CURRENT LITERATURE

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

General and Historical

Britton, W. J. and Hargrave, J. C. Leprosy in the tropics and Australia. Med. J. Aust. **159** (1995) 326–330.

A review covering the natural history and clinical features of leprosy and the impact of the disease in Australia.—Trop. Dis. Bull.

International Federation of Anti-Leprosy Associations. ILEP statement: elimination of leprosy as a public health problem. Lepr. Rev. **65** (1994) 165–166.

The statement was made at the International Federation of Anti-Leprosy Associations (ILEP) Conference held in Hanoi on 4–7 July 1994. The members of ILEP (20 nongovernment donor associations, that are listed at the end of the article) confirm their wish to bring multi drug therapy (MDT) as quickly as possible to every individual with leprosy who needs it. They also consider it important to record the total number of people with leprosy (not only those requiring chemotherapy). Some 2.4 million people were estimated in 1994 to be in need of chemotherapy; there may be some overlap, but around 4 million people have or are at risk of deformity as a result of leprosy according to estimates made in 1992. The possible achievements of the ILEP target of MDT for All and of the WHO target of the Elimination of Leprosy as a Public Health Problem (defined as a prevalence of 1 per 10,000 population) are acknowledged to be historic steps in the battle against leprosy, but the members of ILEP warn that even these achievements will not mean the end of leprosy work. New cases will continue to appear (the number of new cases worldwide has so far not appeared to have declined), fully treated cases may relapse, resurgence of leprosy could occur in areas of low endemicity in the absence of experts in clinical management of the disease, patients with permanent nerve damage have a continuing need of medical services after chemotherapy, and social and economic assistance is often needed by leprosy patients because of social stigma. The members end their statement by stressing their commitment to working against leprosy for many years to come, and stating that they are looking forward to working in partnership with governments, WHO and local associations.—C. A. Brown (Trop. Dis. Bull.)

Chemotherapy

Arbiser, J. L. and Moschella, S. L. Clofazimine: a review of its medical uses and mechanisms of action. J. Am. Acad. Dermatol. **32** 2 Part 1 (1995) 241–247.

Clofazimine has been in clinical use for almost 40 years, but little is known of its mechanism of action. The primary indication for clofazimine is multibacillary leprosy, but it is useful in several infectious and noninfectious diseases, such as atypical mycobacterial infections, rhinoscleroma, pyoderma gangrenosum, necrobiosis lipoidica, severe acne, pustular psoriasis, and discoid lupus erythematosus. Postulated mechanisms of action include intercalation of clofazimine with bacterial DNA and increasing levels of cellular phospholipase A2. Clinical experience, possible mechanisms of action, and side effects of clofazimine are summarized.—Authors' Abstract

Bodmer, T., Zurcher, G., Imboden, P. and Telenti, A. Mutation position and type of substitution in the β-subunit of the RNA

polymerase influence *in-vitro* activity of rifamycins in rifampicin-resistant *Mycobacterium tuberculosis.* J. Antimicrob. Chemother. **35** (1995) 345–348.

Quantitative susceptibility testing for rifampin, rifabutin and rifapentine of 36 *Mycobacterium tuberculosis* isolates with known sequences for the gene encoding for the RNA polymerase β-subunit (*rpo B*) revealed that both mutation position and type of amino acid substitution influence the *in-vitro* activity of rifamycins in rifampin-resistant strains.—Authors' Abstract

Choudhri, S. H., Harris, L., Butany, J. W. and Keystone, J. S. Clofazimine induced cardiotoxicity—a case report. Lepr. Rev. **66** (1995) 63–68.

A 66-year-old Indian male who had been treated for recurrent erythema nodosum leprosum with 300 mg of clofazimine per day for 11 months presented to hospital with a 4-week history of severe gastrointestinal upset. Soon after admission he developed several short runs of ventricular tachycardia with a morphology suggestive of torsade de pointe. The patient had a slightly low magnesium level which was corrected within 2 days; however, his rhythm disturbance persisted for 5 days despite management with intravenous lidocaine. His gastrointestinal symptoms abated 2 weeks after clofazimine was discontinued. Subsequent investigations showed that the patient had a keratopathy and myelin-type figures in his polymorphonuclear white cells similar to that seen with the cardiotoxic drugs chloroquine and amiodarone. It is postulated that clofazimine alone or in conjunction with electrolyte disturbance was responsible for the patient's cardiac arrythmia.—Authors' Summary

Eriksen, T., Bjorkman, S., Roth, C., Fyge, A. and Hoglund, P. Stereospecific determination, chiral inversion *in vitro* and pharmacokinetics in humans of the enantiomers of thalidomide. Chirality **7** (1995) 44–52.

The purposes of this work were (1) to develop a high performance liquid chromatographic (HPLC) assay for the enantiomers of thalidomide in blood, (2) to study their inversion and degradation in human blood, and (3) to study the pharmacokinetics of (+)-(R)- and (−)-(S)-thalidomide after oral administration of the separate enantiomers or of the racemate to healthy male volunteers. The enantiomers of thalidomide were determined by direct resolution on a tribenzoyl cellulose column. Mean rate constants of chiral inversion of (+)-(R)-thalidomide and (−)-(S)-thalidomide in blood at 37°C were 0.30 and 0.31 h(−1), respectively. Rate constants of degradation were 0.17 and 0.18 h(−1). There was rapid interconversion *in vivo* in humans, the (+)-(R)-enantiomer predominating at equilibrium. The pharmacokinetics of (+)-(R)- and (−)-(S)-thalidomide could be characterized by means of two one-compartment models connected by rate constants for chiral inversion. Mean rate constants for *in vivo* inversion were 0.17 h(−1) (R to S) and 0.12 h(−1) (S to R) and for elimination 0.079 h(−1) (R) and 0.24 h(−1) (S), i.e., a considerably faster rate of elimination of the (−)-(S)-enantiomer. Putative differences in therapeutic or adverse effects between (+)-(R)- and (−)-(S)-thalidomide would to a large extent be abolished by rapid interconversion *in vivo.*—Authors' Abstract

Palamanda, J. R., Hickman, D., Ward, A., Sim, E., Romkes Sparks, M. and Unadkat, J. D. Dapsone acetylation by human liver arylamine N-acetyltransferases and interaction with antiopportunistic infection drugs. Drug Metab. Dispos. **23** (1995) 473–477.

Dapsone is used in the treatment of *Pneumocystis carinii* pneumonia, an opportunistic infection that afflicts acquired immunodeficiency syndrome (AIDS) patients. Inhibition of N-acetyltransferase (NAT)-dependent acetylation of dapsone could increase peak plasma concentrations of dapsone and shift the biotransformation pathway to the P450-mediated formation of a toxic metabolite of dapsone, the hydroxylamine. Therefore, we have determined using human liver cytosol and bacterially expressed NATs, the NAT isoform responsible for acetylating dapsone and the potential for antiopportunistic infection drugs to inhibit this metabolic pathway. Formation of monoacetyldiaminodiphenylsulfone

(MADDS) was quantitated by HPLC/UV detection at 270 nm after incubation of dapsone with 100 μM acetyl coenzyme A regenerating system and human liver cytosol. The mean ± S.D. apparent Km for the formation of MADDS in three different human livers predicted to be fast acetylators based on genotyping was 98 ± 17.6 μM, and the V-max was 190 ± 20 pmol/min/mg cytosol protein. Eadie-Hofstee transformation of the substrate velocity data was linear, indicating acetylation by a kinetically single enzyme. Sulfamethazine (250 μM) inhibited dapsone acetylation by 100% and 80%, respectively, at dapsone concentrations of 3 and 100 μM, in both fast- and slow-acetylating liver cytosol preparations; whereas para-aminobenzoic acid (100 μM) did not inhibit MADDS formation at either of these dapsone concentrations. Lineweaver-Burk plots of dapsone acetylation in the presence of 0, 25, and 50 μM sulfamethazine showed an increase in the apparent Km, with increase in sulfamethazine concentration with no change in the V-max, indicating competitive inhibition of dapsone acetylation by sulfamethazine. The apparent Km of dapsone acetylation by bacterially expressed NAT1 and NAT2 enzymes was 687 and 136 μM, respectively. Human liver cytosol preparations, predicted to be slow acetylators based on genotyping, acetylated dapsone at a significantly lower rate when compared with fast acetylator human liver cytosols. At clinically relevant concentrations, pyrimethamine, but not other antiopportunistic infection drugs (atovaquone, sulfadiazine, clarithromycin, trimethoprim, ketoconazole, and fluconazole), significantly but modestly (23%) inhibited MADDS formation in human liver cytosols. These data indicate that NAT2 is the predominant liver NAT isoform acetylating dapsone *in vivo* and that coadministration with anti-opportunistic infection drugs should not significantly inhibit this acetylation pathway.—Authors' Abstract

Wallace, R. J., Brown, B. A., Griffith, D. E., Girard, W. and Tanaka, K. Reduced serum levels of clarithromycin in patients treated with multidrug regimens including rifampin or rifabutin for *Mycobacterium avium-intracellulare* infection. J. Infect. Dis. **171** (1995) 747–750.

The newer macrolides and rifamycins (rifabutin) are major advances for treatment or prophylaxis of disease due to *Mycobacterium avium* complex. Although rifampin and rifabutin are known to induce the hepatic cytochrome P-450 system, their impact on the metabolism of clarithromycin is unknown. Clarithromycin and its major metabolite, 14-OH clarithromycin, were measured in the sera of patients receiving 500 mg twice a day before and after the addition of antituberculous drugs, including 600 mg/day of rifampin or rifabutin. Mean serum levels of clarithromycin given as a single agent were 5.4 ± 2.1 μg/mL. These decreased to 0.7 ± 0.6 μg/mL in patients receiving rifampin and 2.0 1.5 μg/mL in those receiving rifabutin. Mean serum levels of 14-OH clarithromycin were similar in the three groups (1.8–1.9 μg/mL). Rifampin and (to a lesser degree) rifabutin appear to induce the metabolism of clarithromycin.—Authors' Abstract

Waters, M. F. R. Relapse following various types of multidrug therapy in multibacillary leprosy. (Editorial) Lepr. Rev. **66** (1995) 1–9.

The results for multibacillary leprosy (MBL) following the introduction of WHO multidrug therapy (MDT) have been excellent. The epidemic of secondary dapsone resistance has been aborted, and treatment of limited duration (even if continued until smear negativity) has been successfully introduced. Because of the epidemic of dapsone resistance, most thinking leprologists by the early 1970s concluded that some form of MDT was essential, analogous to combined chemotherapy in tuberculosis. The introduction of rifampicin in 1970, and the early results from the pilot Malta project showed that limited duration MDT was a real possibility. WHO MDT has proved very robust under field conditions.

The number of relapses reported to date have been minute, although the majority of patients followed for 8–10 years since release from treatment probably belong to the smear-negative, long-term dapsone monotherapy group; as such their bacterial load of *Mycobacterium leprae* at the start of MDT would have been tiny. In the first 5 years after the introduction of MDT most newly

diagnosed LL and BL patients and most relapse patients (whether from dapsone resistance or from discontinuing treatment) are likely to have been kept on MDT until becoming smear negative; that is for 4–10 years.

Criteria for defining relapse may vary from center to center, and an apparently high relapse rate may reflect too loose a definition (Smith, Jesudasan and Jakeman, personal communication). This editorial has concentrated on centers where relapse has been defined clinically, bacteriologically, and histologically, and where the presence of viable *M. leprae* (proof of bacteriological relapse) has usually been confirmed by multiplication in mouse foot pads. These results suggest that:

1. Risk of relapse is very low in old smear-negative LL and BL patients, some of whom may relapse with localized BT lesions.
2. Risk of relapse is not yet fully known in previously untreated LL and BL patients given WHO MDT until smear negativity, although provisional data suggest that the risk is very small.
3. In previously untreated LL and BL patients treated with WHO MDT for 2 years only, the risk of relapse is related to the pretreatment load of *M. leprae*; the more severe the infection, the greater the risk of relapse.
4. The timing of relapse is important. Very few well-authenticated relapses occur in the first 3 years after release from treatment (RFT), and the claim that the majority occur in the first 5 years is based on absolute numbers reported from large cohorts at 1–4 years, rather than relapse rates. Both from experience with WHO MDT and from other regimens, a follow up of 8-10 years appears essential.
5. There is a great desire among leprosy control experts to give WHO MBL MDT for a set duration of 2 years, especially in "rolling programs" with outside funding. Such short-duration MDT is operationally essential in such circumstances. It has been claimed that by lowering the incidence of tuberculosis (and by inference, of leprosy) by 80%, the endemic of disease should decline. This is almost certainly true. But it is in those areas which previously had the poorest leprosy control which are likely to have the most advanced patients with the highest bacterial loads. Therefore, significant numbers of relapses will almost certainly occur in such circumstances, and therefore an effective residual structure must remain in an area to detect relapses early and to treat them well, both as a duty to the patients and to allay any threat to the credibility of the program. Furthermore, Jamet, Ji, and their colleagues are now proposing that the duration of MDT should be doubled to 4 years in patients with an initial average bacterial index (BI) $\geq$ 4.0 before commencing MDT. This would be a simple measure to implement in high-endemic areas. In low-endemic areas with a declining endemic approaching the WHO "elimination" target, where leprosy may already be tending to persist in "clusters," and where the general health services are likely to be of a high standard, it may well be worth keeping patients on MDT until they achieve smear negativity as originally advised by the WHO Study Group in 1982; this should ensure that the endemic continues to die out at the maximum possible speed, because of minimal relapses. For although the results with WHO MDT are still incomplete, experience in Africa does suggest that the relapse rates after different forms of MDT are usually related both to the initial BI and to the total number of rifampin doses and the total duration over which they are given.—Author's Conclusions

Clinical Sciences

Ahsan, N. and Palmer, B. F. Leprosy-associated renal disease: case report and review of the literature. J. Am. Soc. Nephrol. **5** (1995) 1546–1552.

Leprosy is an infectious disease the principal clinical manifestations of which are anesthetic skin lesions and the development of peripheral neuropathy. The most com-

mon renal manifestation in leprosy patients is glomerulonephritis. Both immunofluorescent and electron microscopic studies suggest that the varied glomerular lesions found in these patients are immune complex mediated. Other renal lesions that have been described include amyloidosis, tubulointerstitial disease, acute renal failure, and functional defects in the absence of identifiable histologic abnormalities. In this report, a patient is described who developed the clinical syndrome of rapidly progressive glomerulonephritis. The renal biopsy showed a diffuse endocapillary proliferative process with electron-dense deposits in the glomerular subendothelial and subepithelial spaces. Organisms consistent with *Mycobacterium leprae* were identified within several of the glomeruli.—Authors' Abstract

Carus, N. H., Raizman, M. B., Williams, D. L. and Baker, A. S. Relapse of *Mycobacterium leprae* infection with ocular manifestations. Clin. Infect. Dis. **20** (1995) 776–780.

A case of ocular leprosy as the manifestation of persistent or relapsed *Mycobacterium leprae* infection similar to 20 years following treatment is reported. The clinical and pathological features of this case are described, and the molecular methods needed to arrive at the definitive diagnosis are examined, If blindness is to be averted, clinicians must have a high index of suspicion for the diagnosis of ocular leprosy when anterior segment changes are noted during ophthalmologic examination of a patient from an area in which *M. leprae* is endemic. The indolent nature of ocular leprosy may require lifelong surveillance and therapy to insure sight preservation.—Authors' Abstract

Chin-A-Lien, R. A. M., Faber, W. R. and Naafs, B. Cyclosporin A treatment in reversal reaction. Trop. Geogr. Med. **46** (1994) 123–124.

The article provides two case histories as examples of the use of cyclosporin A (a potent immunosuppressive drug used in transplantation medicine) for the treatment of reversal reaction in leprosy in patients who have poor tolerance of corticosteroids. Both patients were seen and treated in The Netherlands. The first patient was a 78-year-old man with a clinical diagnosis of midborderline/borderline lepromatous leprosy with a downgrading reaction. Because of his difficulty in controlling his diabetes mellitus, however, prednisolone was considered to be contraindicated. Treatment with cyclosporin A (in addition to multidrug therapy) was started at 5 mg/kg daily for 6 months, tapering off to 2.5 then 1 mg/kg daily. The second patient, a 23-year-old man, had active borderline lepromatous leprosy with reversal reaction and possible erythema nodosum leprosum. Thalidomide and prednisolone were given but 1.5 years later cataract was diagnosed. A further reversal reaction developed 2 months after this diagnosis but, because the cataract might have been caused by the steroid therapy, treatment with cyclosporin A was given, initially at 5 mg/kg daily and tapering off to 1 mg/kg daily. Both patients were monitored monthly for side effects from cyclosporin A: in both the blood pressure and kidney function remained in the normal range. In both patients the improvement in the skin lesions (which was slower than with prednisolone treatment) appeared to be preceded by a decrease in the swelling of the peripheral nerves. The authors state that in their hands cyclosporin A was an effective drug against reversal reactions. The role of the drug will be limited because of its high costs, but in their opinion it could be an alternative therapy for those leprosy patients who do not respond adequately to or cannot tolerate corticosteroid therapy.—C. A. Brown (Trop. Dis. Bull.)

Rojas, V., de Hernandez, O. and Gil, R. Some factors influencing delay in leprosy diagnosis. Bull. PAHO **28** (1994) 56–162.

To study delay in leprosy diagnosis in Cuba, home interviews were conducted with all patients whose cases were diagnosed during 1989–1990 in Guantánamo and Havana, where leprosy prevalences are respectively high and moderate. Data from the two cities showed a significant difference in the average time passing between the first appearance of symptoms and definitive diagnosis, this time being 16.6 months in Havana and 10.7 months in Guantánamo (p

< 0.01). Moreover, the patterns of delay were different. In Havana, the average patient sought medical advice relatively soon (a month after the first symptoms appeared), but the one or more physicians consulted took an average of 15.6 months to arrive at the diagnosis. In contrast, the average Guantánamo physician reached a definitive diagnosis in 5 months, but the average Guantánamo patient waited 5.7 months before visiting the doctor. These observations demonstrate that delayed diagnosis can have quite different causes in different places, and that interventions seeking to reduce such delay need to consider the contributing causes in the particular locale involved. In the case of leprosy diagnosis in Havana and Guantánamo, future interventions in Havana should aim at increasing the physician's level of clinical suspicion, while in Guantánamo they should encourage patients to seek medical care as soon as they begin to notice symptoms.—Trop. Dis. Bull.

Delon, A., Favreliere, S., Couet, W., Courtois, P. H. and Bouquet, S. Rapid and sensitive determination of thalidomide in human plasma by high performance liquid chromatography. J. Liq. Chromatogr. **18** (1995) 297–309.

A sensitive and rapid high-performance liquid chromatographic method using U.V. detection has been developed for the analysis of thalidomide in plasma. This involved a single liquid-solid extraction on Extra-Sep-C8 column in the presence of an internal standard (ciprofloxacin). Analysis was performed by isocratic elution with a mobile phase consisted of 0.01 M aqueous potassium dihydrogen phosphate containing 21% (V/V) acetonitrile and 4.5 mM Heptane sulfonic acid, adjusted to pH 2.3, with U.V. detection at 295 nn. The limit of sensitivity of the assay was 0.06 mg/l. The method was applied to a pharmacokinetic study (50 to 100 mg) in patients with erythema nodusum leprosum (ENL) with a good accuracy (96%–111%) and precision (less than 5.8%).—Authors' Abstract

Grimaud, J., Chapuis, F. and Millan, J. Segmental ulnar nerve conduction velocities in Hansen's disease. Rev. Neurol. (Paris) **150** (1994) 791–795.

In leprosy, ulnar neuritis is considered to be selectively localized at the elbow and is often treated by surgical decompression when pain and/or neurological deficit occurs. The aim of this prospective study is to assess the prevalence, localization and severity of ulnar nerve damage in leprosy. Motor nerve conduction velocity (MNC) was measured at 3 different segments (arm, elbow and forearm) and was expressed both in meters/second (m/s) and percentage of the mean normal values found in our laboratory or as reported in other studies. The patient group consisted of 123 consecutive new leprosy sufferers (228 ulnar nerves only) who attended the Institut de Leprologie Appliquee de Dakar over the period of 1 year. Diagnosis and classification were based on Ridley and Jopling's criteria, including skin and nerve biopsy. Mean MNC was reduced by 13.5 m/s at the arm, 19.8 m/s at the elbow and 7.8 m/s at the forearm as compared to the mean normal values. Increased distal latency as an isolated finding was rare (0.9%). Mean MNC was more reduced in the BL, LL (lepromatous) than in the TT, BT (tuberculoid) subgroups, despite similar disease durations (22.3 ± 18.7 months and 24.2 ± 26.4 months, respectively (n.s.). Using different normal MNC values did not affect the conclusion: we did not see any selective slowing of ulnar MNC at the elbow, suggesting that nerve damage is not primarily related to mechanical factors.—Authors' Abstract

Grimaud, J., Chapuis, F., Verchot, B. and Millan, J. Screening for peripheral neuropathy in patients with leprosy. Rev. Neurol. (Paris) **150** (1994) 785–790.

In leprosy, the early detection of peripheral nerve damage is essential for the prevention of disability. To date, there is no consensus on what is the best clinical test to reveal such abnormalities. In this prospective study, we examined the effectiveness of five clinical tests to assess radial cutaneous nerve (RCN) damage (the most frequently involved). Light touch was assessed by two nylon threads (based on the Semmes-Weinstein monofilaments testing technique) bent on the skin at a pressure of 0.5 gram (N degrees 4 nylon) and 0.2 gram (N degrees 5 nylon). Pinprick and cooling sensations were examined by a needle and a

drop of ether. The nerve thickness was assessed by palpation. Sensory findings were then compared to sensory nerve conduction values of the RCN and a sensitivity analysis was performed.

The patient group consisted of 108 consecutive new leprosy sufferers (138 RCN) who attended the Institut de Leprologie Appliguee de Dakar for 1 year. Diagnosis and classification were based on Ridley and Jopling's criteria (clinical examination, skin smears and biopsy). Normal values were determined among 22 healthy subjects (44 RCN). The best tests in term of sensitivity were palpation (.60), N degrees 5 nylon (.65) and N degrees 5 + palpation (.79). Their positive predictive values were .84 (palpation), .94 (N degrees 5 nylon) and .83 (N degrees 5 + palpation). The best tests in term of area under the curve were palpation (.66), N degrees 5 nylon (.71) and N degrees 5 + palpation (.78). The results remain the same for the lepromatous or tuberculoid leprosy patients. Ulnar and median nerves and different normal sensory nerve conduction values were tested in a sensitivity analysis; the two most sensitive tests remained the same.

Appreciation of nerve thickening and/or abnormal perception of the N degrees 5 nylon thread are the most appropriate diagnostic tools to detect early peripheral nerve damage.—Authors' Abstract

Naafs, B., Chin-A-Lien, R. A. M., Tank B. and van Joost, T. Human immunodeficiency virus and leprosy. Trop. Geogr. Med. **46** (1994) 199–121.

The authors present a short case report for a 35-year-old woman in Holland who was positive for HIV infection and on clinical examination was found to have two inconspicuous skin lesions which were diagnosed histopathologically as due to early borderline lepromatous leprosy in mild downgrading reaction. Some 2–3 weeks after starting on multidrug therapy both lesions had disappeared, leaving only a small hypoanesthetic area on the upper arm. The authors discuss in general terms the lack of increase in the number of cases of leprosy in endemic areas among HIV-infected patients (in contrast to the situation with tuberculosis).—C. A. Brown (Trop. Dis. Bull.)

Owen, B. M. and Stratford, C. J. Assessment of the methods available for testing sensation in leprosy patients in a rural setting. Lepr. Rev. **66** (1995) 55–62.

The aim of this study was to assess the efficacy, practicality and patient understanding of five methods used for testing sensation in leprosy patients in a rural setting. The tests used were the WHO test, cottonwool, pin-prick, monofilaments and the biothesiometer. We concentrated on testing sensation in the hands, and the various tests were carried out on 75 patients and 32 controls, all taken from villagers living at Kindwitwi Leprosy Village, Tanzania. Our results showed that although the WHO, cottonwool and pin-prick tests were all easy to use, cheap and well accepted they were not sensitive enough to be of any practical value. We found that the monofilaments, as well as being cheap and easy to use, had great potential value, since the 2-g monofilament could be used as a threshold value (indicative of leprosy, but not diagnostic) for protective sensation with a combined false-positive and false-negative value of only 4%. Finally, the biothesiometer was found to be a precise test that can accurately identify leprosy patients from controls and identify patients at risk of ulceration. It was, however, associated with its own problems, chiefly those of expense and its need of electricity, although we found this latter problem could be easily and relatively cheaply solved by the use of a solar-powered recharger.—Authors' Summary

Rodriguez Barreras, M., Gonzalez-Abreu Castells, E., Gonzalez Segredo, A. and **Curbelo Santana, L.** [Study of a suspected case of subclinical leprosy infection.] Rev. Leprol. Fontilles **19** (1994) 625–630. (in Spanish)

In this report a case is described in whom antibodies to the *Mycobacterium leprae* specific antigen phenolic glycolipid I were demonstrated. This case was followed up for 5 years, without specific treatment, until the antibody level declined to a normal value. No clinical signs of leprosy were observed during the surveillance period. Therefore, it was assumed that the serological reactivity might have expressed the course of a sub-

clinical infection with *M. leprae.*—Authors' English Summary

Salafia, A. and De Geiking, I. Neuritis and rifampicin. Rev. Leprol. Fontilles **19** (1994) 635–638.

The authors present their observation of neuritis precipitated or aggravated by rifampin in 20 patients all hospitalized except three. The authors believe that, as per literature, none of the drugs used in the MDT programs reach the nerves. However once a nerve is damaged by ischemia, drugs do penetrate and can cause further damage; this explains why many patients asymptomatic before therapy, complain of neural pain after starting the therapy.—Authors' Summary

Terzolo, M., Borretta, G., Ali, A., Cesario, F., Magro, G., Boccuzzi, A., Reimondo, G. and Angeli, A. Misdiagnosis of Cushing's syndrome in a patient receiving rifampicin therapy for tuberculosis. Horm. Metab. Res. **27** (1995) 148–150.

We hereby describe a patient in whom chronic rifampin treatment led to a misdiagnosis of Cushing's syndrome. He had longstanding insulin-dependent diabetes mellitus and active tuberculosis resistant to conventional treatment. The course was complicated by muscle weakness, lower limb atrophy, unstable glycemic control and hypokalemia. Ectopic Cushing's syndrome was suspected on the basis of high urinary free cortisol excretion (UFG) with a blunted circadian profile of serum cortisol and measurable plasma ACTH concentrations. Dynamic endocrine tests and imaging studies were compatible with occult ectopic ACTH syndrome. After substitution of rifampin UFG excretion returned to normal within 2 weeks, as well as the 24-hr cortisol profile and dynamic tests. The present case provides a practical example of the possibility to incorrectly suspecting Cushing's syndrome in patients treated with rifampin, as previously envisaged by pharmacological studies.—Authors' Abstract

Terencio de las Aguas, J. [Leprosy in children.] Rev. Leprol. Fontilles **19** (1994) 639–648. (in Spanish)

The importance of leprosy in children, the frequency in endemic countries and its uncommoness in children under 4 years is reviewed. The most frequent clinical forms are tuberculoid including the children nodular form and the indeterminate with the lepromatous type being exceptional. The importance of the clinical examination in contacts for detection, together with the therapeutical regimens, is described.—Author's English Summary

Waddell, K. M. and Saunderson, P. R. Is leprosy blindness avoidable? The effect of disease type, duration, and treatment on eye damage from leprosy in Uganda. Br. J. Ophthalmol. **79** (1995) 250–256.

The study was designed to measure the prevalence, range, and severity of eye involvement in leprosy patients; to relate this to disease type, duration, and treatment to identify risk factors; and to provide practical guidelines for program managers and field staff on the prevention of blindness. The visual outcome was assessed in a population-based sample of patients in Kasese District, Uganda, followed for up to two decades, and related to disease features and treatment. A total of 678 patients responded to an invitation out of 2715 registered since 1973. Low vision was present in 4.4% of the people and blindness in 1.3%, with 1.5% and 0.6%, respectively, being due to leprosy. Some 12.4% of patients had iritis, of whom 33% had visual loss in one or both eyes, 3.7% of patients had lagophthalmos, and 11.7% had lens opacity. For multibacillary (MB), as opposed to paucibacillary (PB) cases, the adjusted odds ratios were: for iritis 4.6 (95% CI 2.6–8.2), for lagophthalmos 1.4 (0.6–3.2), and for lens opacity 1.7 (1.0–3.0). Potentially sight threatening (PST) lesions were present in 16.8% of patients (95% CI 14.0–19.6).

Levels of eye involvement in this study are low compared with many surveys. Visual loss is uncommon and is more often caused by other diseases; in the present era of multidrug therapy (MDT) it is very unlikely to be caused by leprosy. It is more common with advancing age. PST lesions, especially iritis, may occur in both PB and

MB cases, even if the diagnosis of leprosy is made early and MDT started immediately; they may occur also after completion of MDT. But eye complications need not proceed to loss of sight if treated promptly, and blindness can be avoided. Training of front line staff is therefore crucial.—Authors' Abstract

Immuno-Pathology

Converse, P. J., Haines, V. L., Wondimu, A., Craig, L. E. and Meyers, W. M. Infection of SCID mice with *Mycobacterium leprae* and control with antigen-activated "immune" human peripheral blood mononuclear cells. Infect. Immun. **63** (1995) 1047–1054.

The SCID (severe combined immunodeficient) mouse lacks both B and T cells and tolerates injected mononuclear cells from humans, the principal hosts of *Mycobacterium leprae.* A SCID mouse model of leprosy could be useful to investigate potential vaccine strategies using human cells in a context in which the growth of the organism is monitored. Initial experiments determined that SCID mice are more susceptible than normal mice to infection and dissemination of *M. leprae.* Cells from humans, either BCG-vaccinated or from countries where leprosy is endemic, were stimulated *in vitro* with a number of mycobacterial antigens—whole *M. leprae, M. leprae* cell walls, purified protein derivative of *M. tuberculosis,* and *M. bovis* BCG—and tested for proliferation and production or interleukin-6, tumor necrosis factor-alpha, and gamma-interferon. Cell walls were the most efficient and consistent in inducing all of these activities. *In vitro*-activated human cells retain function better after injection into SCID mice than nonactivated cells. To test the ability of cells to affect the growth of *M. leprae* in the foot pads of SCID mice, cells from a known responder to mycobacterial antigens and from a nonresponder were activated by *M. leprae* cell wall antigens. The cells were harvested and coinjected with fresh *M. leprae* into the right hind foot pads of SCID mice. After 3 months, there was no growth of *M. leprae* in the foot pads of mice coinjected with cells from the mycobacterial antigen responder, while growth was uninhibited in mice receiving cells from the nonresponder. Future experiments will determine requirements for antigen specificity in inhibiting *M. leprae* multiplication.—Authors' Abstract

Cree, I. A., Coghill, G., Subedi, A. M. C., Abbot, N. C., Butlin, S. R., Samson, P. D. and Beck, J. S. Effects of treatment on the histopathology of leprosy. J. Clin. Pathol. **48** (1995) 304–307.

Aims—To identify the histological changes in leprosy skin lesions over the first few weeks after the start of leprosy treatment and to examine their relationship to reversal reaction. Methods—Sequential skin biopsy during treatment with multiple drug therapy. In this study, a series of 28 patients was studied, from whom two or more biopsies were taken at 2-week intervals. Fourteen patients had paucibacillary leprosy (PBL) and 13 had multibacillary leprosy (MBL). Results—In most cases, granuloma fraction and bacterial index (BI) fell during treatment, the BI being less sensitive than the granuloma fraction. Since the biopsies were fixed in buffered formalin and processed through to paraffin wax, little immunohistochemistry was feasible. However, there was strong evidence of immune activation, with increased expression of HLA-DR in the granulomas of MBL and PBL cases: the epidermis also expressed HLA-DR in several patients. Such changes may reflect gamma IFN production from granuloma lymphocytes. Patients with reversal reaction often showed HLA-DR expression on admission which decreased with corticosteroid treatment. Conclusions—The results suggest that activation of cell-mediated immunity in leprosy lesions occurs during treatment with multiple drug therapy and may not be restricted to those with clinical evidence of reversal reaction.—Authors' Abstract

Delabarrera, S., Fink, S., Finiasz, M., Minnucci, F., Valdez, R., Balina, L. M. and Sasiain, M. C. Lack of cytotoxic activity

against *Mycobacterium leprae* 65-kD heat shock protein (hsp) in multibacillary leprosy patients. Clin. Exp. Immunol. **99** (1995) 90–97.

Cytotoxic T cells play an important role in host defense mechanisms, as well as in the immunopathology of leprosy. In this study, we evaluated whether *Mycobacterium leprae* hsp18, hsp65 and *M. tuberculosis* hsp71 could induce cytotoxic T cell activity against autologous macrophages pulsed with these hsp. Paucibacillary (PB) patients and normal controls generated more effector cells than multibacillary (MB) patients with all three hsp tested. There was no crossreactivity between any of the hsp tested. *Mycobacterium leprae* hsp65 induced cytotoxic responses only in those MB patients undergoing an erythema nodosum leprosum (ENL) episode. Although hsp65 and hsp18 induced similar proliferation in MB patients, a high proportion of these patients did not generate cytotoxic effector cells in response to hsp65. Hence, those T cells reacting to hsp65 may play an important role in the control of *M. leprae* infection.—Authors' Abstract

De Wit, T. F. R., Clark-Curtiss, J. E., Abebe, F., Kolk, A. H. J., Janson, A. A. M., Van Agterveld, M. and Thole, J. E. R. A *Mycobacterium leprae*-specific gene encoding an immunologically recognized 45 kDa protein. Mol. Microbiol. **10** (1993) 829–838.

By screening a *Mycobacterium leprae* lambda gt11 expression library with a serum from an Ethiopian lepromatous leprosy (LL) patient a clone was isolated (LL4) belonging to hybridization group II of a panel of previously isolated *M. leprae* clones. Members of this hybridization group encode a serologically recognized 45-kDa protein. The complete DNA sequences of the partially overlapping clones LL4 and L1 (hybridization group III) are presented and these revealed the presence of an open reading frame (ORF) predicting a protein with a molecular size of 42, 448 Da. Southern hybridizations on total genomic DNA of *M. leprae, M. tuberculosis* and eight atypical mycobacteria showed that the LL4 DNA fragment is specific for *M. leprae* DNA even under low-stringency conditions. The *M. leprae* specificity of LL4 DNA was further confirmed by the polymerase chain reaction using four different sets of primers. Western blotting analyses showed that the *M. leprae* 45-kDa protein is frequently recognized by antibodies from leprosy patients and that this recognition is specific since no antibodies could be detected in sera of tuberculosis patients. T-cell proliferation assays also demonstrated T-cell recognition by leprosy patients and healthy contacts of the *M. leprae* 45-kDa protein. The specificity of the LL4 DNA region and the 45-kDa antigen that is encoded by hybridization group III could provide unique tools for the development of *M. leprae*-specific immunological and DNA reagents.—Trop. Dis. Bull.

Filley, E., Thole, J. E. R., Rook, G. A. W., Nagai, S., Waters, M., Drijfhout, J. W., de Wit, T. F. R., de Vries, R. R. P. and Abou-Zeid, C. Identification of an antigenic domain on *Mycobacterium leprae* protein antigen 85B, which is specifically recognized by antibodies from patients with leprosy. J. Infect. Dis. **169** (1994) 162–169.

Sixty-three overlapping 15-oligomer peptides covering the 30-kDa protein antigen 85B of *Mycobacterium leprae* were tested by ELISA to identify epitopes recognized by human antibodies. Serum samples from patients with lepromatous leprosy (LL) reacted mainly with peptides comprising amino acid regions (AA) 206–230, 251–280, and 291–325. Sera of patients with active tuberculosis who responded to the native 30-kDa antigen did not recognize these peptides. The antibody-binding specificity to the defined B-cell regions was evaluated in a blind study with 71 serum samples from patients and household contacts living in Ethiopia where leprosy is endemic. The peptide of AA 256–280 was recognized by 88% of LL patients, 15% of patients with tuberculoid leprosy, and none of the contacts. These findings suggest that there are major linear B-cell epitopes on the *M. leprae* 30-kDa protein that are recognized by lepromin-negative LL patients; whereas lepromin-positive patients respond preferentially to conformational epitopes.—Trop. Dis. Bull.

Gonzalez A. B. and Gonzalez-Abreu, E. Comment: the serodiagnosis of leprosy. Lepr. Rev. **65** (1994) 147–148.

A serological test able to detect the early phase of a leprosy infection would be valuable in leprosy control, because it would be possible to identify—and treat—potentially infectious people before such individuals showed obvious signs of leprosy. The discovery that the glycolipid PGL-I, which is apparently confined to *Mycobacterium leprae,* is antigenic and that antibodies to it are readily detected in sera of people with multibacillary leprosy, seemed to offer such a serological test. This letter is concerned with the usefulness (or otherwise) of a test based on PGL-I for detecting early leprosy; a response to an editorial (P. G. Smith, Leprosy Review, 1992, 63, 97), and the author of the editorial has, in turn, responded to the letter (ibid., 1994, 65, 148-149). The editorial pointed out that although people with elevated antibody levels to PGL-I were (according to several trials) at increased risk from leprosy, the majority of such people never, in fact, develop leprosy, so that the number of "false positives" greatly exceeds the number of potentially infectious people. So the test is not very useful in practice. The letter disputes this view on the grounds that if, in the large Venezuelan trial discussed in the editorial, the "cut-off value" for serological positivity had been differently selected, the ratio between true (serologically positive, subsequently developing leprosy) and false positives (serologically positive but never developing the disease) would be more favorable. However, additional diagnostic methods would still be needed to distinguish those who really have early leprosy. In his response the author of the original editorial reiterates the nonspecific nature of the test for detecting leprosy, and doubts that the proposed addition of a lepromin test would improve the situation.

[It seems clear that PGL-I-based tests are not a general solution to the problem of detecting early leprosy, although they might have some use in situations (as in Cuba) where leprosy is now more-or-less eliminated. At no stage in the discussion is the issue raised of whether serial tests would give more information: would abrupt increases or persistent high values of antibodies to PGL-I efficiently predict subsequent clinical leprosy? Such testing would be more expensive than simple surveys, but the expense might be warranted where leprosy was almost eliminated, if it could be shown to help complete elimination. More information seems needed on this point.]—P. Draper (Trop. Dis. Bull.)

Harkiss, G. D., Cattermole, J., Peterhans, E., Anderson, A., Vogt, H., Dickson, L. and Watt, N. T and B cell responses to mycobacterial 65-kDa heat-shock protein in sheep infected with maedi visna virus. Clin. Immunol. Immunopathol. **74** (1995) 223–230.

Sheep infected with maedi visna virus were tested for immune reactivity to recombinant hsp65 and tuberculin PPD from mycobacteria. The results showed that both naturally and experimentally infected animals had elevated IgM but not IgG or IgA antibodies to hsp65 from *Mycobacterium leprae* or *M. bovis.* In experimentally infected animals, the elevated IgM antibodies appeared in blood from about 3 to 4 weeks postinfection. Increased T-cell proliferative responses to hsp65 and PPD were also found in both naturally and experimentally infected sheep. The T-cell responses to hsp65 were substantially inhibited by antibodies to ovine major histocompatibility complex class II molecules, indicating that the responses were class II restricted. Increased expression of a putative hsp65 molecule was observed in synovial membranes from sheep infected with maedi visna virus and goats infected with the related, caprine arthritis encephalitis virus. The results thus show that lentivirus infection induces T- and B-cell anti-hsp65 immune responses and suggest that synovial inflammation may be due, at least in part, to T- and B-cell recognition of hsp65-like molecules expressed in joints.—Authors' Abstract

Kaleab, B., Wondimu, A., Likassa, R., Woldehawariat, N. and Ivanyi, J. Sustained T-cell activity to *Mycobacterium tuberculosis* specific antigens in "split-anergic" leprosy. Lepr. Rev. **66** (1995) 19–25.

Split anergy represented by delayed-type hypersensitivity skin reaction to tuberculin, but not to leprosin, is known to occur in a distinct proportion of leprosy patients. The

mechanism was originally attributed to *Mycobacterium leprae*-specific suppression of T cells toward common mycobacterial antigens. This study ascertained an alternative explanation, attributing the phenomenon to selective responsiveness to *M. tuberculosis*-specific epitopes. Indeed, the results of blood T-cell proliferative responses in 11 split-anergic patients showed normal responsiveness to the *M. tuberculosis*-specific 38-kDa lipoprotein and peptide 71-91 of the 16-kDa antigen but diminished responsiveness to two common mycobacterial antigens, represented by the 65-kDa heat-shock protein and the fibronectin-binding Ag85 complex, as compared with leprosin-responsive patients and healthy contacts. These findings support the hypothesis that split anergy is due to selective recognition of *M. tuberculosis*-specific epitopes and deletion of T cells reacting to shared mycobacterial antigens.—Authors' Summary

Karopoulos, C., Rowley, M. J., Handley, C. J. and Strugnell, R. A. Antibody reactivity to mycobacterial 65 kDa heat shock protein: relevance to autoimmunity. J. Autoimmun. **8** (1995) 235–248.

Reactivity to the mycobacterial 65-kDa heat-shock protein (hsp65) has been implicated in the pathogenesis of adjuvant arthritis in the rat, and may be involved in the pathogenesis of rheumatoid arthritis or other autoimmune diseases in humans. Accordingly this study sought quantitative or qualitative differences in the antibody reactivity to hsp65 between normal controls, patients with the multisystem autoimmune diseases, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) and patients with the mycobacterial infections, tuberculosis (TB) and leprosy.

Levels of antibodies to recombinant hsp65 in serum were measured by ELISA in normal subjects and in patients with RA, SLE, TB or leprosy. Antibody reactivity was examined by Western blotting using polypeptide fragments of hsp65 derived by recombinant DNA techniques, or by digestion with trypsin or cyanogen bromide (CNBr). Reactivity to a synthetic peptide, the adjuvant arthritis T-cell epitope of hsf65 (180–188), was tested by ELISA. High levels of antibodies to full length recombinant hsp65 from *Mycobacterium bovis* were present in all the groups tested. By Western blot analysis, most reactivity with intact hsp65 was retained in a 32-kDa tryptic fragment, judged by sequencing and size estimations to represent amino acid residues 118—similar to 388. This sequence included a major T-cell epitope for adjuvant arthritis (180–188), but these nine amino acids were not essential for B-cell reactivity since most sera also reacted with residues 188–540 which lack the T-cell epitope. Moreover, the 180–188 synthetic peptide was unreactive by ELISA, and did not inhibit reactivity with the intact recombinant hsp65.

In conclusion, most individuals had antibodies to mycobacterial hsp65, presumably resulting from previous bacterial infections. The magnitude of the response was unrelated to the occurrence of systemic autoimmune disease, and the pattern of antibody reactivity with recombinant and proteolytic fragments of hsp65 suggests that the major B-cell epitope is conformational and consists of discontinuous regions of the molecule.—Authors' Abstract

Klatser, P. R. Serology of leprosy. Trop. Geogr. Med. **46** (1994) 115–118.

The author gives a brief overview of the usefulness of serology in diagnosis, prognosis, and epidemiology of leprosy. He stresses that serological results can help toward the diagnosis of leprosy but need to be interpreted in combination with other diagnostic information. Serological tests can be of particular support for diagnosis in the early stages of disease, for follow up after treatment, and for the early detection of relapses after treatment (to distinguish them from reversal reactions). Seroepidemiological studies have revealed that infection with *Mycobacterium leprae* is more prevalent than disease caused by the organism, and such studies may be of value in the monitoring of changes in intensity of infection resulting from control measures. Simplified tests that can be standardized are urgently needed.—C. A. Brown (Trop. Dis. Bull.)

Parkash, O., Chaturvedi, V., Girdhar, B. K. and Sengupta, U. A study on performance of two serological assays for diagnosis of leprosy patients. Lepr. Rev. **66** (1995) 26–30.

We compared two serological tests for the diagnosis of leprosy to test their performances. The tests include the serum antibody competition test (SACT) for the detection of antibodies to *Mycobacterium leprae*-specific epitope on 35-KDa protein molecule, and *M. leprae* gelatin particle agglutination assay (MLPA), for the detection of antiphenolic glycolipid-I (PGL-I) antibodies. In both the assays a higher serological positivity was seen among multibacillary (MB) patients than those in paucibacillary (PB) patients. Taking all leprosy patients together, the sensitivity of SACT (59.7%) was observed to be statistically comparable to that of MLPA (66.9%). However, SACT proved to be more specific (97.7%) than MLPA (75.0%). The agreement between these two assays was observed to be moderate.—Authors' Summary

Sheela, R., Shankernarayan, N. P., Ramu, G. and Muthukkaruppan, V. R. IgG subclass antibodies to mycobacterial sonicate and recombinant antigens in leprosy. Lepr. Rev. **66** (1995) 10–18.

In this study the IgG subclass antibodies to sonicated preparations of *Mycobacterium leprae* (leprosin A) and BCG (BCG-S) as well as to purified recombinant 65-kDa protein of *M. leprae* (rML65) were analyzed in sera from leprosy patients and healthy household contacts (HFC) and noncontacts (HNC) in a leprosy-endemic population. In LBI + (lepromatous bacterial index positive) patients, IgG3 was predominant in the responses to sonicated antigens of *M. leprae*. Following chemotherapy, IgG3 responses were reduced while IgG2 levels were increased. On the other hand, IgG response to rML65 was dominated by IgG1 in all the patient and control groups. Interestingly, the level of antileprosin A IgG antibody in erythema nodosum leprosum (ENL) was similar to that of lepromatous groups, while the level of anti-rML65 IgG antibody was significantly reduced in ENL. IgG4 antibodies to the antigens studied were only at low levels in all groups, including ENL. Significant differences were observed between HNC and HFC in the pattern of IgG subclass antibodies to sonicated antigens, even though their antigen-specific IgG levels were similar. While HNC showed equivalent proportion of IgG1 and IgG2 in their responses to leprosin A and BCG-S, HFC showed a specific increase in IgG1 levels, suggesting that both groups are distinctly different. Further studies are required to elucidate the functional significance of IgG subclass pattern in pathogenesis and the mechanism of immunoregulation resulting in the high levels of IgG1 and IgG3 antibodies to *M. leprae* protein antigens in lepromatous leprosy.—Authors' Summary

Ulrich, M., Rodriguez, V., Centeno, M. and Convit, J. Differing antibody IgG isotypes in the polar forms of leprosy and cutaneous leishmaniasis characterized by antigen-specific T cell anergy. Clin. Exp. Immunol. **100** (1995) 54–58.

Leprosy and American cutaneous leishmaniasis are tropical diseases which present a spectrum of clinical and immunological manifestations. Lepromatous leprosy and diffuse cutaneous leishmaniasis are the severe, progressive polar forms of disease characterized by persistent T-cell anergy. Relative concentrations of antibodies belonging to the four IgG isotypes have been determined in these forms of disease as well as active visceral leishmaniasis, which presents transitory T-cell anergy. Leishmania-specific IgG4 antibodies predominated in 19/20 sera from patients with diffuse cutaneous leishmaniasis, and IgG1 antibodies predominated in 9/10 cases of untreated visceral leishmaniasis. The predominant IgG isotype of *Mycobacterium leprae*-specific antibodies in untreated lepromatous leprosy was remarkably variable (IgG1, IgG2, IgG3 and IgG4 in 8, 6, 2 and 1 sera, respectively). Differing IgG antibody isotypes have been associated with distinct CD4+ T-cell helper subpopulations and their characteristic lymphokine profiles in several pathologies. These results suggest that T-cell anergy in chronic intracellular infections may be associated with as yet undefined mechanisms which modulate reported T-helper cell-lymphokine isotype relationships.—Authors' Abstract

Vila, L. M., Haftel, H. M., Park, H. S., Lin, M. S., Romzek, N. C., Hanash, S. M. and Holoshitz, J. Expansion of mycobacterium-reactive gamma delta T cells by a subset of memory helper T cells. Infect. Immun. **63** (1995) 1211–1217.

Human gamma delta T cells expressing the V gamma 9/V delta 2 T-cell receptor have been previously found to proliferate in response to certain microorganisms and to expand throughout life, presumably because of extrathymic activation by foreign antigens. *In vitro* expansion of V gamma 9/V delta 2 cells by mycobacteria has been previously shown to be dependent on accessory cells. In order to gain an insight into the mechanisms involved in the expansion of these cells, we have undertaken to identify the peripheral blood subset of cells on which proliferation of V gamma 9/V delta 2 cells in response to mycobacteria is dependent. Contrary to their role in antigen presentation to alpha beta T cells, professional antigen-presenting cells, such as monocytes, B cells, and dendritic cells, were unable to provide the cellular support for the expansion of V gamma 9/V delta 2 cells. Selective depletion of T-cell subsets, as well as the use of highly purified T-cell populations, indicated that the only subset of peripheral blood cells that could expand V gamma 9/V delta 2 cells were CD4+ CD45RO+ CD7−, alpha beta T cells. These cells underwent distinct intracellular signaling events after stimulation with the mycobacterial antigen. Expansion of V gamma 9/V delta 2 cells by alpha beta T cells was dependent on cell-cell contact. This is the first evidence that a small subset of the memory helper T-cell population is exclusively responsible for the peripheral expansion of V gamma 9/V delta 2 cells. These data illustrate a unique aspect of antigen recognition by gamma delta T cells and provide new means to study their immune defense role.—Authors' Abstract

Wang, X. H., Golkar, L., Uyemura, K., *et al.* T cells bearing Vβ6 T cell receptors in the cell-mediated immune response to *Mycobacterium leprae.* J. Immunol. **151** (1993) 7105–7116.

To identify predominant specific T-cell subpopulations in leprosy lesions, the TCR-β chain repertoire was simultaneously studied in skin-biopsy specimens and PBMC from both immunologically resistant tuberculoid leprosy and susceptible lepromatous leprosy patients in The Philippines. This was accomplished by obtaining RNA from lesions and PBMC, synthesizing cDNA, and performing the polymerase chain reaction (PCR). [The authors] found that TCR gene subfamilies Vβ6.1 through Vβ6.4 (Vβ6.1–4) were strikingly over-represented in lesions vs. PBMC of 7 of 9 tuberculoid patients but only 1 of 9 lepromatous patients. Similarly, Vβ6.5/6.8/6.9 subfamilies were predominant in 4 of 9 tuberculoid patients, but 0 of the 9 lepromatous patients. To explore the influence of the complementarity-determining region 3 (CDR3) in selection of T cells expressing Vβ6 TCR, the authors sequenced the Vβ6.1–4Cβ PCR products derived from the lesions and PBMC of two tuberculoid patients. From the analysis of deduced amino acid sequences, the authors found conserved amino acid residues and amino acid motifs in the CDR3 region of the lesion-derived sequences from each patient. The authors' data suggest that the nominal Ag select T cells bearing Vβ6 TCR in the cell-mediated immune response to *Mycobacterium leprae.*—Trop. Dis. Bull.

Microbiology

Chaturvedi, V., Singh, N. B. and Sinha, S. Immunoreactive antigens of a candidate leprosy vaccine: *Mycobacterium habana.* Lepr. Rev. **66** (1995) 31–38.

Mycobacterium habana (*M. simiae* serovar-1) is a candidate vaccine for mycobacterial infections on the basis of the protection shown by this strain. We prepared three fractions of *M. habana,* i.e., the cell wall (CW), the cell membrane (CM) and the cytosol (CS). Protein antigens of these fractions were resolved by SDS-PAGE and subsequently probed with the sera of leprosy

and tuberculosis patients and also antiBCG antibodies.

We saw three major protein bands at ≃ 33 kD in the CW, ≃ 38 kD in the CM and ≃ 22 kD in the cytosol (CS) after Coomassie blue staining of the gels. Pool leprosy patients' serum had identified proteins of ≃ 26 kD in CW, ≃ 35 and ≃ 18 kD in CM and ≃ 24 kD in the CS which have not been seen by the TB patient's serum pool. Pool serum of tuberculosis patients has identified 1 protein at ≃ 10 kD in the CW and a broad band between 20 and 24 kD and 1 at ≃ 4 kD in the CM which have not been visualized in the pool leprosy patient's serum lane. The proteins of *M. habana* which are recognized only by leprosy antisera or only by tuberculosis antisera could be exploited for developing diagnostic agents against these infections.—Authors' Summary

Dabbs, E. R., Yazawa, K., Mikami, Y., Miyaji, M., Norisaki, N., Iwasaki, S. and Furihata, K. Ribosylation by mycobacterial strains as a new mechanism of rifampin inactivation. Antimicrob. Agents Chemother. **39** (1995) 1007–1009.

Several fast-growing *Mycobacterium* strains were found to inactivate rifampin. Two inactivated compounds (RIP-Ma and RIP-Mb) produced by these organisms were different from previously reported derivatives, i.e., phosphorylated or glucosylated derivatives, of the antibiotic. The structures of RIP-Ma and RIP-Mb were determined to be those of 3-formyl-23-[*O*-(α-D-ribofuranosyl)]rifamycin SV and 23-[*O*-(α-D-ribofuranosyl)]rifampin, respectively. To our knowledge, this is the first known example of ribosylation as a mechanism of antibiotic inactivation.—Authors' Abstract

Hermans, P. W. M., Abebe, F., Kuteyi, V. I. O., Kolk, A. H. J., Thole, J. E. R. and Harboe, M. Molecular and immunological characterization of the highly conserved antigen 84 from *Mycobacterium tuberculosis* and *Mycobacterium leprae.* Infect. Immun. **63** (1995) 954–960.

Crossed immunoelectrophoresis (CIE) has been used to develop a reference system for classifying mycobacterial antigens. The subsequent use of specific antibodies allowed further determination of antigens by molecular weight. The monoclonal antibody F126-2, originally raised against a 34-kDa antigen of *Mycobacterium kansasii,* reacted with antigen 84 (Ag84) in the CIE reference system for *M. bovis* BCG and to *M. tuberculosis.* To characterize Ag84, we screened a lambda gt11 gene library from *M. tuberculosis* with antibody F126-2 and identified the encoding gene. The corresponding *M. leprae* Ag84 gene was subsequently selected from a cosmid library, using the *M. tuberculosis* gene as a probe. Both genes were expressed as 34-kDa proteins in *Escherichia coli,* and the recombinant proteins indeed corresponded to Ag84 in the CIE reference system. The derived amino acid sequences of the *M. tuberculosis* and *M. leprae* proteins showed 85% identity, which indicates that Ag84 constitutes a group of highly conserved mycobacterial antigens. Antibodies of almost 60% of lepromatous leprosy patients responded to Ag84, indicating that the protein is highly immunogenic following infection in multibacillary leprosy.—Authors' Abstract

Khoo, K. H., Dell, A., Morris, H. R., Brennan, P. J. and Chatterjee, D. Structural definition of acylated phosphatidylinositol mannosides from *Mycobacterium tuberculosis:* definition of a common anchor for lipomannan and lipoarabinomannan. Glycobiology **5** (1995) 117–127.

Based on chemical analysis, we have previously concluded that the biologically important lipoarabinomannan (LAM) and lipomannan (LM) from *Mycobacterium* are multiglycosylated forms of the phosphatidylinositol mannosides (PIMs), the characteristic cell envelope mannophosphoinositides of mycobacteria. Using definitive analytical techniques, we have now re-examined the reported multiacylated nature of PIMs in order to gain a better insight into their possible roles as biosynthethic precursors of LM and LAM. High-sensitivity fast atom bombardment-mass spectrometry analyses of the perdeuteroacetyl and permethyl derivatives of PIMs from *Mycobacterium tuberculosis* and *M. leprae* enabled us to define the exact fatty acyl compositions of the multiacylated, heterogeneous PIM families, notably the dimannoside (PIM(2)) and the hexamannoside (PIM(6)).

Specifically, in conjunction with other chemical and gas chromatography-mass spectrometry (GC-MS) analyses, the additional C16 fatty acyl substituent on PIM(2) and its lyse form were defined as attached to the C6 position of mannose. We also present evidence for triacylated mannophosphoinositide as a common lipid anchor for both LM and LAM, and further postulate that acylation of PIM(2) may constitute a key regulatory step in their biosynthesis.—Authors' Abstract

Kong, D. Q. and Kunimoto, D. Y. Secretion of human interleukin 2 by recombinant *Mycobacterium bovis* BCG. Infect. Immun. **63** (1995) 799–803.

The human interleukin 2 (huIL-2) gene was introduced into *Mycobacterium bovis* BCG by using the integrative vector pMV306. To express and secrete huIL-2 from BCG, two different plasmids, CI and CII, were made. In CI, the huIL-2-encoding region was under the control of the alpha-antigen promoter of BCG; in CII, the expression of huIL-2 was regulated by the heat-shock protein 60 promoter. A signal peptide sequence isolated from the naturally secreted alpha-antigen of BCG was inserted between the promoter and huIL-2-encoding region to facilitate secretion. Both huIL-2 expression plasmids were integrated into the BCG genome when introduced into the BCG Pasteur strain by electroporation. Approximately 150 U of huIL-2 was secreted into the medium of a BCG CII culture, while the BCG-CI cells secreted approximately one-sixth of that amount. When the IL-2-expressing BCG strain BCG-CII was injected intravenously into BALB/c mice, the number of BCG cells in the spleens of these mice was significantly less than the number in the control mice. The decreased number of IL-2-expressing BCG cells is likely due to the augmentation of the host immune response by the secreted huIL-2, although the exact mechanism is not known.—Authors' Abstract

McAdam, R. A., Weisbrod, T. R., Martin, J., Scuderi, J. D., Brown, A. M., Cirillo, J. D., Bloom, B. R. and Jacobs, W. R. *In vivo* growth characteristics of leucine and methionine auxotrophic mutants of *Mycobacterium bovis* BCG generated by transposon mutagenesis. Infect. Immun. **63** (1995) 1004–1012.

Insertional mutagenesis in *Mycobacterium bovis* BCG, a member of the slow-growing *M. tuberculosis* complex, was accomplished with transposons engineered from the *M. smegmatis* insertion element IS1096. Transposons were created by placing a kanamycin-resistance gene in several different positions in IS1096, and the resulting transposons were electroporated into BCG on nonreplicating plasmids. These analyses demonstrated that only one of the two open reading frames was necessary for transposition. A library of insertions was generated. Southern analysis of 23 kanamycin-resistant clones revealed that the transposons had inserted directly, with no evidence of cointegrate formation, into different restriction fragments in each clone. Sequence analysis of nine of the clones revealed junctional direct 8-bp repeats with only a slight similarity in target sites. These results suggest that IS1096-derived transposons transposed into the BCG genome in a relatively random fashion. Three auxotrophs, two for leucine and one for methionine, were isolated from the library of transposon insertions in BCG. They were characterized by sequencing and found to be homologous to the leuD gene of *Escherichia coli* and a sulfate-binding protein of cyanobacteria, respectively. When inoculated intravenously into C57BL/6 mice, the leucine auxotrophs, in contrast to the parent BCG strain or the methionine auxotroph, showed an inability to grow *in vivo* and were cleared within 7 weeks from the lungs and spleen.—Authors' Abstract

Portaels, F., ed. Integration of traditional and modern methods in the control of leprosy and in the study of mycobacterial taxonomy (13th IWGMT Conference). Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 1–96.

This supplement contains material presented at an international colloquium held in Antwerp Belgium, in December 1992. In honor of the retirement of Professor S. R. Pattyn the first day of the meeting dealt with leprosy control methods, then the following 3 days doubled as the 13th conference of

the International Working Group on Mycobacterial Taxonomy (IWGMT). After a short introduction and appreciation of Professor Pattyn by the editor, D. M. Scollard (pp. 5–11) discusses new dimensions in the immunopathological spectrum of leprosy. D. E. Minnikin, *et al.* (pp. 13–24) next describe an integrated procedure for the direct detection of characteristic lipids in tuberculosis patients, and then Minnikin with other colleagues (pp. 25–34) outlines the identification of the leprosy bacillus and related mycobacteria by analysis of mycocerosate profiles. Enzyme electrophoresis in taxonomy of mycobacteria is discussed by M. Ridell (pp. 35–39), and finally, as the last of the leading papers, the rapid, early and specific diagnosis of tuberculosis and other mycobacterial diseases in Burundi is examined by T. Barihuta, *et al.* (pp. 41–51). The supplement is completed by abstracts of 23 oral presentations and of a further 18 poster presentations, an 8-page summary of the 13th conference on the genus *Mycobacterium* of the IWGMT, and a 1-page report of a task group on special problems in laboratory aspects of tuberculosis in developing countries.—C. A. Brown (Trop. Dis. Bull.)

Smith, A. W. Stationery phase induction in *Escherichia coli*—new targets for antimicrobial therapy? J. Antimicrob. Chemother. **35** (1995) 359–361.

Bacteria are remarkably able to survive for prolonged periods, even in the apparent absence of available nutrients. Accumulating evidence indicates that certain gram-negative species do not form survival bodies, such as spores, but survive by the induction of specific genes during late logarithmic and early stationary phase. This article reviews this evidence and offers the hypothesis that the physiology of starved stationary-phase bacteria *in vitro* could be highly relevant to growth *in vivo,* particularly in chronic infections where cells may be nutrient-limited and dividing slowly or not at all. In so doing, these bacteria could present new targets for antimicrobial therapy.—From the Article

Experimental Infections

Gidoh, M., Matsuki, G., Tsutsumi, S., Hidaka, T. and Nakamura, S. Inhibition of the multiplication of *Mycobacterium leprae* in nude mice by intermittent administration of a new rifamycin derivative, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (KRM-1648) combined with sparfloxacin. Lepr. Rev. **66** (1995) 39–47.

Inhibition of the multiplication of *Mycobacterium leprae* in the foot pads of nude mice by the oral administration of sparfloxacin, a new quinolone, and 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin (KRM-1648), selected from a series of newly synthesized benzoxazinorifamycins, was studied. When the two drugs were administered alternately at intervals of 3 or 4 days, (i.e., each drug was administered once weekly), or simultaneously once weekly, between 3 and 5 months after inoculation of nude mice with *M. leprae,* 10 mg sparfloxacin and 0.6 mg KRM-1648 per kg bodyweight were sufficient to prevent multiplication of the organisms. Only partial inhibition of multiplication was achieved by alternate administration of 5 mg sparfloxacin and 0.3 mg KRM-1648 per kg, as was the case for 20 mg sparfloxacin per kg or 1 mg KRM-1648, each drug administered alone once weekly. The addition to these two drugs of dapsone, administered in the diet in a concentration of 0.001 g per 100 g, enhanced their effect. The potential usefulness of multidrug regimens including these compounds is considered.—Authors' Summary

Gosselin, D., Turcotte, R. and Lemieux, S. Cellular target of *in vitro*-induced suppressor cells derived from the spleen of *Mycobacterium lepraemurium* infected mice and role of IFN-gamma in their development. J. Leukoc. Biol. **57** (1995) 122–128.

Unfractionated spleen cells from C3H mice infected a few weeks before with *Mycobacterium lepraemurium* developed a

suppressor activity after overnight culture. This requires contact of plastic adherent cells with nonadherent cells distinct from T, B, or natural killer cells. The present study demonstrates that anti-interferon-gamma monoclonal antibody and indomethacin totally abrogate the expression, although not the induction, of this activity. Furthermore, culture-induced suppressor cells selectively inhibit T lymphocyte proliferation, probably by altering the generation of interleukin-2 (IL-2) responsiveness through reduction of the affinity and density of high-affinity IL-2 receptors on activated cells. These and other previously determined properties of culture-induced suppressor cells, similar to those of adherent suppressor cells detected in freshly harvested spleen cells at a later stage of *M. lepraemurium* infection, suggest a common precursor. If so, the present observations should help in defining a strategy to prevent the impairment of cell-mediated immunity in infected mice.—Authors' Abstract

Epidemiology and Prevention

Abel, L., Lap, V. D., Oberti, J., Van Thuc, N., Van Cua, V., Guilloud Bataille, M., Schurr, E. and Lagrange, P. H. Complex segregation analysis of leprosy in Southern Vietnam. Genet. Epidemiol. **12** (1995) 63–82.

To investigate the nature of the genetic component controlling susceptibility to leprosy and its subtypes, 402 nuclear families were ascertained through a leprosy patient followed at the Dermatology Hospital in Ho Chi Minh City, Vietnam; 285 families were of Vietnamese origin and 117 were of Chinese origin with a higher proportion of lepromatous forms among Chinese patients. Segregation analyses were conducted using the model developed by Abel and Bonney [(1990) Genet Epidemiol 7:391–407], which accounted for variable age of onset and time-dependent covariates. Three phenotypes were considered: leprosy *per se* (all forms of leprosy together), nonlepromatous leprosy, and lepromatous leprosy. For each phenotype, analyses were performed on the whole sample and separately on the Vietnamese and the Chinese families. The results showed that a single Mendelian gene could not account for the familial distributions of leprosy *per se* and its two subtypes in the whole sample. However, these results were different according to the ethnic origin of the families. In the Vietnamese subsample, there was evidence for a codominant major gene with residual familial dependences for the leprosy *per se* phenotype, and borderline rejection of the Mendelian transmission hypothesis for the nonlepromatous phenotype. In Chinese families, strong rejection of Mendelian transmission was obtained in the analysis of leprosy *per se,* and no evidence for a familial component in the distribution of the nonlepromatous phenotype was observed. For the lepromatous phenotype, the discrimination between models was poor, and no definitive conclusion could be reached. Referring to immunological data, we suggest that these results could be explained by a heterogeneity in the definition of the lepromatous phenotype. It is likely that progress in the understanding of the genetic components involved in the expression of leprosy will come from a better definition of the phenotype under study, and immunological studies are ongoing in this population to investigate this hypothesis.—Authors' Abstract

Arora, P. N. and Grover, S. Changing trends of leprosy in armed forces. Med. J. Armed Forces India **50** (1994) 259–260.

A total of 651 cases of leprosy were hospitalized from January 1987 to December 1992. Each patient underwent hemogram, total and differential white cell count, urinalysis, liver function tests, skin slit smear for acid-fret bacilli and skin biopsy. Nerve conduction studies, electromyographic studies and nerve/nerve sheath biopsies were undertaken as and when indicated. These patients were managed with multidrug therapy. Paucibacillary (PB) leprosy accounted for 476 (73.1%) cases which comprised indeterminate leprosy 90 (13.8%), tuberculoid leprosy 14 (2.2%), borderline tuberculoid leprosy 310 (47.6%) and neuritic leprosy 62 (9.5%). The remaining 175 patients

(26.9%) were multibacillary (MB) which included borderline leprosy 9 (1.4%), borderline lepromatous leprosy 129 (19.8%) and lepromatous leprosy 37 (5.7%) patients. There were total 153 patients in 1987. This number declined to 44 in 1992. PB declined from 113 in 1987 to 39 in 1992 and MB cases from 40 to 5.—Trop. Dis. Bull.

Carrazana Hernandez, G. B. and Ferra Torres, T. M. [Leprosy incidence during monotherapy and multidrug therapy in Camaguey, Cuba, 1984–1993.] Rev. Leprol. Fontilles **19** (1994) 603–612. (in Spanish)

A comparative study of the incidence of leprosy in Camagüey, Cuba, was carried out during the last 5 years of monodrug therapy (1984–1988) and the first with multidrug therapy (1989–1993).

The epidemiological indexes analyzed were the incidence and its rates per 100,000 inhabitants, the percentage of clinic forms, sex and age groups.

The results of the study have demostrated a diminution of the incidence of leprosy (from 121 to 81 cases) and its rates (maximum from 11.5 to minimum of 2.7 × 100,000 inhabitants); a displacement toward the predominance of multibacillary forms (from 48.7 to 63.0%); from a predominance of the indeterminate form in the monodrug therapy stage (27.3%) evolved to the minimum percentage (7.4%) among all clinic forms during the MDT; a slight predominance of female (55.0%); a minimum affectation under 15 years (average 1.5%) and a higher affectation in the group of 35-64 and more than 65 years age group.—Authors' English Summary

Jaffré, Y. and Moumouni, A. [The importance of socio-cultural data concerning the accessibility of heath care and the observance of treatment in leprosy; the example of the Zarma in Niger.] Bull. Soc. Pathol. Ex. **87** (1994) 283–288. (in French)

From an epidemiological point of view leprosy remains a problem of public health. Various factors influence the accessibility to health care and the observance of treatment. The study carried out in Niger shows that beyond the sigmatism associated with the disease, the most important factors concern the difference between scientific and popular etiology and semiology. In the face of such problems it is necessary, from a theoretical point of view, to make anthropological enquiries based on the theme of the different representations of this pathology, and from a practical point of view, to examine the possibility of the participation of former patients in public health teams.—Authors' English Summary

Jakeman, P., Jakeman, N. R. P. and Singay, J. Trends in leprosy in the Kingdom of Bhutan, 1982–1992. Lepr. Rev. **66** (1995) 69–75.

An evaluation program was undertaken 11 years after the introduction of multidrug therapy (MDT) into Bhutan, by examining the case notes of 3239 leprosy patients who had been under treatment at any time during the period. The registered prevalence was found to have fallen markedly, as expected, and this had been accompanied by a clear fall in the case detection rate as well. The lepromatous rate among new patients rose considerably, giving epidemiological hope that the disease may be coming under control. However, no concomitant fall in the proportion of child cases was seen. The disability rate at detection rose slightly, although numbers were small. New cases were increasingly likely to have more highly positive skin smears, and to be self-reported. Program planners should give thought to the implications of these findings.—Authors' Summary

Noordeen, S. K. Elimination of leprosy as a public health problem: progress and prospects. Bull. WHO **73** (1995) 1–6.

Leprosy is still an important problem in about 80 countries of Asia, Africa and Latin America, some 2.4 million persons being estimated to have the disease in 1994. The WHO-recommended standard multidrug therapy (MDT) was introduced in the 1980s and has been shown to be effective in combating the disease. Experiences based on many thousands of patients treated with MDT over the past decade indicate extremely low relapse rates (cumulative relapse rates around 1%). By the end of 1993, some 5.6 million patients had been cured,

and the global cumulative MDT coverage of registered patients had reached 89%. The number of registered cases fell from 5.4 million in 1985 to 1.7 million in 1994.

The significant progress made in leprosy control enabled the World Health Assembly in 1991 to set a goal for eliminating leprosy as a public health problem by the year 2000. One important epidemiological factor is that leprosy is very unevenly distributed: 80% of the problem is confined to only five countries and 92% to just 25 countries. The elimination strategy envisages identifying and treating with MDT a total of about 5 million cases from 1994 to the year 2000. The cost of dealing with these cases has been estimated at US$420 million, including US$150 million for the drugs.—Authors' Abstract

Orege, P. A., Fine, P. E. M., Lucas, S. B., Obura, M., Okelo, C., Okuku, P. and Were, M. A case control study on human immunodeficiency virus-1 (HIV-1) infection as a risk factor for tuberculosis and leprosy in western Kenya. Tuber. Lung Dis. **74** (1993) 377–381.

A case control study was undertaken in western Kenya from April 1989 to August 1990 to evaluate HIV-1 infection as a risk factor for tuberculosis and leprosy. The study involved 144 newly diagnosed, sputum smear-positive tuberculosis cases with 432 age-, sex-, and neighborhood-matched controls, and 132 diagnosed leprosy cases with 384 matched controls. Odds ratio obtained by conditional logistic regression (matched) analysis were 4.9 (95% CI 2.6, 6.8) and 1.8 (95% CI 0.9, 3.2), for the association between HIV-1 and tuberculosis and leprosy, respectively. Approximately 31% of tuberculosis cases among males, and 11% of cases among females, were attributable to HIV infection.—Trop. Dis. Bull.

Shaw, M. A., Atkinson, S., Dockrell, H., Hussain, R., Lins-Lainson, Z., Shaw, J., Ramos, F., Silveira, F., Mehdi, S. Q., Kaukab, F., Khaliq, S., Chiang, T. and Blackwell, J. An RFLP map for 2q33-q37 from multicase mycobacterial and leishmanial disease families: no evidence for an *Lsh/Ity/Bcg* gene homologue influencing susceptibility to leprosy. Ann. Hum. Genet. **57** (1993) 251–271.

In order to determine whether a human homologue to the murine macrophage resistance gene *Lsh/Ity/Bcg* influences susceptibility to human disease, multicase families for the three diseases caused by *Mycobacterium leprae, M. tuberculosis* and *Leishmania* sp. were collected, and linkage analysis performed using a panel of markers in the region of human chromosome 2q33–q37 known to be conserved with the *Lsh/Ity/Bcg*-containing region of murine chromosome. 1. Because of the paucity of available polymorphic markers/linkage information for 2q33–q37, data from 35 multicase leprosy, TB and visceral leishmaniasis families (310 individuals) were first pooled to produce a detailed RFLP map of the region. Peak LOD scores well in excess of 3 were observed for linkage between adjacent pairs of a more proximal (2q33–q35) set of markers CRYGP1, MAP2, FN1, TNP1, VIL1 and DES, and between adjacent pairs of a more distal (2q35–q37) set COL6A3, D2S55 and D2S3. These peak LOD scores and the corresponding values for 0 were used in the MAP92 program to generate a multiple two-point map with gene order/map intervals (cM) of: CRYGP1-4.65-MAP2-3.45-FN1-5.95-TNP1-3.41-VIL1-3.01-DES-20.14-COL6A-10.91-D2S55-3.67-D2S3. Although local support for the placement of loci in this order was weak (LOD < 2, except for DES COL6A3 where LOD $= 6.02$), the map is consistent with the gene order for those loci (*Cryg, Fn-1, Tp-1, Vil, Des, Col6a3*) previously mapped in the mouse. Data from 17 multicase leprosy families (149 individuals) were further analyzed for linkage between a putative disease susceptibility locus (DSL) controlling susceptibility to leprosy *per se* and each of the marker loci. Assuming 100% penetrance for the susceptibility allele, no positive LOD score was obtained for linkage between the DSL and any of the marker genes. The data provided convincing evidence (LOD scores < -2) that a DSL does not fall within 10–20 cM of CRYGP1, MAP2, TNP1, VIL1, DES or D2S55, or within 5–10 cM of FN1, COL6A3 or D2S3. This effectively excludes a putative DSL controlling susceptibility to leprosy *per se* from the entire region 2q33–q37. Even with reduced penetrance (80% and 60%) for the susceptibility allele, the data argue against

a putative DSL within the region TNP1-DES where the murine *Lsh/Ity/Bcg* gene is located. Analysis of the data for these loci using affected pedigree-member linkage analysis also failed to provide evidence for co-segregation of these markers with susceptibility to disease *per se*. Nor could any evidence be found for a gene in this region controlling susceptibility to the tuberculoid form of disease, or to T-cell responder phenotypes for proliferative responses to the mycobacterial antigens purified protein derivative (PPD) or *M. leprae* soluble antigen (MLSA). The RFLP map generated is a detailed genetic map of the region 2q33–q37, mostly comprising genes encoding molecules of known function. This provides an anchor map and a set of typed families around which new highly polymorphic microsatellite markers can be ordered.—Trop. Dis. Bull.

Rehabilitation

Arolkar, S. K. and Antia, N. H. Vascular surgery of the posterior tibial compartment for plantar ulceration in leprosy. Lepr. Rev. **66** (1995) 48–54.

Traditional surgical decompression of the posterior tibial nerve yields equivocal results. The authors postulate that the posterior tibial artery is the most compromised structure in the neurovascular compartment and that the best surgical results in healing of plantar ulcers are achieved by the rechannelling of the blood flow in the posterior tibial artery during posterior tibial neurovascular compartment surgery. This procedure has been of benefit to patients with plantar ulcers of greater than 7-10 years' duration in whom all other modes of healing had failed. It has been undertaken as an outpatient procedure under local anesthesia, supported by postoperative vasodilator drugs. The use of tourniquet, antibiotics and surgical interference with the ulcer *per se* was eschewed. A report of 156 patients is presented with follow up of up to 6 years for the earlier cases.—Authors' Summary

Other Mycobacterial Diseases and Rèlated Entities

Adams, L. B., Mason, C. M., Kolls, J. K., Scollard, D., Krahenbuhl, J. L. and Nelson, S. Exacerbation of acute and chronic murine tuberculosis by administration of tumor necrosis factor receptor-expressing adenovirus. J. Infect. Dis. **171** (1995) 400–405.

Tumor necrosis factor (TNF) plays a pivotal role in inflammatory phenomena that culminate in either pathogenesis or resistance in mycobacterial disease. The regulatory role of TNF in murine tuberculosis was examined by administering a recombinant adenovirus encoding a fusion protein consisting of the human 55-kDa TNF receptor extracellular domain and the mouse IgG heavy chain domain (AdTNFR). During acute infections with *Mycobacterium tuberculosis,* AdTNFR pretreatment induced elevated mycobacterial burdens of 1 log in the tissues of H37Ra-infected mice and 2 log 10^{10} (spleen and liver) and 4 log 10^{10} (lungs) in H37Rv-infected mice. In mice infected chronically with H37Rv, AdTNFR treatment induced a 3-log 10^{10} increase of *M. tuberculosis* in the lungs, in which a tuberculous bronchopneumonia developed with numerous acid-fast bacilli visible in alveoli and bronchi. Administration of AdTNFR may serve as a useful model for studying the pathogenesis and chemotherapy of progressive primary tuberculosis.—Authors' Abstract

Amicosante, M., Richeldi, L., Trenti, G., Paone, G., Campa, M., Bisetti, A. and Saltini, C. Inactivation of polymerase inhibitors for *Mycobacterium tuberculosis* DNA amplification in sputum by using capture resin. J. Clin. Microbiol. **33** (1995) 629–630.

Endogenous polymerase chain reaction (PCR) inhibitors hamper DNA amplification of *Mycobacterium tuberculosis* DNA in sputum samples. Use of an anion-binding resin (GeneReleaser [GR]) improved PCR sensitivity from 77% (27 of 35 culture-positive samples) with phenol-chloroform extraction (P-C) alone to 91% (GR without P-C; $p > 0.05$) and 100% (P-C and GR combined; $p < 0.05$).—Authors' Abstract

Blackwell, J. M., Barton, C. H., White, J. K., Roach, T. I. A., Shaw, M. A., Whitehead, S. H., Mock, B. A., Searle, S., Williams, H. and Baker, A. M. Genetic regulation of leishmanial and mycobacterial infections: the Lsh/Ity/Bcg gene story continues. Immunol. Lett. **43** (1994) 99–107.

A common basis to genetic regulation of leishmanial and mycobacterial infections is provided by the action of the murine Lsh/Ity/Bcg gene in controlling the priming/activation of macrophages for antimicrobial activity. This relies on the TNF-alpha-dependent sustained expression of the inducible nitric oxide synthase (iNOS) gene responsible for the generation of large amounts of toxic nitric oxide (NO). The Lsh/Ity/Bcg gene has many pleiotropic effects, including differential expression of the early response gene KC following stimulation of macrophages with bacterial lipopolysaccharide (LPS) and mycobacterial lipoarabinomannan (LAM). The major signal transduction pathway involved in KC induction requires the generation of low levels of NO via constitutive nitric oxide synthase (cNOS) activity, leading to activation of guanylate cyclase and the cGMP-dependent kinase pathway. NO, therefore, appears to provide a common link between the early influence of Lsh in regulating the expression of genes which mediate many pleiotropic effects, and the later production of NO as the final effector mechanism for kill. The recently cloned candidate for Lsh/Ity/Bcg, designated Nramp for natural resistance associated macrophage protein, encodes a polytopic integral membrane protein that has structural features common to prokaryotic and eukaryotic transporters and includes a conserved binding-protein-dependent transport motif which may be involved in interaction with peripheral ATP-binding subunits. The N-terminal sequence also carries a proline/serine rich putative SH3 binding domain, consistent with a role for tyrosine kinases in regulating Nramp function. This is also supported by the demonstration that ligation of beta(1) integrins, which signal via tyrosine kinases, by plating of macrophages onto extracellular matrix proteins is sufficient to mediate differential TNF-alpha release by macrophages from congenic Lsh resistant and susceptible mice. Transfection studies with the resistant allele demonstrate that Nramp plays a role, either directly or as an additional pleiotropic effect, in interferon-gamma/LPS upregulated L-arginine transport across the macrophage membrane, thus providing the substrate required to generate NO for both signal transduction and antimicrobial activity. Nra,mp also shows 55–58% sequence similarity with the yeast genes SMF1 and SMF2, which influence protein import into mitochondria. A high degree of conservation over the region of Nramp which contains the susceptible Nramp mutation indicates a possible common function at the level of protein translocation across membranes of intracellular compartments. Analysis of human NRAMP has identified a novel 3X9 nucleotide repeat in the putative SH3 binding domain, with a rare second allele bearing a 2X9 nucleotide repeat occurring at low frequency in the Brazilian population. Studies in progress will attempt to determine the function of human Nramp, and hence to identify its role in parallel activation pathways in man. This is of particular interest in the light of studies demonstrating (i) that NO generated by iNOS is not used for antimicrobial activity in human macrophages, and (ii) that the iNOS gene itself and the interferon-gamma-inducible JAK tyrosine kinases are candidates for two other major genes, Sell and Scl2, identified and mapped in mice for their role in controlling different leishmanial resistance phenotypes.

Further analysis of genetic regulation of pathways leading to iNOS-mediated NO production may provide the key to understanding why human macrophages do not use this as an antimicrobial pathway, and may also provide the basis for development of novel immunotherapeutic strategies for disease control.—Authors' Abstract

Boesen, H., Jensen, B. N., Wilcke, T. and Andersen, P. Human T-cell responses to secreted antigen fractions of *Mycobacterium tuberculosis*. Infect. Immun. **63** (1995) 1491–1497.

The T-cell response of human donors to secreted antigen fractions of *Mycobacterium tuberculosis* was investigated. The donors were divided into five groups: active pulmonary tuberculosis (TB) patients with minimal and with advanced disease, *M. bovis* BCG-vaccinated donors with and without contact with TB patients, and non-vaccinated individuals. We found that patients with active minimal TB responded powerfully to secreted antigens contained in a short-term culture filtrate, The response to secreted antigens was mediated by CD4(+) Th-1-like lymphocytes, and the gamma-interferon release by these cells was markedly higher in patients with active minimal TB than in healthy BCG-vaccinated donors, Patients with active advanced disease exhibited depressed responses to all preparations tested, The specificity of the response to secreted antigens was investigated by stimulating lymphocytes with narrow-molecular-mass fractions of short-term culture filtrate obtained by the multi-elution technique. Considerable heterogeneity was found within the donor groups. Patients with active minimal TB recognized multiple secreted targets but, interestingly, six of eight patients demonstrated a predominant recognition of a low-mass (<10-kDa) protein fraction which induced high levels of gamma-interferon release *in vitro*. Only a few of 12 previously characterized secreted antigens were recognized by T cells isolated from TB patients, suggesting the existence of a number of as yet undefined antigenic targets among secreted antigens.—Authors' Abstract

Bothamley, G. H., Gibbs, J. H., Beck, J. S., Schreuder, G. M. T., de Vries, R. R. P., Grange, J. M., Ivanyi, J. and Kardjito, T. Delayed hypersensitivity and HLA in smear-positive pulmonary tuberculosis. Int. Arch. Allerg. Immunol. **106** (1995) 38–45.

Tuberculin responses were quantified by induration of the skin, velocity of blood flow in dermal microcirculation and composition of the cellular infiltrate in 125 patients with tuberculosis and 39 healthy controls. The diameters of the tuberculin responses were greater in HLA-DR15-positive patients than in DR15-negative patients. The density of infiltrating CD4+-positive cells showed a positive correlation with induration in DR15-negative subjects ($r = 0.54$). A fraction of DR15-positive patients gave large tuberculin responses ($\geq$ 15 mm) but with few CD4+ cells in the test site ($< 500/mm^2$); these patients had a greater percentage of cells in the diffuse dermal infiltrate than in the perivascular region, greater blood flow velocities in the tuberculin response but more frequently with central relative slowing of blood flow and had higher total IgG and specific antimycobacterial antibody levels compared to other DR15-positive patients. If the inflammatory infiltrate in the lungs parallels that in the tuberculin skin test, the lack of immunocompetent cells and tissue hypoxia could permit pulmonary cavitation and explain the association of HLA-DR15 with smear-positive pulmonary tuberculosis.—Authors' Abstract

Boughton, B. J., Sheehan, T. M. T., Wood, J., O'Brien, D., Butler, M., Simpson, A. and Hale, K. A. High-performance liquid chromatographic assay of plasma thalidomide: stabilization of specimens and determination of a tentative therapeutic range for chronic graft-versus-host disease. Ann. Clin. Biochem. **32** Part 1 (1995) 79–83.

Thalidomide is now widely used to treat chronic graft-versus-host disease, but its use is associated with non-teratogenic side effects such as peripheral neuropathy. To examine the value of monitoring plasma concentrations of the drug in such patients, we have developed a high-performance liquid chromatographic (HPLC) assay. The method uses 0.5 mL plasma, is linear to 10 mg/L and had a detection limit of 0.2 mg/L. Thalidomide in plasma specimens was unstable at physiological pH but could be stabilized for several weeks by simple acidification. We describe a protocol for monitoring patients treated with thalidomide which permits convenient transportation and storage of specimens and report, provisionally, that

plasma concentrations in the range 1–7 mg/L are therapeutically effective in chronic graft-versus-host disease without adverse side effects.—Authors' Abstract

Brewer, T. F. and Colditz, G. A. Relationship between bacille Calmette-Guerin (BCG) strains and the efficacy of BCG vaccine in the prevention of tuberculosis. Clin. Infect. Dis. **20** (1995) 126–135.

Bacille Calmette-Guerin (BCG) vaccination for the prevention of tuberculosis has been used in humans since 1921, Furthermore, for > 60 years it has been possible to separate BCG strains (defined here as a BCG vaccine maintained in a particular laboratory and used in a particular trial or set of trials) on the basis of *in vitro* and *in vivo* tests. Investigators have concluded that differences in the BCG strains used in efficacy trials on humans may be responsible for the wide range in levels of protection from tuberculosis reported in those trials. We review the development of the separate strains used in the trials included in a recent meta-analysis and examine data for and against the protective efficacy of different BCG strains, The difficulties in correlating results of *in vitro* and *in vivo* tests with protective efficacy in humans are discussed. The limited data available from human studies suggest that the BCG strain used for vaccination is not a significant determinant of the overall efficacy in the prevention of tuberculosis.—Authors' Abstract

Chan, C. H. S., Lai, C. K. W., Leung, J. C. K. and Ho, A. S. S. Elevated interleukin-2 receptor level in patients with active pulmonary tuberculosis and the changes following antituberculosis chemotherapy. Eur. Respir. J. **8** (1995) 70–73.

Soluble interleukin-2 receptor (sIL-2R) is a marker of T-lymphocyte activation. We have undertaken a study to examine the serum sIL-2R levels in patients with pulmonary tuberculosis (TB) and the changes following anti-TB chemotherapy.

Forty-four patients with pulmonary TB or tuberculosis pleural effusion were recruited. Serum was collected from the patients before and at 1, 2, 4 and 6 months after initiation of anti-TB chemotherapy. Serum sIL-2R level was measured by an enzyme immunoassay: The mean sIL-2R level before treatment was 1452 ± 103 (SEM) U ml^{-1}, which was significantly higher than that of healthy control subjects (374 ± 30 U ml^{-1}). There was no significant change in the sIL-2R level at 1 month, but there was a gradual reduction from the second month onward. At the sixth month the mean sIL-2R level was 1080 ± 81 U ml^{-1}, which was significantly lower than that before treatment. However, despite clinical improvement, the sIL-2R levels at the sixth month were still significantly higher than those of control subjects.

We conclude that sIL-2R levels were elevated in patients with pulmonary TB and there was a gradual reduction following anti-TB chemotherapy. However, the sIL-2R levels were still higher than control subjects at completion of treatment, suggesting a delayed resolution of the inflammation in patients with pulmonary TB.—Authors' Abstract

Chan, J., Tanaka, K., Carroll, D., Flynn, J. and Bloom, B. R. Effects of nitric oxide synthase inhibitors on murine infection with *Mycobacterium tuberculosis.* Infect. Immun. **63** (1995) 736–740.

We have recently demonstrated that the macrophage L-arginine-dependent cytotoxic pathway effectively kills the virulent Erdman strain of *Mycobacterium tuberculosis in vitro* via the generation of toxic reactive nitrogen intermediates by the enzyme nitric oxide synthase. This report demonstrates that two distinct inhibitors of nitric oxide synthase (aminoguanidine and N-G-monomethyl-L-arginine) render similar deleterious effects on tuberculous infection in mice, as assessed by mortality, bacterial burden, and pathological tissue damage, thus confirming the importance of reactive nitrogen intermediates in resistance against *M. tuberculosis.*—Authors' Abstract

Child, J., Andrews, J. M. and Wise, R. Pharmacokinetics and tissue penetration of the new fluoroquinolone grepafloxacin. Antimicrob. Agents Chemother. **39** (1995) 513–515.

A single 400-mg oral dose of grepafloxacin (OPC-17116) was given to each of six healthy male volunteers, and the concen-

trations of the drug in plasma, cantharides-induced inflammatory fluid, and urine were measured over the subsequent 12 hr. The mean peak concentration in plasma of 1.5 μg/ml was attained at a mean time of 2.0 hr postdose. The mean peak concentration in inflammatory fluid of 1.1 μg/ml was attained at a mean time of 4.8 hr postdose. The mean elimination half-life in plasma was 5.2 hr, and that in inflammatory fluid was 12.7 hr. The overall penetration into inflammatory fluid was 180.6% (or 133% if one aberrant result from one volunteer is excluded). Recovery of the drug in urine during the first 24 hr postdose was 8.3% of the administered dose. Our results indicate that a once- or twice-daily dosage of grepafloxacin should be adequate to treat systemic infections caused by most bacterial pathogens.—Authors' Abstract

Cho, S.-N., van der Vliet, G. M. E., Park, S., Baik, S.-H., Kim, S.-K., Chong, Y., Kolk, A. H. J., Klatser, P. R. and Kim, J.-D. Colorimetric microwell plate hybridization assay for detection of amplified *Mycobacterium tuberculosis* DNA from sputum samples. J. Clin. Microb. **33** (1995) 752–754.

We developed a colorimetric microwell plate hybridization assay (CoMPHA) for the specific detection of 5′-biotinylated amplified *Mycobacterium tuberculosis* DNA. The optical densities of the CoMPHA corresponded to the initial amounts of purified template DNA. Here, we show that the CoMPHA is useful in distinguishing the PCR-positive and PCR-negative samples.—Authors' Abstract

Cocito, C. and van Linden, F. Composition and immunoreactivity of the A60 complex and other cell fractions from *Mycobacterium bovis* BCG. Scand. J. Immunol. **41** (1995) 179–187.

Surface static cultures of *Mycobacterium bovis* BCG contained cells embedded in an extracellular matrix, whose mechanical removal yielded free cells that were pressure disrupted and fractionated into cytoplasm and walls. Cell envelopes were either mechanically disrupted or extracted with detergents. Intracellular and extracellular fractions were analysed for proteins, polysaccharides, and antigen 60 (A60), a major complex immunodominant in tuberculosis. A60 was present in extracellular matrix, cytoplasm and walls: it represented a substantial portion of the proteins and polysaccharides of these fractions. While the protein/polysaccharide ratio varied according to the origin of A60 preparations, the electrophoretic patterns of A60 proteins (which accounted for the immunogenicity of the complex) remained unchanged. Western blots pointed to the proteins present within the 29–45-kDa range as the A60 components endowed with the highest immunogenicity level. Since the most heavily stained protein bands in SDS-PAGE patterns were located outside the region best recognized by antisera, a striking discordance was found between concentration and immunogenicity patterns of A60 proteins. The electrophoretic patterns of A60- and non-A60-proteins from cytoplasm were also different. A60 complexes in dot blots and some electrophoresed A60 proteins reacted with monoclonal antibodies directed against lipoarabinomannan (LAM), a highly immunogenic polymer of cell envelope. This contaminating compound was removed from A60 with organic solvents and detergents. SDS-PAGE and Western blot patterns of proteins from delipidated A60 were similar to those of native A60 proteins.—Authors' Abstract

Cole, C. H., Roger, P. C. J., Pritchard, S., Phillips, G. and Chan, K. W. Thalidomide in the management of chronic graft-versus-host disease in children following bone marrow transplantation. Bone Marrow Transplant. **14** (1994) 937–942.

Chronic graft-versus-host disease (GVHD) is the major complication in patients surviving > 100 days postallogeneic bone marrow transplantation and occurs in 30% of pediatric patients. It is most prevalent 1–2 years post-transplant. Treatment involves corticosteroids and other immunosuppressive therapy which may affect growth and increase the likelihood of infectious complications. We report five children with severe corticosteroid-dependent chronic GVHD treated with thalidomide 12–25 mg/kg/day. Response to therapy was based on resolution of symptoms of chronic GVHD

and withdrawal of other immunosuppressive therapy. All the children showed clinical response to thalidomide with cessation or diminution in other immunosuppressive medication. Side effects were minimal and no patient developed peripheral neuropathy. All patients are alive 48–65 months post-transplantation. Thalidomide is a safe and effective drug for the treatment of chronic GVHD in children and may avoid the use of long-term corticosteroid therapy.—Authors' Abstract

Cooper, A. M., Roberts, A. D., Rhoades, E. R., Callahan, J. E., Getzy, D. M. and Orme, I. M. The role of interleukin-12 in acquired immunity to *Mycobacterium tuberculosis* infection. Immunology **84** (1995) 423–432.

The early phase of acquired cellular immunity to *Mycobacterium tuberculosis* infection is mediated by the emergence of protective CD4 T lymphocytes that secrete cytokines including interferon-gamma (IFN-gamma), a molecule which is pivotal in the expression of resistance to tuberculosis. Recent evidence demonstrates that infection with *M. tuberculosis* induces peripheral blood mononuclear cells to release the cytokine interleukin-12 (IL-12), a molecule that promotes the emergence of T-helper type-1 (Th1), IFN-gamma-producing T cells. We demonstrate here that IL-12 mRNA expression was induced by *M. tuberculosis* infection both *in vivo* and *in vitro* and that exogenous administration of IL-12 to mice transiently resulted in increased resistance to the infection. IL-12 also increased the production of IFN-gamma by both splenocytes derived from infected animals treated *in vivo* and by antigen-stimulated CD4 cells from untreated infected animals, with maximal effects at times associated with the expansion of antigen-specific CD4 T cells *in vivo*. In the absence of a T-cell response, as seen in SCID mice or nude mice, IL-12 only slightly augmented the moderate bacteriostatic capacity of these immunocomprised mice. Neutralization of IL-12 by specific monoclonal antibodies resulted in a reduction in granuloma integrity and slowing of the capacity of the animal to control bacterial growth.—Authors' Abstract

Corkill, M., Stephens, J. and Bitter, M. Fine needle aspiration cytology of mycobacterial spindle cell pseudotumor. Acta Cytol. **39** (1995) 125–128.

We report a case of mycobacterial spindle cell pseudotumor (MSP) in a lymph node from an acquired immuno-deficiency syndrome patient diagnosed by fine needle aspiration (FNA). The FNA cytology was characterized by spindle cell proliferation without the typical foamy histiocytes usually seen in mycobacterial infections and mimicked a mesenchymal neoplasm, particularly Kaposi's sarcoma. This case illustrates the importance of including MSP in the differential diagnosis of lymph node FNAs from immunocompromised patients, particularly those that show spindle cell proliferation suspicious for Kaposi's sarcoma or another mesenchymal neoplasm.—Authors' Abstract

Deng, L. Y., Mikusova, K., Robuck, K. G., Scherman, M., Brennan, P. J. and McNeil, M. R. Recognition of multiple effects of ethambutol on metabolism of mycobacterial cell envelope. Antimicrob. Agents Chemother. **39** (1995) 694–701.

Ethambutol is known to rapidly inhibit biosynthesis of the arabinan component of the mycobacterial cell wall core polymer, arabinogalactan (K. Takayama and J. O. Kilburn, Antimicrob. Agents Chemother. 33:1493–1499, 1989). This effect was confirmed, and it was also shown that ethambutol inhibits biosynthesis of the arabinan of lipoarabinomannan, a lipopolysaccharide noncovalently associated with the cell wall core. In contrast to cell wall core arabinan, which is completely inhibited by ethambutol, synthesis of the arabinan of lipoarabinomannan was only partially affected, demonstrating a differential effect on arabinan synthesis in the two locales. Further studies of the effect of ethambutol on cell wall biosynthesis revealed that the synthesis of galactan in the cell wall core is strongly inhibited by the drug. In addition, ethambutol treatment resulted in the cleavage of arabinosyl residues present in the mycobacterial cell wall; more than 50% of the arabinan in the cell wall core was removed from the wall 1 hr after addition of the drug to growing mycobacterial cultures. In con-

trast, galactan, was not released from the cell wall during ethambutol treatment. The natural function of the arabinosyl-releasing enzyme remains unknown, but its action in combination with inhibition of synthesis during ethambutol treatment results in severe disruption of the mycobacterial cell wall. Accordingly, ethambutol-induced damage to the cell wall provides a ready molecular explanation for the known synergetic effects of ethambutol with other chemotherapeutic agents. Nevertheless, the initial direct effect of ethambutol remains to be elucidated.—Authors' Abstract

Dessen, A., Quemard, A., Blanchard, J. S., Jacobs, W. R. and Sacchettini, J. C. Crystal structure and function of the isoniazid target of *Mycobacterium tuberculosis*. Science **267** (1995) 1638–1641.

Resistance to isoniazid in *Mycobacterium tuberculosis* can be mediated by substitution of alanine for serine 94 in the InhA protein, the drug's primary target. InhA was shown to catalyze the beta-nicotinamide adenine dinucleotide (NADH)-specific reduction of 2-trans-enoyl-acyl carrier protein, an essential step in fatty acid elongation. Kinetic analyses suggested that isoniazid resistance is due to a decreased affinity of the mutant protein for NADH. The three-dimensional structures of wild-type and mutant InhA, refined to 2.2 and 2.7 angstroms, respectively, revealed that drug resistance is directly related to a perturbation in the hydrogen-bonding network that stabilizes NADH binding.—Authors' Abstract

Emler, S., Bottger, E. C., Broers, B., Cassis, I., Perrin, L. and Hirschel, B. Growth-deficient mycobacteria in patients with AIDS: diagnosis by analysis of DNA amplified from blood or tissue. Clin. Infect. Dis. **20** (1995) 772–775.

Amplification and sequencing of mycobacterial ribosomal RNA genes (16S rDNA) may permit the detection of growth-deficient species (i.e., those exhibiting no growth or those whose growth is delayed for more than 12 weeks). Of blood samples from 26 patients with AIDS and a liver sample from one additional AIDS patient, three samples (two of blood and the one of liver) were positive by polymerase chain reaction only; cultures of these three samples remained negative for more than 12 weeks. Analysis of amplified 16S rDNA from blood revealed a sequence characteristic of *Mycobacterium genavense* in the first case, in which one of many previous blood cultures had also been positive for *M. genavense*. The sequences found in the second and third cases were characteristic of *M. avium*. The sample from the second patient was a liver biopsy specimen in which acid-fast bacilli were visualized; the culture of this specimen yielded *M. avium* after 7 months. The third sample was a blood sample from a patient in whom a relapse of treated *M. avium* infection was suspected. These results indicate that amplification and sequencing of mycobacterial 16S rDNA may permit early diagnosis and provide a rationale for treatment of infections due to growth-deficient mycobacteria.—Authors' Abstract

Eriksen, N., Kumar, S. B., Fukuchi, K., Martin, G. M. and Benditt, E. P. Molecular mimicry: histone H3 and mycobacterial protein epitopes. Proc. Natl. Acad. Sci. U.S.A. **92** (1995) 2150–2153.

A 15-kDa protein detected initially in amyloidotic ileum from a transgenic mouse and subsequently in control (nontransgenic) ileum by various polyclonal rabbit antiserums applied to electroblots of extracts derived from these tissues was identified by partial sequence analysis as histone H3. Antiserums were made against immunogens unrelated to the histone, but they recognized calf thymus histone H3 (14.7 kDa) on Western blots. The bacterial component of the Freund's medium used as an adjuvant for the immunogens was either *Mycobacterium butyricum* or *M. smegmatis*. Absorption tests with histone H3 and sonicated *M. butyricum* substantiated the presence of anti-histone H3 activity in the antiserums. These findings indicate that the two mycobacterium species make a protein with epitopes perceived as nonself by recipient rabbits but sufficiently similar to epitopes of mammalian histone H3 that the rabbits produced antibodies crossreactive with the histone.—Authors' Abstract

Fazal, N., Lammas, D. A., Rahelu, M., Pithie, A. D., Gaston, J. S. H. and Kumararatne, D. S. Lysis of human macrophages by cytolytic CD4+ T cells fails to affect survival of intracellular *Mycobacterium bovis* bacille Calmette-Guerin (BCG). Clin. Exp. Immunol. **99** (1995) 82–89.

Human CD4+, mycobacteria-specific, cytolytic T cell clones were used to lyse BCG-infected macrophages, and the effect on the subsequent growth and viability of the organisms was examined. The survival of released bacteria following cell lysis was assessed by both H-3-uridine labelling and colony-forming unit estimation. The results indicate that even when effective antigen-specific or lectin-mediated cytolysis of the infected macrophages was achieved, there was no evidence for a direct mycobactericidal effect on the intracellular bacteria. This remained the case even if the period of coculture of T cells and macrophages was extended up to 48 hr. Pretreatment of the macrophages with interferon-gamma was not able to act together with T cell-mediated lysis to produce inhibition of mycobacterial growth.—Authors' Abstract

Greenburg, S. S., Xie, J. M., Kolls, J., Mason, C. and Didier, P. Rapid induction of mRNA for nitric oxide synthase II in rat alveolar macrophages by intratracheal administration of *Mycobacterium tuberculosis* and *Mycobacterium avium.* Proc. Soc. Exp. Biol. Med. **209** (1995) 46–53.

Mycobacterium avium complex (MAC) organisms are among the most common bacterial cause of disseminated infection in patients with acquired immune deficiency syndrome (AIDS). An increase in the incidence of virulent *M. tuberculosis* (MTB) is also occurring throughout the world. *In vitro* data suggest that nitric oxide (NO) may be important in restricting the growth of MAC. However, the ability of MTB to stimulate NO production and the susceptibility of MTB to the bactericidal activity of NO produced by murine alveolar macrophages (AM) is controversial. This study tested the hypothesis that *in vivo* administration of heat-killed MAC (strain tao and 101) and human virulent MTB (strain F1) to rats stimulated NO production by rat AM, *ex vivo.* We show that heat-killed MTB instilled into rat lungs rapidly induced mRNA for NO synthase (iNOS) II in AM obtained by bronchoalveolar ravage (BAL). In contrast, expression of AM iNOS mRNA was only found in 40% of the rats given MAC. Moreover, the change in iNOS mRNA in the AM obtained from rats given MTB and MAC correlated with the production of the reactive nitrogen intermediates (RNI) NO2- and NO3- in BAL fluid, lung homogenate, and the spontaneous generation of RNI by isolated AM *ex vivo* and occurred without measurable increases in DAL fluid tumor necrosis factor-alpha. L-N-G-monomethylarginine (50 mg/kg, i.p.) given 30 min before MAC or MTB attenuated the increase in RNI in lung homogenates and BAL fluid. This is the first demonstration that *in vivo* exposure to MTB results in rapid upregulation of gene expression for iNOS which is associated with functional RNI production by rat AM. These results show that MTB human virulent strain 1 has the ability to rapidly upregulate iNOS mRNA in AM. If human AM generate NO from L-arginine by either iNOS or other NADPH oxidases then NO may play a role in the overall host-defense response of the lung to MAC and MTS.—Authors' Abstract

Hanano, R. and Kaufmann, S. H. E. Nitric oxide production and mycobacterial growth inhibition by murine alveolar macrophages: the sequence of rIFN-gamma stimulation and *Mycobacterium bovis* BCG infection determines macrophage activation. Immunol. Lett. **45** (1995) 23–27.

Prestimulation with recombinant-interferon-gamma (rIFN-gamma) followed by *Mycobacterium bovis* BCG infection induced high nitric oxide production and potent mycobacterial growth inhibition in murine alveolar macrophages. Reversal of the sequence of treatments caused opposite effects, suggesting that a sequential two-step process comprising first rIFN-gamma stimulation and second mycobacterial infection is operative in the activation of alveolar macrophages.—Authors' Abstract

Hasan, A., Childerstone, A., Pervin, K., Shinnick, T., Mizushima, Y., Vanderzee, R., Vaughan, R. and Lehner, T. Recognition of a unique peptide epitope of the mycobacterial and human heat shock protein 65-60 antigen by T cells of patients with recurrent oral ulcers. Clin. Exp. Immunol. **99** (1995) 392–397.

T-cell epitopes of the 65-kDa heat shock protein (hsp) were investigated in patients with recurrent oral ulcers (ROU). Peripheral blood mononuclear cells were stimulated with overlapping synthetic peptide (15(ers)) derived from the sequence of the 65-kD hsp of *Mycobacterium tuberculosis.* Specific lymphoproliferative responses were stimulated only with peptide 91–105 in ROU, compared with healthy or disease controls ($p < 0.01$). This was confirmed by studying 760 short-term cell lines generated with the 65-kDa hsp and then stimulated with the peptides. The frequency of short-term cells lines responding to peptide 91-105 in ROU was significantly greater than in healthy ($p < 0.0001$) or disease controls ($p < 0.01$). A comparative investigation with the homologous human 60-kDa hsp peptide 116–130 also showed significantly greater lymphoproliferative responses in ROU than in healthy ($p < 0.01$) or disease controls ($p < 0.001$). The potential involvement of the T-cell epitope 91–105 in the pathogenesis of ROU is supported by finding a significant increase in the lymphoproliferative responses stimulated with peptide 91–105 during the stage of ulceration, compared with remission in 9/11 patients studied sequentially ($p < 0.05$). The results suggest that oral ulceration might be initiated by the microbial hsp peptide 91–105 stimulating the mucosal Langerhans' cells, which may generate autoreactive T-cell clones primed to the homologous peptide 116–130.—Authors' Abstract

Horwitz, M. A., Lee, B. W. E., Dillon, B. J. and Harth, G. Protective immunity against tuberculosis induced by vaccination with major extracellular proteins of *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U.S.A. **92** (1995) 1530–1534.

Tuberculosis, caused by the intracellular pathogen *Mycobacterium tuberculosis,* is the world's leading cause of death in humans from a single infectious agent. A safe and effective vaccine against this scourge is urgently needed. This study demonstrates that immunization with the 30-kDa major secretory protein, alone or in combination with other abundant extracellular proteins of *M. tuberculosis,* induces strong cell-mediated immune responses and substantial protective immunity against aerosol challenge with virulent *M. tuberculosis* bacilli in the highly susceptible guinea pig model of pulmonary tuberculosis. Protection is manifested by decreased clinical illness including decreased weight loss, reduced mortality, and decreased growth of *M. tuberculosis* in the lungs and spleens of immunized animals compared with sham-immunized controls. This study demonstrates that purified major extracellular proteins of *M. tuberculosis* are candidate components of a subunit vaccine against tuberculosis and provides compelling support for the concept that extracellular proteins of intracellular pathogens are key immunoprotective molecules.—Authors' Abstract

Jagannath, C., Reddy, M. V., Kailasam, S., O'Sullivan, J. F. and Gangadharam, P. R. J. Chemotherapeutic activity of clofazimine and its analogues against *Mycobacterium tuberculosis: in vitro,* intracellular and *in vivo* studies. Am. J. Respir. Crit. Care Med. **151** (1995) 1083–1086.

Clofazimine (CFM), a riminophenazine drug, is primarily used in therapy for leprosy and *Mycobacterium avium* infections. With an objective of identifying drugs active against *M. tuberculosis,* including those with multidrug resistance, we investigated CFM and nine of its chemical analogs. Among these, B746 and B4101 had better activity than CFM against six drug-susceptible and nine single/multiple drug-resistant *M. tuberculosis* strains. B746 also showed slightly better activity than CFM against intracellular *M. tuberculosis* in J774A.1 macrophages and was comparable to CFM in its *in vivo* activity against experimental tuberculosis in C57BL/6 mice. Interestingly, it caused less pigmentation in internal organs.—Authors' Abstract

Klegerman, M. E., Oner, F., Morris, P., Son, K. and Groves, M. J. Isolation of a fibro-

nectin-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(R) 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 less than or equal to 6.5 kDa 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

McKenna, M. T., McCray, E. and Onorato, I. The epidemiology of tuberculosis among foreign-born persons in the United States, 1986 to 1993. N. Engl. J. Med. **332** (1995) 1071–1076.

One third of the world's population is infected with *Mycobacterium tuberculosis,* and in the developed countries immigration is a major force that sustains the incidence of tuberculosis. We studied the effects of immigration on the epidemiology of tuberculosis and its recent resurgence in the United States.

We analyzed data from the national tuberculosis reporting system of the Centers for Disease Control and Prevention (U.S.A.). Since 1986 reports of tuberculosis have included the patient's country of origin. Population estimates for foreign-born persons were derived from special samples from the 1980 and 1990 censuses. The proportion of persons reported to have tuberculosis who were foreign-born increased from 21.6% (4925 cases) in 1986 to 29.6% (7346 cases) in 1993. For the entire 8-year period, most foreign-born patients with tuberculosis were from Latin America (43.9%; 21, 115 cases) and Southeast Asia (34.6%; 16, 643 cases). Among foreign-born persons the incidence rate was almost quadruple the rate for native residents of the United States (30.6 vs 8.1 per 100,000 person-years), and 55 percent of immigrants with tuberculosis had the condition diagnosed in their first 5 years in the United States. Immigration has had an increasingly important effect on the epidemiology of tuberculosis in the United States. It will be difficult to eliminate tuberculosis without better efforts to prevent and control it among immigrants and greater efforts to control it in the countries from which they come.—Authors' Abstract

Millar, D. S., Withey, S. J., Tizard, M. L. V., Ford, J. G. and Hermon Taylor, J. Solid-phase hybridization capture of low-abundance target DNA sequences: application to the polymerase chain reaction detection of *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp. *silvaticum.* Analyt. Biochem. **226** (1995) 325–330.

Polymerase chain reaction (PCR) has been widely applied to the detection of microorganisms. Overall sensitivity of PCR tests may be substantially reduced due to a large excess of nontarget DNA and inhibitory substances in the sample. We used a 5′-biotinylated 513-bp probe from the 3′ region of the IS 900 element specific for *Mycobacterium paratuberculosis* (Mptb) to capture target Mptb DNA from crude sample DNA extracts. Captured target DNA was separated using streptavidin-coated magnetic particles (Dynal). Since the IS 900 element shares homology over this region with IS 902 in *M. avium* subsp. *silvaticum* (Mavs), target DNA from this other pathogen was also retained. Highly specific PCR for the detection of either organism directed to the 5′ regions of IS 900 or IS 902 was then performed directly on the solid phase. Hybridization capture of target DNA using sequence adjacent to the desired specific PCR site applied to Mptb increased overall sensitivity of detection in tissue and fecal extracts 10- to 100-fold. False-positives due to contamination artifact were substantially excluded since the capture probe did not retain amplicons from the detection PCR. Development of the method to involve covalent 5′ immobilization of capture probes on heat-resistant polymers should, in the future, provide a simple system with broad potential applications.—Authors' Abstract

Morris, S., Bai, G. H., Suffys, P., Portillo Gomez, L., Fairchok, M. and Rouse, D. Molecular mechanisms of multiple drug

resistance in clinical isolates of *Mycobacterium tuberculosis*. J. Infect. Dis. **171** (1995) 954–960.

The molecular mechanisms of resistance to streptomycin, rifampin, and isoniazid in 53 *Mycobacterium tuberculosis* clinical isolates were examined. Twenty-five of 44 streptomycin-resistant strains had mutations in the rpsL gene and 5 of these had rus gene perturbations. The region of the rpoB gene that is associated with resistance to rifampin was altered in 28 of 29 rifampin-resistant strains. Mutations in known genetic markers of isoniazid resistance were detected in 25 of 42 isoniazid-resistant isolates: 20 strains had katG gene alterations and 5 had perturbations in the inhA operon. Of the 20 multiply resistant strains with reduced sensitivity to streptomycin, rifampin, and isoniazid, 11 had mutations in genetic markers associated with resistance to each of these three drugs. These studies suggest that the primary mechanism of multiple-drug resistance in tuberculosis is the accumulation of mutations in individual drug target genes.—Authors' Abstract

Norden, M. A., Kurzynski, T. A., Bownds, S. E., Callister, S. M. and Schell, R. F. Rapid susceptibility testing of *Mycobacterium tuberculosis* (H37Ra) by flow cytometry. J. Clin. Microbiol. **33** (1995) 1231–1237.

The resurgence of tuberculosis has caused considerable effort to be focused on the development of rapid methods for determining the susceptibility of *Mycobacterium tuberculosis* to antimycobacterial agents. We demonstrated that susceptibility testing of *M. tuberculosis* can be accomplished rapidly by using flow cytometry. Results of tests were available within 24 hr after *M. tuberculosis* organisms were incubated with ethambutol, isoniazid, rifampin, or streptomycin. The method was based on the ability of viable *M. tuberculosis* organisms to hydrolyze fluorescein diacetate (FDA) and the detection of fluorescent mycobacteria by flow cytometric analysis. The assay system also did not require multiplication of the mycobacteria. In contrast, *M. tuberculosis* organisms exposed to antimycobacterial agents hydrolyzed significantly less FDA. The use of flow cytometry and FDA staining shows considerable promise as a rapid method for obtaining susceptibility test results.—Authors' Abstract

Onyebujoh, P. C., Abdulmumini, T., Robinson, S., Rook, G. A. W. and Stanford, J. L. Immunotherapy with *Mycobacterium vaccae* as an addition to chemotherapy for the treatment of pulmonary tuberculosis under difficult conditions in Africa. Respir. Med. **89** (1995) 199–207.

A study to assess the impact of immunotherapy with *Mycobacterium vaccae* on the treatment of pulmonary tuberculosis was conducted under existing conditions in Kano, a large city in northern Nigeria. While, it did not quite meet all the criteria of a well-controlled randomized or double-blind trial, the study produced results suggestive of a successful intervention. Immunotherapy with *M. vaccae* had a beneficial in influence on clinical recovery and survival, whether given after 1, 2 or 3 weeks of chemotherapy, according to an assessment made 10–14 months after treatment.

Approximately 3 weeks (19.8 days) after the onset of chemotherapy (SHRZ), 73% of the patients who received immunotherapy and 19% of those who received placebo (chemotherapy alone) had become sputum negative by microscopy for acid-fast bacilli (AFB). Similarly, a mean fall in erythrocyte sedimentation rate (ESR) of 25.4 ± 2.50 mm and 4.0 ± 2.29 mm was observed in the immunotherapy and placebo recipients, respectively, at the same time of assessment. When weight was assessed in the two groups, it was observed that 3 weeks after starting chemotherapy, the recipients of immunotherapy had a mean weight gain of 2.90 ± 0.24 kg while placebo recipients had a mean weight gain of only 0.55 ± 0.17 kg. These parameters were re-evaluated 10–14 months later. They showed that 11% of the recipients of the active intervention and 84.6% of placebo recipients still had demonstrable AFB in their sputum. The mean weight gain had increased to 7.91 ± 1.03 kg and 2.04 ± 0.94 kg in the immunotherapy and placebo recipients, respectively. The recorded mortality among those traced in this second follow up was 40% for the placebo recipients and 0% for the recipients of immunotherapy.

The impact of immunotherapy is discussed against the backdrop of a high mortality rate from tuberculosis, resulting from the absence of the most basic of anti-TB medication in the hospital, a preponderance of fake drugs in the open markets and local chemist stores as well as the rising seroprevalence of HIV and AIDS.—Authors' Abstract

Rastogi, N., Goh, K. S., Ruiz, P. and Casal, M. *In vitro* activity of roxithromycin against the *Mycobacterium tuberculosis* complex. Antimicrob. Agents Chemother. **39** (1995) 1162–1165.

Roxithromycin has recently been shown to possess significant *in vitro* activity against a variety of atypical mycobacteria such as the *Mycobacterium avium* complex, *M. scrofulaceum, M. szulgai, M. malmoense, M. xenopi, M. marinum,* and *M. kansasii* and rare pathogens like *M. chelonei* and *M. fortuitum.* In the present investigation, screening of its *in vitro* activity was further extended by testing it against 34 strains belonging to the *M. tuberculosis* complex (including *M. tuberculosis, M. africanum, M. bovis,* and *M. bovis* BCG). The MICs were determined by the radiometric BACTEC 460-TB methodology at pHs of both 6.8 and 7.4, as well as with 7H10 agar medium by the 1% proportion method. With the exception of *M. bovis* BCG (MIC ranges 0.5 to 4 μg/ml at pH 6.8 and 0.25 to 2 μg/ml at pH 7.4), MICs for all of the isolates were significantly greater (MIC ranges 32 to > 64 μg/ml at pH 6.8 and 16 to > 32 μg/ml at pH 7.4) than those reported previously for atypical mycobacteria. Roxithromycin MICs of 64 or > 64 μg/ml for all of the *M. tuberculosis* isolates screened were found by the 7H10 agar medium method. Roxithromycin, however, showed a pH-dependent bactericidal effect against *M. tuberculosis* because the drug was relatively more active when it was used at pH 7.4 than when it was used at pH 6.8. We conclude that roxithromycin *per se* is not a drug of choice for the treatment of *M. tuberculosis* infection or disease; however, considering its pharmacokinetics, eventual antitubercle bacillus activity in an *in vivo* system cannot yet be excluded. We suggest that the use of roxithromycin in chemoprophylactic regimens for the prevention of opportunistic infections (including *M. avium* complex infections) in patients with AIDS should be carefully monitored, and patients should be enrolled in such a regimen only after it has been excluded that the patient has an underlying infection or disease caused by *M. tuberculosis.*—Authors' Abstract

Rastogi, N., Labrousse, V. and Bryskier, A. Intracellular activities of roxithromycin used alone and in association with other drugs against *Mycobacterium avium* complex in human macrophages. Antimicrob. Agents Chemother. **39** (1995) 976–978.

Recent reports have shown that roxithromycin possesses significant activity against atypical mycobacteria, including the *Mycobacterium avium* complex (MAC), and that its extracellular anti-MAC activity is further enhanced in two- or three-drug combinations with ethambutol, rifampin, amikacin, ofloxacin, and clofazimine. In accordance with the above data, the anti-MAC potential of roxithromycin used alone and in combination with the above-mentioned antituberculous drugs was screened intracellularly against five clinical MAC isolates (from both human immunodeficiency virus-positive and human immunodeficiency virus-negative patients), phagocytized by human monocyte-derived macrophages. The results showed that roxithromycin used alone and within clinically achievable levels was active against all of the MAC isolates tested. Screening of two-drug combinations showed that both rifampin and clofazimine further increased the intracellular activity of roxithromycin against all five isolates by 35% to 80% (ethambutol, ofloxacin, and amikacin resulted in increased intracellular activity against one, two, and four isolates, respectively). For the three-drug combinations, the combination of roxithromycin plus ethambutol used with rifampin or clofazimine was the most uniformly active against all five MAC isolates, with activity increases of 42% to 90%, followed by roxithromycin plus ethambutol used with amikacin, which resulted in activity increases of 15% to 90%. The overall level of intracellular killing after 5 days of drug addition, in comparison with growth in untreated controls, varied from 1 to 3 log units depending on the individual MAC isolate and/

or drug combination used.—Authors' Abstract

Silver, R. F., Wallis, R. S. and Ellner, J. J. Mapping of T cell epitopes of the 30-kDa ex-antigen of *Mycobacterium bovis* strain bacillus Calmette-Guerin in purified protein derivative (PPD)-positive individuals. J. Immunol. **154** (1995) 4665–4674.

The fibronectin-binding 30-kDa alpha antigen (Ag) is a major secretory protein of growing mycobacteria that stimulates *in vitro* lymphocyte blastogenesis in most healthy purified protein derivative-positive individuals, but only a minority of patients with active tuberculosis. T-cell epitopes of the alpha Ag were assessed using blastogenic responses of PBMC from 12 healthy purified protein derivative-positive subjects to a set of synthetic peptides based on the 325-amino acid sequence of the alpha Ag of *Mycobacterium bovis* BCG. Because epitope-specific precursor cells are infrequent and randomly distributed, we used Poisson analysis to determine positive responses to 10 μg/ml of each peptide in 12 replicate culture wells. Seven immunodominant regions of the alpha Ag were identified. Each subject responded to at least one of the two most dominant epitopes, which correspond to amino acids 131-155 and 233-257 (from N terminus). Peptides of these two epitopes induced production of IFN-gamma by sorted CD4+ T cells. The immunodominant peptides may have use as components of a vaccine and as tools to study the evolution of the immune response to *M. tuberculosis.* The two most dominant epitopes both occur in regions of the alpha Ag that differ from those of the atypical pathogens *M. avium* and *M. kansasii.* In addition, the *M. bovis* epitope of amino acids 133-155 differs from that of *M. tuberculosis* by a single amino acid. It may be possible to exploit the sequence differences for development of diagnostic tests with increased specificity.—Authors' Abstract

Struillou, L., Cohen, Y., Lounis, N., Bertrand, G., Grosset, J., Vilde, J.-L., Pocidalo, J.-J. and Perronne, C. Activities of roxithromycin against *Mycobacterium avium* infections in human macrophages and C57BL/6 mice. Antimicrob. Agents Chemother. **39** (1995) 878–881.

The activity of roxithromycin against three clinical isolates of *Mycobacterium avium* was compared with that of clarithromycin both in a model of infection of human monocyte-derived macrophages and in a model of established infection of C57BL/6 mice. In the cell culture model, roxithromycin and clarithromycin were bactericidal for strains MO-1 and N-92159 and bacteriostatic for strain N-93043. For the three strains, the differences between the intracellular activities of roxithromycin and clarithromycin were not significant after 7 days of treatment. Mice were infected with the MO-1 strain. Drugs were given by gavage at a dosage of 200 mg/kg of body weight 6 days per week for 16 weeks starting 5 weeks after infection. At the end of treatment, clarithromycin was more effective than roxithromycin in lungs; roxithromycin was as effective as clarithromycin in spleens. Thus, the activity of roxithromycin was comparable to that of clarithromycin both *in vitro* and *in vivo.*—Authors' Abstract

Von Reyn, C. F., Jacobs, N. J., Arbeit, R. D., Maslow, J. N. and Niemczyk, S. Polyclonal *Mycobacterium avium* infections in patients with AIDS: variations in antimicrobial susceptibilities of different strains of *M. avium* isolated from the same patient. J. Clin. Microbiol. **33** (1995) 1008–1010.

Broth microdilution MICs were determined for pairs of strains isolated from five AIDS patients with polyclonal *Mycobacterium avium* infection. Four (80%) of the five patients were infected simultaneously with strains having different antimicrobial susceptibility patterns. These findings have implications for the interpretation of susceptibility data in *M. avium* prophylaxis and treatment trials.—Authors' Abstract

Wilson, T. M., de Lisle, G. W. and Collins, D. M. Effect of *inhA* and *katG* on isoniazid resistance and virulence of *Mycobacterium bovis.* Mol. Microbiol. **15** (1995) 1009–1015.

Isoniazid (INH) resistance of the *Mycobacterium tuberculosis* complex (MtbC) is associated with both loss of catalase activity and mutation of the *inhA* gene. However, the relative contributions of these changes

to resistance and to the loss of virulence for guinea pigs is unknown. In this study, a virulent strain of *Mycobacterium bovis,* a member of the MtbC, was exposed to increasing concentrations of INH. Two INH-resistant strains were produced which had lost catalase activity. Strain WAg405, which had a higher resistance to INH, also had a mutation in the *inhA* gene. This demonstrated that loss of catalase activity and mutation of *inhA* had a cumulative effect on INH resistance. When a functional *katG* gene was integrated into the genome of WAg405 the INH resistance was greatly reduced. This indicated that most of the resistance had been caused by loss of catalase activity. While the parent INH-sensitive strain was virulent for guinea pigs, the INH-resistant strains were significantly less virulent. Integration of a functional *katG* gene into the most resistant strain restored full virulence. This clearly established that *katG* is a virulence factor for *M. bovis* and that mutation of the *inhA* gene has no effect on virulence.—Authors' Summary