y. S., Wilson, R. W., Rajagopalan, M. and Wallace, R. J.** PCR amplification and restriction endonuclease analysis of a 65-kDa heat shock protein gene sequence for taxonomic separation of rapidly growing mycobacteria. J. Clin. Microbiol. **33** (1995) 149–153.

A total of 129 reference and clinical strains of rapidly growing mycobacteria (RGM) belonging to 10 taxonomic groups were studied for restriction fragment length polymorphism patterns from a PCR-amplified 439-bp segment of the 65-kDa heat shock protein (HSP) gene. Of 24 endonucleases evaluated, restriction fragment length polymorphism patterns produced by HaeIII and BstEII and then by AciI and CfoI gave the best separation. Sixty percent of all RGM taxa studied were differentiated by HaeIII digests alone. Single unique patterns were observed with HaeIII and/or BstEII for *Mycobacterium fortuitum* (100%), *M. chelonae* (94%), *M. abscessus* (96%), *M. smegmatis* (100%), *M. mucogenicum* (formerly the *M. chelonae*-like organism) (100%), and the sorbitol-negative third biovariant of *M. fortuitum* (100%). Evidence is presented in support of two subgroups within *M. peregrinum, M. smegmatis,* and the unnamed third biovariant of *M. fortuitum* (sorbitol positive and sorbitol negative). PCR-based technology provides a rapid, accurate system for the identification of clinically important species of RGM which should be particularly useful for reference laboratories.—Authors' Abstract

**Tebas, P., Sultan, F., Wallace, R. J. and Fraser, V.** Rapid development of resistance to clarithromycin following monotherapy for disseminated *Mycobacterium chelonae* infection in a heart transplant patient. Clin. Infect. Dis. **20** (1995) 443–444.

*Mycobacterium chelonae* (formerly known as *M. chelonae* subspecies *chelonae*) is a rapidly growing mycobacterium that can cause disseminated infections, especially in immunocompromised hosts. The bacterium is typically resistant to antimicrobial agents; less than 20% of *M. chelonae* isolates are susceptible to trimethoprim-sulfamethoxazole, doxycycline, erythromycin, or ciprofloxacin. Findings in a recent study suggested that clarithromycin may be the drug of choice for the treatment of cutaneous (disseminated) disease due to *M. chelonae*. We describe a 60-year-old heart transplant patient with disseminated *M. chelonae* infection for whom monotherapy with clarithromycin failed because of the rapid development of resistance to the drug.—Authors' Abstract

**Toossi, Z., Young, T. G., Averill, L. E., Hamilton, B. D., Shiratsuchi, H. and Ellner, J. J.** Induction of transforming growth factor beta 1 by purified protein derivative of *Mycobacterium tuberculosis*. Infect. Immun. **63** (1995) 224–228.

We examined the ability of purified protein derivative (PPD) of *Mycobacterium tuberculosis* to induce transforming growth factor beta 1 (TGF-beta 1), a potent immunosuppressive and macrophage-deactivating molecule, in blood monocytes from healthy individuals. TBF-beta 1 activity in PPD-induced monocyte supernatants was identified by Western immunoblot analysis and was not inhibited by polymyxin B, an inhibitor of bacterial lipopolysaccharide (LPS). Furthermore, PPD at equivalent amounts in weight to LPS was as potent in stimulation of monocyte production of TGF-beta 1 at 24 hr of culture, as quantified by enzyme-linked immunosorbent assay. The inducing effect of PPD, in contrast to that of LPS, was sustained at later time points of culture (72 hr). PPD enhanced the constitutive expression of TGF-beta 1 steady-state mRNA in monocytes at 24 and 48 hr of culture. In contrast, neither mycobacterial heat shock protein (64-kDa protein of *M. bovis*) nor LPS induced TGF-beta 1 mRNA. Decay studies suggested a transcriptional rather than a posttranscriptional effect of PPD on TGF-beta 1 gene expression.—Authors' Abstract

**Tortoli, E., Piersimoni, C., Bacosi, D., Bartoloni, A., Betti, F., Bono, L., Burrini, C., Desio, G., Lacchini, C., Mantella, A., Orsi, P. G., Penati, V., Simonetti, M. T. and Bottger, E. C.** Isolation of the newly described species *Mycobacterium celatum* from AIDS patients. J. Clin. Microbiol. **33** (1995) 137–140.

*Mycobacterium celatum* is a recently described species which, on the basis of conventional tests, may be misidentified as *Mycobacterium xenopi* or as belonging to the *M. avium* complex. Only genomic sequencing or high-performance liquid chromatography of cell wall mycolic acids can presently allow a correct identification of this *Mycobacterium*. Two cases of infection due to *M. celatum*, in AIDS patients, are described here. The quantitative susceptibility pattern of the isolates to a wide spectrum of drugs is also reported.—Authors' Abstract

**Tsukaguchi, K., Balaji, K. N. and Boom, W. H.** CD4+ alpha beta T cell and gamma delta T cell responses to *Mycobacterium tuberculosis*—similarities and differences in Ag recognition, cytotoxic effector function, and cytokine production. J. Immunol. **154** (1995) 1786–1796.

CD4+ and gamma delta T cells are activated readily by *Mycobacterium tuberculosis*. To examine their role in the human immune response to *M. tuberculosis*, CD4+ and gamma delta T cells from healthy tuberculin-positive donor were studied for patterns of Ag recognition, cytotoxicity, and cytokine production in response to *M. tuberculosis*-infected mononuclear phagocytes. Both T cell subsets responded to intact *M. tuberculosis* and its cytosolic Ags. However, CD4+ and gamma delta T cells differed in the range of cytosolic Ags recognized: reactivity to a wide m.w. range of Ags for CD4+ T cells, and a restricted pattern for gamma delta T cells, with domi-

nance of Ags of 10 to 15 kDa. Both T cell subsets were equally cytotoxic for *M. tuberculosis*-infected monocytes. Furthermore, both CD4+ and gamma delta T cells produced large amounts of IFN-gamma; mean pg/ml of IFN-gamma in supernatants was 2458 ± 213 for CD4+ and 2349 ± 245 for gamma delta T cells. By filter-spot ELISA (ELISPOT), the frequency of IFN-gamma-secreting gamma delta T cells was one-half of that of CD4+ T cells in response to *M. tuberculosis,* suggesting that gamma delta T cells on a per cell basis were more efficient producers of IFN-gamma than CD4+ T cells. In contrast, CD4+ T cells produced more IL-2 than gamma delta T cells, which correlated with diminished T cell proliferation of gamma delta T cells compared with CD4+ T cells. These results indicate that CD4+ and gamma delta T-cell subsets have similar effector functions (cytotoxicity, IFN-gamma production) in response to *M. tuberculosis*-infected macrophages, despite differences in the Ags recognized, IL-2 production, and efficiency of IFN-gamma production.—Authors' Abstract

**Ulstrup, J. C., Jeansson, S., Wiker, H. G. and Harboe, M.** Relationship of secretion pattern of MPB70 homology with osteoblast-specific factor 2 to osteitis following *Mycobacterium bovis* BCG vaccination. Infect. Immun. **63** (1995) 672–675.

Significant homology was found between MPB70 and each of four repeat domains of osteoblast-specific factor 2 (OSF-2). Two internal homology regions within each repeat domain of OSF-2 presumed to be related to the active site(s) of this bone adhesion molecule showed the highest homology. A literature search concerning osteitis after *Mycobacterium bovis* BCG vaccination in neonates revealed that MPB70-high-producer substrains were associated with an increased incidence of osteitis following vaccination. These observations indicate that the function of MPB70 is related to the interaction between bacilli and the host following vaccination or infection with mycobacteria.—Authors' Abstract

**Uthoff, K., Zehr, K. J., Gaudin, P. B., Kumar, P., Cho, P. W., Vogelsang, G., Hruban, R. H., Baumgartner, W. A. and Stuart, R. S.** Thalidomide as replacement for steroids in immunosuppression after lung transplantation. Ann. Thorac. Surg. **59** (1995) 277–282.

Steroids have been implicated in postoperative complications after lung transplantation: infections, delayed wound healing, and poor bronchial anastomotic healing. Thalidomide (alpha-phthalimidoglutarimide), a sedative drug with known immunomodulatory properties, was used to replace corticosteroids after canine lung transplantation. Fifteen mongrel dogs underwent single-lung transplantation: group I (N = 5) received cyclosporin A (20 mg/kg twice a day), azathioprine (2.5 mg/kg once a day), and thalidomide (50 mg/kg twice a day). Group II (N = 5) received standard immunosuppression of cyclosporin A (20 mg/kg twice a day), azathioprine (2.5 mg/kg once a day), and prednisone (2 mg/kg once a day), and group III (N = 5) received cyclosporin A (10 mg/kg twice a day), azathioprine (2.5 mg/kg once a day), and thalidomide (50 mg/kg twice a day). Open lung biopsy and bronchoscopy were performed weekly until sacrifice on day 28. Serum thalidomide and cyclosporin A levels were followed up weekly. Group I showed essentially no rejection until week 2 and minimal rejection (grade 1) until day 28. Group II had moderate rejection (grade 2) of the graft at all time points. Group III animals had moderate to severe rejection (grades 3 to 4) after 21 days ($p < 0.05$ for group I versus groups II and III). The number of clinically evident episodes of pneumonia was also significantly lower in group I than in groups II and III ($p < 0.05$). We conclude that thalidomide appears to replace corticosteroids effectively in early postoperative immunosuppression after lung transplantation and is associated with a decreased incidence of pneumonia. It was not efficacious in combination with low-dose cyclosporin A. This drug may have a significant impact after clinical lung transplantation by reducing steroid-associated complications.—Authors' Abstract

**Vilanova, M., Ribeiro, A., Carneiro, J. and Arala Chaves, M.** The effects of thalidomide treatment on autoimmune-prone

NZB and MRL mice are consistent with stimulation of the central immune system. 2. Scand. J. Immunol. **40** (1994) 543–548.

We describe here some immunomodulatory effects of thalidomide on autoimmune-prone mice. The highly increased synthesis of splenic IgM in NZB mice, of splenic and lymph node IgG of different subclasses in MRL/n mice, and of splenic and lymph node IgG1 in MRL/lpr mice was markedly inhibited by thalidomide treatment. After a single treatment with 3 mg of thalidomide, the following changes were observed in NZB mice: i) an initial decrease in the numbers of large CD5+ mu(high), and in the numbers of total CD5+ mu−, CD5− mu(high) CD5+ mu(high) lymphocyte populations of the pleural cavity followed by a late increase in the numbers of large cells of the three cell populations; ii) a consistent increase in the numbers of a CD5(low) mu(low) pleural lymphoid population; iii) a consistent reduction in the numbers of splenic large CD5+ B cells and an oscillatory increase in the number of cells with CD5− phenotype; iv) a late reduction in the numbers of splenic total CD5+ B cells. These results are consistent with the notion that thalidomide controls a disease-associated expansion of B cells in autoimmune prone mouse strains through a stimulatory effect of the drug on the immune system.—Authors' Abstract

**Wagner, B., Fattorini, L., Wagner, M., Jin, S.-H., Stracke, R., Amicosante, G., Franceschini, N. and Orefici, G.** Antigenic properties and immunoelectron microscopic localization of *Mycobacterium fortuitum* β-lactamase. Antimicrob. Agents Chemother. **39** (1995) 739–745.

*Mycobacterium fortuitum* is a fast-growing *Mycobacterium* species which produces a β-lactamase involved in the intrinsic resistance of the microorganism to β-lactam antibiotics. An anti-β-lactamase serum against the purified enzyme was raised in rabbits. Antibody binding was specific for native β-lactamase, and enzyme activity was partially inhibited by the serum; furthermore, crossreactions with denatured class A β-lactamases were observed. This serum was used as a probe in immunogold labeling for the localization of the cell-bound β-lactamase in both the low-level producer ATCC 19542 (parental strain) and the overproducer mutant D316. By the combination of pre-embedding immunogold labeling and replica technique, it was shown that the β-lactamase was uniformly distributed on the whole external cell surface, where it appeared to be associated with a Tween 80-removable capsule-like material. Compared with the parental strain, a much higher level of expression of surface enzyme was observed in strain D316. Surface labeling was more intense in the stationary phase of growth than in exponentially growing cells. The data obtained are interpreted in the context of the intrinsic resistance of *M. fortuitum* to β-lactam antibiotics.—Authors' Abstract

**Wallace, R. J.** Recent changes in taxonomy and disease manifestations of the rapidly growing mycobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 953–960.

Rapidly growing mycobacteria are a complex group of environmental organisms that cause human disease. Eight taxonomic groups of pathogens are recognized of which three, the *Mycobacterium chelonae*-like organism (MCLO) and the two *M. fortuitum* third biovariant groups, have yet to receive names pending DNA-DNA homology studies. Clinical disease due to all eight taxons usually consists of skin or soft tissue infections following trauma. Nosocomial disease has become increasingly important, pseudoepidemics associated with contaminated automated endoscopic washing machines being the most recently described manifestation. The development of DNA fingerprinting technology, especially pulsed-field gel electrophoresis, has improved the possibilities of studying the epidemiology of these infections. Pulmonary disease, primarily due to *M. abscessus*, and disseminated cutaneous disease, due to *M. chelonae*, have recently been characterized. Imipenem and the newer macrolide clarithromycin represent important new therapeutic agents active against these species. Other important therapeutic agents include amikacin and the newer quinolones, which have

variable degrees of susceptibility within different taxonomic groups, necessitating susceptibility testing.—Authors' Abstract

**Whelen, A. C., Felmlee, T. A., Hunt, J. M., Williams, D. L., Roberts, G. D., Stockman, L. and Persing, D. H.** Direct genotypic detection of *Mycobacterium tuberculosis* rifampin resistance in clinical specimens by using single-tube heminested PCR. J. Clin. Microbiol. **33** (1995) 556–561.

Recent analysis of the gene encoding the beta subunit of *Mycobacterium tuberculosis* RNA polymerase (*rpoB*) has demonstrated a small region that harbors the mutations most frequently associated with rifampin resistance. Earlier reports have described a high degree of sequence conservation of *rpoB* among mycobacteria other than *M. tuberculosis* and other GC-rich bacteria that can lead to false-positive amplification when applied directly to clinical specimens. We developed reagents for PCR amplification that are based on signature nucleotides discovered by comparative sequence analysis of the *rpoB* genes of organisms phylogenetically related to *M. tuberculosis*. The specificities of the reagents were challenged with 20 isolates of multiple-drug-resistant *M. tuberculosis* and more than 20 species of mycobacteria other than *M. tuberculosis* and other GC-rich organisms. A single-tube heminested PCR protocol was devised to obtain sensitivity equal to those of an IS*6110*-based PCR assay and culture in spiked sputum experiments. The assay correctly identified 21 of 24 (87.5%) culture-positive specimens, 13 of which were acid-fast smear-negative, in a panel of 51 clinical specimens. Three specimens that were false-positive initially were negative upon repeat testing when the assay was modified to eliminate the potential for aerosol carryover of the first-round amplification product during the open-tube addition of the second set of reaction reagents. This assay is the most sensitive and specific test to date for the direct detection of *M. tuberculosis rpoB* in clinical specimens. This rapid PCR-based assay can be used for the simultaneous identification of *M. tuberculosis* and its rifampin susceptibility genotype.—Authors' Abstract

**Yang, Z. H., de Haas, P. E. W., van Soolingen, D., van Embden, J. D. A. and Andersen, A. B.** Restriction fragment length polymorphism of *Mycobacterium tuberculosis* strains isolated from Greenland during 1992: evidence of tuberculosis transmission between Greenland and Denmark. J. Clin. Microbiol. **32** (1994) 3018–3025.

In order to describe the transmission of tuberculosis (TB) at the clonal level in a defined geographic region during a certain period of time, all isolates of *Mycobacterium tuberculosis* collected during 1992 from Greenland were subjected to analyses of DNA restriction fragment length polymorphism (RFLP). The RFLP patterns obtained by probing the genomic DNA with the repetitive insertion segment IS*6110* revealed a high degree of similarity among the isolates, indicating a relatively high transmission rate and a close relationship between the individual *M. tuberculosis* clones. This was further confirmed by reprobing the Southern blots with two more-stable genetic markers, IS*1081* and the DR sequence. The RFLP patterns were compared with those of more-stable genetic markers, IS*1081* and the DR sequence. The RFLP patterns were compared with those of 245 *M. tuberculosis* strains collected from Denmark during the same period (representing 91% of all new, bacteriologically verified cases of TB in Denmark in 1992). One of the three prevalent IS*6110*-defined clusters was traced to a group of immigrants from Greenland living in a small, defined geographical region in Denmark and to a group of Danish citizens either with known contact with these immigrants or, in other cases, with a record of previous travel or working activities in Greenland. The study showed that the present technique is extremely helpful in monitoring the spread of TB and thereby also contributing to improved disease control.—Authors' Abstract

**Zhong, Y.-H., Broser, M., Cohen, H., Bodkin, M., Law, K., Reibman, J. and Rom, W. N.** Enhanced interleukin-8 release and gene expression in macrophages after exposure to *Mycobacterium tuberculosis* and its components. J. Clin. Invest. **95** (1995) 586–592.

*Mycobacterium tuberculosis* infection is accompanied by acute and chronic inflammatory infiltrates associated with necrotizing granulomas in lung tissue. The cellular infiltrate is characterized by inflammatory cells which include neutrophils, lymphocytes, and macrophages. In animal and *in vitro* models of mycobacterial infection, cytokines including tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interferon gamma (IFN-$\gamma$), and interleukin-1$\beta$ (IL-1$\beta$) participate in granulomatous inflammation. We hypothesized that interleukin-8, a potent chemoattractant for neutrophils and lymphocytes, could be released by activated alveolar macrophages after exposure to *M. tuberculosis* or its components and contribute to granulomatous lung inflammation. A quantitative immunoassay revealed that IL-8 protein release was significantly elevated in supernatants of macrophages and in lavage fluid obtained from patients with pulmonary tuberculosis compared to normal controls. In addition, Northern blots demonstrated striking upregulation of IL-8 mRNA in macrophages from these patients. *M. tuberculosis* and its cell wall components lipoarabinomannan (LAM), lipomannan (LM), and phosphoinositolmannoside (PIM) stimulated IL-8 protein release and mRNA expression in vitro from alveolar macrophages, but deacylated LAM did not. Neutralizing antibodies to TNF-$\alpha$ and/or IL-1-$\alpha$ and $\beta$ blocked 83% of the stimulation. IL-8 synthesis and release is an early response of macrophages after phagocytosis of *M. tuberculosis.* Its production serves to attract both acute and chronic inflammatory cells of active infection and thus participates in the process of containment of the pathogen.—Authors' Abstract