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CURRENT LITERATURE

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

General and Historical

Spanier, J. M. [The Netherlands Foundation for Leprosy Control: medical work under adverse conditions.] Nederlands Tijdschrift Geneeskunde **135** (1991) 2183–2185.

The work of the Dutch Institute for Prevention of Leprosy (Nederlandse Stichting voor Leprabestrijding, NSL) in implementing the 1982 WHO recommendation of multiple drug therapy is outlined. Over the next few years, the NSL intends to combat leprosy and tuberculosis concomitantly (on a worldwide basis, tuberculosis is more frequent). In Africa a relationship has been shown between increasing frequencies of tuberculosis and HIV seropositivity. Africa remains a problem in terms of leprosy, but other areas of the world are also seriously affected: half of all registered patients are in India. The author claims that the NSL, a publicly-funded body, has the approval of the Dutch who contributed about £4 million in 1990.—W. Houston (Trop. Dis. Bull.)

Chemotherapy

Almeida, J. G. *Leprosy: Short-term Treatment—A Quantitative Scientific Basis for Anti-microbial Chemotherapy in Control Programmes.* Kettering, U.K.: Kinaird Print, 1990, 106 pp.

This publication by Plateau, UK (a "non-profit imprint, publishing refereed medical monographs") is sub-titled "A quantitative scientific basis for anti-microbial chemotherapy in control programmes" and is essentially a presentation in book form of the author's analyses, as presented to the University of London for the degree of Doctor of Philosophy in 1991, supplemented by his publications in the medical and scientific press from 1983 onward. The author thoughtfully includes both a scientific abstract and a layperson's summary (translated into French, German and Italian), explaining the main content and conclusions of his work on the chemotherapy of leprosy during the past few years, namely: 1) dapsone monotherapy is adequate for all but a small and identifiable minority of patients; 2) dapsone may be given as "short-term" treatment (e.g., for 3 years); 3) the introduction of multiple drug therapy instead of short-term dapsone therapy would waste the bulk of resources devoted to leprosy; and 4) self-healing patients, whose risk of disability might well be increased by drugs that inhibit *Mycobacterium leprae,* are common and probably identifiable. The chapter headings of the main body of the text (nearly 100 pages) include: introduction; dapsone monotherapy: quantitative analysis of bacteriological outcome; dapsone resistance: magnitude of the problem; the principle of antimicrobial imbalance in multiple drug treatment; the minimum duration of drugs theoretically required for bacteriological cure with MDT; prognosis of tuberculoid infections with treatment that excludes antimicrobial chemotherapy; the recurrent variable cost of antimicrobial chemotherapy against *M. leprae* in India; conclusions and recommendations. There is an extremely comprehensive reference list, followed by appendices describing the mathematical basis for the quantitative techniques used in the text. There are a considerable number of addenda and errata in the copy received and many tables and figures have been past-

ed over, either by way of revision, or perhaps because of the receipt of late data. In a few instances, for example, under "Measurement of bacterial proportion viable" on page 9, this process has interfered with the original continuity of the text.

[Dr. Almeida's observations in this important field of leprosy are always interesting, and it is valuable to have them collected together in a single publication of this kind. Apart from published articles, his numerous attacks on the scientific basis for the development and widespread implementation of MDT, as advised by WHO in 1982, have from time to time appeared in the correspondence columns of leprosy journals, followed by authoritative rebuttals of his views (see: "Dapsone susceptibility of *M. leprae* before and after 1977," *International Journal of Leprosy,* 1987, 55, 726, with reply in the same journal, 1988, 56, 326, and "Comment: recent advances in the antimicrobial chemotherapy of leprosy," *Leprosy Review,* 1992, 63, 83, with reply in the same issue). Nevertheless, his assertions are invariably stimulating and provocative and many remain as disconcerting today as they were when he first made them in the early 1980s. Whether, at this stage, they will have any influence on established policies with regard to MDT is doubtful. But one thing has to be acknowledged, and that is the extent to which his carefully thought-out conclusions on this subject have helped to prevent us falling too easily into the trap of unthinking acceptance of all facets of "perceived wisdom."]—A. C. McDougall (Trop. Dis. Bull.)

Bozeman, P. M., Learn, D. B. and Thomas, E. L. Inhibition of the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase by dapsone. Biochem. Pharmacol. **44** (1992) 553–563.

Dapsone (4, 4′-diaminodiphenylsulfone) is an antimicrobial substance that also has antiinflammatory activity, which has been attributed to inhibition of the leukocyte enzyme myeloperoxidase (MPO). We observed that dapsone was a much better inhibitor of the eosinophil peroxidase (EPO) in an assay that measured peroxidase-catalyzed oxidation of tetramethylbenzidine at pH 5.4. To clarify the specificity and pH dependence of dapsone inhibition of the purified enzymes under more physiologic conditions, we studied peroxidase-catalyzed oxidation of chloride to the antimicrobial and cytotoxic agent hypochlorous acid. Taurine was added as a trap for hypochlorous acid, to prevent inactivation of the enzymes or chlorination of dapsone by hypochlorous acid. Dapsone was much more effective as an inhibitor of both MPO and EPO when chloride rather than tetramethylbenzidine was the substrate. Inhibition of both enzymes was greater at neutral pH than at acid pH (pH 7 vs pH 5), but EPO was more sensitive to inhibition than MPO regardless of the pH. Inhibition was increased by lowering chloride, raising hydrogen peroxide, or lowering the enzyme concentration. Inhibition was accompanied by irreversible loss of enzyme activity, which was correlated with loss of the heme absorption spectrum, indicating chemical modification of the enzyme active site. EPO, but not MPO, was partially protected against inactivation by adding physiologic levels of bromide along with chloride. The results suggest that dapsone could prevent MPO- and EPO-mediated tissue injury at sites where the peroxidase enzymes are secreted and diluted into the neutral pH environment of the tissue interstitial space. Dapsone might not inhibit peroxidase-mediated antimicrobial activity, which occurs at high enzyme concentrations in the acid environment of phagolysosomes.—Authors' Abstract

Coleman, M. D., Rhodes, L. E., Scott, A. K., Verbov, J. L., Friedmann, P. S., Breckenridge, A. M. and Park, B. K. The use of cimetidine to reduce dapsone-dependent methaemoglobinaemia in dermatitis herpetiformis patients. Br. J. Clin. Pharmacol. **34** (1992) 244–249.

We have attempted to reduce dapsone-dependent methemoglobinemia formation in six dermatitis herpetiformis patients stabilized on dapsone by the co-administration of cimetidine. In comparison with control, i.e., dapsone alone, methemoglobinemia due to dapsone fell by 27.3 ± 6.7% and 26.6 ± 5.6% the first and second weeks after commencement of cimetidine administration. The normally cyanotic appearance of the

patient on the highest dose of dapsone (35 mg/d^{-1}) underwent marked improvement. There was a significant increase in the trough plasma concentration of dapsone (2.8 ± 0.8 × 10^{-5}% dose ml^{-1}) at day 21 in the presence of cimetidine compared with control (day 7 = 1.9 ± 0.6 × 10^{-5}% dose ml^{-1}, $p < 0.01$). During the period of the study, dapsone-mediated control of the dermatitis herpetiformis in all six patients was unchanged. Trough plasma concentrations of monoacetyl dapsone were significantly increased ($p < 0.05$) at day 21 (1.9 ± 1.0 × 10^{-5}% dose ml^{-1}) compared with day 7 (1.6 ± 0.9 × 10^{-5}% dose ml^{-1}: control). Over a 12-hr period, 20.6 ± 8.9% (day 0) of a dose of dapsone was detectable in urine as dapsone hydroxylamine. Significantly less dapsone hydroxylamine was recovered from urine at day 14 (15.0 ± 8.4) in the presence of cimetidine, compared with day 0 (control: $p < 0.05$). The co-administration of cimetidine may be of value in increasing patient tolerance to dapsone, a widely used, effective, but comparatively toxic drug.—Authors' Abstract

Freerksen, E. and Seydel, J. K. Critical comments on the treatment of leprosy and other mycobacterial infections with clofazimine. Arznemittel Forschung **42** (1992) 1243–1245.

The usefulness of clofazimine (CLO, CAS 2030-63-9) in the treatment of mycobacterial infections with special emphasis on treatment of leprosy is critically discussed. Skin discoloration which decreases compliance, placenta passage, excretion in mother's milk which endanger the embryo or baby, respectively, saturation kinetics in absorption and difficulties to determine free drug concentration are severe problems. The observed antagonism in the combination of CLO with other drugs, especially with dapsone, is another argument against its application in the therapy of mycobacterial infections. In Germany, CLO has not been approved by the Bundesgesundheitsamt.—Authors' Abstract

Gelber, R. H., Iranmanesh, A., Murray, L., Siu, P. and Tsang, M. Activities of various quinolone antibiotics against *Mycobacterium leprae* in infected mice. Antimicrob. Agents Chemother. **36** (1992) 2544–2547.

Previously, pefloxacin and ofloxacin were found to be active against *Mycobacterium leprae in vitro,* in experimental animals, and in clinical trials of lepromatous leprosy patients. In this study, we compared certain more recently developed fluoroquinolones (lomefloxacin, PD 124816, WIN 57273, temafloxacin, and sparfloxacin) with pefloxacin and ofloxacin in *M. leprae*-infected mice at doses of 50, 150, and 300 mg/kg given five times weekly. All seven of the fluoroquinolones studied were active against *M. leprae*; temafloxacin and sparfloxacin were the most active, being fully bactericidal at all three dosage schedules. Additionally, sparfloxacin was found to be fully bactericidal at 15 and 30 mg/kg given five times weekly.—Authors' Abstract

Hebert, M. F., Roberts, J. P., Prueksaritanont, T. and Benet, L. Z. Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. Clin. Pharmacol. Thera. **52** (1992) 453–457.

The pharmakinetics of cyclosporine was studied in six healthy volunteers after administration of the drug orally (10 mg/kg) and intravenously (3 mg/kg) with and without concomitant rifampin administration. Both blood and plasma (separated at 37°C) samples were analyzed for cyclosporine concentration. For blood and plasma, respectively, clearances of cyclosporine were calculated to be 0.30 and 0.55 L/hr/kg, values for volume of distribution at steady state were 1.31 and 1.68 L/kg, and bioavailabilities were 27% and 33% during the pre-rifampin phase. Post-rifampin phase clearances of cyclosporine were 0.42 and 0.79 L/hr/kg, values for volume of distribution at steady state were 1.36 and 1.35 L/kg, and bioavailabilities were 10% and 9% for blood and plasma, respectively. Rifampin not only induces the hepatic metabolism of cyclosporine but also decreases its bioavailability to a greater extent than would be predicted by the increased metabolism. The decreased bioavailability most probably can be explained by an induction of intestinal cytochrome P450 enzymes, which appears to be

markedly greater than the induction of hepatic metabolism.—Authors' Abstract

Kashyap, A., Saha, K. and Sehgal, V. N. Reconstruction of anti-leprosy drug depleted complement haemolytic activity by addition of zymosan-treated sera (a source of C142) and C(rat)EDTA (a source of C3-C9). Int. J. Immunopharmacol. **14** (1992) 1409–1414.

This paper describes the mechanism of *in vitro* interaction of human serum complement system with antileprosy drugs (dapsone and clofazimine) and antilepra reaction drugs such as chloroquine. These drugs could inhibit the complement-mediated lysis of erythrocytes both via direct and alternative pathways, but only at hypertherapeutic doses. Attempts were made to restore the drug-depleted complement-mediated lysis of erythrocytes by adding zymosan-treated guinea-pig sera (a source of C142) and also by adding C(rat)EDTA sera (a source of C3-C9). Destroyed complement-mediated hemolytic activity by dapsone could be restored by early complement (C142) components, while complement-mediated hemolytic activity blocked by clofazimine could be regenerated by adding both late (C3-C9) and early (C142) complement component. However, chloroquine-mediated inhibition of the complement-mediated hemolysis activity could not be appreciably restored by adding both early and late complement reagents.—Authors' Abstract

Katoch, K., Natarajan, M., Bhatia, A. S. and Yadav, V. S. Treatment of paucibacillary leprosy with a regimen containing rifampicin, dapsone and prothionamide. Indian J. Lepr. **64** (1992) 303–312.

Ninety paucibacillary leprosy patients having indeterminate (I), tuberculoid (TT) and borderline tuberculoid (BT) type of leprosy with bacterial index (BI) of less than 2 on the Ridley scale were treated with rifampin (RFM) 600 mg once a month, dapsone (DDS) 100 mg daily and prothionamide (PTH) 250 mg daily. Treatment was stopped at the end of 6 months. The patients tolerated the drugs fairly well and in only two patients the drugs had to be stopped (in one due to jaundice and in the other due to gastric intolerance). About 6% of patients had early reactions which subsided with additional steroid therapy. The inactivity rate was 60% at 6 months, and this improved to 96% at 12 months. No cases of late reactions and relapses were encountered in the limited follow-up period of 6 months; a longer follow-up is necessary for ascertaining the relapse rates. The preliminary results, however, suggest that the addition of prothionamide to the standard WHO paucibacillary regimen is well tolerated with increased inactivity rate and fewer instances of late reactions.—Authors' Abstract

Kolars, J. C., Schmiedlin-Ren, P., Schuetz, J. D., Fang, C. and Watkins, P. B. Identification of rifampin-inducible P450-IIIA4 (CYP3A4) in human small bowel enterocytes. J. Clin. Invest. **90** (1992) 1871–1878.

Enzymes within the P450IIIA (CYP3A) subfamily appear to account for significant "first pass" metabolism of some drugs in the intestine. To identify which of the known P450IIIA genes are expressed in intestine, enterocyte RNA was hybridized on Northern blots with synthetic oligonucleotides complementary to hypervariable regions of hepatic P450IIIA4, P450IIIA5, and P450IIIA7 cDNAs. Hybridization was detected only with the P450IIIA4-specific oligonucleotide. The identity of the hybridizing mRNA was confirmed to be P450IIIA4 by direct sequencing of a DNA fragment amplified from enterocyte cDNA by the polymerase chain reaction. To determine if enterocyte P450IIIA4 is inducible, biopsies of small bowel mucosa were obtained from five volunteers before and after they received 7 days of treatment with rifampin, a known inducer of P450IIIA4 in liver. Rifampin treatment resulted in a five- or eight-fold mean increase ($p < 0.05$) in the biopsy concentration of P450IIIA4 mRNA when normalized for content of sucrase isomaltase or intestinal fatty acid binding protein mRNAs, respectively. Rifampin also induced P450IIIA immunoreactive protein in enterocytes in each of the subjects, as judged by immunohistochemistry, and resulted in a 10-fold increase in P450IIIA4-specific catalytic activity (erythromycin *N*-demethylation) in the one patient studied. Our

identification of inducible P450IIIA4 in enterocytes may in part account for drug interactions characteristic of P450IIIA4 substrates and suggests a strategy for controlling entry into the body of a major class of xenobiotics.—Authors' Abstract

Rastogi, N. and Goh, K. S. Effect of pH on radiometric MICs of clarithromycin against 18 species of mycobacteria. Antimicrob. Agents Chemother. **36** (1992) 2841–2842.

The antimycobacterial spectrum of clarithromycin, a new macrolide drug, was screened against 26 strains belonging to 18 species of mycobacteria by determining radiometric MICs by BACTEC methodology at three different pHs, i.e., pH 6.0, 6.8, and 7.4. The MICs obtained at pH 7.4 were 1 to 2 or more dilutions lower in some of the species than those obtained at pH 6.8; whereas they were significantly higher at pH 6.0. The numbers of species for which MICs were below the C(max) levels of clarithromycin (i.e., 4 μg/ml) at different pHs were 8 of 18 species at pH 6.0, 13 of 18 species at pH 6.8, and 14 of 18 species at pH 7.4. The drug possessed promising activity against such potential pathogens as *Mycobacterium avium, M. scrofulaceum, M. szulgai, M. malmoense, M. xenopi, M. marinum,* and *M. kansasii* and rare pathogens like *M. chelonei.*—Authors' Abstract

Rastogi, N., Goh, K. S. and Labrousse, V. Activity of clarithromycin compared with those of other drugs against *Mycobacterium paratuberculosis* and further enhancement of its extracellular and intracellular activities by ethambutol. Antimicrob. Agents Chemother. **36** (1992) 2843–2846.

Radiometric MICs of clarithromycin, a new macrolide drug, were determined against five mycobactin-dependent strains of *Mycobacterium paratuberculosis* (including two Crohn's disease clinical isolates) and compared with those of other drugs which included rifampin, ethambutol, amikacin, ofloxacin, ciprofloxacin, and sparfloxacin. Among the drugs screened, clarithromycin was the drug for which MICs were lowest against the five strains tested. Since MICs were significantly below the reported C(max) levels (about 4 μg/ml), the intracellular activity of clarithromycin against the type strain of *M. paratuberculosis* maintained in cultured macrophages was screened. Clarithromycin was able to kill the initial inoculum by more than 1 log within 7 days, and this activity was further potentiated by ethambutol. Extracellular drug combination screened by using sublethal concentrations of the drugs showed that ethambutol was able to enhance clarithromycin activity in three out of four *M. paratuberculosis* strains instead of only one out of four strains (or none in the case of ofloxacin) when associated with other drugs. These results suggest that clarithromycin may be fruitful to treat human disease in which *M. paratuberculosis* may be etiologically involved.—Authors' Abstract

van Brakel, W. H. Multi-drug therapy, leprosy and leprosy control in west Nepal. J. Nepal Med. Assoc. **29** (1991) 1–9.

This is an excellent account of the implementation of multiple drug therapy (MDT, as advised by WHO in 1982), in the Western and Mid-Western Regions of Nepal, starting in 1982, where 8000 patients have already completed their regimens. By 1989/1990, 6101 were still on treatment, of whom 1628 were taking dapsone monotherapy. In general, the results have been excellent, with significant reduction in prevalence rates, good compliance and few relapses. Attention is however drawn to problems that have arisen with the change from a vertical to an integrated approach, using the basic health services, due to inadequate planning, staff training and supervision.—A. C. McDougall (Trop. Dis. Bull.)

Van Rensburg, C. E. J., Joone, G. K., O'Sullivan, J. F. and Anderson, R. Antimicrobial activities of clofazimine and B669 are mediated by lysophospholipids. Antimicrob. Agents Chemother. **36** (1992) 2729–2735.

The susceptibilities of a range of gram-positive and gram-negative microbial

pathogens to clofazimine and its analog B669 (0.1 to 32 μg/ml), as well as the effects of these agents on membrane phospholipid metabolism in *Staphylococcus aureus* and *Escherichia coli*, have been investigated *in vitro*. Gram-positive bacteria were found to be generally susceptible to these agents; whereas gram-negative organisms were uniformly resistant. Exposure of *S. aureus* to both agents (1 to 5 μg/ml), especially B669, caused dose-related enhancement of the activity of phospholipase A2, according to an increase in the release of H-3-radiolabeled arachidonate and lysophosphatidylethanolamine ([H-3]LPE) from bacterial-membrane phospholipids. Treatment of *E. coli* with the riminophenazines also increased the release of [H-3]arachidonate and [H-3]LPE. Growth of gram-positive but not gram-negative bacteria was inhibited by LPE and lysophosphatidylcholine. Moreover, coincubation with alpha-tocopherol (vitamin E), a lysophospholipid complex-forming agent, or with lysophospholipase protected gram-positive bacteria against the riminophenazines as well as against lysophospholipids. The results from this study are consistent with a mechanism whereby lysophospholipids mediate the activities of the two drugs.—Authors' Abstract

Clinical Sciences

Ghosh, S. and Biswas, A. Digital blood circulation in paucibacillary leprosy. Indian J. Dermatol. Venereol. Leprol. **58** (1992) 164–168.

Digital vascular flow in paucibacillary leprosy was assessed by photoplethysmography (PPG). In PPG, infra-red light emitting diode and an adjacent photosensor detect the blood flow in cutaneous capillaries, as represented graphically on a strip-chart. In 29 (6F, 23M) untreated paucibacillary leprosy patients (7 TT, 16 BT and 6 purely neural type) having ailment on limb/limbs, suffering for 5 months to 12 years, PPG recordings were done by applying the device to the distal phalanges of all the digits of four limbs serially with velcro-strap at an ambient temperature of 28°C–30°C and humidity of 60–70%. Diminished digital vascular flow of the affected limb/limbs was seen in long-standing cases suffering for > 2 years irrespective of the type, site, morphology or phase (reactional) of the disease. This may be mostly due to specific vascular changes rather than reactional or functional changes. Specific vascular changes are terminal arteritis, vasculitis of vasa nervorum or truncular arteritis, i.e., tuberculoid lesions of the major arteries. Two patients, suffering for 1.5 years, paradoxically showed increased digital blood flow which is probably due to reactive hyperemia or paralytic vasodilation as a result of autosympathectomy.—Authors' Abstract

Hussein, N., Chiang, T., Ehsan, Q. and Hussain, R. Intraocular pressure decrease in household contacts of patients with Hansen's disease and endemic control subjects. Am. J. Ophthalmol. **114** (1992) 479–483.

We compared the intraocular pressure in 150 urban household contacts of patients with Hansen's disease and 132 endemic control subjects from an urban population in Karachi, Pakistan, who were matched in regard to race, age, gender, and socioeconomic status. The mean intraocular pressure in the upright position was 12.6 mm Hg in the right eye and 12.9 mm Hg in the left eye in household contacts of patients with Hansen's disease and 15.3 mm Hg in the right eye and 15.4 mm Hg in the left eye in the endemic control population ($p < 0.0005$). The postural change in intraocular pressure from the upright to supine position was 1.7 mm Hg in the right eye and 2.1 mm Hg in the left eye in the household contacts group and 1.3 mm Hg in the right eye and 1.5 mm Hg in the left eye in the endemic control group ($p < 0.0006$). Our findings, especially in view of past observations in intraocular pressure changes in patients with Hansen's disease, raise serious questions

about possible early ocular susceptibility to *Mycobacterium leprae* infection and about public health issues, including possible early indication of disease.—Authors' Abstract

Jennekens, F. G. I. and Jennekens-Schinkel, A. Neurological examination of patients suffering from leprosy: is it worthwhile? Lepr. Rev. **63** (1992) 269–276.

We examined 28 male leprosy patients to discover if a more extensive neurological investigation than usual would be worthwhile in diagnosis and/or management. Our findings were fully compatible with what might be expected from a mononeuritis multiplex, either due to leprosy or other causes. The following observations are noteworthy. Changes of position sense and a decrease of some tendon reflexes were present in a minority of the patients. In soles of the feet, considered to be anesthetic or hypesthetic, some residual pain sensation could occasionally be detected. Functional testing of at least one muscle group (m. triceps surae) appeared to be more reliable than manual testing according to MRC criteria. We concluded that an extensive neurological examination is probably not required for diagnosis. It does provide, however, more accurate information on the extent of damage to the peripheral nervous system, which may be important for management and for assessment of treatment effects. The use of a myometer is advocated.—Authors' Summary

Kubeyinje, E. P. and Onunu, A. N. Leprosy among university and high school students in Benin City, Nigeria: an eight year review. Trop. Geogr. Med. **44** (1992) 537–547.

Thirty-six cases of leprosy were seen among university and high school students in the Skin Clinic of the University of Benin Teaching Hospital during an 8 year period (1982–1989). The male-to-female ratio was 2 to 1. Borderline tuberculoid and tuberculoid leprosy were the commonest forms of presentation, seen in 66.6% of all cases. Borderline lepromatous and lepromatous leprosy were seen in 22%, while indeterminate in 11% of cases. There has been a gradual reduction of new cases from a peak of about 8 cases in 1983 to 1 in 1989. Most patients complied with treatment [with dapsone, rifampin and clofazimine] and were able to continue their education with little or no hindrance.—AS (Trop. Dis. Bull.)

Lapinsky, S. E., Baynes, R. D., Schulz, E. J., MacPhail, A. P., Mendelow, B., Lewis, D. and Bothwell, T. H. Anaemia, iron-related measurements and erythropoietin levels in untreated patients with active leprosy. J. Int. Med. **232** (1992) 273–278.

The mechanisms responsible for anemia in leprosy were studied prior to the institution of therapy in 56 patients with active disease. Hematological indices, iron-related measurements, inflammatory markers and erythropoietin levels were assessed, with bone-marrow studies being performed on anemic patients. Anemia was more common in the patients with lepromatous leprosy (85.7%) than it was in the rest of the group (19%). The lepromatous group exhibited the disordered iron transport of the anemia of chronic disorders in that they had a significantly lower mean serum iron level ($p < 0.05$), and a mildly raised serum ferritin concentration. Anemic lepromatous patients also showed a blunted erythropoietin response compared with controls with noninflammatory anemia. A subgroup of five anemic subjects displayed apparently adequate transport of iron to the erythroid marrow (normal percentage transferrin saturations and appropriate sideroblast counts) and the blunted erythropoietin response appeared to be the dominant factor in the pathogenesis of their anemia. Analysis of inflammatory markers revealed that while the erythrocyte sedimentation rate was very high in the lepromatous subjects, there was no concomitant rise in C-reactive protein concentration. This suggests the presence of a disordered, cytokine-mediated, acute phase response in the condition.—Authors' Abstract

Myint, T., Thet, A. T., Htoon, M. T. and Win, M. A comparative KAP study of leprosy patients and members of the community in Hlaing and Laung-Lon Townships [Myanmar (Burma)]. Indian J. Lepr. **64** (1992) 313–324.

A knowledge, attitudes and practices (KAP) study was conducted in the peri-ur-

ban Hlaing and rural Laung-Lon Townships in Myanmar. It was found that both the leprosy patients as well as community members were still not sure about the cause of leprosy. Social stigma of leprosy encountered by patients needs to be addressed especially in peri-urban areas. It was also found that the patient's understanding of treatment regularity was still very unsatisfactory, for which health education measures need to be introduced.—Authors' Abstract

Namafoyane, N. A., Jacyk, W. K. and Lotz, B. P. Primary neuritic leprosy in a black South African. Lepr. Rev. **63** (1992) 277–281.

A case of primary neuritic leprosy in a black South African is described in which the multiple peripheral nerves were affected. The clinical picture and electrophysiological studies are in keeping with a picture of mononeuritis multiplex. Selective involvement of the facial nerve branches with normal blink reflex latencies was observed. The biopsy of the sural nerve disclosed features most consistent with borderline leprosy.—Authors' Summary

Rosa, H., Costa, A. P. V. F., Ferraz, M. L., Pedroza, S. C., Andrade, A. L. S. S., Martelli, C. M. T. and Zicker, F. Association between leprosy and hepatitis-B infection—a survey in Goiania, Central Brazil. Rev. Inst. Med. Trop. São Paulo **34** (1992) 421–426.

This investigation presents the results of hepatitis-B virus (HBV) screening among leprosy patients conducted in central Brazil as a preliminary information for a HBV vaccination program. The main objectives were to assess the seroprevalence of HBV serum markers among lepromatous patients and to analyze institutionalization as a risk factor for HBV infection in this population. Two groups of lepromatous patients were studied, 83 outpatients and 171 institutionalized ones. Screening for HBV serum markers included the detection of HBsAg, anti-HBV by radioimmune assay (RIA). The prevalence of carrier state (HBsAg) was 4.8% and 8.8% among outpatients and institutionalized, respectively ($p > 0.05$). Seroprevalence of exposure (all markers) was statistically significantly different between outpatients (16.9%) and institutionalized ones (50.3%). Institutionalized patients had an almost fourfold risk of HBV infection when compared to the outpatients, and the highest risks were among patients with more than 21 years of residence in the colony, after adjusting for age and sex.—Authors' Abstract

Soni, N. K. Leprosy of the tongue. Indian J. Lepr. **64** (1992) 325–330.

Ten out of the 25 lepromatous leprosy patients studied showed clinical evidence of involvement of the tongue, and they presented with various symptoms such as loss of taste, stiffness of tongue, bleeding, pain, etc. Various types of lesions ranging from small nodules to granuloma formation, ulceration, macroglossia and fissured cracked tongue were noted. The tongue lesions were found to be related to the severity of leprosy.—Authors' Abstract

Talwar, S., Jha, P. K. and Tiwari, V. D. Neuritic leprosy: epidemiology and therapeutic responsiveness. Lepr. Rev. **63** (1992) 263–268.

We studied epidemiology, progression and therapeutic responsiveness in 62 cases of neuritic leprosy. Numbness was the main presenting symptom. Mononeuritis involving the ulnar nerve, followed by the common peroneal nerve was the commonest presentation. The lepromin test was positive in 34 cases while a slit-skin smear was negative in all cases. We treated 20 of these cases with dapsone monotherapy and 5 cases (25%) developed a skin lesion after an average duration of 3 months' treatment. We treated 42 cases with a combination of dapsone and rifampin, and 3 cases (7%) developed a skin lesion after an average duration of 2–6 months. The subsequent diagnosis in cases developing skin lesions was borderline-lepromatous in 1 case, borderline-tuberculoid in 4 cases, tuberculoid in 2 cases and indeterminate in 1 case.—Authors' Summary

Tzourio, C., Said, G. and Millan, J. Asymptomatic nerve hypertrophy in lepromatous leprosy—a clinical, electrophysiological and morphological study. J. Neurol. **239** (1992) 367–374.

In order to learn more about early nerve lesions observed in leprosy, we performed a clinical, electrophysiological and morphological study in seven patients with untreated lepromatous leprosy, palpably enlarged radial cutaneous nerve and preserved sensation in the corresponding territory. The conduction velocity of the cutaneous radial nerve, which was decreased in all patients, did not significantly differ from that of a group of patients with lepromatous leprosy, hypertrophy of the radial cutaneous nerve and sensory loss. In contrast, the sensory action potential was significantly lower in patients with sensory loss, which demonstrates that axon loss is more important than demyelination in producing sensory loss. In all patients nerve enlargement was due to thickening of the epineurium and of the perineurium subsequent to inflammatory infiltrates and proliferation of fibroblasts and perineurial cells. In several fascicles, the inflammatory infiltrates and the infected cells infiltrated endoneurial connective tissue septa and blood vessels. *Mycobacterium leprae* were abundant in perineurial cells, fibroblasts, macrophages, Schwann cells and endothelial cells, and lymphocytic vasculitis present in all cases. The average density of myelinated fibers was 2600 with a SD of 880 fibers/mm^2 (control: 7700 fibers/mm^2), with marked differences between individual fascicles, versus 420 fibers/mm^2 in patients with nerve hypertrophy and sensory loss (range 0–2080 fibers/mm^2). Single fiber preparations showed that segmental demyelination predominated in two patients, axonal degeneration in one, while inflammatory infiltrates and proliferation of connective tissue adhering to individual fibers were prominent in the others. Both infection of Schwann cells and secretory products released by mononuclear cells involved in the inflammatory process are likely to play a role in the lesions of nerve fibers observed in early stages of lepromatous leprosy.—Authors' Abstract

Vaishnavi, C., Agnihotri, N., Ganguly, N. K., Kaur, S. and Kumar, B. Acute phase reactants in leprosy. Microbiol. Immunol. **35** (1991) 975–980.

The 74 untreated leprosy patients were examined at a leprosy clinic in Chandigarh, India. C-reactive protein was detected by latex agglutination in serum from 19 patients (25.6%): none of the 3 patients with polar tuberculoid leprosy was positive although positive results were obtained throughout the rest of the leprosy spectrum. As shown by ELISA, all groups of the leprosy patients had significantly raised levels of plasma fibronectin compared with a control group of 5 healthy volunteers.—C. A. Brown (Trop. Dis. Bull.)

van Brakel, W. H., de Soldenhoff, R. and McDougall, A. C. The allocation of leprosy patients into paucibacillary and multibacillary groups for multidrug therapy, taking into account the number of body areas affected by skin, or skin and nerve lesions. Lepr. Rev. **63** (1992) 231–246.

In Nepal, the setting up and maintaining of reliable services for slit-skin smears have proven difficult. A clinical classification system for leprosy has therefore been developed to assist in the allocation of patients to either paucibacillary or multibacillary groups for the purposes of multiple drug therapy (MDT), using 9 body areas: head (1), arms (2), legs (2), trunk (4). Patients with more than two areas of the body affected are grouped as multibacillary (MB) and those with only one or two areas affected are paucibacillary (PB). Using a computer simulation model and the data of 53 patients registered at Green Pastures Hospital (GPH) in Pokhara and 703 field patients from the Western Region, different clinical classification systems were evaluated with regard to their sensitivity, specificity, and predictive value for MB or PB classification, as compared with the histological classification for the GPH cases and the bacteriological classification for the field patients. The sensitivity and specificity of the body area system in present use were 93% and 39%, respectively. The low specificity is due to MB overclassification. The sensitivity of the WHO classification system without skin smear facilities is 73% (the difference with the body area system is significant: $p < 0.05$, McNemar's test). Our histology findings confirm previous publications indicating that, while some borderline-tuberculoid (BT) patients may outwardly have a "PB appearance" and be skin-smear negative,

their nerve biopsy and sometimes skin biopsy may show a "MB" picture. This is the first publication discussing a "body area system" for the purpose described, including diagrams of the areas used. In Nepal it has proved easy to use and teach and its use may be justified in other control programs which implement MDT, particularly if slit-skin smear services are unreliable or nonexistent.—Authors' Summary

Immuno-Pathology

Abbot, N. C., Beck, J. S., Carnochan, F. M. T., Lowe, J. G. and Gibbs, J. H. Circulatory adaptation to the increased metabolism in the skin at the site of the tuberculin reaction. Int. J. Microcircul. Clin. Exp. **11** (1992) 383–401.

The sequence of changes at the site of a positive tuberculin test response was studied in 19 healthy young adults who had been immunized with BCG in childhood. The development of erythema preceded that of induration and both were most intense at 48–72 hr. The strongest reactions showed higher laser Doppler (LD) flux at the periphery than at the center (central relative slowing). All showed a substantial reduction in steady-state (ss) tcpO2 from 24 hr onward and the oxygen consumption rate (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$), calculated from the rate of fall in tcpO2 during temporary cuff occlusion of arterial input, was raised (greater than twofold) throughout the period of study (to 96 hr). The density of lymphocytes and macrophages in the inflammatory infiltrate in the dermis was related to the fall in tcpO2·ss and to the extent of thickening of the dermis. These experiments showed that the previously healthy dermal microcirculation can adapt to temporary increase in metabolic demands of leukocytes emigrated from the circulation into the tissue: in intense delayed hypersensitivity (DHS) reactions there are considerable hypoxia and respiratory debt, but maintenance of viability in the short term. It is likely that similar adaptations occur in the period of establishment of microbial infection.—Authors' Abstract

Al-Attiyah, R., Moreno, C. and Rook, G. A. W. TNF-alpha-mediated tissue damage in mouse footpads primed with mycobacterial preparations. Res. Immunol. **143** (1992) 601–610.

Tissue sites involved in certain types of inflammation become sensitive to the destructive effect of a subsequent injection of tumor necrosis factor alpha (TNF-alpha). To try to further delineate the cascade of effector and regulatory events controlling this activity, a new model is described and its main properties characterized. C57BL/GrFa mice received mycobacterial products subcutaneously in the foot pads. Recombinant TNF-alpha was injected 24 hr later into the same sites. To assess the tissue-destructive effect of TNF-alpha in these "primed" foot pads, swelling and hemoglobin content of injected foot pads were measured 16 hr and 20 hr, respectively, after the injection of TNF-alpha. When loaded with either *Escherichia coli* LPS (10-μg) or *Mycobacterium vaccae* soluble sonicate (17-μg), foot pads were reactive to the subsequent injection of 1-μg recombinant TNF-alpha, as assessed by both swelling and hemoglobin content. When C57BL/GrFa mice received 10^9 autoclaved *M. vaccae* subcutaneously in the back 10 days before the foot pad was "primed" with soluble *M. vaccae* sonicate, the destructive effect of TNF-alpha was significantly enhanced, becoming 5–10-fold greater than that seen in sites "primed" with an optimal dose of LPS. This higher reactivity was abrogated by a single dose of anti-CD4 given just before the injection of TNF-alpha. This local reactivity to TNF-alpha of skin sites loaded with mycobacterial products is compared to the local LPS-dependent Schwartzman reaction, and the relevance of this assay as a model with which to delineate the mechanisms of tissue damage in tuberculosis is discussed.—Authors' Abstract

Bäckström, B. T., Harris, D. P., Prestidge, R. L. and Watson, J. D. Genetic control of immune responses to the 18-kDa protein of *Mycobacterium leprae*; different

T_H1 subsets may be involved in proliferative and delayed-type hypersensitivity responses. Cell. Immunol. **142** (1992) 264–274.

In vitro and *in vivo* responses to the 18-kDa protein of *Mycobacterium leprae* have been analyzed in different strains of mice. Lymphocytes from BALB/cJ (H-2^d), BALB.B (H-2^b), B10.BR (H-2^k), and B10.M (H-2^f) mice primed with 18-kDa protein yielded high T-cell proliferative responses, while those from C57BL/10J (H-2^b) mice yielded lower responses. Both H-2 and non-H-2 genes contributed to the magnitude of responsiveness. F_1 mice from high and low responder strains showed high responsiveness to the 18-kDa protein. Supernatants from lymph node cell cultures prepared from 18-kDa protein-immunized BALB/cJ, B10.BR, and C57BL/10J mice contained IL-2 but no IL-4, indicating that activated T cells from both high and low responder mice were of a T_H1 phenotype. Cell cultures from low responder C57BL/10J mice produced less IL-2 than those from high responders. The low responsiveness to the 18-kDa protein in proliferative assays might be due to a low frequency of antigen-specific T cells in the C57BL/10J mouse strain. BALB/cJ, C57BL/10J, and F_1 (BALB/cJ × B10.BR) mouse strains were tested for *in vivo* delayed-type hypersensitivity (DTH) reactions to the 18-kDa protein. All strains, including C57BL/10J, were high DTH responders. Although DTH effector cells and 18-kDa protein-specific proliferative T cells belong to the T_H1 subset, our data comparing high and low responder status indicate that distinct T_H1 subpopulations are stimulated in response to the 18-kDa protein of *M. leprae.*—Authors' Abstract

Bermudez, L. E., Wu, M., Petrofsky, M. and Young, L. S. Interleukin-6 antagonizes tumor necrosis factor-mediated mycobacteriostatic and mycobactericidal activities in macrophages. Infect. Immun. **60** (1992) 4245–4252.

Interleukin-6 (IL-6) is a cytokine produced by a number of cells, including macrophages, and is directly involved in the inflammatory response. The production of IL-6 can be stimulated by monokines such as IL-1 and tumor necrosis factor (TNF). *Mycobacterium avium* complex organisms frequently cause disseminated disease in patients with AIDS. *M. avium* is an intracellular bacterium that mainly infects macrophages. Treatment of *M. avium*-infected macrophage monolayers with recombinant IL-6 decreased the ability of TNF to activate cultured macrophages to inhibit growth of or kill intracellular *M. avium* (68% ± 14% decrease in intracellular killing compared with that in monolayers not treated with IL-6). To further evaluate whether this effect was dependent on the down regulation of membrane receptors to TNF, we examined ^{125}I-TNF binding to macrophages previously exposed to IL-6: the expression of TNF receptors was decreased by 78% ± 9%. The effect of IL-6 on TNF receptors was observed after 4 hr and was reversible. Infection of macrophages with different *M. avium* serovars was associated with release of IL-6, and IL-6 production peaked at 48 hr after infection in concentrations ranging from 328 ± 87 ng/10^5 cells to 907 ± 224 ng/10^5 cells. IL-6 did not have any influence on the rate of growth of the tested strains of *M. avium* within or outside macrophages. These results suggest that release of IL-6 by *M. avium*-infected macrophages may influence the host's immune response and the outcome of the disease.—Authors' Abstract

Bothamley, G., Beck, J. S., Britton, W., Elsaghier, A. and Ivanyi, J. Antibodies to *Mycobacterium tuberculosis*-specific epitopes in lepromatous leprosy. Clin. Exper. Immunol. **86** (1991) 426–432.

Sera from patients with leprosy or tuberculosis and healthy subjects have been analyzed for the presence of antibodies to species-specific mycobacterial epitopes, 4 different viruses and 5 autoantigens. Antibodies to the *Mycobacterium leprae*-specific 35-kDa protein and phenolic glycolipid-I epitopes were not present in patients with active pulmonary tuberculosis. In contrast, antibody levels to species-specific epitopes of the 38-kDa and 14-kDa antigens of *M. tuberculosis* were significantly elevated in patients with lepromatous leprosy. Neither of the two antigens is crossreactive with *M. leprae* at the B-cell level. However, it was considered that crossreactive helper T cells could recall the response of *M. tuberculosis*-

specific memory B cells, which had been primed through prior self-healing tuberculosis infection. As an alternative explanation, the possible role of polyclonal B cell stimulation was considered. This seemed unlikely, however, since: a) antibody levels to autoantigens, except anti-smooth muscle, were not elevated, and b) antibody levels to four distinct viruses, unlike those to all mycobacterial epitopes, showed no correlation with titers, to *M. tuberculosis*-specific epitopes.—H. Dockrell (Trop. Dis. Bull.)

Chatterjee, B. R. Leprosy immunopathogenesis and vaccine development. Indian J. Lepr. **64** (1992) 359–374.

The truly effective immunity against intracellular parasites, including mycobacteria, is mediated by monocytelmacrophages, and in the immunologically responding (resistant) host these phagocytes need minimal antigenic stimulus, specific or nonspecific, to become activated and be microbicidal. T-cell-mediated delayed hypersensitivity (DTH) causes tissue damage and destruction, which is particularly unwelcome in leprosy because of its nerve-damaging potential. Gamma interferon (INF-γ), the terminal lymphokine of a DTH response, promotes mycobacterial survival and growth. There are T cells (TH_1 subtypes) that produce DH response either independent of or only partly dependent on INF-γ; this type of DH peaking at 24 hr appears similar to the Jones-Mote type rather than to the tuberculin type of DTH peaking at 48–72 hr and is devoid of the necrotic component of tuberculin type of DTH. *Mycobacterium leprae* antigens normally elicit this Jones-Mote type of DH. Suppressor T cells are associated with a protective immune response, while helper T cells mediating DTH are harmful. In view of this immunobiology, it would appear that pathogenic mycobacteria that generate a tuberculin type DTH response should not be used as immunogens in leprosy.—Authors' Abstract

D'Souza, D., Thomas, I. M. and Das, B. C. Effect of inhibitor of poly (ADP-ribose) polymerase in blood lymphocyte cultures of untreated leprosy patients. Mut. Res. **284** (1992) 251–255.

Poly (ADP-ribose) polymerase is a cellular repair enzyme synthesized following damage to DNA. 3-Aminobenzamide (3-AB) is an inhibitor of this repair enzyme. To study repair efficiency in leprosy patients, who usually show a significantly higher frequency of spontaneous chromosome aberrations and sister-chromatid exchanges (SCEs), their blood lymphocyte cultures were treated with 3-AB. A marginal increase in the frequency of chromosome aberrations was observed following treatment with 3-AB in controls as well as in patient groups. There was also no significant difference in the frequency of SCEs in control cultures with or without 3-AB. A significant increase in the frequency of SCEs was observed in lymphocyte cultures of paucibacillary (PB) and multibacillary (MB) patients treated with 3-AB when compared with controls. Observation of a significant increase in the frequency of SCEs in 3-AB-treated cultures over the untreated value indicates that DNA damage caused in leprosy patients following mycobacterial infection is not repaired because of the presence of the inhibitor of repair enzyme.—Authors' Abstract

Douglas, J. T., Steven, L. M., Hirsch, D. S., Fujiwara, T., Nelson, K. E. Madarang, M. G. and Cellona, R. V. Evaluation of four semi-synthetic *Mycobacterium leprae* antigens with sera from healthy populations in endemic and non-endemic areas. Lepr. Rev. **63** (1992) 199–210.

In order to determine the frequency of occurrence of antibodies to semisynthetic antigens of *Mycobacterium leprae* in clinically healthy nonpatient populations and to establish a "baseline" for comparison with antibody frequencies in both patients with a history of leprosy and their contacts, ELISAs were conducted using representative sera from two areas: a leprosy-endemic area, Cebu City, Philippines, and a nonendemic area for leprosy, Chicago, Illinois, U.S.A. These sera were tested, by an indirect IgM ELISA, for the presence of antibodies reacting with four semisynthetic antigens based on the phenolic glycolipid-I antigen of *M. leprae*: ND-O-BSA (natural disaccharide with octyl linkage to bovine serum albumin), NT-O-BSA (natural trisaccharide

with octyl linkage to BSA), ND-P-BSA (natural disaccharide with phenolic ring linkage to BSA) and NT-P-BSA (natural trisaccharide with phenolic ring linkage to BSA). Using an OD reading ≥ 0.16 as positive, the antigen with the lowest background seroreactivity was ND-O-BSA,which reacted with 5/398 (1.3%) sera from Cebu, and 3/426 (0.7%) sera from Chicago. A total of 10 (2.5%) of 398 sera from the endemic area reacted with at least one antigen and 5 (1.3%) sera reacted with all four semisynthetic antigens. Of the 426 sera from Chicago, 12 (2.8%) were reactive with at least one antigen and 3 (0.7%) were reactive with all four semisynthetic antigens. Mean ELISA values for the 22 positive sera for each antigen ranged from 0.17 to 0.3 OD units, while the mean values for all sera in each area ranged from 0.01 to 0.04 OD units for all four antigens. Reactivity of 14 of the positive sera to some antigens, but not all four semisynthetic antigens, indicated that the carrier and linker arms might be associated with this background reactivity. Investigation of alternative linker arms and carriers is warranted. We conclude that nonspecific background reactivity to the semisynthetic antigens representing the PG-I molecule of *M. leprae* is 0.7%–1.3%, based on a ≥ 0.16 OD cutoff value. From these data it was concluded that reactivity in individuals free of leprosy was low enough to warrant use of these antigens in a diagnostic setting, such as screening household contacts and highly endemic populations. When incidence and prevalence of leprosy are low, testing with these antigens would not be cost effective, unless applied to high-risk individuals. Serological screening with these antigens might be useful in detecting and differentiating bacteriological relapse, types 1 or 2 reactions, early detection of leprosy, and monitoring treatment in endemic areas.—Authors' Summary

Filley, E. A., Bull, H. A., Dowd, P. M. and Rook, G. A. W. The effect of *Mycobacterium tuberculosis* on the susceptibility of human cells to the stimulatory and toxic effects of tumour necrosis factor. Immunology **77** (1992) 505–509.

It has previously been shown that the inherently tumor necrosis factor-alpha (TNF-alpha)-sensitive L929 murine fibroblast cell line becomes much more sensitive to the cytotoxic effect of this cytokine after exposure to *Mycobacterium tuberculosis* in culture. In this study it is now shown that normal human cells of types likely to be involved in tuberculosis lesions are affected in a similar way. Growth of normal human fibroblasts is usually stimulated by TNF-alpha *in vitro,* but after exposure to *M. tuberculosis* or to extracts of this organism, these cells are killed rather than stimulated by subsequent exposure to TNF-alpha. Similarly, human endothelial cells become susceptible to doses of TNF-alpha which do not normally affect viability. Moreover this enhancement of sensitivity to TNF-alpha is not confined to its toxicity. Endothelial cells and HeLa cells exposed to *M. tuberculosis* express increased levels of ICAM-1 after subsequent exposure to TNF-alpha, implying synergy between the two stimuli. It is suggested that these effects contribute to the ability of *M. tuberculosis* to distort the normal protective role of TNF-alpha so that the cytokine becomes detrimental to the host.—Authors' Abstract

Furney, S. K., Skinner, P. S., Roberts, A. D., Appelberg, R. and Orme, I. M. Capacity of *Mycobacterium avium* isolates to grow well or poorly in murine macrophages resides in their ability to induce secretion of tumor necrosis factor. Infect. Immun. **60** (1992) 4410–4413.

The results of this study show that clinical isolates of *Mycobacterium avium* fall into two categories in terms of their capacity to grow within murine bone-marrow-derived macrophage cultures: those that grow progressively and those that are incapable of growing within such cells. Members of the first category were invariably of the smooth-transparent colonial type, while most of the second were of the smooth-domed type. In addition, this paper shows that although all isolates induced tumor necrosis factor (TNF) secretion by host cells to some extent, this production was always delayed in isolates that subsequently grew well in the host cells. This observation, coupled with the demonstration that the growth of the latter isolates was inhibited by the exogenous addition of TNF, leads us to hypothesize that

the ability of a given isolate to somehow avoid host macrophage TNF production early during the course of the infection is a key factor in the pathogenesis of the disease.—Authors' Abstract

Ganju, L., Batra, H. V., Talwar, G. P. and Mukherjee, R. A rapid latex agglutination test for detection of antibodies in tuberculosis and Hansen's disease. J. Immunoassay **12** (1991) 579–595.

A sonicate of a rapidly growing mycobacterial organism (*Mycobacterium w*) with antigenic epitopes crossreactive with *M. leprae* and *M. tuberculosis* was coated onto latex beads. Positive agglutination reactions were observed with sera from 79% of 108 patients with lepromatous leprosy and from 86% of 84 patients with pulmonary tuberculosis. Apparently healthy control subjects from a nonendemic area yielded 4.3% positive results and from an endemic area 8.8%. The antigen-coated latex beads are stated to be stable for 6 months if kept at 4°C and provide a simple, rapid test which the authors claim can be used even in rural areas of developing countries.—B. W. Allen (Trop. Dis. Bull.)

Geluk, A., Van Meijgaarden, K. E., Janson, A. A. M., Drijfhout, J. W., Meloen, R. H., de Vries, R. R. P. and Ottenhoff, T. H. M. Functional analysis of DR17-(DR3)-restricted mycobacterial T cell epitopes reveals DR17-binding motif and enables the design of allele-specific competitor peptides. J. Immunol. **149** (1992) 2864–2871.

We have previously shown that p3-13 (KTIAYDEEARR) of the 65-kDa heat-shock protein (hsp65) of *Mycobacterium tuberculosis* and *M. leprae* is selected as an important T-cell epitope in HLA-DR17+ individuals, by selectively binding to (a pocket in) DR17 molecules, the major subset of the DR3 specificity. We have now further studied the interaction between p3-13, HLA-DR17 and four different TCR (Vβ5.1, Vβ1, and Vβ4) by using T-cell stimulation assays, direct peptide-DR binding assays, and a large panel (N = 240) of single amino acid substitution analogs of p3-13. We find that residues 5(I) and 8(D) of p3-13 are important DR17 binding residues; whereas the residues that interact with the TCR vary slightly for each DR17-restricted clone. By using N- and C-terminal truncated derivatives of p2-20 we defined the minimal peptide length for both HLA-DR17 binding and T-cell activation: the minimal peptide that bound to DR17 was seven amino acids long; whereas the minimal peptide that activated T-cell proliferation was eight amino acids in length. Furthermore, two new DR17-restricted epitopes were identified on hsp70 and hsp18 of *M. leprae.* Alignment of the critical DR17-binding residues 5(I) and 8(D) of p3-13 with these two novel epitopes and two other DR17-binding peptides revealed the presence of highly conserved amino acids at positions n and n + 3 with I, L, and V at position n and D and E at position n + 3. D and E are particularly likely to interact with the DR17-specific, positively charged pocket that we have defined earlier. Based on these results, a set of single amino acid substituted analogs that failed to activate these T-cell clones but still bound specifically to DR17 was defined and tested for their ability to inhibit T cell activation by p3-13 or other DR17-restricted epitopes. Those peptides were able to inhibit the response to p3-13 as well as other DR17-restricted mycobacterial epitopes in an allele-specific manner, and are anticipated to be of potential use for immunotherapeutic and vaccine design strategies.—Authors' Abstract

Kaufmann, S. H. E., Gulle, H., Daugelat, S. and Schoel, B. Tuberculosis and leprosy—attempts to identify T-cell antigens of potential value for vaccine design. Scand. J. Immunol. **36** (1992) 85–90.

Tuberculosis and leprosy are chronic bacterial infectious diseases which represent major health problems worldwide. It is generally accepted that, on the one hand, effective vaccination strategies are required for satisfactory control of these diseases and, on the other hand, that currently available vaccination measures are insufficient for this purpose. Ideally, a subunit vaccine should be designed which is composed of one or a few protective antigens. In this brief treatise our approach toward the identification of antigens with potential value for vaccine design is described. It comprises high-reso-

lution fractionation by two-dimensional gel electrophoresis, transfer of separated fractions by electroelution, and testing of separated fractions with viable T cells and accessory cells. Using this approach we find: a) multiple antigens are recognized by T cells from leprosy and tuberculosis patients as well as healthy contacts, b) apparently, suppressive antigens exist in leprosy, c) an antigen cluster which is apparently indicative for immunity against *Mycobacterium tuberculosis* is present among secreted proteins. We hope that further improvement of this methodology will help in the rational design of subunit vaccines against tuberculosis and leprosy.—Authors' Abstract

Khanolkar-Young, S., Kolk, A. H. J., Andersen, A. B., Bennedsen, J., Brennan, P. J., Rivoire, B., Kuijper, S., McAdam, K. P. W. J., Abe, C., Batra, H. V., Chaparas, S. D., Damiani, G., Singh, M. and Engers, H. D. Results of the 3rd Immunology of Leprosy/Immunology of Tuberculosis Antimycobacterial Monoclonal Antibody Workshop. Infect. Immun. **60** (1992) 3925–3927.

An international workshop was sponsored by the World Health Organization to screen new antimycobacterial monoclonal antibodies and to identify antibodies which could be recommended as standard reagents giving consistent results under differing assay conditions. Fifty-eight antibodies were submitted to the workshop by eight independent laboratories. Nineteen of the antibodies recognized antigens distinct from those identified in earlier workshops, defining at least 10 new protein antigens. Monoclonal antibodies characterized in the workshop provide a set of convenient reagents for further characterization of mycobacterial antigens.—Authors' Abstract

Launois, P., Huygen, K., De Bruyn, J., *et al.* T cell response to purified filtrate antigen 85 from *Mycobacterium bovis* bacilli Calmette-Guérin (BCG) in leprosy patients. Clin. Exp. Immunol. **86** (1991) 286–290.

T-cell proliferations and IFN-γ production of peripheral blood mononuclear cells from 25 healthy controls and 39 leprosy patients [in Senegal] were tested against BCG-bacilli and culture filtrate, *Mycobacterium leprae* and purified antigen 85 (the major secreted 30–32 kDa protein antigen) from *M. bovis* strain BCG. In lepromin-negative healthy controls, blastogenesis was low to *M. leprae* and completely negative to antigen 85. IFN-γ levels were very low, close to detection limits. In all lepromin-positive controls, significant proliferation and IFN-γ secretion were found in response to *M. leprae* and antigen 85. In the group of lepromatous leprosy (LL) patients, 25/29 of patients [with either positive (13) or negative (12) lymphoproliferative response to BCG] were unreactive to *M. leprae* or to antigen 85. Four LL patients with positive T-cell response to BCG responded with detectable lymphoproliferative response and IFN-γ secretion to antigen 85. All tuberculoid (TT) leprosy patients responded to BCG, *M. leprae* and antigen 85. Hence, T cells from leprosy patients and controls demonstrate a marked parallelism of responsiveness toward whole *M. leprae* and purified antigen 85 from *M. bovis* BCG, suggesting strong crossreactivity between the two species and underlining the biological importance of such secreted antigens.—AS (Trop. Dis. Bull.)

Li, M.-H., *et al.* [Comparison between Ml- and Ms-ELISA in the determination of serum antibodies in leprosy. China Lepr. J. **8** (1992) 78–81. (in Chinese)

The antibodies in the sera have been determined with Ml- and Ms-ELISA in 146 cases of leprosy (MB 121, PB 25), 256 contacts with leprosy patients, 158 school children in an endemic area, 28 TB cases and 98 healthy persons. The results showed that the ELISAs with both antigens are highly sensitive (> 90%) and specific (> 90%) and there is a positive correlation between them ($\gamma = 0.82$, $p < 0.001$). Among leprosy patients and health controls, the coincidence rate of the two tests is over 90%. In leprosy patients, the mean OD value and positive rate are related to the type of their disease, showing gradual increase from PB to MB, but Ms-ELISA has the higher crossreaction. These methods are simple and rapid, the antigens used may be made by oneself, and

the needed material and reagents may be bought in the domestic market. Therefore, when antigen specific to *Mycobacterium leprae* is insufficient, these methods can be used in survey of leprosy and affect evaluation of treatment.—Authors' English Abstract

Lussow, A. R., Barrios, C., Van Embden, J., *et al.* Mycobacterial heat-shock proteins as carrier molecules. Eur. J. Immunol. **21** (1991) 2297–2302.

[The authors] have previously shown that the priming of mice with live *Mycobacterium tuberculosis* var. *bovis* (bacillus Calmette-Guérin, BCG) and immunization with the repetitive malaria synthetic peptide $(NANP)_{40}$ conjugated to purified protein derivative (PPD) led to the induction of high and long-lasting titers of antipeptide IgG antibodies, overcoming the requirement of adjuvants and the genetic restriction of the antibody response to the peptide. This initial work led us to the following observations. BCG had to be live for priming to lead to the induction of anti-peptide antibodies. Surprisingly, priming with other living microorganisms which chronically infect the macrophage (e.g., *Salmonella typhimurium* and *Leishmania major*) also induced antipeptide antibodies in mice immunized with PPD-$(NANP)_{40}$ conjugate. It was, thus, hypothesized that molecules expressed during active infection and also known to be highly conserved between species, namely, the heat-shock proteins (hsp), could mediate the T-cell sensitization required for the production of antipeptide antibodies. In fact, when the PPD portion of the conjugate was replaced by a highly purified recombinant protein corresponding to the 65-kDa (GroEL-type) hsp of *M. bovis,* this resulted in the production of anti-(NANP) IgG antibodies in BCG-primed mice, irrespective of the major histocompatibility complex-controlled responsiveness to the (NANP) sequence itself. Further, similar induction of antipeptide antibody response was also obtained with a recombinant 70-kDA (DnaK-type) hsp of *M. tuberculosis,* but not with a small molecular mass (18 kDa) of *M. leprae.* Finally, an adjuvant-free carrier effect for antipeptide IgG antibody production in BCG-primed mice was also exerted by the GroEL hsp of *Escherichia coli.* This finding that hsp can act as carrier molecules without requiring conventional adjuvants is of potential importance in the development of vaccine strategies.—AS (Trop. Dis. Bull.)

Momotani, E., Wuscher, N., Ravisse, P. and Rastogi, N. Immunohistochemical identification of ferritin, lactoferrin and transferrin in leprosy lesions of human skin biopsies. J. Comp. Pathol. **106** (1992) 213–220.

Granulomatous lesions of human leprosy contained ferritin and lactoferrin but little or no transferrin, as demonstrated by the avidin-biotin complex immunoperoxidase method. Lactoferrin was found in the neutrophils. These results suggested that the cells of the host mononuclear phagocyte system in leprosy granulomas provide an adequate nutritional environment for iron acquisition by *Mycobacteria leprae.* A possible role of iron-binding proteins in the granulomas is discussed in relation to previous data on bovine paratuberculous granulomas.—Authors' Summary

Mullins, R. J., Roche, P., Adams, E., Jones, P., Chen, S., Theuvenet, W. and Basten, A. Limiting dilution analysis in leprosy. Immunol. Cell Biol. **70** (1992) 277–290.

Peripheral blood mononuclear cells (PBM) obtained from leprosy patients and healthy controls were cultured with *Mycobacterium leprae* and the control antigens, BCG and SKSD. Parallel cultures were supplemented with additional interleukin-2 (IL-2). On the basis of the level of response to *M. leprae,* leprosy patients could be divided into low, intermediate and high responders. The addition of IL-2 resulted in enhanced proliferation to antigen only by cells from intermediate responders. This effect was neither antigen specific nor was it confined to cells from leprosy patients. When limiting dilution analyses were performed on cells from 26 patients across the leprosy spectrum, no *M. leprae*-reactive lymphocytes were detected in cells from subjects with lepromatous disease. The precursor fre-

quency for cultures containing *M. leprae* plus IL-2 was no greater than that of cultures containing IL-2 alone, thereby excluding the possibility of clonal anergy reversible with IL-2. This was observed in both untreated patients and those on long-term treatment, which made sequestration of antigen-reactive cells within leprosy lesions an unlikely explanation. On the other hand, *M. leprae*-reactive lymphocytes were detected in patients with tuberculoid and borderline tuberculoid disease and in two subjects with borderline lepromatous leprosy in type I reversal reaction. IL-2 reactive cells were detected in all patients regardless of clinical classification. Three "suppressor" curves were obtained but were not confined to cells from lepromatous patients. Taken together, these findings suggest that the nonresponsiveness to *M. leprae* characteristic of the great majority of multibacillary patients is due to an absence of antigen-sensitive T cells.—Authors' Abstract

Neubert, R., Nogueira, A. C. and Neubert, D. Thalidomide and the immune system. 2. Changes in receptors on blood cells of a healthy volunteer. Life Sci. **51** (1992) 2107–2116.

Thalidomide (Thd) was given in two trials (total daily dose: 5 or 8 mg Thd/kg body weight, respectively) for 5 and 3 days to a healthy male volunteer, and various receptors were analyzed on white blood cells before, during and after (up to 30 days) the treatment period. There were neither marked deviations in the absolute number of total leukocytes nor in the percentage of total lymphocytes or monocytes throughout the study period. The most pronounced changes were observed in the surface receptors on CD4 ("helper cells") cells and leukocytes bearing the CD11b (Mac 1) and other integrin and adhesion receptors. Other changes included shifts in the ratio cytotoxic cells/suppressor cells as well as a reduction of the receptor density (passage from bright to dim) in T-helper cells bearing CD45RO "memory" markers. Simultaneously, the number of B cells was found to be increased as was the percentage of some adhesion receptors on CD8+ cells. Unlike in previous experiments in which Thd was administered to marmoset monkeys, no effect could be seen in cells bearing the CD2 (LFA-2) epitope.—Authors' Abstract

Porcelli, S., Morita, C. R. and Brenner, M. B. CD1b restricts the response of human CD4−8− T lymphocytes to a microbial antigen. (Letter) Nature **360** (1992) 593–594.

Molecules encoded by the human CD1 locus on chromosome 1 are recognized by selected CD4−8− T-cell clones expressing either $\alpha\beta$ or $\gamma\delta$ T-cell antigen receptors. The known structural resemblance of CD1 molecules to antigen-presenting molecules encoded by major histocompatibility complex (MHC) genes on human chromosome 6 suggested that CD1 may represent a family of antigen-presenting molecules separate from those encoded in the MHC. Here we report that the proliferative and cytotoxic responses of human CD4−8− $\alpha\beta$TCR+ T cells specific for *Mycobacterium tuberculosis* can be restricted by CD1b, one of the four identified protein products of the CD1 locus. The responses of these T cells to *M. tuberculosis* seemed not to involve MHC-encoded molecules, but were absolutely dependent on the expression of CD1b by the antigen-presenting cell and involved an antigen-processing requirement similar to that seen in MHC class II-restricted antigen presentation. These results provide, to our knowledge, the first direct evidence for the proposed antigen-presenting function of CD1 molecules and suggest that the CD1 family plays a role in cell-mediated immunity to microbial pathogens.—Authors' Abstract

Rambukkana, A., Das, P. K., Burggraaf, J. D., Yong, S., Faber, W. R., Thole, J. E. R. and Harboe, M. Heterogeneity of monoclonal antibody-reactive epitopes on mycobacterial 30-kilodalton-region proteins and the secreted antigen-85 complex and demonstration of antigen-85B on the *Mycobacterium leprae* cell wall surface. Infect. Immun. **60** (1992) 5172–5181.

Proteins of the antigen 85 complex in the 30-kDa region secreted by live mycobacteria are important in the immune response against mycobacterial infections and may play an important biological role in the host-

parasite interaction. In the present study, we have characterized epitopes of the 30-kDa-region proteins and the antigen 85 complex by using a panel of 13 monoclonal antibodies (MAbs) reacting with these antigens, six of which have not been described before. By using five previously characterized related secreted proteins of *Mycobacterium tuberculosis,* MFT44 (85A), MPT59 (85B), MPT45 (85C), MPT51 (27 kDa), and MPT64 (26 kDa), we have identified at least 10 different MAb-reactive epitopes on the proteins of the antigen 85 complex. A heterogeneous distribution of epitopes was observed within the components of the antigen 85 complex. Two distinct epitopes specific for antigen 85B and two other epitopes restricted to the 85A and 85B components were recognized. Two of them were shared with a previously unidentified 27-kDa protein present in *M. tuberculosis* culture fluid from which all MPT proteins were derived. The rest of the MAb-reactive epitopes were found to be present mostly in antigens 85A and 85B and to a lesser extent in antigen 85C. None of these MAbs recognized component 85C alone nor did they bind to the related MPT51 and MPT64 proteins. Interestingly, most of the MAbs reacted with purified native proteins of the antigen 85 complex but not to them in their denatured forms. In contrast, reactivity of the MAbs with the cytosol fraction of *M. tuberculosis* in immunoblotting revealed that they bound to a closely related cytosolic 30-kDa protein(s) even when they were denatured. Heterogeneity of these MAb-reactive epitopes of the antigen 85 complex was further evident as they were found to be distributed in various patterns among 19 different mycobacterial species. By using fusion proteins of the *M. leprae* 30/31-kDa antigen 85 complex, we have localized at least six different epitopes within amino acid residues 55 to 266 of the *M. leprae* antigen 85 complex. Finally, by immunohistochemical analysis, we have demonstrated the *in situ* expression of one of the novel MAb-reactive epitopes specific for antigen 85B on the cell wall surface of *M. leprae* within macrophages in lepromatous leprosy lesions and thus provide direct evidence for the presence of the B component of the antigen 85 complex on the surface of intact *M. leprae.*—Authors' Abstract

Rastogi, N., Labrousse, V. and DeSousa, J. P. C. Mycobacterial growth and ultrastructure in mouse L-929 fibroblasts and bone marrow-derived macrophages—evidence that infected fibroblasts secrete mediators capable of modulating bacterial growth in macrophages. Curr. Microbiol. **25** (1992) 203–213.

The intracellular growth kinetics of *Mycobacterium avium* and H37Rv (virulent) and H37Ra (avirulent) strains of *M. tuberculosis* were compared by use of both the professional (mouse bone marrow-derived macrophages, BMMO) and nonprofessional (mouse L-929 fibroblast cell line) phagocytes. The results obtained showed that all the mycobacterial strains grew more actively in fibroblasts than in BMMO. This difference was paralleled by lesser acid phosphatase (AcP) labeling of noninfected fibroblasts and the observation that upon infection both the proportion of AcP-positive cells and AcP content were higher in BMMO than in L-cells during the 7 days of infection. In parallel experiments, intracellular growth of *M. tuberculosis* H37Rv and *M. avium* was compared inside BMMO from both the Bcg(s) (C57BL/6) and BCg(r) (DBA-2) mice, which were matured and differentiated with either an L-cell-conditioned medium (LCM) obtained from control, noninfected L-929 cells, or an LCM obtained with *M. tuberculosis*- or *M. avium*-infected L-cells. Upon mycobacterial infection, fibroblasts were able to secrete mediators that stimulated the BMMO to better control the infection by pathogenic mycobacteria. These results are discussed in terms of the mycobacteria-fibroblast interactions and their eventual role in the immune modulation of the host's response to invading mycobacteria.—Authors' Abstract

Richard, L., Forget, A. and Turcotte, R. A role for gamma interferon, tumor necrosis factors, and soluble T-cell receptors in the depressed blastogenic response of spleen cells of *Mycobacterium lepraemurium*-infected mice. Infect. Immun. **59** (1991) 3387–3392.

Spleen cells of *Mycobacterium lepraemurium*-infected mice were cultured on petri dishes coated with mycobacterial antigens, and antigen-reactive cells were

isolated. Upon incubation in mitogen- or antigen-free culture medium, these cells released mediators capable of depressing the *in vitro* proliferative response of normal splenocytes to specific antigen and to concanavalin A and lipopolysaccharide. One of these mediators was identified with gamma interferon (IFN-γ), mainly on the basis that treatment of supernatants with monoclonal anti-IFN-γ antibodies markedly reduced the suppressive activity contained therein. Detectable levels of tumor necrosis factor alpha (TNF-α) and TNF-β were present in spleen cell culture supernatants of infected mice. Moreover, low doses of recombinant TNF-α and TNF-β were found to potentiate the suppressive activity of exogenous IFN-γ. Soluble T-cell receptors beta were also detected in the culture supernatants. The elimination of these molecules with monoclonal anti-T-cell receptor beta (F23.1) antibodies immobilized on a plastic surface partially reversed the depression of the response to mycobacterial antigen but did not affect the response to mitogens. These results revealed the complex nature of suppressor mediators that are produced by mycobacterial antigen-reactive cells and that regulate the *in vitro* proliferative response.—Authors' Abstract

Schauf, V., Holobaugh, P., Miller, P. and Mittal, K. Sensitization *in vitro* of human peripheral blood mononuclear cells to phenolic glycolipid 1 of *Mycobacterium leprae* in liposomes. Cell. Immunol. **137** (1991) 81–87.

Peripheral blood mononuclear cells from individuals not previously exposed to leprosy were immunized *in vitro* with *Mycobacterium leprae* phenolic glycolipid (PGL-I) encapsulated in liposomes. The cells were incubated with the antigen in flasks for 4 or 11 days, harvested and recultured in tissue-culture plates for 48 hr; incorporation of tritiated thymidine was then measured in an 18-hr assay. Fifteen out of 25 cell donors showed significant lymphocyte proliferation after 11-, but not after 4-days' culture with liposomes containing PGL-I. Two donors showed suppression of the response (compared with diluent control wells) by the PGL-I-containing liposomes. The control antigen, KLH, induced sensitization in a higher proportion of donors (21 of 25); suppression was observed in 8 donors, usually at the lower antigen concentrations. In general, primary lymphocyte proliferation responses increased with increasing concentration of antigen. There was no relationship between sensitization and the presence of HLA-DR2, shown in some studies to be associated with tuberculoid leprosy. The authors believe that this system has great potential for immunogenetic studies, or for investigating the primary responses to vaccines.—H. Dockrell (Trop. Dis. Bull.)

Shetty, V. P., Mukherjee, R. and Antia, N. H. Ultrastructural study of mouse dorsal root ganglion cultures infected long term with *M. leprae.* Indian J. Lepr. **64** (1992) 293–301.

Ultrastructural changes in the mouse dorsal root ganglion cultures infected long-term with viable *Mycobacterium leprae* were studied. Subtle cytomorphological changes and loss of axons noted in the long-term infected cultures were correlated to early events in the nerve damage.—Authors' Abstract

Sullivan, L., Sano, S., Pirmez, C., *et al.* Expression of adhesion molecules in leprosy lesions. Infect. Immun. **59** (1991) 4154–4160.

The paper reports on the distribution of the intercellular adhesion molecule 1 (ICAM-1) and the ICAM-1 ligand, LFA-1, across the leprosy spectrum. Skin biopsies were examined by immunocytochemistry and *in situ* hybridization. The overlying epidermis showed ICAM-1 expression and contained lymphocytes expressing LFA-1, in leprosy lesions with strong delayed-type hypersensitivity. These include tuberculoid leprosy, reversal reactions, and also ENL. Expression of ICAM-1 and LFA-1 was minimal in lepromatous leprosy. Lymphocytes with mRNA coding for γ-interferon and tumor necrosis factor correlated with ICAM-1 expression. The outcome of infection with *Mycobacterium leprae* correlates with cytokine and adhesion molecule expression. The initial interaction of T cells and antigen-presenting cells may well take place first, the subsequent ICAM-1 expression serving to amplify the immunopathological processes.—S. B. Lucas (Trop. Dis. Bull.)

Uyemura, K., Ohmen, J. D., Grisso, C. L., Sieling, P. A., Wyzykowski, R., Reisinger, D. M., Rea, T. M. and Modlin, R. L. Limited T-cell receptor beta-chain diversity of a T-helper cell type-1-like response to *Mycobacterium leprae.* Infect. Immun. **60** (1992) 4542–4548.

Delayed-type hypersensitivity (DTH) is the standard measure of T-cell responsiveness to infectious organisms. For leprosy, the Mitsuda reaction, a local immune response to cutaneous challenge with *Mycobacterium leprae,* is considered to represent a measure of DTH against the pathogen. We analyzed the diversity of the T-cell receptor beta-chain repertoire in Mitsuda reactions to determine the breadth of the mycobacterial antigens involved. The polymerase chain reaction was used to compare Vbeta usage in the Mitsuda reaction T-cell lines established and unstimulated peripheral blood. These molecular analyses revealed a skewed T-cell receptor Vbeta gene usage in the Mitsuda reaction and in T-cell lines from lesions. To examine the reactivity of T cells from these lesions, T-cell lines were tested against the available native and recombinant antigens of *M. leprae.* T-cell lines derived from Mitsuda reactions responded more strongly to the 10-kDa *M. leprae* antigen, a homolog of GroES in *Escherichia coli,* than to other *M. leprae* proteins. T-cell lines were also shown to proliferate strongly in response to the 17- and 3-kDa proteins. The pattern of the lymphokine mRNA of these cells was reminiscent of the pattern of murine T(H)1 cells, positive for interleukin-2 and gamma-interferon and weakly positive for interleukin-4. These data indicate that a limited array of T cells, perhaps recognizing stress proteins, secretes a type 1 lymphokine profile in the DTH response to mycobacteria.—Authors' Abstract

van Schooten, W. C. A., Ko, J. L., van der Stoep, N., Haanen, J. B. A. G., Pickering, L., de Vries, R. R. P. and van den Elsen, P. T-cell receptor β-chain gene usage in the T-cell recognition of *Mycobacterium leprae* antigens in one tuberculoid leprosy patient. Proc. Natl. Acad. Sci. U.S.A. **89** (1992) 11,244–11,248.

The β chain of the T-cell antigen receptor present on 20 T-cell clones isolated from a tuberculoid leprosy patient was studied by gene rearrangement and PCR analysis. These T-cell clones all responded to *Mycobacterium leprae*-encoded protein antigens, and 8 of them specifically recognized peptides of the mycobacterial 65-kDa heat shock polypeptide (65hsp). All T-cell clones studied were HLA-DR-restricted (DR2 or -3). In the DR3-restricted group, 7 of 10 used a β-chain variable region Vβ5 gene family member; whereas in the DR2-restricted group, 2 of 10 T-cell clones used a Vβ5 gene segment and 5 used the Vβ18 gene segment. The deduced amino acid sequences of the β chain from 8 T-cell clones have revealed that 3 of 4 DR3-restricted T-cell clones expressed the Vβ5.1 gene segment; whereas the fourth DR3-restricted T-cell clone employed a Vβ5 family member not previously described. The Vβ5.1-positive T-cell clones all recognized the same 65hsp peptide from residues 2 to 12. The N-D-N segment (where D is diversity) of the junctional region of these T-cell clones was very similar, despite different β-chain joining gene segments. Of the 4 DR2-restricted T-cell clones investigated, 3 used the Vβ18 gene segment and recognized the 65hsp peptide from residues 418 to 427. In conclusion, within this panel of *M. leprae*-reactive T-cell clones, the DR3-restricted T-cell clones mainly used a Vβ5 gene segment; whereas the DR2-restricted clones employed preferentially the Vβ18 gene segment.—Authors' Abstract

Vandenakker, T. H. W., Naafs, B., Kolk, A. H. J., De Glopper-Van Der Veer, E., Chin A Lien, R. A. M. and Van Joost, T. Similarity between mycobacterial and human epidermal antigens. Br. J. Dermatol. **127** (1992) 352–358.

Eight out of 17 mouse anti-*Mycobacterium leprae* monoclonal antibodies (MAb) were previously observed to react with human nerve and skin antigenic determinants in cryostat sections, using an indirect immunoperoxidase technique. These observations suggested that antigenic mimicry may be involved in the development of the clinical manifestations of leprosy. In the present study we have extended our earlier findings by investigating sera from leprosy patients and MAb using Western blot technique. It was observed that 30 sera and their corresponding F(ab')2 fragments from iso-

lated IgG fractions of both tuberculoid and lepromatous patients reacted with 40–50 epidermal proteins of molecular weights (MW) ranging from 10 to 130 kDa. Sera from 14 controls, however, showed similar reactivity patterns. Absorption of 9 patient and control sera with *M. tuberculosis, M. marinum* and *M. kansasii* resulted in the removal of several components of different MW in 9, 4 and 3 cases, respectively. No consistent differences between sera from leprosy patients and controls were observed. Four out of 8 MAb against *M. leprae* which reacted with determinants in human epidermis and/or dermis in skin cryostat sections reacted with epidermal proteins of MW higher than 39 kDa in Western blot. Four MAb which showed reactivity in cryostat sections did not react in Western blot. Another 4 MAb did react with human epidermal proteins in Western blot but did not react in cryostat sections, indicating that the MAb were reacting with different epitopes in the two systems. Five MAb did not react with human epidermal proteins either in cryostat sections or in Western blot. Because all sera that were investigated contained antibodies against antigenic determinants of epidermal proteins, some of which are shared with *M. leprae* and cultivatable environmental mycobacteria, it is tempting to speculate that antigenic mimicry could be involved in autoimmune skin diseases which are induced and/or maintained by environmental micro-organisms.—Authors' Abstract

Wang, X.-H., Ohmen, J. D., Uyemura, K., Rea, T. H., Kronenberg, M. and Modlin, R. L. Selection of T lymphocytes bearing limited T-cell receptor β chains in the response to a human pathogen. Proc. Natl. Acad. Sci. U.S.A. **90** (1993) 188–192.

Delayed-type hypersensitivity (DTH) is a classic measure of T-cell responsiveness to foreign antigen. To estimate the extent of the T-cell repertoire in the DTH response to a human pathogen, we measured T-cell receptor (TCR) β-chain variable-region (V_β) gene usage in reversal reactions in leprosy. Reversal reactions represent naturally occurring DTH responses in leprosy, in which augmentation of T-cell responses to *Mycobacterium leprae* is concomitant with clearance of bacilli from lesions. T cells using the V_β6-, V_β12-, V_β14-, and V_β19-encoded TCRs were strikingly overrepresented in the lesions of patients as compared to blood and pre-DTH lesions from the same individuals. Furthermore, these data indicate a possible association between the predominant expression of a V_β gene segment in lesions and the major histocompatibility complex class II haplotype of the individual. V_β6 was prominent in the lesions of four patients who were DR15, a marker of resistance in leprosy infection. Sequence analysis of V_β6 TCRs showed frequent use of V_β6.1 and J_β2.7 gene segments and a conserved amino acid motif in the V–J junction in a reversal-reaction lesion, but not in blood from the same patient. The limited TCR repertoire expressed by the infiltrating T cells suggests that a limited set of antigens is recognized in the DTH response to a human pathogen. We suggest that the mechanism by which major histocompatibility complex haplotype influences DTH in this disease involves the presentation of specific peptides, with subsequent selection of specific TCRs followed by local oligoclonal expansion.—Authors' Abstract

Microbiology

Anes, E., Portugal, I. and Monizpereira, J. Insertion into the *Mycobacterium smegmatis* genome of the aph gene through lysogenization with the temperate mycobacteriophage Ms6. FEMS Microbiol. Lett. **95** (1992) 21–26.

A derivative of the temperate mycobacteriophage Ms6 containing the aph gene from transposon Tn5 was constructed. In the transductants the aph gene was integrated in the bacterial genome. The aph gene is stably maintained in the absence of pos-

itive selection after more than 150 generations. The results presented in this report show that Ms6 can be used as a vehicle for the integration of foreign DNA into the *Mycobacterium smegmatis* genome.—Authors' Abstract

Barclay, R., Furst, V. and Smith, I. A simple and rapid method for the detection and identification of mycobacteria using mycobactin. J. Med. Microbiol. **37** (1992) 286–290.

A system was developed for the identification of mycobacteria, such as *Mycobacterium tuberculosis* and *M. avium,* by thin layer chromatography of Fe-55-labeled mycobactin. Approximately 2 × 10^3 mycobacteria were detected within 24 hr and little operator time or skill was required. *M. avium, M. intracellulare* and *M. scrofulaceum* were found to have lower requirements for iron than other mycobacteria, and this may influence their growth in host organisms.—Authors' Abstract

Dekesel, M., Gilot, P., Coene, M. and Cocito, C. Composition and immunological properties of the protein fraction of A36, a major antigen complex of *Mycobacterium paratuberculosis.* Scand. J. Immunol. **36** (1992) 201–212.

TMA (thermostable macromolecular antigens) are major mycobacterial complexes present in all mycobacteria. We have purified A36, the TMA complex of *Mycobacterium paratuberculosis,* the etiological agent of paratuberculosis (Johne's disease), and shown, by the immune electron microscopy approach, its presentation at the cell surface. The immunodominance of the A36 complex in Johne's disease was suggested by comparative ELISA analysis of infected bovine sera, using either A36 or *M. paratuberculosis* total soluble sonicate as antigens. The crossreactivity of TMA complexes from different mycobacteria was evaluated by immunoenzymometric measurements. Percentage of shared epitopes was high for the couple *M. paratuberculosis-M. avium,* and somewhat lower for the couple *M. paratuberculosis.-M. bovis.* Immunological kinship between *M. paratuberculosis* and *M. leprae* was suggested by the finding that out of 11 anti-*M. leprae* monoclonals, four crossreacted with A36 proteins. The specificity missing at the level of the whole A36 complex was sought at the level of its protein components. Comparative immunoblot analysis of electrophoresed A36 proteins indicated three of them to contain epitopes not shared by *M. bovis* proteins, and one of them to contain epitopes specific with respect to *M. avium, M. bovis* and *M. phlei.* The latter component, a 34-kDa protein, could be an ideal reagent for a serological test for Johne's disease, being immunodominant in infected cattle and endowed with species-specific epitopes.—Authors' Abstract

Fiallo, P., Williams, D. L., Chan, G. P. and Gillis, T. P. Effects of fixation on polymerase chain reaction detection of *Mycobacterium leprae.* J. Clin. Microbiol. **30** (1992) 3095–3098.

The effects of standard fixatives (10% neutral buffered formalin, ethanol and mercury based) on the detection of *Mycobacterium leprae* DNA by the polymerase chain reaction (PCR) were studied. Mercury-based fixatives (Zenker's and Carnoy-Lebrun's fluids) strongly inhibited PCR amplification of *M. leprae* DNA. Ten percent neutral buffered formalin was inhibitory, but significant inhibition was observed only when fixation times exceeded 24 hr. Ethanol-based fixatives provided the best medium for holding specimens for subsequent PCR with both free bacilli and skin-biopsy specimens containing *M. leprae.* The *M. leprae*-specific, 360-bp region of the 18-kDa protein gene could be amplified from paraffin-embedded sections of formalin-fixed skin-biopsy specimens from patients with either multibacillary or paucibacillary infections when proper fixation conditions were used. Results of the study demonstrate that tissues properly fixed with two standard fixatives (10% neutral buffered formalin and 50% or 70% ethanol) can be analyzed by PCR for the presence of *M. leprae* with no loss in specificity and only minimal diminution in sensitivity compared with the specificities and sensitivities obtained by use of freshly prepared, unfixed specimens.—Authors' Abstract

Herbert, D. and Prabhakar, R. Observations on the cultivation of *M. leprae* and *M. tuberculosis* in medium "V" and "V1." Indian J. Lepr. **64** (1992) 341–347.

Skin scrapings from five different active sites were collected from 14 leprosy patients and inoculated into medium V. Skin scrapings from three leprosy patients were inoculated into medium V 1. All of the cultures were incubated at 8–10°C. *Mycobacterium tuberculosis* $H_{37}Rv$, pretreatment isolates and streptomycin-resistant strains were inoculated into medium V, with and without antibiotics, and incubated at 8–10°C as well as 37°C. Smears were made from the *M. leprae* and *M. tuberculosis* cultures at 0 hours and at different time points. The number of bacilli in the smears were counted. There was no increase in the number of *M. leprae* or *M. tuberculosis* in any of the cultures.—Authors' Abstract

Kempsell, K. E., Ji, Y. E., Estrada, I. C. E., Colston, M. J. and Cox, R. A. The nucleotide sequence of the promoter, 16S rRNA and spacer region of the ribosomal RNA operon of *Mycobacterium leprae* precursor rRNA and comparison with *Mycobacterium leprae* precursor rRNA. J. Gen. Microbiol. **138** (1992) 1717–1727.

Mycobacterium tuberculosis H37Rv has a single rrn (ribosomal RNA) operon. The operon was cloned and a region of 1536 nucleotides was sequenced, starting 621 bp upstream from the 5′ end of the 16S rRNA coding region and continuing to the start of the 23S rRNA coding region. The 16S rRNA sequence inferred from the gene sequence was found to differ in one position from *M. bovis* (nucleotide 1443) and from *M. microti* (nucleotide 427). A single putative promoter was identified on the basis of similarities with the sequence of rrn operons of *Bacillus subtilis* and *Escherichia coli*. The regions of similarity include a - 35 box, a - 10 box, a stringent response element, antitermination signals, potential RNAase III processing sites and features of precursor rRNA secondary structure. Sequences upstream from the 5′-end of *M. leprae* 16S rRNA were also investigated. Homologous schemes of secondary structure were deduced for precursor rRNA of both *M. tuberculosis* and *M. leprae*; although the principal features are common to both species there are notable differences.—Authors' Abstract

Morris, S. L., Nair, J. and Rouse, D. A. The catalase-peroxidase of *Mycobacterium intracellulare*: nucleotide sequence analysis and expression in *Escherichia coli*. J. Gen. Microbiol. **138** (1992) 2363–2370.

The activation of catalase genes in response to oxidative stress may contribute to the intracellular survival of mycobacteria. In this report, the nucleotide sequence of a mycobacterial catalase gene is described. The deduced protein sequence of this *Mycobacterium intracellulare* gene (MI85) was 60% identical to the *Escherichia coli* hydroperoxidase I (HPI) protein, 59% identical to the *Salmonella typhimurium* (HPI) catalase, and 47% identical to a *Bacillus stearothermophilus* peroxidase. The MI85 protein, expressed in *E. coli*, has also been shown to have peroxidase and catalase activities. Furthermore, Southern blot hybridizations, which demonstrated that a MI85 gene probe hybridizes with chromosomal DNA from 13 different strains of mycobacteria, suggest that this catalase-peroxidase gene is prevalent in the mycobacterial genus. The availability of catalase gene probes should permit an evaluation, at the molecular level, of the role of catalase in mycobacterial pathogenesis.—Authors' Abstract

Murray, A., Winter, N., Lagranderie, M., Hill, D. F., Rauzier, J., Timm, J., Leclerc, C., Moriarty, K. M., Gheorghiu, M. and Gicquel, B. Expression of *Escherichia coli* β-galactosidase in *Mycobacterium bovis* BCG using an expression system isolated from *Mycobacterium paratuberculosis* which induced humoral and cellular immune responses. Molec. Microbiol. **6** (1992) 3331–3342.

A promoter sequence, P_{AN}, was isolated from *Mycobacterium paratuberculosis* and characterized. This promoter lies adjacent to, and outside, the 3′ end of an IS*900* insertion element. IS*900* contains an open reading frame, ORF2, on the complementary strand which codes for the putative transposase of this insertion sequence. A DNA fragment containing P_{AN} and part of

ORF2 was fused to the *lacZ* gene and inserted into the replicative shuttle vector pRR3. *Mycobacterium smegmatis* and *Mycobacterium bovis* BCG (BCG) transformed with this plasmid exhibited β-galactosidase activity. However, *lacZ* was only expressed in *Escherichia coli* under the control of P_{AN}, when ORF2 was deleted. Immunization of mice with the recombinant *M. bovis* BCG expressing *lacZ* resulted in the induction of a high humoral and cellular response directed against β-galactosidase. The P_{AN}-ORF2 expression system may prove to be particularly useful for cloning and expression of heterologous genes in the BCG vaccine strain.—Authors' Summary

Patil, M. A., Katoch, V. M., Venkatesan, K., Sharma, V. D., Shivannavar, C. T., Kanaujia, G. V. and Agrawal, B. M. Correlation between inhibitory effect of quinolones and mycolic acid metabolism in mycobacteria. Indian J. Lepr. **64** (1992) 331–340.

Mycolic acids are important components having a significant role in maintaining the rigidity of the mycobacterial cell wall. They could also be the barrier for penetration of certain drugs into the bacterial cell. A novel *in vitro* model system was established for assessing the effect of ciprofloxacin on mycolic acid metabolism in pathogenic mycobacteria *kansasii* (which has a similar mycolic acid pattern to that from *M. leprae*) and the effect of norfloxacin in *M. intracellulare.* These test mycobacteria were exposed in their mid-logarithmic phase of growth to 0.5, 1, 2, 3, 4, 5 and 6 μg/ml of ciprofloxacin and norfloxacin respectively for 1, 2 and 24 hr. Ciprofloxacin completely inhibited the synthesis of mycolates in *M. kansasii* at 3, 4 and 5 μg/ml; whereas norfloxacin exhibited its maximum inhibitory action on mycolic acids in *M. intracellulare* at 6 μg/ml for all the durations of exposure. Inhibition of mycolates directly correlated with bacterial viability which was estimated by colony forming units. The effect of quinolones on mycolic acid metabolism appears to be direct and not secondary to DNA gyrase. The results obtained from this study and our previous findings show that mycolic acid metabolism is affected by various groups of drugs, whose primary sites of activity may be different. The findings of the present study may have significant therapeutic implications in leprosy and other mycobacterial diseases.—Authors' Abstract

Prabhakarn, K., Harris, E. B. and Randhawa, B. A unique type of GABA binding by *Mycobacterium leprae.* Microbios **70** (1992) 139–144.

Neurotropism is one of the unusual properties of *Mycobacterium leprae.* The organism contains glutamic acid decarboxylase that generates gamma-amino-butyric acid (GABA) which is an inhibitory neurotransmitter. The binding of GABA by *M. leprae in vitro* was studied by using H-3-GABA as substrate. The bacteria had high-affinity binding sites for the amino acid. The uptake was a specific saturable process with a K(m) of 66.7 pM, pH optimum of 7.3 and a temperature optimum of 37°C. The binding did not seem to be time-dependent, being complete in about 5 min. None of the known antagonists and agonists of GABA uptake by neurons showed any significant effect on *M. leprae*; the receptors in the bacteria are apparently of a non-neuronal type, and different from those reported in spermatozoa and *Pseudomonas.*—Authors' Abstract

Rambukkana, A., Das, P. K., Burggraaf, J. D., Faber, W. R., Teeling, P., Krieg, S., Thole, J. E. R. and Harboe, M. Identification and characterization of epitopes shared between the mycobacterial 65-kilodalton heat shock protein and the actively secreted antigen-85 complex—their *in situ* expression on the cell wall surface of *Mycobacterium leprae.* Infect. Immun. **60** (1992) 4517–4527.

Both mycobacterial hsp65 and the actively secreted antigen 85 complex of 30-kDa region proteins are considered to be major immune targets in mycobacterial diseases. In this study, by using a novel series of monoclonal antibodies (MAbs) directed to these antigens, we identified and partially characterized three unique epitopes (Rb2, Pe12, and A2h11) that are shared between mycobacterial hsp65 and the individual components of the antigen 85 complex. Dot blot assays with native purified proteins revealed that all three MAbs are strongly bound to hsp65 and antigens 85A (MPT44)

and 85B (MPT59), while a weak reaction or no reaction was found with antigen 85C (MPT45). Immunoblotting showed that MAb Rb2 reacted strongly with both hsp65 and the antigen 85 complex proteins; whereas MAbs Pe12 and A2h11 reacted strongly with the former but weakly with the latter. Moreover, these MAbs did not react with other closely related MPT51 and MPT64 secreted proteins. Further characterization of these epitopes was performed by using recombinant fusion and truncated proteins of *Mycobacterium bovis* BCG hsp65 (MbaA) and the *M. leprae* 30- and 31-kDa antigen 85 complex fusion proteins. In hsp65, Rb2-Pe12- and A2h11-reactive epitopes were found to reside in the C-terminal region of amino acid residues 479 to 540 and 303 to 424, respectively. In the *M. leprae* 30 and 31-kDa antigen 85 complex, all three epitopes were located in an N-terminal region of amino acid residues 55 to 266, one of the known fibronectin-binding sites of the *M. leprae* antigen 85 complex. Comparison of these MAb-reactive amino acid sequence regions between mycobacterial hsp65 and the components of the antigen 85 complex revealed that these regions show certain amino acid sequence identities. Furthermore, by immunoperoxidase and immunogold ultracytochemistry, we demonstrated that Rb2-, Pe12-, and A2h11-reactive epitopes are expressed both on the cell wall surface and in the cytosol of *M. leprae* bacilli within the lesions of lepromatous leprosy patients and in *M. leprae*-infected armadillo liver tissue.—Authors' Abstract

Trias, J., Jarlier, V. and Benz, R. Porins in the cell wall of mycobacteria. Science **258** (1992) 1479–1480.

The cell wall of mycobacteria is an efficient permeability barrier that makes mycobacteria naturally resistant to most antibiotics. Liposome swelling assays and planar bilayer experiments were used to investigate the diffusion process of hydrophilic molecules through the cell wall of *Mycobacterium chelonae* and identify the main hydrophilic pathway. A 59-kilodalton cell wall protein formed a water-filled channel with a diameter of 2.2 nanometers and an average single-channel conductance equal to 2.7 nanosiemens in 1 M potassium chloride. These results suggest that porins can be found in the cell wall of a gram-positive bacterium. A better knowledge of the hydrophilic pathways should help in the design of more effective antimycobacterial agents.—Authors' Abstract

Von Pessolani, M. C. and Brennan, P. J. *Mycobacterium leprae* produces extracellular homologs of the antigen-85 complex. Infect. Immun. **60** (1992) 4452–4459.

The antigen 85 complex is a set of at least three closely related secreted proteins (85A, 85B, and 85C) of 30 to 32 kDa produced by *Mycobacterium tuberculosis* and other mycobacteria. Their prominence in *M. leprae,* the one obligate intracellular pathogen of the genus, had been assumed on the basis of immunological evidence and proof of the existence of the gene encoding the 85B protein of the complex. We have now observed the production of this family of proteins by *M. leprae* through analysis of various fractions by Western blotting (immunoblotting) with monospecific rabbit antisera raised against the individual *M. bovis* BCG 85A, 85B, and 85C proteins. A predominant crossreactive band with an apparent molecular mass of 30 kDa was detected in extracts of nondisrupted whole *M. leprae* and in soluble fractions prepared from the tissues of *M. leprae*-infected armadillos. Further studies of the subcellular distribution of this protein within the bacterium confirmed that it is secreted by the organism, an observation that explains past difficulties in detecting the antigen 85 complex in *M. leprae.* Confirmation that the *M. leprae* product is a member of the antigen 85 complex was obtained by comparison of peptide fingerprints with those from the BCG product. The pattern of reactivity of the *M. leprae* antigen 85 complex with anti-*M. bovis* BCG 85B serum, as well as two-dimensional electrophoresis, established that the 85B component was the predominant member of the complex in *M. leprae.* The fibronectin-binding capacity of the *M. leprae* and BCG 85 complexes was reinvestigated by new approaches and is questioned. Nevertheless, the results obtained with the native proteins

reinforce previous reports, derived primarily from the use of homologous proteins, that the antigen 85 complex is one of the dominant protein immunogens of the leprosy bacillus.—Authors' Abstract

Watanabe, M., Kudoh, S., Yamada, Y., Iguchi, K. and Minnikin, D. E. A new glycolipid from *Mycobacterium avium-Mycobacterium intracellulare* complex. Biochim. Biophys. Acta **1165** (1992) 52–60.

From a nonpolar lipid fraction of *Mycobacterium avium-M. intracellulare* complex cell mass, a new glycolipid was obtained, which was shown to be 5-mycoloyl-beta-arabinofuranosyl-(1→2)-5-mycoloyl-alpha-arabinofuranosyl-(1→1')-glycerol. When examined by TLC, all the 12 strains of this species tested, including clinical isolates, were found to contain this glycolipid. But the glycolipid was not detected in *M. bovis* BCG or *M. tuberculosis* H37Rv.—Authors' Abstract

Williams, D. L., Gillis, T. P., Fiallo, P., Job, C. K., Gelber, R. H., Hill, C. and Izumi, S. Detection of *Mycobacterium leprae* and the potential for monitoring antileprosy drug therapy directly from skin biopsies by PCR. Mol. Cell. Probes **6** (1992) 401–410.

An improved protocol for polymerase chain reaction (PCR) analysis of *Mycobacterium leprae*-infected tissues, based on enzymatic lysis, has been developed and used to demonstrate the feasibility of using PCR for detecting *M. leprae* in routine skin biopsies taken from leprosy patients throughout the clinical spectrum. Of 92 multibacillary patients tested, 99% were PCR-positive using gel electrophoresis or DNA hybridization to detect the amplified product. Similar analysis of paucibacillary patients, in which only 1 of 27 biopsies had demonstrable acid-fast bacilli microscopically, gave a positivity rate of 74%. No PCR signals were demonstrated from skin biopsies from seven patients with nonleprosy dermatoses and one AIDS patient with a disseminated atypical mycobacteriosis. Evaluation of leprosy patients with antileprosy drug therapy prior to biopsy demonstrated that PCR signals were either greatly diminished or absent after 2 months of continuous antibiotic therapy. PCR was also able to detect the presence of *M. leprae* in tissues of patients receiving antibacterial therapy when patients were suspected of harboring drug-resistant *M. leprae*.—Authors' Abstract

Experimental Infections

Lu, X.-H., *et al.* [Nude mouse foot pad model of leprosy infection.] China Lepr. J. **8** (1992) 71–73. (in Chinese)

Leprosy bacilli obtained from the skin lesions of LL and BL leprosy patients before and after treatment have been inoculated into the right rear foot pad of BALB/c nude mice, and then the bacilli collected from the inoculated foot pad have been secondarily inoculated. The bacilli recovered 18 months after inoculation showed an increase of 1000 to 2000 times in quantity and dissemination in the noninoculated feet, ears and apex of the nose. One year after the inoculation the foot pads have swollen. The growth curves of the bacilli recovered from primarily and secondarily inoculated mice are almost concordant. Two strains of leprosy bacilli obtained from treated patients had been inoculated into the mice without growth. Through culture in test tube and foot pad inoculation, the recovered bacilli from nude mice tissues have been preliminarily identified as the human leprosy bacilli.—Authors' English Abstract

Shi, Z.-R., *et al.* [Intraocular experimental infection with *M. leprae* in nude rats.] China Lepr. J. **8** (1992) 75–78. (in Chinese)

Ten Rowett nude rats were inoculated intraocularly with *Mycobacterium leprae* suspension. The suspension was prepared with a concentration of 1.19×10^7AFB/ml (with 6×10^6 living AFB) in normal saline, sonicated for 2 min before the inoculation. The

inoculations were made after general anesthesia of the animals. At the lateral inferior quadrant of the cornea 0.03 ml of the bacilli suspension was injected into the anterior chamber of the right eyes of animals 1–6 and 0.01 ml of the suspension into the anterior chamber of the right eyes of 7–10 animals. Into the vitreous humor of the left eye of each animal 0.03 ml of the suspension was injected. During the inoculations some leakage of the suspension was noticed but the amount of the leaked fluid was not able to be measured.

Nine rats (one died just after the inoculation) were sacrificed at the 4th to 10th week after the inoculation, and the eyes of the animals were enucleated and fixed. Paraffin sections of the eyes were made and stained with hematoxylin and eosin and the Fite-Faraco method.

Due to trauma to the very large lens during inoculation, phacogenic endophthalmitis occurred in 9 eyes of the 9 animals, of which 6 were inoculated in the anterior chambers and 3 in the vitreous humor. In these eyes the lens capsule was broken and around the lens material leaked into the vitreous body there was dense infiltration of lymphocytes, macrophages, sometimes with giant cells, and neutrophils. There were newly formed fibrous tissues around the infiltrating cells and the fibrous tissue was extended to the ciliary body and the retina. The retina was edematous, folding or atrophic. Chronic inflammatory cells were infiltrating the iris and ciliary body in various numbers.

Of the 9 eyes with intact lens, for 3 the bacilli were inoculated into anterior chamber and for 6 into vitreous humor. In these eyes lymphocyte and small macrophage infiltration usually of mild degree was seen in the intraocular tissues. No granulomatous lesion was seen. AFB were searched for and found in almost all the eyes, whether with or without phacogenic endophthalmitis, and almost all the bacilli were of the granular form.

In view of the above findings, it seems that it is not adequate to produce a model of human eye leprosy in these nude rats through the intraocular inoculation of *M. leprae* for further work.—Authors' English Abstract

Suárez-Moreno, O. [Experience with the mouse foot pad technique to obtain *Mycobacterium leprae.*] Rev. Leprol. Fontilles **18** (1992) 511–515.

The study was in two stages: in the first stage were studied five types of isogenic mice and nonisogenic mice to determine which one was the best for leprosy bacillus multiplication in the mouse foot pad. In a type in which bacilli growth proved to be poor, thymectomy and ipsilateral lymphadenectomy were tried in stage two of the study obtaining in this way a larger number of bacilli compared to the type that produced the greatest amount of bacteria in the first stage. The utilization of immunodepressed mice by popliteal lymphadenectomy is recommended to achieve a higher harvest in *Mycobacterium leprae* multiplication.—Authors' English Summary

Wang, H. [Influence of feeding on the multiplication of *M. leprae* in the mouse foot pad.] China Lepr. J. **8** (1992) 73–75. (in Chinese)

The effect of diet on the multiplication of *Mycobacterium leprae* was assessed by the mouse foot pad technique. The results showed that the multiplications of *M. leprae* were different between the experimental group and control group of mice. The multiplication of *M. leprae* in that group fed on special low iodine diet was more vigorous by far than in the group fed on routine diet. It seems that the lack of some nutrition components could decrease nonspecific immunity of the animal. As far as this experiment, the hypothesis that the diet richer in iodine might increase the susceptibility to leprosy cannot be proved.—Authors' English Abstract

Epidemiology and Prevention

Baker, D. M. and Nguyen, V. T. J. S. BCG vaccine and leprosy. (Letter) Lancet **339** (1992) 1236.

Referring to a previous report that BCG vaccination has provided ≥ 50% protection against leprosy in northern Malawi, these correspondents now present supporting data from southern Malawi. They identified 145 current cases of leprosy from the Balaka Lepra Control Project and matched each case with two randomly selected community control subjects for age, sex and educational status. Independent assessors looked for signs of a BCG scar as evidence of BCG vaccination in both groups of people. In the unvaccinated people the odds ratio for having leprosy was 2.75. The correspondents calculate that BCG vaccination thus gave 64.3% protection against leprosy (95% CI 41.1–77.2%, $p < 0.001$). Although admitting that BCG has produced variable degrees of protection against leprosy in different geographical locations, the correspondents support the concept of continuation of BCG vaccination programs for leprosy control.—C. A. Brown (Trop. Dis. Bull.)

Cartel, J.-L., Boutin, J.-P., Spiegel, A., Glaziou, P., Plichart, R., Cardines, R. and Grosset, J.-H. Leprosy in French Polynesia. Epidemiological trends between 1946 and 1987. Lepr. Rev. **63** (1992) 211–222.

The analysis of computerized data (OMSLEP system) on patients from French Polynesia followed since 1940 has shown a decrease in the mean annual detection rates for leprosy, all forms combined, from 24.73 per 100,000 inhabitants in 1946 to 8.1 per 100,000 in 1987 ($y = -0.49x + 45.83$; $p < 0.05$). In fact, the decrease was significant ($y = -1.18x + 83.54$; $p < 0.05$) during the first half of the study period (1946–1966), but not during the second half (1967–1987). Similarly, a significant decrease in all of the specific mean annual detection rates (according to the form of leprosy and to the sex and age of patients), in the proportion of multibacillary patients among the total of newly detected cases, and in the proportion of all patients with disabilities at the onset of leprosy was observed only during the first half of the study period (1946–1966). Nevertheless, when comparing age-specific cumulative detection rates, calculated by 10-year age groups over the period 1946–1966, to those of the period 1967–1987, an ageing of the leprosy population was noted. Finally, the decrease of mean annual detection rates was greater in the smaller populations of remote islands than in the population of Tahiti, the main island, where 70% of the total population were living during the study period. This decline was shown to correspond to an effective improvement of the leprosy situation which could be attributed, among other factors (such as economic development and systematic BCG vaccination), to the implementation of a control program for leprosy in 1950. The introduction in 1982 of multidrug therapy for all patients suffering active leprosy has raised the hope of a subsequent decline of leprosy in French Polynesia in the near future.—Authors' Summary

Cartel, J.-L., Spiegel, A., Ngoc, L. N., Moulia-Pelat, J.-P., Martin, P. M. V. and Grosset, J.-H. Leprosy in French Polynesia. The possible impact of multidrug therapy on epidemiological trends. Lepr. Rev. **63** (1992) 223–230.

In 1982, following the recommendations of a WHO study group, multidrug therapy (MDT) was introduced into French Polynesia to treat all patients suffering from active leprosy, and—only on request—those still on dapsone monotherapy. After 5 years, a clear-cut decrease of prevalence and mean annual detection rates for leprosy (except for detection rates among children aged less than 15 years, many of such cases being detected early by increased household contact training) has been observed. There was also a decrease in the proportion of newly detected cases with disabilities. During the 21-year period preceding the introduction of MDT into the control program, mean annual detection rates for leprosy had remained stable, and this led to the consid-

eration that such a decrease was due neither to the natural decline of the disease nor to the economic improvement of the country. Our results, together with the fact that, to date, the relapse rate was nil in the Polynesian patients put on MDT, strongly suggest that the implementation of MDT has resulted in a decrease of detection rates for leprosy which may be a consequence of a decrease in the transmission of the disease.—Authors' Summary

Castro Chavez, R., Carrazana Hernández, G. B. and Ferrá Torres, T. M. [Correlation between vaccination with BCG and the clinical forms of leprosy.] Rev. Leprol. Fontilles **18** (1992) 475–480. (in Spanish)

The correlation between BCG vaccination and the clinical forms of leprosy was studied in 43 leprosy patients; 8 of them (18.6%) had a history of BCG vaccination and 35 (81.4%) did not. Sixty percent of the not previously vaccinated cases developed multibacillary clinical forms; 87.5% of the vaccinated patients presented paucibacillary clinical forms. BCG vaccination seems to give certain protection against the development of future multibacillary leprosy clinical forms.—Authors' English Summary

Day, R., Lever, P. and Asri, M. Leprosy control in 7 districts of South Sulawesi, Indonesia, 1986–91. Lepr. Rev. **63** (1992) 247–254.

This paper describes the leprosy control program in seven districts of the South Sulawesi Province in Indonesia. This province is reported to have the highest prevalence of leprosy in the country. The program started in 1986 with re-registration of all patients on the cumulative registers. Strict criteria for admission of patients to MDT were initially applied. In 1990 it appeared that these criteria had been too strict, thus necessitating a second re-registration of patients still on DDS monotherapy. More flexible criteria for admission to MDT led to an increase in MDT coverage from 45% to 78% within 6 months. By April 1991, 5 years after the start of the program, the registered prevalence had decreased from 4:4 per 1000 in 1986 to 1:6 per 1000; the coverage with MDT had increased from 6% in 1986 to 78%, and the case detection rate remained stable (around 4 per 10,000) after an initial increase at the start of the program.—Authors' Summary

Gómez C., A., Cantillo, L., López S., S., Zamora, D., Medine, M. E. and Tórrez, D. [A focus of leprosy in San Francisco Libre, Nicaragua—1990.] Rev. Leprol. Fontilles **18** (1992) 481–509. (in Spanish)

In a survey carried out in 1989, a high prevalence rate (4.36/1000) of leprosy cases was demonstrated in 15 year olds or less of the Free San Francisco district, a leprosy infected area next to the city of Managua, Nicaragua, and one of the poorest areas of the country. Based on those results a clinical-epidemiological study was carried on with the aim of early detection in leprosy, basically on school-children of the communities affected by this disease and close contacts of patients discovered during the school survey. Also studied is the relation between the quality of life and this disease, including attitudes and perceptions of the community toward this disease. The results obtained were higher than the rates of the previous study with an index of 19.2/1000, indicating a hyperendemic. The identification of the areas with a higher concentration of cases is associated with the worse socioeconomic conditions. This situation needs an urgent establishment of an antileprosy program with medical and social-economic intervention for the eradication of leprosy in the district of Free San Francisco.—Authors' English Summary

International Meeting on Epidemiology of Leprosy in Relation to Control held in Jakarta, Indonesia, 17–21 June 1992. Lepr. Rev. **63** Supplement (1992) 123S.

Following are the major conclusions and recommendations of the meeting.

The WHO International Meeting on Epidemiology of Leprosy in Relation to Control reviewed the state-of-the-art of epidemiology and discussed issues relevant to leprosy control, particularly in terms of changing needs following the widespread implementation of multidrug therapy (MDT) and the declining trends in prevalence of the disease. The following are the

major conclusions and recommendations of the meeting.

1. The need for rapid assessment of the leprosy prevalence situation, particularly before introducing MDT, was recognized. As sample surveys are not feasible in most situations other methods of estimating prevalence through application of extrapolation factors were considered suitable.

2. The need for making predictions for the future trends was recognized for the purpose of planning, including resource mobilization, evaluation and research. It was recommended that methods for prediction of trends, including simulation models, should be further developed through a multidisciplinary effort and by making optimal use of existing epidemiological data sets.

3. The leprosy evaluation indicators developed at earlier WHO meetings were reviewed and six essential indicators were recommended for routine programs. Optional indicators were also recommended for more developed control programs.

4. The meeting identified some of the more important research areas in epidemiology of leprosy including risk-factor studies for the disease and leprosy-related disabilities, the influence of HIV infection in leprosy, cost-effectiveness of different vaccine and therapy strategies, and correlates for protective effect of vaccines.

5. In view of the leprosy elimination goal adopted by the World Health Assembly and the rapidly changing situation the meeting strongly recommended strengthening of epidemiological capabilities of leprosy control programs. It also recommended that problems at the operational level of leprosy control should be solved through systematic approaches such as health system research.

Kher, S. K., Rao, M. R., Raghunath, D. and Chattopadhyay, S. P. HLA-linked genetic control of leprosy. Med. J. Armed Forces India **48** (1992) 116–118.

Association of certain human leukocyte antigen (HLA) types in various diseases is well established, notably in ankylosing spondylitis and narcolepsy. Elaborate HLA typing was carried out in [110] leprosy patients and [236] controls of Indocaucasoid stock to look for any association. There was no predisposition of leprosy *per se* to any particular HLA type. However, the type of leprosy was significantly influenced by HLA type. DR3 predisposed to tuberculoid type, relative risk (RR 4.26), and DQW1 to lepromatous leprosy (RR 9.04). It seems that HLA class II molecules are products of immune response genes to *Mycobacterium leprae*.—AS (Trop. Dis. Bull.)

Noordeen, S. K., Lopez Bravo, L. and Sundaresan, T. K. Estimated number of leprosy cases in the world. Bull. WHO **70** (1992) 7–10.

Planning for disease control requires estimates of the number of leprosy patients from local to global levels. From the mid-sixties to the mid-eighties, global estimates appeared to be constant at between 10 and 12 million. The introduction of multidrug therapy (MDT) in many countries and the consequent reduction of prevalence of the disease have necessitated a reassessment of the global estimate. Based on available information and its interpretation, the number of leprosy cases in the world in 1991 has been estimated at 5.5 million. The number of individuals with deformities due to leprosy, including persons now cured of the disease, has been estimated at between 2 and 3 million.—Authors' Abstract

Rani, R., Zaheer, S. A. and Mukherjee, R. Do human leukocyte antigens have a role to play in differential manifestation of multibacillary leprosy—a study on multibacillary leprosy patients from India. Tissue Antigens **40** (1992) 124–127.

One-hundred-eighteen multibacillary leprosy patients with differential manifestations were studied for the antigens they expressed at MHC loci to investigate the role of human leukocyte antigens in the differential response to the same causative agent. While the lepromatous leprosy (LL) patients showed a significant increase of Bw60, DR2, DRw8 and DQw1, borderline lepromatous (BL) patients had Bw52, DR9 and DQw7 significantly more often as compared to the normal controls. A comparison of LL, BL and midborderline (BB) patients showed a significantly higher frequency of Bw60 in LL patients as compared to the BL. However, Bw52, Bw53, DR9 and DQw7 were found significantly more often in the

BL patients as compared to the LL patients but the difference failed to reach significance after pc. A comparison of HLA antigens in BB patients with those of either the LL or BL patients did not show any significant differences.—Authors' Abstract

Smith, P. G. Revised estimates of global leprosy numbers. (Editorial) Lepr. Rev. **62** (1992) 317–318.

Increased optimism in leprosy control efforts have come about in recent years because of the implementation of multidrug therapy (MDT), the setting of the goal by the World Health Assembly to eliminate leprosy as a public health problem by the year 2000, and the recent dramatic downward revision of the number of leprosy cases in the world from 10–12 million in the last few decades to a new figure of 5.5 million. In revising these figures the definition of an individual with leprosy has been changed and this is responsible, in considerable part, for the reduced number of cases reported. The new figure of 5.5 million cases is defined as those individuals still requiring chemotherapy. In situations in which the rates of a disease are not changing very much over time, prevalence = incidence * average duration of disease. Current estimates of prevalence are reduced from former estimates to a large part because MDT has reduced the duration of the disease compared to long term dapsone monotherapy in the past. It remains to be seen whether or not MDT will actually impact on disease transmission and be responsible for falls in incidence. Current trends in that direction began years before the application of MDT in many areas and may well be due simply to improvements in socioeconomic conditions and enhanced BCG coverages in many populations. It may well be premature to plan that leprosy will cease to be a public health problem by the year 2000.—RCH

Sundaresan, T. K. Issues involved in the rapid assessment of the leprosy problem. Lepr. Rev. **63** Supplement (1992) 11S–20S.

Sample surveys for estimation can prove very expensive and time-consuming because of the enormous sample sizes usually required. Where sample surveys have to be undertaken, diagnoses should be limited to detecting a case of leprosy, without attempting skin smears, etc., in order to classify by types. Usually enough knowledge is available on the approximate proportion of multibacillary (MB) cases in most communities, and this knowledge could be utilized for estimating the caseload by types of leprosy. Again intensive tracing of nonrespondents could be limited to either males or females depending on convenience, and well-known sex ratios among patients utilized for deriving estimates for the other sex. The type of rapid methods of estimation depends on three types of situations: (1) before multidrug therapy (MDT); (2) 5 years or more after MDT; and (3) less than 5 years after MDT. In the first situation one or more of the following methods are suggested: (i) extrapolation from registered cases; (ii) extrapolation from child prevalence; and (iii) conducting rapid village surveys. In situations where MDT has been introduced for 5 years or more the registered cases plus a small number, depending on local experience, would seem to be adequate. When MDT was introduced less than 5 years before, it is suggested that the prevalence rates be obtained by statistical interpolation drawing on the experience from areas which have had more than 5 years of MDT.—Author's Summary

Vardy, D. A., Pappo, O., Zlotogorski, A., Benmeir, P. and Leviatan, A. Leprosy acquired in Israel. Israel J. Med. Sci. **27** (1991) 218–220.

This is an excellent account of a 60-year-old man who developed multibacillary borderline leprosy in Israel, having never been out of the country except for a brief trip to Vienna and London after the onset of his symptoms. Prior to diagnosis in the Dermatology Clinic of Hadassah University Hospital, he had been diagnosed as having erythema annulare centrifugum and his illness in fact began with a subtle erythematous eruption on various parts of the body 8 years earlier. The diagnosis was confirmed by biopsy and he responded satisfactorily to multiple drug therapy (rifampin, dapsone

and clofazimine). As the authors rightly point out this case is a reminder that, although uncommon, leprosy does indeed exist in Israel, and should be kept in mind in the differential diagnosis of cutaneous and neurological diseases, especially if not conforming to usual patterns.—A. C. McDougall (Trop. Dis. Bull.)

Rehabilitation

Chen, X.-S., et al. [Stepwise regression analysis of the factors affecting the disability of leprosy.] China Lepr. J. **8** (1992) 67–70. (in Chinese)

Stepwise regression analysis for factors affecting the disability of leprosy has been done using data obtained from 320 leprosy patients (241 male and 79 female) with disability in Yangzhou City and Dongtai City of Jiangsu Province. The arithmetic disability index (ADI) and weighted disability index (WDI) were used as the quantity index of the disability and nine factors were analyzed on microcomputer. The results showed that the significant factors affecting disability were the same by these two indices, including frequency of leprosy reaction, leprosy type (0 for PB and 1 for MB), patient's age and delay period (from the onset to treatment). The equations of multiple regression were ADI = −0.1732 + 0.0154 × (age) + 0; 5135 × (leprosy type) + 0.0147 × (delay period) + 0.2281 × (the reaction) and WDI = 0.4471 + 0.0147 × (age) + 0.3877 × (leprosy type) + 0.0109 × (delay period) + 0.1809 × (the reaction), and their multiple correlation coefficients are 0.6816 and 0.6608, respectively. For preventing leprosy disability, the early case-finding and immediate treatment with MDT as well as the effective prevention and control of leprosy reaction all are very important.—Authors' English Abstract

Kartikeyan, S. and Chaturvedi, R. M. Pattern of leprosy deformities among agricultural labourers in an endemic district: a pilot study. Indian J. Lepr. **64** (1992) 375–379.

A study of 1338 leprosy-affected agricultural laborers in an endemic district revealed that 12% had deformities. The patient's sex, type of disease, duration and educational status seemed to influence the pattern of leprosy deformities. The patients continued working despite deformities in order to avoid financial dependence on their family members and loss of dignity.—Authors' Abstract

Weber, M. W., Van Soest, A., Neff, G., Chiang, T. and Pfau, R. Results of surgical procedures for the correction of foot-drop and of lagophthalmus due to leprosy. Lepr. Rev. **63** (1992) 2555–262.

Leprosy mutilations of the muscles and skeleton can be relieved by reconstructive surgery, but evaluation of the results of these operations is seldom undertaken. Between 1975 and 1984, 59 leprosy patients were operated on at the Marie Adelaide Leprosy Centre, Karachi, Pakistan, for lagophthalmus with the transposition of the temporalis muscle and for foot-drop with the transposition of the posterior tibial muscle. We were able to re-examine 39 patients: tibialis posterior transposition was performed 25 times, and temporalis transposition was carried out 33 times; 18 of the 25 patients with the tibialis posterior transposition were pleased with the result, 7 were not: 21 patients could extend their feet above the neutral position; 24 of the patients with the temporalis transposition were satisfied, 9 were not: complete closure was demonstrated in 21 eyes; persistent corneal damage was noted in 15 eyes; 12 of the 23 male patients cared for themselves, 16 lived with their families; 7 of the 8 female patients lived with their families. The results of the rehabilitation, in relation to the degree of mutilation, are considered satisfactory for a developing country. These surgical procedures give a good result, provided they are followed by intensive physiotherapy.—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Alugapalli, S. and Larson, L. Secondary fatty alcohols of *Mycobacterium xenopi*. J. Gen. Microbiol. **138** (1992) 2499–2502.

Secondary alcohols of *Mycobacterium xenopi* were studied by gas chromatography and gas chromatography–mass spectrometry. Mycobacterial cells were hydrolyzed and the liberated alcohols separated by extraction and analyzed both underivatized and as trimethylsilyl-, and methyl ether- and pentafluorobenzoyl derivatives. Seven straight-chain secondary alcohols containing from 18 to 24 carbon atoms and two branched-chain secondary alcohols with 21 and 23 carbon atoms were present in all of the studied strains.—Authors' Abstract

Andersen, P., Askgaard, D., Gottschau, A., Bennedsen, J., Nagai, S. and Heron, I. Identification of immunodominant antigens during infection with *Mycobacterium tuberculosis*. Scand. J. Immunol. **36** (1992) 823–831.

T lymphocytes isolated from mice infected with *Mycobacterium tuberculosis* respond vigorously to proteins secreted by the bacilli and these antigens may be of importance in the generation of protective immunity against the disease. In this study, short-term culture filtrate (ST-CF), which constitutes a complex mixture of secreted proteins, was fractionated by a modified preparative SDS-PAGE technique. The ability of each fraction to be recognized by T cells isolated from infected mice was evaluated by quantifying proliferation and IFN-gamma production in cell cultures. Two molecular mass regions 4–11 and 26–35 kDa were found to possess marked stimulatory properties. Four potent single antigens were mapped within the stimulatory regions. These purified antigens stimulated T cells isolated from mice at the height of a tuberculous infection to produce large amounts of IFN-gamma. Two of these stimulatory antigens belonged to the antigen 85 complex.—Authors' Abstract

Ausina, V., Luquin, M., Barcelo, M. G., Laneélle, M. A., Lévy-Frébault, V., Belda, F. and Prats, G. *Mycobacterium alvei* sp-nov. Int. J. System. Bacteriol. **42** (1992) 529–535.

A new species of rapidly growing, nonphotochromogenic mycobacteria, *Mycobacterium alvei*, is described. The inclusion of this organism in the genus *Mycobacterium* is based on its acid fastness, its mycolate pattern, and its G+C content. A study of six strains showed that they form a homogeneous group with an internal phenotypic similarity value of 97 ± 2.22%. DNA relatedness studies showed that the six *M. alvei* strains which we studied form a single DNA hybridization group which is less than 49% related to 14 other species of the genus *Mycobacterium*; the DELTAT(m) values determined for the strains which exhibited higher levels of DNA homology were all greater than 7.9°C. A lipid analysis showed that tuberculostearic acid was present. Docosanoic and tetracosanoic acid methyl esters were detected as mycolic acid cleavage products. All six isolates which we tested contained alpha-mycolic acids and relatively large amounts of a new kind of mycolic acid containing a methoxy group at omega-1 position, a characteristic that has not been described previously in mycobacteria. Strain CR-21 is the type strain; a culture of this strain has been deposited in the Collection Nationale de Cultures de Microorganismes de l'Institut Pasteur, Paris, France, as strain CEP 103464.—Authors' Abstract

Besra, G. S., Bolton, R. C., McNeil, M. R., Riddel, M., Simpson, K. E., Glushka, J., Van Halbeek, H., Brennan, P. J. and Minnikin, D. E. Structural elucidation of a novel family of acyltrehaloses from *Mycobacterium tuberculosis*. Biochemistry **31** (1992) 9832–9837.

Analysis of the lipids of *Mycobacterium tuberculosis* H37Rv, by both normal- and reverse-phase thin-layer chromatography, revealed a series of novel glycolipids based on 2,3-di-O-acyltrehalose. The structures of these acylated trehaloses were elucidated by a combination of gas chromatography–mass spectrometry, H-1, C-13, two-dimensional

H-1-H-1, and H-1-C-13 nuclear magnetic resonance spectrometry. The fatty acyl substituents were mainly of three types: saturated straight-chain C16-C19 acids; C21-C25 "mycosanoic acids" and C24-C28 "mycolipanolic acids." Analysis of one of the major 2,3-di-O-acyltrehaloses by two-dimensional H-1-chemical shift correlated and H-1-detected heteronuclear multiple-bond correlation spectroscopy established that the C18 saturated straight-chain acyl group was located at the 2 position and that the C24 mycosanoyl substituent was at the 3 position of the same "right-hand" glucosyl residue. At least six molecular species differing only in their fatty acid content comprised this family of di-O-acylated trehaloses. We regard these acyltrehaloses as elemental forms of the multiglycosylated acyltrehaloses (the lipooligosaccharides) perhaps due to an inability of the majority of isolates of virulent tubercle bacilli to glycosylate core acyltrehaloses. The acyltrehaloses are minor but consistent components of virulent *M. tuberculosis* and apparently the basis of the specific serological activity long associated with its lipid fractions.—Authors' Abstract

Biehle, J. and Cavalieri, S. J. In vitro susceptibility of *Mycobacterium kansasii* to clarithromycin. Antimicrob. Agents Chemother. **36** (1992) 2039–2041.

The MICs of the macrolide, clarithromycin, for 31 clinical isolates of *Mycobacterium kansasii* were determined by three different methods. The methods employed were the proportion resistance method on 7H10 agar, the radiometric (BACTEC) method, and the T100 method of datum analysis. All methods gave similar results. The MICs were in a narrow range from 0.16 to 0.50 μg/ml, with the MICs for 90% of isolates tested of 0.50 μg/ml for the agar dilution and radiometric methods and 0.37 μg/ml for the T100 method. The MBCs were determined for nine representative isolates by the radiometric broth method. The MBCs were equal to the MICs for four isolates, and the MBCs were twofold higher than the MICs for five isolates. Killing of 99.9% of the bacterial population was achieved at a clarithromycin concentration of 2.0 μg/ml for all nine isolates tested.—Authors' Abstract

Bollet, C., Delamballerie, X., Zandotti, C., Vignoli, C., Gevaudan, M. J. and Demicco, P. Detection and identification of *Mycobacterium tuberculosis, M. bovis* BCG, and *M. avium* by 2-step polymerase chain reaction—comparison with ELISA using A60 antigen. Microbiologica **15** (1992) 345–350.

We propose a rapid two-step polymerase chain reaction (PCR) to amplify a 767-bp sequence present in the gene coding for the 65-kDa antigen of mycobacteria. The high G+C content (80%) permitted annealing to occur at 70°C, enhancing the specificity. The amplified fragment contains a restriction site for differentiation between *Mycobacterium tuberculosis, M. bovis*/BCG, and *M. avium.* Complete diagnosis can be achieved in less than 4 hr without labeled probe or nucleic acid transfer.—Authors' Abstract

Boom, W. H., Chervenak, K. A., Mincek, M. A. and Ellner, J. J. Role of the mononuclear phagocyte as an antigen-presenting cell for human γδ T cells activated by live *Mycobacterium tuberculosis.* Infect. Immun. **60** (1992) 3480–3488.

Gamma-delta (γδ) T cells, both human and murine, have been found to be highly responsive to mycobacterial antigens. However, the role and function of γδ T cells in the immune response to *Mycobacterium tuberculosis* remain largely unknown. In earlier studies, we demonstrated that monocytes infected with live *M. tuberculosis* were particularly effective inducers of human peripheral blood γδ T cells. The present studies were performed to further characterize the interaction between human mononuclear phagocytes, γδ T cells, and live *M. tuberculosis,* in comparison with CD4+ T cells. First, we found that resting γδ T cells expanded *in vitro* by live *M. tuberculosis* were specific for *M. tuberculosis,* and that heat killing and washing the mycobacteria removed the antigen(s) for γδ T cells. In contrast, the heat-killed mycobacteria retained significant antigenicity for CD4+ T cells. Second, live *M. tuberculosis*-expanded γδ T cells from healthy tuberculin-positive

donors did not respond significantly to the antigens in *M. tuberculosis* culture filtrate, including the 65- and 71-kDa mycobacterial heat-shock proteins. Third, the activation of γδ T cells by live mycobacteria was dependent on antigen-presenting cells, and mononuclear phagocytes were found to be very efficient antigen-presenting cells both for resting peripheral blood γδ T cells and for activated expanded γδ T cells. The mononuclear phagocyte carried the necessary costimulatory factors necessary for γδ T-cell proliferation. Fourth, the antigen repertoire and HLA requirements for CD4+ memory T cells and those for γδ T cells appear to be quite distinct from each other. CD4+ T cells recognized both soluble protein antigens and whole organisms in a class II major histocompatibility complex-restricted manner; whereas γδ T cells appeared to recognize only constituents associated with the whole organism and were not restricted by class I or class II major histocompatibility complex molecules. Finally, the assay system described to expand and purify responding CD4+ and γδ T cells after stimulation with live *M. tuberculosis* represented a simple approach to the direct comparison of these two T-cell populations in the interaction with mononuclear phagocytes infected with *M. tuberculosis*. Such studies provide insight not only into the relative roles of human CD4+ and γδ T cells in the human immune response to intracellular bacterial pathogens such as *M. tuberculosis* but also into the basic biologic role of human γδ T cells in antimicrobial immunity.—Authors' Abstract

Brown, B. A., Wallace, R. J. and Onyi, G. O. Activities of clarithromycin against 8 slowly growing species of nontuberculous mycobacteria, determined by using a broth microdilution MIC system. Antimicrob. Agents Chemother. **36** (1992) 1987–1990.

MICs of clarithromycin against 324 clinical isolates belonging to eight species of slowly growing nontuberculous mycobacteria were inoculated into twofold drug dilutions in Middlebrook 7H9 broth (pH corrected to 7.4) and then incubated at 30°C for 7 days for *Mycobacterium marinum* and for 14 days for all other species. The MIC for 90% of the strains (MIC90) was ≤ 0.5 μg/ml for isolates of *M. gordonae* (6 strains), *M. scrofulaceum* (5 strains), *M. szulgai* (6 strains), and *M. kansasii* (35 strains). MICs for *M. marinum* (25 strains) and *M. avium* complex (237 strains) were higher, but 100% and 89% of the strains, respectively, were susceptible to ≤ 4 μg/ml. In contrast, MICs for 5 of 6 *M. simiae* strains were > 8 μg/ml, and the range of MICs for *M. chromogenicum* varied from ≤ 0.125 to 8 μg/ml. For the 237 isolates of *M. avium* complex, the MIC50 was 2 μg/ml and for the MIC90 was 8 μg/ml. MICs for most isolates (77%) were in the 1–4 μg/ml range. For the 80 isolates in this group known to be from AIDS patients, the MIC50 was 4 μg/ml. These MIC studies combined with preliminary clinical trials suggest that clarithromycin may be useful for drug therapy of most species of the slowly growing nontuberculous mycobacteria except *M. simiae*.—Authors' Abstract

Butler, W. R., Thibert, L. and Kilburn, J. O. Identification of *Mycobacterium avium* complex strains and some similar species by high-performance liquid chromatography. J. Clin. Microbiol. **30** (1992) 2698–2704.

Strains of *Mycobacterium avium, M. intracellulare, M. scrofulaceum, M. xenopi,* and *M. gordonae* were identified by high-performance liquid chromatography (HPLC) analysis of mycolic acids as bromophenacyl esters. HPLC criteria were used to develop a flow chart identification scheme, which was evaluated in our laboratory with a set of 234 strains representing five species and a hitherto undescribed species. Correct identifications of *M. gordonae* and *M. xenopi* were easily made. Flow chart differentiation of *M. avium, M. intracellulare,* and *M. scrofulaceum* was done with 97.9%, 97.5%, and 89.2% accuracies, respectively. Independent evaluation of the flow chart at a separate laboratory demonstrated an overall identification accuracy of 97% for *M. avium* complex. Strains that have been described biochemically as being intermediate between *M. avium–M. intracellulare* and *M. scrofulaceum* were identified as one or the other of these known species. Strains which were negative with the species-spe-

cific radioactive probe for *M. avium* complex but which were positive with the nonradioactive SNAP X probe were usually identified as *M. intracellulare* and *M. scrofulaceum* but rarely as *M. avium.*—Authors' Abstract

Chawla, P. K., Klapper, P. J., Kamholz, S. L., Pollack, A. H. and Heurich, A. E. Drug-resistant tuberculosis in an urban population including patients at risk for human immunodeficiency virus infection. Ann. Rev. Respir. Dis. **146** (1992) 280–284.

In the past 5 years, an increased incidence of tuberculosis has been noted in the United States. Simultaneously, the population infected with human immunodeficiency virus-type I (HIV-I) and the number of cases of acquired immunodeficiency syndrome (AIDS) have increased. Selected areas of the United States have also reported increases in the frequency of drug-resistant isolates of *Mycobacterium tuberculosis.* Because our institution serves a population in which tuberculosis, AIDS, and drug-resistant isolates of *M. tuberculosis* are frequently encountered, we sought to better define interrelationships among these factors by retrospectively reviewing the demographic, clinical, bacteriologic, and radiologic data for all adult patients in whom *M. tuberculosis* was isolated from a culture of respiratory-tract secretions during a 1-year period (June 1, 1988 to May 31, 1989). Two-hundred-forty-six patients were thus identified; 66.5% were U.S. born blacks, and 62.6% were 17 to 40 years of age. Risk factors for HIV infection were present in 106 patients.

The overall resistance rate (one or more drugs) = 30.9%, with primary resistance = 22.6% (35 of 155) and secondary resistance = 49.2% (29 of 59). In addition, 12 resistant isolates were found in 32 patients whose prior treatment status was indeterminate. Of the resistant isolates, 56.6% (43 of 76) were multiply resistant. Isoniazid resistance was noted in 90.7% (69 of 76) and rifampin resistance was noted in 50% (38 of 76) of the resistant isolates. No significant differences in the overall frequency of resistance were noted in patients at risk for HIV infection compared with those without these risks. Prior treatment with antituberculosis medications was highly predictive of drug resistance ($p < 0.01$). The finding of a positive direct Ziehl-Neelsen stain of respiratory-tract secretions was strongly correlated with pansensitive disease ($p < 0.03$). In areas with a high prevalence of single-drug- and multidrug-resistant tuberculosis, the adoption of an initial four-drug therapeutic regimen (isoniazid, rifampin, pyrazinamide, ethambutol) must be recommended.—Authors' Abstract

Cho, S.-N., Shin, J. S., Daffé, M., Chong, Y., Kim, S. K. and Kim, J. D. Production of monoclonal antibody to a phenolic glycolipid of *Mycobacterium tuberculosis* and its use in detection of the antigen in clinical isolates. J. Clin. Microbiol. **30** (1992) 3065–3069.

A monoclonal antibody (MAbIII604) specific to phenolic glycolipid Tb (PGL-Tb), a *Mycobacterium tuberculosis*-specific antigen, was produced and used in the detection of the antigen. MAbIII604 reacted with the PGL-Tb antigen but not with other phenolic glycolipids from *M. leprae, M. bovis,* and *M. kansasii,* thus indicating the specificity of the monoclonal antibody to PGL-Tb. A dot enzyme-linked immunosorbent assay with MAbIII604 was employed to detect the PGL-Tb antigen in lipids purified from *M. tuberculosis* clinical isolates. Of 50 isolates, 32 (64.0%) showed clear evidence of the PGL-Tb antigen by the dot enzyme-linked immunosorbent assay, but there were marked variations in the intensities and sizes of spots. This suggests differences in PGL-Tb antigen production among *M. tuberculosis* strains even when they are grown in the same culture media and conditions. This was most evident from the fact that in only eight (16.0%) of the isolates examined was the PGL-Tb antigen detectable by thin-layer chromatography, which is much less sensitive for the detection of glycolipid antigens. This study shows that monoclonal antibodies specific to PGL-Tb are useful in detecting the antigen in lipid extracts and that there is a marked variation in the PGL-Tb production among *M. tuberculosis* clinical isolates.—Authors' Abstract

Chu, C. Q., Field, M., Andrew, E., Haskard, D., Feldmann, M. and Maini, R. N. Detection of cytokines at the site of tuberculin-induced delayed-type hypersensitivity in man. Clin. Exp. Immunol. **90** (1992) 522–529.

Cytokines are chiefly local mediators which play an important role in the regulation of the cell–cell interactions which may be involved in the development of the delayed-type hypersensitivity (DTH) reaction. Using immunohistochemical techniques, the presence of IL-1α, IL-1β, IL-6, interferon-gamma (IFN-gamma) and tumor necrosis factor-alpha (TNF-alpha) in the skin in tuberculin-purified protein derivative (PPD)-induced DTH reactions was investigated in six normal individuals. Cells staining for these cytokines were first observed 6 hr after PPD challenge, and they were detected throughout the duration of the 7-day experiment. The number of cells staining for IFN-gamma reached a peak at 48 hr, where 33% of the total aggregate cells were positive, but declined thereafter to 3% at day 7. On the other hand, the number of cells staining for TNF-alpha and IL-1 persisted at high levels throughout the observation period of 7 days (e.g., at 48 hr and thereafter, about 40% cells positive for TNF-alpha and 20% for IL-1α and IL-1β). Double immunofluorescence and staining on sequential sections showed that IFN-gamma-staining cells were CD3+ T cells; TNF-alpha, IL-1 and IL-6 staining cells were mainly of the CD68+ macrophages/monocytes; and that 80% of the CD1a+ cells (Langerhans-like cells) in the dermis contained TNF-alpha and IL-1. The presence of these cytokines at the site of inflammation suggests that they may be locally produced by the inflammatory cells. Their persistence during the reaction suggests that they are intimately associated with this response, and are involved in the development of the reaction.—Authors' Abstract

Cohen, Y., Perronne, C., Truffot-Pernot, C., Grosset, J., Vilde, J. L. and Pocidalo, J. J. Activities of WIN-57273, minocycline, clarithromycin, and 14-hydroxy-clarithromycin against *Mycobacterium avium* complex in human macrophages. Antimicrob. Agents Chemother. **36** (1992) 2104–2107.

The activities of the fluoroquinolone WIN-57273, 14-OH clarithromycin (a human metabolite of clarithromycin), and minocycline against two virulent strains of *Mycobacterium avium* complex were evaluated in a model of intracellular infection and compared with that of clarithromycin. Human monocyte-derived macrophages were infected at day 6 of culture. Intracellular CFU at 60 min and intracellular and supernatant CFU on days 4 and 7 were counted after inoculation. The concentrations used, which were equal to peak levels in serum, were 3 μg of WIN-57273 per ml (MICs for the two strains, 1 μg/ml), 4 μg of 14-OH clarithromycin per ml (MICs, 8 and 2 μg/ml, respectively, at pH 7.4), 4 μg of minocycline per ml (MICs, 64 and 32 μg/ml, respectively), and 4 μg of clarithromycin per ml (MICs, 2 and 0.5 μg/ml, respectively, at pH 7.4). On day 7, compared with controls, WIN-57273, minocycline ($p < 0.02$), clarithromycin, or different combinations of clarithromycin and the other drugs ($p < 0.001$) slowed the intracellular replication of strain MO-1. 14-OH clarithromycin ($p < 0.02$), clarithromycin ($p < 0.02$), 14-OH clarithromycin plus clarithromycin ($p < 0.01$), clarithromycin plus minocycline, or clarithromycin plus minocycline plus 14-OH clarithromycin ($p < 0.001$) slowed the intracellular replication of strain LV-2. WIN-57273 was less effective than clarithromycin against strain MO-1 ($p < 0.05$). Clarithromycin plus 14-OH clarithromycin plus minocycline ($p < 0.02$) was more effective than clarithromycin alone against strain LV-2. Thus, clarithromycin plus minocycline, which corresponds in humans to three active molecules, may exhibit a better efficacy than clarithromycin in this model.—Authors' Abstract

Coyle, M. B., Carlson, L. D. C., Wallis, C. K., Leonard, R. B., Raisys, V. A., Kilburn, J. O., Samadpour, M. and Bottger, E. C. Laboratory aspects of *Mycobacterium genavense*, a proposed species isolated from AIDS patients. J. Clin. Microbiol. **30** (1992) 3206–3212.

"*Mycobacterium genavense*" is a proposed new species recently reported to cause disseminated infections in 18 patients with AIDS in Europe. We have recovered "*M. genavense*" as slowly growing fastidious mycobacteria in blood cultures of seven patients with AIDS. In the original studies of "*M. genavense*," the fastidious organism grew only in BACTEC 13A vials. The Seattle, Washington, isolates of "*M. genavense*" also failed to grow when subcultured from 13A vials to routine solid media, but dysgonic colonies were produced on Middlebrook 7H11 agar supplemented with mycobactin J. The mycolic acid pattern of patients' isolates closely resembled that of the type strain of *M. simiae* when analyzed by one- and two-dimensional thin-layer chromatography and by high-performance liquid chromatography. Whole-cell fatty-acid analyses by gas-liquid chromatography distinguished the isolates from *M. simiae* but misidentified them as *M. fortuitum*. Sequence determinations of the hypervariable regions of the 16S rRNA gene indicate that these organisms belong to the recently proposed new species "*M. genavense*." Growth from Middlebrook 7H11 agar supplemented with mycobactin J consistently yielded positive tests for catalase (semiquantitative and at 68°C), pyrazinamidase, and urease which enable mycobacteriology laboratories to presumptively identify "*M. genavense*" without nucleic acid analyses. The failure of "*M. genavense*" to grow on conventional mycobacterial solid media suggests that mycobacterial blood cultures should include a broth medium incubated for at least 8 weeks.—Authors' Abstract

Crowle, A. J., Ross, E. R., Cohn, D. L., Gilden, J. and May, M. H. Comparison of the abilities of *Mycobacterium avium* and *M. intracellulare* to infect and multiply in cultured human macrophages from normal and human immunodeficiency virus-infected subjects. Infect. Immun. **60** (1992) 3697–3703.

Patients with AIDS commonly develop disseminated infections with *Mycobacterium avium* (MA) but not its close relative, *M. intracellulare* (MI). In non-AIDS patients who have these infections, the two species are about equally distributed. The higher incidence of infection with MA than with MI in AIDS patients might be due to the selective susceptibility of these patients to MA. This possibility was tested by comparing the abilities of MA and MI to infect and replicate in cultured macrophages from normal subjects and from patients with AIDS-related complex or AIDS. The macrophages were cultured in medium supplemented with 1% or 5% normal or patient sera or with 1% defined serum substitute. Replication of MA (serovar 4) or MI (serovars 16 and 17) in the macrophages was measured by CFU counts made from lysed samples of the macrophages taken at 0, 4, and 7 days after macrophage infection. MA and MI in infected normal macrophages which were cultured in normal serum replicated in these macrophages at similar rates. MA but not MI multiplied abnormally rapidly in patient macrophages cultured in either normal serum or patient serum. The accelerated growth of MA in patient macrophages was macrophage dependent, because patient sera did not change the rate of MA replication in culture medium lacking macrophages. However, patient sera did increase the permissiveness of normal macrophages to MA but not to MI. These results suggest that a selective increased susceptibility to MA compared with a retained normal resistance to MI in human-immunodeficiency-virus-infected patients as they progress from AIDS-related complex to AIDS accounts for the higher prevalence of MA than MI infection in AIDS patients. The results also indicate that the mechanisms of native resistance in human macrophages to MA and MI are different.—Authors' Abstract

Dalovisio, J. R., Stetter, M. and Mikota-wells, S. Rhinoceros' rhinorrhea—cause of an outbreak of infection due to airborne *Mycobacterium bovis* in zookeepers. Clin. Infect. Dis. **15** (1992) 598–600.

Seven of 24 zookeepers exposed to a Southern white rhinoceros infected with *Mycobacterium bovis* were presumably infected via aerosols generated in the cleaning of the barn for the rhinoceros. All demonstrated conversion by the intermediate-

strength purified-protein-derivative skin test, but none had clinical illness. In certain occupational settings like zoos and abattoirs, exposure to *M. bovis* may be an occupational hazard, and routine periodic tuberculin screening should be performed.—Authors' Abstract

Das, S., Cheng, S. H., Lowrie, D. B., Walker, K. B., Mitchison, D. A., Vallishayee, R. S. and Narayanan, P. R. The pattern of mycobacterial antigen recognition in sera from Mantoux-negative individuals is essentially unaffected by bacille Calmette-Guerin (BCG) vaccination in either South India or London. Clin. Exp. Immunol. **89** (1992) 402–406.

Paired sera were obtained before and 8 weeks after routine BCG vaccination from 20 PPD-S Mantoux-negative individuals who were living adjacent to the Chingleput BCG vaccine trial area in Tamil Nadu, South India, and from seven Mantoux-negative school-children in London, UK. Most subjects became Mantoux-positive after vaccination. In ELISA tests against soluble extracts of BCG or *Mycobacterium tuberculosis* H37Rv or against PPD-S, pre-vaccination antibody titers of South Indian subjects were about twice those of British subjects but there was no increase in titer of antibodies after vaccination of either population. Western blotting showed that even before vaccination, and even in British subjects, antibodies were present that recognized numerous antigenic components in extracts of BCG and *M. tuberculosis.* There was no consistent difference between band patterns with South Indian and British subjects and any effect of vaccination on the patterns was minimal.—Authors' Summary

Dastidar, S. G. and Chakrabarty, A. N. Viability of acid-fast bacilli from gamma-irradiated and UV-irradiated lepromatous armadillo tissues infected with *Mycobacterium leprae.* Indian J. Med. Res. [A] **95** (1992) 263–269.

Gamma-irradiated splenic homogenates of armadillos infected with *Mycobacterium leprae* proved sterile by conventional tests and media. However, on media for chemoautotrophy, these could repeatedly grow as a single type of acid-fast nocardioform bacterium like the unirradiated specimens, although with a much reduced count. In the slide culture, transition from the initial AFB/coccoid bodies to sporulating mycelia and granules in the final stage could be observed sequentially. The gamma-irradiated tissue specimens failed to yield any other mycobacterium/corynebacterium tested according to standard protocols.—Authors' Abstract

Decock, K. M., Soro, B., Coulibaly, I. M. and Lucas, S. B. Tuberculosis and HIV infection in sub-saharan Africa. JAMA **268** (1992) 1581–1587.

Objectives.–To review the epidemiologic, clinical, and pathological characteristics and the public health implications of human immunodeficiency virus (HIV)-associated tuberculosis in sub-saharan Africa.

Data Sources.—Published medical literature (English and French) and proceedings of international and African conferences on the acquired immunodeficiency syndrome (AIDS).

Study Selection.—Selection by the authors of articles most pertinent to HIV infection and tuberculosis in Africa and internationally.

Data Extraction.—Direct reporting of quantitative data (e.g., HIV seroprevalence levels) and of qualitative descriptions and conclusions from selected literature.

Data Synthesis.—High rates (20% to 67%) of HIV infection in patients with tuberculosis have been reported from east, west, central, and southern Africa. An increase in tuberculosis cases has been reported at the same time as the emergence of AIDS in several countries. Autopsies in Abidjan, Ivory Coast (Cote d'Ivoire) have shown tuberculosis as the most frequent opportunistic infection in patients dying of AIDS. Clinical differences in patients with tuberculosis who were HIV-positive and HIV-negative are reviewed, the most important being a greatly increased mortality rate in HIV-associated disease. Access to HIV testing is required for firm diagnosis, for clinical care and counseling, and for public health surveillance.

Conclusions.—The epidemiology of tuberculosis has been profoundly influenced by the epidemic of HIV infection in sub-saharan Africa. Greatly increased human and material resources are required for this neglected problem in international health.—Authors' Abstract

Desousa, J. P. C. and Rastogi, N. Comparative ability of human monocytes and macrophages to control the intracellular growth of *Mycobacterium avium* and *Mycobacterium tuberculosis*—effect of interferon-gamma and indomethacin. FEMS Microbiol. Lett. **89** (1992) 329–334.

Intracellular growth of *Mycobacterium avium* and *M. tuberculosis* H37Rv was compared both in human peripheral blood monocytes and in cultured macrophages. The cells were treated with 300 U of human recombinant interferon-gamma (IFN-gamma) either 48 hr prior to phagocytosis or after infection. In some cases, indomethacin (IND, a potent inhibitor of prostaglandin-E2 synthesis) was added immediately after infection of macrophages. IFN-gamma pretreatment of monocytes resulted in about 50% lesser uptake of both pathogens, but had no effect in macrophages. Macrophages, as compared to monocytes, were more permissive to *M. avium* growth, suggesting that monocytes may be innately more efficient in controlling the intracellular growth of this pathogen. About tenfold higher growth of *M. avium* as compared to *M. tuberculosis* was observed in both culture systems. IFN-gamma-treatment alone did not confer any anti-*M. avium* activity to monocytes and macrophages alike and addition of IND did not change this unresponsiveness. In the case of *M. tuberculosis,* the IFN-gamma treatment alone endowed both monocytes and macrophages with significant bacteriostatic activity which was further potentiated by the addition of IND. These observations show innate differences in the ability of human monocytes and macrophages to control the growth of two major mycobacterial pathogens and the immunoregulatory mechanisms involved.—Authors' Abstract

Doran, T. J., Hodgson, A. L. M., Davies, J. K. and Radford, A. J. Characterisation of a novel repetitive DNA sequence from *Mycobacterium bovis.* FEMS Microbiol. Lett. **96** (1992) 179–186.

We report characterization of three copies of a novel repeat sequence isolated from a *Mycobacterium bovis* genomic library. The repeat occurs within open reading frames, potentially encoding a conserved tandem array of a pentapeptide sequence with the consensus X-Gly-Asn-X-Gly. The tandem array is present up to five times in *M. bovis,* and it is proposed that they may occur in a family of genes expressing functionally related proteins. We postulate that these proteins may play a role in binding of *M. bovis* to host cell receptors.—Authors' Abstract

Elsaghier, A., Lathigra, R. and Ivanyi, J. Localisation of linear epitopes at the carboxy-terminal end of the mycobacterial 71 kDa heat shock protein. Mol. Immunol. **29** (1992) 1153–1156.

Four distinct linear epitopes localized within species-specific sequences at the carboxy-terminal end of the 71-kDa heat-shock protein of *Mycobacterium tuberculosis* have been identified by scanning 94 overlapping peptides with 13 human sera. One epitope ("C") of entirely *M. tuberculosis*-specific core sequence (GEAGPG) has been found immunogenic in smear-negative tuberculosis, but not in nontuberculous mycobacterial diseases. This peptide appears to be a valuable candidate for further serodiagnostic evaluation.—Authors' Abstract

Elsaghier, A., Prantera, C., Moreno, C. and Ivanyi, J. Antibodies to *Mycobacterium paratuberculosis*-specific protein antigens in Crohn's disease. Clin. Exp. Immunol. **90** (1992) 503–508.

The possible role of infection with *Mycobacterium paratuberculosis* (MAP) for the etiopathogenesis of Crohn's disease (CD) has been a matter of long-term controversy. In addition to similarities with the pathology of ruminant paratuberculosis, DNA fingerprinting confirmed the organism isolated from gut tissue, but the specificity of the immune repertoire has not as yet been evaluated. We report here on a serological study of 29 patients with CD, 20 patients with

ulcerative colitis, and 18 healthy control subjects, using three antigens attributed with species-specificity and selective immunogenicity following MAP infection. Antibodies binding to the 38-kDa band of MAP extract were demonstrable by the Western blot technique in 57% of CD patients. Antibody levels to the 24-kDa (p24BCD) cathodic bands, determined by competition ELISA using a monospecific murine antiserum, and to the 18-kDa protease-resistant purified bacterioferritin, detected by standard ELISA, were significantly elevated in 53% of CD patients. However, these three antibody specificities tested in individual CD patients did not show any correlation with each other. Thus, 18% of patients were positive for all three specificities, while 84% had antibodies to at least one of the specific antigens. Although the exact proportion of affected patients is yet to be defined, the serological results obtained support the view that MAP infection may play an etiological role in Crohn's disease.—Authors' Abstract

Esaguy, N., Macedo, P. M., Castro, A. P. and Aguas, A. P. Acquisition of autoimmunity genes by New Zealand mice is associated with natural resistance to infection by mycobacteria. J. Autoimmun. **5** (1992) 641–651.

New Zealand (NZ) mouse strains comprise both autoimmune and nonautoimmune animals: NZ black (NZB) mice and the F_1 hybrid (NZB/W) of NZB and NZ white (NZW) mice show spontaneous autoimmune disease by 6 months of age and die before the first year of age from renal disease, while NZW mice do not show autoimmune disorders. We investigated whether the autoimmunity-prone NZ animals (NZB and NZB/W) differ from the nonautoimmune NZW mice in susceptibility/resistance to mycobacterial infection. The three groups of NZ mice were infected by intraperitoneal inoculation of 10^8 colony forming units (cfu) of *Mycobacterium avium*. The *M. avium* infection was induced in 3-month-old mice (i.e., before NZB and NZB/W mice develop autoimmune disease) and studied for 4 months. Infected NZB and NZB/W mice showed evidence of renal disease at 2 and 4 months of infection (but not as 1 month). The nonautoimmune NZW mice were found to be susceptible to *M. avium* since they allowed massive proliferation (4–5 log growth) of the bacilli in liver and spleen. In contrast, both groups of autoimmunity-prone mice (NZB and NZB/W) were resistant to *M. avium* since their mycobacterial loads remained below the value of the initial inoculum. We conclude that in NZ mice the acquisition of autoimmunity genes is associated with expression of natural resistance to mycobacterial infection. This is consistent with the view that autoimmunity genes may have been evolutionarily selected because of their association with increased resistance of the host to infections by intracellular parasites.—Authors' Abstract

Evans, K. D., Nakasone, A. S., Sutherland, P. A., Delamaza, L. M. and Peterson, E. M. Identification of *Mycobacterium tuberculosis* and *Mycobacterium avium–intracellulare* directly from primary BACTEC cultures by using acridinium-ester-labeled DNA probes. J. Clin. Microbiol. **30** (1992) 2427–2431.

Identification of members of the *Mycobacterium tuberculosis* complex and the *M. avium–M. intracellulare* complex (MAC) directly from primary BACTEC cultures was evaluated by using acridinium-ester-labeled DNA probes (AccuProbe; GenProbe, Inc., San Diego, California, U.S.A.). In preliminary experiments, blood present in samples was found to interfere with the assay because of nonspecific chemiluminescence, which was measured in relative light units (RLUs). There was a direct relationship between the age of the culture and the number of nonspecific RLUs. A protocol using 1% sodium dodecyl sulfate–5 mM EDTA to treat BACTEC broth cultures which, with specimens containing blood, gave on the average a ninefold reduction in nonspecific chemiluminescence was developed. By using this treatment protocol, 120 specimens were tested directly from BACTEC broth cultures with an AccuProbe for the *M. tuberculosis* complex and/or the MAC. In order to establish the background of the specimen, the patient sample was assayed without probe. The criteria for the inclusion of BACTEC cultures in the evaluation were a growth index of ≥ 100 and a positive

smear for acid-fast bacilli directly from the BACTEC broth. For the 120 cultures tested, if a hybridization result of ≥ 30,000 RLUs was considered positive, the sensitivities for detecting the *M. tuberculosis* complex and the MAC were 47% and 90%, respectively, with a specificity of 100% for both. However, if a ratio of the RLUs obtained with the MAC or the *M. tuberculosis* complex probe to those obtained with the specimen background of ≥ 20% was considered positive, this gave 77% sensitivity and 100% specificity for BACTEC cultures containing *M. tuberculosis* complex isolates and 96% sensitivity and 100% specificity for those growing MAC isolates.—Authors' Abstract

Fauvilledufaux, M., Vanfleteren, B., Dewit, L., Vincke, J. P., Van Vooren, J. P., Yates, M. D., Serruys, E. and Content, J. Rapid detection of tuberculous and non-tuberculous mycobacteria by polymerase chain reaction amplification of a 162 bp DNA fragment from antigen-85. Eur. J. Clin. Microb. Infect. Dis. **11** (1992) 797–803.

A polymerase chain reaction (PCR) assay was developed for detection of mycobacteria using amplification of a 162 bp region of the genes coding for the mycobacterial antigen 85 complex. Strains belonging to the *Mycobacterium tuberculosis* complex were further differentiated from nontuberculous mycobacteria by hybridization of the PCR-derived Southern blot with an internal oligonucleotide probe and washing under stringent conditions. The method allowed rapid and sensitive detection of mycobacterial DNA in uncultured clinical samples. PCR results obtained for *M. tuberculosis* in 206 specimens from 180 untreated patients gave a sensitivity of 93.9% and a specificity of 94.3% compared with the culture. PCR detected DNA from *M. tuberculosis* in seven samples from patients with clinically evident tuberculosis in whom culture was negative. The results suggest that this PCR assay could be used for early and specific diagnosis of tuberculosis.—Authors' Abstract

Flynn, J. L., Goldstein, M. M., Triebold, K. J., Koller, B. and Bloom, B. R. Major histocompatibility complex class I-restricted T cells are required for resistance to *Mycobacterium tuberculosis* infection. Proc. Natl. Acad. Sci. U.S.A. **89** (1992) 12013–12017.

Mice with a targeted disruption in the β_2-microglobulin (β_2m) gene, which lack major histocompatibility complex class I molecules and consequently fail to develop functional CD8 T cells, provided a useful model for assessing the role of class I-restricted T cells in resistance to infection with virulent *Mycobacterium tuberculosis.* Of mutant β_2m−/− mice infected with virulent 10^6 *M. tuberculosis,* 70% were dead or moribund after 6 weeks, while all control mice expressing the β_2m gene remained alive for >20 weeks. Granuloma formation occurred in mutant and control mice, but far greater numbers of tubercle bacilli were present in the lungs of mutant mice than in controls, and caseating necrosis was seen only in β_2m−/− lungs. In contrast, no differences were seen in the course of infection of mutant and control mice with an avirulent vaccine strain, bacille Calmette-Guérin (BCG). Immunization with BCG vaccine prolonged survival of β_2m−/− mice after challenge with *M. tuberculosis* for 4 weeks but did not protect them from death. These data indicate that functional CD8 T cells, and possibly T cells bearing $\gamma\delta$ antigen receptor, are a necessary component of a protective immune response to *M. tuberculosis* in mice.—Authors' Abstract

Fuursted, K., Askgaard, D. and Faber, V. Susceptibility of strains of the *Mycobacterium tuberculosis* complex to fusidic acid. APMIS **100** (1992) 663–667.

The activity of fusidic acid was studied in 40 strains of *Mycobacterium tuberculosis* (of which 20 strains were mono- or multiresistant to standard antituberculosis drugs) and 10 strains of *M. bovis.* Minimum inhibitory concentration (MIC) was determined by the radiometric (BACTEC) broth method. The MIC for the 50 strains varied between 8 mg/l and 32 mg/l, with a MIC90 of 16 mg/l for *M. tuberculosis* and a MIC90 of 32 mg/l for *M. bovis.* Minimal bactericidal concentration (MBC, defined as the lowest concentration of fusidic acid which killed 99% or more of the population) varied between 32 mg/l and 500 mg/l, with a

MBC90 of 250 mg/l for *M. tuberculosis* and 500 mg/l for *M. bovis.* No cross-resistance to other antituberculosis drugs (ethambutol, isoniazid, rifampin, streptomycin, pyrazinamide, ofloxacin, ciprofloxacin) was observed as strains resistant to one or more standard antituberculosis drugs were as susceptible to fusidic acid as sensitive strains of *M. tuberculosis.* No synergism or antagonism could be demonstrated when fusidic acid was combined with either ethambutol, isoniazid, rifampin or streptomycin against strains of *M. tuberculosis* resistant to one or more standard antituberculosis drugs. Addition of pooled human serum to the medium increased both MIC and MBC by factors of 4 and 8 at serum concentrations of 10% and 50%, respectively. Single-step mutation to high-level resistance to fusidic acid at a frequency of $< 1.7 \times 10^{-8}$ could be readily selected at four times the MIC. These fusidic-acid-resistant organisms had a generation time 2.0–2.7 times longer than their parent organisms.—Authors' Abstract

Gautier, N., Marin, L. M. L., Lanéelle, M. A. and Daffé, M. Structure of mycoside-F, a family of trehalose-containing glycolipids of *Mycobacterium fortuitum.* FEMS Microbiol. Lett. **98** (1992) 81–87.

Nuclear magnetic resonance spectroscopy, fast-atom bombardment mass spectrometry, gas chromatography–mass spectrometry, as well as chemical degradations were used to elucidate the structure of the major glycolipids of *Mycobacterium fortuitum.* Three main glycoconjugates were detected and their structures established as 2,3-diacyl, 2,3,4- and 2,3,6-triacyl trehalose. The characteristic infrared spectrum which led to their original designation as mycoside-F, a family of glycolipids limited in distribution to *M. fortuitum,* was due to the nature of the fatty acyl substituents identified primarily as 2-methyl-octadecen-2-oyl. The antigenic glycolipids typified the biovar. *fortuitum,* thus allowing its easy recognition from the C-mycoside glycopeptidolipid-containing biovar. *peregrinum.*—Authors' Abstract

Gilot, P., DeKesel, M., Coene, M. and Cocito, C. Induction of cellular immune reactions by A36, an antigen complex of *Mycobacterium paratuberculosis*—comparison of A36 and Johnin components. Scand. J. Immunol. **36** (1992) 811–821.

Paratuberculosis (Johne's disease) is a chronic enteritis syndrome of ruminants, which is due to infection by *Mycobacterium paratuberculosis.* Cutaneous testing with proteins extracted from a mycobacterial culture fluid (johnin-PPD) is currently used to evaluate the cellular immune status. We have compared the components of johnin-PPD with those of the A36 complex, a thermostable macromolecular antigen (TMA) present in the cytoplasm and associated with the cell wall of *M. paratuberculosis.* The presence in the johnin-PPD of 15 A36 components has been shown by Western blotting. Moreover, monoclonal antibodies, which bind respectively to the 65-kDa *M. leprae* heat-shock protein, the 28-kDa *M. leprae* superoxide dismutase, and *M. tuberculosis* lipoarabinomannan, recognized components of the johnin-PPD. The ability of A36 to trigger delayed hypersensitivity reactions in sensitized rabbits, and to induce the proliferation of T lymphocytes from the lymph nodes of A36-sensitized mice, matched that of johnin-PPD. The homology levels of T epitopes between A36 and the TMA complexes of *M. phlei, M. bovis, M. tuberculosis* and *M. avium* were estimated, in a lymphoproliferation assay, to be 51%, 52%, 59% and 94%, respectively. A strong crossreactivity of A36 with an *M. leprae* sonicate was also observed by cutaneous testing. The A36 components within the 45.2–26.8-kDa and the 21.6–19.8-kDa ranges were proved to induce the proliferation of T lymphocytes from sensitized mice. This work supports the possible use of the A36 complex, and of some of its components, for cutaneous tests and lymphocyte proliferation assays, in order to monitor cellular immunity in Johne's disease.—Authors' Abstract

Harboe, M. and Wiker, H. G. The 38-kDa protein of *Mycobacterium tuberculosis*—a review. J. Infect. Dis. **166** (1992) 784–884.

This review illustrates that the 38-kDa protein is one of the most important anti-

gens of *Mycobacterium tuberculosis.* It is actively secreted but partly attached to the surface of the mycobacterial cell by a lipid tail that may also be responsible for binding of carbohydrate to the protein. It is a major constituent of *M. tuberculosis* culture fluid after growth on the synthetic Sauton medium and occurs in bacille Calmette-Guerin in far lower concentrations. The protein induces B- and T-cell responses with high specificity for infection with *M. tuberculosis* and is a prime candidate for development of new diagnostic reagents for tuberculosis.—Authors' Abstract

Hardie, R. M. and Watson, J. M. *Mycobacterium bovis* in England and Wales—past, present and future. Epidemiol. Infect. **109** (1992) 23–33.

This report reviews the literature concerning tuberculosis resulting from infection with *Mycobacterium bovis* in man and cattle and summarizes data derived from surveillance of *M. bovis* in England and Wales from 1986 to 1990. Of the 228 isolates of *M. bovis* examined in this period, 122 (53%) were from patients aged over 60 years and are largely the result of reactivation of infection acquired prior to the institution of control measures. However, eight isolates (3.5%) were from patients aged less than 30 years. The potential sources for these presumed primary infections include the few remaining cattle infected with *M. bovis* or infectious human cases in the United Kingdom. However, infections acquired abroad, especially in immigrants, may account for some of these cases. Outbreaks of tuberculosis due to *M. bovis* continue to occur in cattle. Wild animals, particularly badgers, have been implicated as reservoirs of the infection. However, man may also prove to be an important reservoir of *M. bovis* for cattle as well as humans.—Authors' Abstract

Heym, B. and Cole, S. T. Isolation and characterization of isoniazid-resistant mutants of *Mycobacterium smegmatis* and *M. aurum.* Res. Microbiol. **143** (1992) 721–730.

INH-resistant mutants of *Mycobacterium aurum* and *M. smegmatis* were isolated and characterized in an attempt to provide fresh insight into the activity of isoniazid (INH), a key antibiotic in the treatment of tuberculosis. In both cases, high levels of resistance were accompanied by slower growth rate, by loss of peroxidase and reduced catalase activities, although mycolic acid production was unaffected. A gene homologous to the katG gene of *M. tubercuulosis,* encoding peroxidase-catalase, was detected in wild-type and INH-resistant strains and it appears that INH resistance may stem from the loss of its product.—Authors' Abstract

Hinshelwood, S. and Stoker, N. G. Cloning of mycobacterial histidine synthesis genes by complementation of a *Mycobacterium smegmatis* auxotroph. Molec. Microbiol. **6** (1992) 2887–2895.

Histidine-requiring auxotrophs of *Mycobacterium smegmatis* were isolated following *N*-methyl-*N*-nitro-*N*-nitrosoguanidine treatment. One of these mutants, *his5,* was transformed with an *M. smegmatis* shuttle cosmid library, and complementing clones were isolated at a frequency of approximately 1%. A 2.3 kb fragment was subcloned and sequenced, and found to contain the start of an operon including the *hisD* gene and part of the *hisC* gene. No *hisG* gene was detected upstream of *hisD,* suggesting that the regulation of histidine biosynthesis in mycobacteria may differ from that of *Escherichia coli.* The strategy used here will allow the molecular genetics of complex mycobacterial-specific biosynthetic pathways involved in the virulence of pathogenic species to be studied.—Authors' Summary

Iredell, J., Whitby, M. and Blacklock, Z. *Mycobacterium marinum* infection—epidemiology and presentation in Queensland (Australia) 1971–1990. Med. J. Australia **157** (1992) 596–598.

The objective of the study was to perform an evaluation of the clinical and epidemiological features of *Mycobacterium marinum* infection in Queensland. Laboratory identification and *in-vitro* susceptibility tests

of 29 isolates from the Queensland Health Department Tuberculosis Reference Laboratory were retrospectively gathered and followed up by contacting referring practitioners and obtaining clinical details of the patients involved. Subjects were 29 patients from whom *M. marinum* was isolated, with a male:female ratio of 3.1:1, and a mean age of 47.4 years. Of 26 patients for whom adequate information was available, 12 had evidence of involvement of deep tissues (including 2 cases of arthritis) and 5 suffered sporotrichoid spread of infection. The delay between onset of symptoms and consultation with a medical practitioner was 5 months (range, 2 weeks to 2 years), with a further mean delay to definitive diagnosis of 4.4 weeks. Cure was apparent in 22 of 23 cases. Chemotherapy alone was adequate in 11 cases, as was surgical intervention in 3, while a combination approach was successful in 8 cases. Trimethoprim/sulfamethoxazole was successful in 7 of 9 cases and combination rifampin and ethambutol in 6 of 7. Tetracyclines were employed as single-agent therapy in 9 patients and were effective in 7. Synovitis was a common presenting feature of *M. marinum* infection in Queensland patients. Occupational and recreational exposure to salt or fresh water was common, and although this history was available to practitioners a mean delay to definitive diagnosis of 4.4 weeks still occurred. The data suggest that chemotherapy alone is often adequate, even with deep tissue involvement. Combinations of conventional antimycobacterial drugs may be the therapy of choice, especially for serious infections, although success was recorded with trimethoprim/sulfamethoxazole alone.—Authors' Abstract

Jackson, K., Sievers, A., Ross, B. C. and Dwyer, B. Isolation of a fastidious *Mycobacterium* species from two AIDS patients. J. Clin. Microbiol. **30** (1992) 2934–2937.

Two strains of fastidious mycobacteria were isolated from two patients with AIDS and clinical disease suggestive of *Mycobacterium avium* complex infection. Acid-fast bacilli were isolated from blood and bone marrow of both patients in BACTEC 12B and/or 13A media. The acid-fast bacilli failed to grow on subculture to routine Löwenstein-Jensen medium containing pyruvate and egg yolk agar. After several attempts, the strain from one patient was finally cultured on Middlebrook 7H9 medium with agar, charcoal, and yeast extract 13 months after the initial specimens were received in the laboratory. The second patient's strain was cultured on the same medium 6 weeks postinoculation with fresh BACTEC fluid and 5 months after specimen collection. Routine biochemical and growth tests were performed on these isolates but failed to give definitive identifications. 16S rRNA gene sequencing suggested that the organisms share at least 98.9% homology with *M. simiae.* Even greater homology (99.86%) was found with the recently described species "*M. genavense.*" Recognition of the fastidious nature of some mycobacteria that infect AIDS patients is important in the treatment of infections in these patients and in understanding the epidemiology of atypical mycobacterial infections. It is suggested that a liquid culture medium such as BACTEC be employed for primary isolation of mycobacteria from AIDS patients and that subculture to the charcoal medium described here be carried out for those organisms that fail to grow on subculture to routine media.—Authors' Abstract

Ji, B.-H., Lounis, N., Truffot-Pernot, C. and Grosset, J. Selection of resistant mutants of *Mycobacterium avium* in beige mice by clarithromycin monotherapy. Antimicrob. Agents Chemother. **36** (1992) 2839–2840.

Beige mice were inoculated intravenously with $10^{7.90}$ CFU of *Mycobacterium avium* 101. Among the untreated control mice, when the mean CFU per spleen increased to a level greater than 10^8, small numbers of organisms resistant to clarithromycin (CLARI) were isolated from some of the spleens; the frequency of CLARI-resistant mutants was estimated to be between 10^8 and 10^9. In mice treated with 200 mg of CLARI per kg of body weight six times weekly, however, CLARI-resistant organisms were isolated from the spleens of all

mice examined after treatment for 8 weeks; the mean CFU per spleen and the frequency of resistant mutants were significantly greater than those of control mice and increased further after treatment for 16 weeks. The MICs of CLARI against the resistant organisms isolated from both control and treated mice were ≥ 512 μg/ml.—Authors' Abstract

Kawamura, I., Tsukada, H., Yoshikawa, H., Fujita, M., Nomoto, K. and Mitsuyama, M. IFN-γ-producing ability as a possible marker for the protective T cells against *Mycobacterium bovis* BCG in mice. J. Immunol. **148** (1992) 2887–2893.

Four weeks after the subcutaneous injection of mice with 10^5 viable BCG, the ability of PPD to elicit delayed-type hypersensitivity (DTH) in the mouse foot pads was at its peak. At the same time interval after infection, splenic T cells from these mice could transfer to recipient mice both DTH and specific resistance to challenge infection with BCG. In contrast, T cells from mice immunized with 10^7 killed BCG could transfer DTH but not resistance to challenge BCG. In response to PPD the culture supernatants of T cells from both groups of mice had similar enhanced levels of IL-2, but only the former had increased numbers of IFN-γ-producing cells and a significantly greater level of IFN-γ. From these and other results the authors conclude that protective CD4+ T cells generated by live BCG are characterized by the ability to produce IFN-γ after stimulation with specific antigen.—C. A. Brown (Trop. Dis. Bull.)

Kazda, J., Muller, H. J., Stackebandt, E., Daffé, M., Muller, K. and Pitulle, C. *Mycobacterium madagascariense* Sp-Nov. Int. J. System. Bacteriol. **42** (1992) 524–528.

Strains of a new type of rapidly growing, scotochromogenic mycobacterium were isolated from sphagnum vegetation in Madagascar. These strains grew at 31°C and 22°C but not at 37°C, possessed catalase, acid phosphatase, and arylsulfatase activities, split urea and pyrazinamide, hydrolyzed Tween, and produced acid from glucose, L-arabinose, fructose, mannitol, rhamnose, sorbitol, xylose, and trehalose. Furthermore, they metabolized iron and possessed putrescine oxidase activity but did not reduce nitrate. The internal similarity level of the strains, as determined by taxonomic methods, was 92.50%. The phylogenetic relationships of strain P2T (T = type strain) with members of the genus *Mycobacterium*, as determined by comparing the 16S rRNA primary structure of this strain with the 16S rRNA primary structures of 41 other mycobacterial species, indicated that strain P2T belongs to a separate line of descent within a cluster that includes *M. phlei, M. smegmatis, M. confluentis, M. flavescens*, and *M. thermoresistibile*. Hence, the new strains are considered members of a new species of nonpathogenic, rapidly growing mycobacteria, for which we propose the name *Mycobacterium madagascariense*. Strain P2 is the type strain; a culture of this strain has been deposited in the American Type Culture Collection as strain ATCC 49865.—Authors' Abstract

Kemper, C. A. Rifabutin and the *Mycobacterium avium* complex (MAC). Infect. Dis. Alert (Nov. 15 1992) 30–32.

Rifabutin, an ansamycin antibiotic derived from rifamycin-S, reduces the incidence of *Mycobacterium avium* complex bacteremia by more than 50% in patients with AIDS.—Author's Synopsis

Kent, R. J., Bakhtiar, M. and Shanson, D. C. The in-vitro bactericidal activities of combinations of antimicrobial agents against clinical isolates of *Mycobacterium avium–intracellulare*. J. Antimicrob. Chemother. **30** (1992) 643–650.

The *in-vitro* activities of five antimicrobial agents (rifabutin, clarithromycin, ethambutol, ciprofloxacin and amikacin), alone and in combination, were evaluted against 21 strains of *Mycobacterium avium–intracellulare* isolated from patients with AIDS. The combined activities of these agents were studied on solid medium by a full checkerboard method. Synergy was demonstrated most frequently (28%–71%

of isolates) with those combinations that included ethambutol. In killing curve experiments where double and triple combinations of agents were tested against two of the strains, 99% kill was achieved in 7 days at concentrations well below those that are attainable in serum. However, an additive rather than a synergic effect was seen in most instances. Although ciprofloxacin alone had the greatest bactericidal activity against these two strains, its activity was antagonized in the presence of rifabutin; this antagonism became inapparent when a third agent was added. Demonstration of bactericidal activity in broth culture may be more relevant than the results of susceptibility testing on solid medium when choosing antimicrobial therapy for patients with this infection.—Authors' Abstract

Klemens, S. P. and Cynamon, M. H. Activity of rifapentine against *Mycobacterium avium* infection in beige mice. J. Antimicrob. Chemother. **29** (1992) 555–561.

The activity of rifapentine (MDL 473) was evaluated in the beige (C57BL/6J-bg^j/bg^j) mouse model of disseminated *Mycobacterium avium* infection. Approximately 10^7 cfu of *M. avium,* serotype 1, were given i.v. Seven days later treatment was started with intraperitoneal rifapentine at 20 mg/kg of body weight. Treatment was given daily for 5 days followed by twice weekly for 3 weeks. The mice were killed 2 days after the last dose. Spleens, livers and lungs were homogenized and cfu/organ determined. Analysis of variance and Tukey honestly significant difference tests indicated that rifapentine reduced cfu in each of the organs compared with untreated controls. A dose-response experiment was performed with a daily rifapentine dose of 10, 20 or 40 mg/kg administered intraperitoneally. Dose-related reductions in cfu counts were observed in each of the organs. The activity of oral rifapentine at 20 mg/kg was demonstrated in a comparative experiment with rifampin at 20, 40 or 60 mg/kg. Rifapentine significantly reduced cfu counts in organs compared with rifampin. Rifapentine should be considered for further evaluation in the treatment of *M. avium* complex infection in humans.—Authors' Abstract

Klemens, S. P., Destefano, M. S. and Cynamon, M. H. Activity of clarithromycin against *Mycobacterium avium* complex infection in beige mice. Antimicrob. Agents Chemother. **36** (1992) 2413–2417.

The activity of clarithromycin alone and in combination with other antimycobacterial agents was evaluated in the beige (C57BL/6J bg(j)/bg(j)) mouse model of disseminated *Mycobacterium avium* complex (MAC) infection. A dose-response experiment was performed with clarithromycin at 50, 100, 200, or 300 mg/kg of body weight administered daily by gavage to mice infected with approximately 10^7 viable MAC. A dose-related reduction in spleen and liver cell counts was noted with treatment at 50, 100, and 200 mg/kg. The difference in cell counts between treatment at 200 and 300 mg/kg was not significant. Clarithromycin at 200 mg/kg of body weight was found to have activity against three additional MAC isolates (MICs for the isolates ranged from 1 to 4 μg/ml by broth dilution). Clarithromycin at 200 mg/kg in combination with amikacin, ethambutol, temafloxacin, or rifampin did not result in increased activity beyond that seen with clarithromycin alone. Clarithromycin in combination with clofazimine or rifabutin resulted in an increase in activity beyond that seen with clarithromycin alone. The combination of clarithromycin with clofazimine or rifabutin should be considered for evaluation in the treatment of human MAC infections.—Authors' Abstract

Kolk, A. H. J., Schuitema, A. R. J., Kuijper, S., Van Leeuwen, J., Hermans, P. W. M., Van Embden, J. D. A. and Hartskeerl, R. A. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a nonradioactive detection system. J. Clin. Microbiol. **30** (1992) 2567–2575.

A test based on the polymerase chain reaction (PCR) was developed for the detection of the *Mycobacterium tuberculosis* complex in clinical samples. In this test, a 245-bp sequence of the insertion element IS986 was amplified and detected by agarose gel electrophoresis in the presence of ethidium bromide and by Southern blot and dot blot hybridization by using a 188-bp

digoxigenin-labeled probe. We tested clinical specimens from 227 patients suspected of having tuberculosis. These included 102 cerebrospinal fluid, 48 sputum, 18 pleural fluid, 5 bronchoalveolar lavage, 18 blood, 7 pus, 8 bone marrow, and 6 urine samples and 15 tissue biopsy specimens. We also tested sputum samples from 75 patients with diseases other than tuberculosis. Sputum samples were first decontaminated, and all samples were treated with proteinase K-detergent solution to extract the DNA. Part of each sample was spiked with *M. tuberculosis* to provide a semiquantitative assay and to control for the loss of mycobacteria or interference with the PCR which may cause false-negative results. One femtogram of *M. tuberculosis* DNA could be detected. PCR was positive for all 32 culture-positive (for *M. tuberculosis*) and Ziehl-Neelsen staining (ZN)-positive samples, 10 of 12 culture-positive and ZN-negative samples, and all 4 culture-negative and ZN-positive samples. PCR detected *M. tuberculosis* complex bacteria in 35 of 178 culture- and ZN-negative samples. Clinical data supported the diagnosis of tuberculosis in the majority of the 35 patients from whom those samples were obtained.—Authors' Abstract

Koryakin, V. A., Zeliger, L. R. and Galenko, N. N. [Tarivid in the combined treatment of pulmonary tuberculosis patients.] Probl. Tuberk. **9** (1991) 38–40. (in Russian)

The effectiveness of tarivid, a new drug having a broad spectrum of antimicrobial action and antituberculous effect, was studied. The drug was given to 29 patients with newly detected destructive pulmonary tuberculosis and bacillary excretion. Eight patients had microbacterial resistance to streptomycin, rifampin and isoniazid, 12 had tolerance to them; in 9 patients tuberculosis was complicated by a nonspecific inflammatory process. The course of treatment ranged from 3 weeks to 3 months. The drug was well tolerated. Its effect was manifested by reduction of intoxication, resolution of the inflammatory and pericavitary pulmonary lesions and bacilli absence. Tarivid can be considered an efficacious drug in the multimodality therapy of tuberculosis patients.—Authors' English Abstract

Lebrun, L., Espinasse, F., Poveda, J. D. and Lévy-Frebault, V. V. Evaluation of nonradioactive DNA probes for identification of mycobacteria. J. Clin. Microbiol. **30** (1992) 2476–2478.

Commercial chemiluminescent DNA probes (Accuprobe; Gen-Probe, San Diego, California, U.S.A.) for the identification of *Mycobacterium tuberculosis* (MTB) complex, *M. avium* complex (MAC), *M. gordonae,* and *M. kansasii* were evaluated with 134 clinical isolates. These included 36 MTB complex, 40 MAC, 27 *M. gordonae,* 9 *M. kansasii,* and 22 *Mycobacterium* spp. The specificity was 100% for the four probes. The sensitivity was 100% for the MTB complex and *M. gordonae* probes and 95.2% for the MAC probe. Five of the nine *M. kansasii* isolates tested were not detected with the probe.—Authors' Abstract

Lee, J. D., Shin, K. H., Cho, S.-N., Lee, M. G., Yang, W. I., Park, C. Y., Yoo, H. S., Lee, J. T. and Awh, O. D. Immunoscintigraphy in the detection of tuberculosis with radiolabelled antibody fragment against *Mycobacterium bovis* bacillus Calmette-Guérin—a preliminary study in a rabbit model. Eur. J. Nucl. Med. **19** (1992) 1011–1015.

Immunoscintigraphy with radiolabeled monoclonal antibodies is widely used to detect solid tumors, but only a few trials have been carried out concerning the specific *in vivo* localization of an inflammatory process. The purpose of this study was to investigate the detectability of tuberculous foci utilizing this method with radiolabeled bacillus Calmette-Guérin (BCG)-specific F(ab')2 in rabbits. All of the tuberculous lesions (N = 8) were clearly visualized on serial scintigraphy for up to 48 hr after injection of the antibody. Immunohistochemical and Ziel-Neelsen staining of the tuberculous lesions confirmed the presence of the tuberculous antigens and bacilli. It failed to demonstrate any sustained retention of the BCG-specific antibody fragment in the control group with syphilitic orchitis (N = 2). Therefore, the specific *in vivo* localization of tuberculosis is feasible by immunoscintigraphy.—Authors' Abstract

Majumdar, S., Flasher, D., Friend, D. S., Nassos, P., Yajko, D., Hadley, W. K. and Duzgunes, N. Efficacies of liposome-encapsulated streptomycin and ciprofloxacin against *Mycobacterium avium–M. intracellulare* complex infections in human peripheral blood monocyte/macrophages. Antimicrob. Agents Chemother. **36** (1992) 2808–2815.

Current treatments of disseminated infection caused by the *Mycobacterium avium–M. intracellulare* complex (MAC) are generally ineffective. Liposome-mediated delivery of antibiotics to MAC-infected tissues *in vivo* can enhance the efficacy of the drugs. We investigated the therapeutic efficacies of liposome-encapsulated streptomycin and ciprofloxacin against growth of the MAC inside human peripheral blood monocyte/macrophages. Treatment was initiated 24 hr after infection of macrophages with the MAC and stopped after 20 hr, and the cells were incubated for another 7 days. The antimycobacterial activity of streptomycin was enhanced when the drug was delivered to macrophages in liposome-encapsulated form, reducing the CFU about threefold more than the free drug did throughout the concentration range studied (10 to 50 μg/ml). With 50 μg of encapsulated streptomycin per ml, the CFU were reduced to 11% of the initial level of infection. Liposome-encapsulated ciprofloxacin was at least 50 times more effective against the intracellular bacteria than was the free drug: at a concentration of 0.1 μg/ml, liposome-encapsulated ciprofloxacin had greater antimycobacterial activity than the free drug at 5 μg/ml. With liposome-encapsulated ciprofloxacin at 5 μg/ml, the CFU were reduced by more than 1000-fold at the end of the 7-day incubation period, compared with untreated controls. These results suggest that liposome-encapsulated ciprofloxacin or other fluoroquinolones may be effective against MAC infections *in vivo*.—Authors' Abstract

Marin, L. M. L., Laneelle, M. A., Prome, D., Laneelle, G., Prome, J. C. and Daffe, M. Structure of a novel sulfate containing mycobacterial glycolipid. Biochemistry **31** (1992) 11106–11111.

We described previously the unusual structures of the two major C-mycoside glycopeptidolipids from *Mycobacterium fortuitum* biovar. *peregrinum*. More polar glycolipids, potentially more interesting in terms of antigenicity, were also present in the strains. A combination of FAB mass spectrometry, NMR, chemical analyses, and radiolabeling was successfully applied to these glycolipids to arrive at the unexpected and novel structure for the more polar compound. This consisted of the "orthodox" basic structure of the apolar C-mycosides, modified at the alaninol end by the presence of a sulfate group on position 2 of a 3,4-di-O-methylrhamnosyl residue. This novel and second class of sulfate-containing mycobacterial glycolipid may provide a chemical basis for the differentiation and classification of members of the *M. fortuitum* complex, the main group causing human diseases among the many fast-growing mycobacteria widely distributed in nature.—Authors' Abstract

Mathur, M. and Kolattukudy, P. E. Molecular cloning and sequencing of the gene for mycocerosic acid synthase, a novel fatty acid elongating multifunctional enzyme, from *Mycobacterium tuberculosis* var. *bovis* bacillus Calmette-Guérin. J. Biolog. Chem. **267** (1992) 19388–19395.

Mycocerosyl lipids are found uniquely in the cell walls of pathogenic mycobacteria. Mycocerosic acid synthase (MAS) is a multifunctional protein which catalyzes elongation of *n*-fatty acyl-CoA with methylmalonyl-CoA as the elongating agent. To understand how the various domains that catalyze the reactions involved in chain elongation are organized, *mas* gene from *Mycobacterium tuberculosis bovis* BCG was cloned. A λgt11 library of *Alu*I partially digested genomic DNA from the organism was screened with an oligonucleotide probe designed from the N-terminal amino acid sequence of purified MAS. Using terminal segments of inserts from positive clones as the probe, the library was rescreened and the process was repeated. Sequencing of four overlapping clones revealed a contiguous sequence of 9699 base pair(s) (bp) of mycobacterial genome containing a 6330-bp open reading frame that could code for a

protein of 2100 amino acids with a molecular mass of 225,437 daltons. The authenticity of the open reading frame as that of MAS was verified by correspondence of the amino acid sequences deduced from the gene with the directly determined amino acid sequences of the N terminus and three different internal peptide fragments. By comparing the MAS amino acid sequence with the sequences in the active site regions of known fatty acid synthases and polyketide synthases the functional domains in MAS were identified. This analysis showed that the domains were organized in the following order: β-ketoacyl synthase, acyl transferase, dehydratase-enoyl reductase, β-ketoreductase, acyl carrier protein; no thioesterase-like domain could be found. These results establish MAS as the first case of an elongating multifunctional enzyme composed of two identical subunits that resemble the vertebrate fatty acid synthase in size, subunit structure, and linear organization of functional domains. Southern and Western blot analyses showed absence of *mas* gene and encoded proteins in *M. smegmatis* and *Escherichia coli.* This result is consistent with the report that mycocerosic acid is present only in pathogenic mycobacteria.—Authors' Abstract.

McFadden, J., Collins, J., Beaman, B., Arthur, M. and Gitnick, G. Mycobacteria in Crohn's disease—DNA probes identify the wood pigeon strain of *Mycobacterium avium* and *Mycobacterium paratuberculosis* from human tissue. J. Clin. Microbiol. **30** (1992) 3070–3073.

Mycobacterium paratuberculosis is known to cause Johne's disease, a granulomatous ileitis in ruminants, and may be involved in some cases of Crohn's disease. Like *M. paratuberculosis,* the wood pigeon strain of *M. avium* may also show mycobactin dependence on primary isolation that is attenuated on further subculturing. A wood pigeon strain, *M. avium* restriction fragment length polymorphism (RFLP) type A/I, is also capable of causing granulomatous ileitis in experimental animal models but is not known to cause disease in humans. *M. avium* RFLP type A is associated with disease in immunocompromised hosts. Three DNA probes, pMB22 and the two subclones pMB22/S4 and pMB/S12, were found to be capable of distinguishing among *M. paratuberculosis, M. avium* type A, and *M. avium* type A/I (wood pigeon strain) on the basis of RFLPs. These DNA probes were used to identify two mycobacterial isolates (*M. paratuberculosis* and *M. avium* type A/I, wood pigeon strain) derived from the intestinal tissues of two patients with Crohn's disease. In addition, the wood pigeon strain of *M. avium* was identified from a patient with ulcerative colitis, and *M. avium* RFLP type A was identified from a patient with colonic carcinoma. This is the first time that *M. avium* A/I (wood pigeon strain) is known to have been isolated from human tissue. There are too few isolates to speculate about the etiological significance of mycobacteria and inflammatory bowel disease, but it is reasonable to conjecture that *M. paratuberculosis* may be responsible for some cases of Crohn's disease and that the wood pigeon strain of *M. avium* may also be an inflammatory bowel disease pathogen in humans.—Authors' Abstract

Meylan, P. R. A., Richman, D. D. and Kornbluth, R. C. Reduced intracellular growth of mycobacteria in human macrophages cultivated at physiologic oxygen pressure. Am. Rev. Respir. Dis. **145** (1992) 947–953.

The growth of mycobacteria in human macrophages was examined at an ambient concentration of oxygen (5% CO_2 and 95% air, corresponding to 20% O_2 or 140 mm Hg PO_2) and at a concentration corresponding to tissue levels (5% O_2 and 5% CO_2 in nitrogen balance, corresponding to 36 mm Hg PO_2). Compared with the higher PO_2 level, macrophages cultivated at lower PO_2 level spread more widely and had an increased glycolytic and decreased oxidative metabolism. Upon PMA stimulation, they displayed a better preserved ability to produce superoxide anion and to respond to IFN-γ priming by increased superoxide anion production. When infected with either *Mycobacterium tuberculosis* or *M. avium,* macrophages cultured with the lower PO_2 level permitted significantly less growth than those cultured at the higher PO_2 level. From Day 0 to Day 7, *M. tuberculosis* grew an average of 0.39 and 1.17 log cfu in mac-

rophages cultured at lower and higher PO_2, respectively ($p < 0.0001$). From Day 0 to Day 3 of infection, *M. avium* decreased in macrophages cultured at lower PO_2 on average by 0.19 log cfu but grew by 0.34 log cfu in macrophages cultured at higher PO_2 ($p = 0.0001$). Mycobacteria grew equally well in macrophage-free media at either PO_2. Crude lymphokines, rIFN-γ, or rTNF-α did not consistently affect the growth of mycobacteria in macrophages at either high or low oxygen conditions. In conclusion, mycobacteria displayed a reduced growth when cultivated in macrophages at a physiologic PO_2 that did not reduce the growth of extracellular bacteria. This effect of PO_2 on macrophage antimycobacterial power might explain the preferential localization of tuberculous lesions in body areas with high tissue PO_2, such as the lung apex.—Authors' Abstract

Millership, S. E. and Want, S. V. Whole-cell protein electrophoresis for typing *Mycobacterium tuberculosis.* J. Clin. Microbiol. **30** (1992) 2784–2787.

A method of discriminating between strains of *Mycobacterium tuberculosis* by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis of whole-cell proteins combined with a sensitive silver stain is described. Thirty-five isolates of *M. tuberculosis* and five isolates from other species of *Mycobacterium* were examined, including serial isolates from the same patients and isolates from a small cluster of hospital cases. Different species of *Mycobacterium* were clearly distinguished, and within the species *M. tuberculosis,* different fingerprints were obtained, allowing discrimination of some strains from different patients. The reproducibility and discrimination of the technique are discussed.—Authors' Abstract

Moss, M. T., Sanderson, J. D., Tizard, M. L. V., Hermon-Taylor, J., Elzaatari, F. A. K., Markesich, D. C. and Graham, D. Y. Polymerase chain reaction detection of *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp *silvaticum* in long term cultures from Crohn's disease and control tissues. Gut **33** (1992) 1209–1213.

Thirty one cultures were established in MG3 medium from the intestinal tissues of 29 patients, including 18 with Crohn's disease, 5 with ulcerative colitis, and 6 noninflammatory bowel disease controls. All cultures grew either acid-fast bacilli or uncharacterized spheroplasts. Pellets from these cultures were coded and assayed blind for *Mycobacterium paratuberculosis* and *M. avium* subsp *silvaticum* using IS900- and IS902-PCR (polymerase chain reaction) assays, respectively. IS900 and IS902 are multicopy DNA insertion elements specific for these two organisms. Six Crohn's disease cultures and a single noninflammatory bowel disease control were positive for *M. paratuberculosis.* A further six cultures were positive for *M. avium* subsp *silvaticum,* of which two each were from Crohn's disease, ulcerative colitis, and noninflammatory bowel disease controls. The intensity of the IS900-PCR signals indicated very low numbers of *M. paratuberculosis* organisms and bore no relation to visible spheroplastic or bacillary mycobacterial growth. The results suggest that *M. paratuberculosis* isolated from man exists in a form which hardly replicates if at all when cultured in MG3 medium *in vitro,* and are consistent with the involvement of this known animal enteric pathogen in a proportion of chronic enteritis in man.—Authors' Abstract

Nolan, C. M. Failure of therapy for tuberculosis in human immunodeficiency virus infection. Am. J. Med. Sci. **304** (1992) 168–173.

Optimum treatment of tuberculosis in persons with human immunodeficiency virus (HIV) infection is still being defined. Tuberculosis treatment failure in an HIV-infected patient is described and 10 similar cases from the medical literature are reviewed to search for common patterns associated with an adverse outcome of therapy in this setting. Six patients were poorly compliant. In nine patients, the subsequent episode of tuberculosis was disseminated or extrapulmonary; in four the central nervous system was involved. In five patients, a problem with rifampin usage was encountered: Three had rifampin-resistant *Mycobacterium tuberculosis,* one experienced an adverse reaction to rifampin, leading to

withdrawal from the regimen after 1 week, and one was receiving a drug that may interfere with rifampin's antimycobacterial effect. This case report and literature review suggest that particular attention should be directed toward ensuring that patients with HIV infection comply with treatment of tuberculosis. For the majority of patients, the already stretched resources available for the treatment of tuberculosis and HIV infection should be devoted to compliance enhancement rather than to more prolonged or intensive drug regimens. However, it should be emphasized that patients with disseminated tuberculosis or central nervous system disease and those who are not able to receive rifampin because of drug resistance or an adverse reaction should be managed individually.—Authors' Abstract

Nunn, P., Brindle, R., Carpenter, L., Odhiambo, J., Wasunna, K., Newnham, R., Githui, W., Gathua, S., Omwega, M. and McAdam, K. Cohort study of human immunodeficiency virus infection in patients with tuberculosis in Nairobi, Kenya—analysis of early (6-month) mortality. Am. Rev. Respir. Dis. **146** (1992) 849–854.

Retrospective studies suggest that the mortality rate from HIV-1 associated tuberculosis is greater than that from tuberculosis alone, but it is not clear if this is due to failure of antituberculosis treatment or to the complications of HIV-1 infection. We have carried out a prospective cohort study of patients with tuberculosis in Nairobi, Kenya, to compare mortality rates, risk factors, and causes of death in HIV-1-positive and HIV-1-negative patients. One-hundred-seven HIV-1-positive and 174 HIV-1-negative patients with tuberculosis attending two tuberculosis treatment centers in Nairobi were enrolled and followed monthly. Mortality was significantly higher in HIV-1-positive than in HIV-1-negative patients within 6 months of the start of antituberculosis treatment after adjustment for age, sex, and education (rate ratio = 3.8; 95% confidence interval, 1.7 to 8.1; $p < 0.001$). Most of the excess mortality occurred after the first month of treatment and was due to nontuberculous infections. Predictors for mortality differed greatly between HIV-1-positive and HIV-1-negative patients. Mortality was greater in HIV-1 positive patients treated with a "standard" regimen for tuberculosis than in HIV-1-positive patients receiving a "short-course" regimen ($p = 0.08$ when adjusted for all independent risk factors). Tuberculosis control programs in developing countries need to implement "short-course" regimens and train health workers to recognize and treat nontuberculous infections to maintain their effectiveness in the face of the HIV epidemic.—Authors' Abstract

Pal, P. G. and Horwitz, M. A. Immunization with extracellular proteins of *Mycobacterium tuberculosis* includes cell-mediated immune responses and substantial protective immunity in a guinea pig model of pulmonary tuberculosis. Infect. Immun. **60** (1992) 4781–4792.

We have studied the capacity of a selected fraction of *Mycobacterium tuberculosis* extracellular proteins (EP) released into broth culture by mid-logarithmic-growth-phase organisms to induce cell-mediated immune responses and protective immunity in a guinea pig model of pulmonary tuberculosis. Guinea pigs infected with *M. tuberculosis* by aerosol but not uninfected control guinea pigs exhibit strong cell-mediated immune responses to EP, manifest by dose-dependent cutaneous delayed-type hypersensitivity and splenic lymphocyte proliferation. Guinea pigs immunized subcutaneously with EP but not sham-immunized control guinea pigs also develop strong cell-mediated immune responses to EP, manifest by dose-dependent cutaneous delayed-type hypersensitivity and splenic lymphocyte proliferation. EP is nonlethal and nontoxic to guinea pigs upon subcutaneous immunization. Guinea pigs immunized with EP and then challenged with aerosolized *M. tuberculosis* exhibit protective immunity. In five independent experiments, EP-immunized guinea pigs were consistently protected against clinical illness, including weight loss. Compared with EP-immunized guinea pigs, sham-immunized control guinea pigs lost $12.9 \pm 2.0\%$ (mean $\pm$ SE) of their total body weight. EP-immunized guinea pigs also had a 10-fold reduction in viable *M. tuberculosis* bacilli

in their lungs and spleens ($p = 0.004$ and 0.001, respectively) compared with sham-immunized control animals. In the two experiments in which some guinea pigs died after aerosol challenge, EP-immunized animals were protected from death. Whereas all 12 (100%) EP-immunized guinea pigs survived challenge with aerosolized *M. tuberculosis,* only 6 of 12 (50%) sham-immunized control guinea pigs survived challenge ($p = 0.007$, Fisher exact test). This study demonstrates that actively growing *M. tuberculosis* cells release immunoprotective molecules extracellularly, that a subunit vaccine against tuberculosis is feasible, and that extracellular molecules of *M. tuberculosis* are potential candidates for a subunit vaccine.—Authors' Abstract

Perrone, C., Cohan, Y., Truffot-Pernot, C., Grosset, J., Vildé, J.-L. and Pocidalo, J.-J. Sparfloxacin, ethambutol, and cortisol receptor inhibitor RU-40 555 treatment for disseminated *Mycobacterium avium* complex infection of normal C57BL/6 mice. Antimicrob. Agents Chemother. **36** (1992) 2408–2412.

Sparfloxacin (50 mg/kg of body weight given subcutaneously each day), alone or in combination with ethambutol (50 mg/kg given subcutaneously each day), was examined for its therapeutic efficacy against experimental infection induced with the *Mycobacterium avium* complex in normal C57BL/6 mice. In addition, the potential anti-infective role of RU-40 555 (100 mg/kg given intraperitoneally each day), a drug that inhibits the cortisol receptors, was examined in the same model. Treatments were started 24 hr after intravenous bacterial challenge and were continued for 21 days. Compared with controls, sparfloxacin or ethambutol decreased the CFU counts in spleens and lungs ($p < 0.001$). The sparfloxacin plus ethambutol combination was more effective than sparfloxacin alone in spleens ($p < 0.001$) but not in lungs. The sparfloxacin plus ethambutol plus RU-40 555 combination was more effective than the sparfloxacin plus ethambutol combination in spleens and lungs ($p < 0.001$). Thus, in this model, RU-40 555 enhanced the antibacterial activities of the antibiotics tested. Results of the study showed that normal C57BL/6 mice infected with the *M. avium* complex can be used for the evaluation of antimicrobial agents.—Authors' Abstract

Pithie, A. D., Rahelu, M., Kumararatne, D. S., Drysdale, P., Gaston, J. S. H., Iles, P. B., Innes, J. A. and Ellis, C. J. Generation of cytolytic T-cells in individuals infected by *Mycobacterium tuberculosis* and vaccinated with BCG. Thorax **47** (1992) 695–701.

Macrophage activation by cytokines provides only a partial explanation of antimycobacterial immunity in man. Because cytolytic T lymphocytes have been shown to contribute to immunity in animal models of intracellular infection, the generation of mycobacterial antigen specific cytotoxic T cells was examined in the peripheral blood of patients with tuberculosis. Subjects comprised 36 patients with active tuberculosis (18 newly diagnosed) and 32 healthy volunteers, of whom 25 had had BCG vaccination and 7 were Mantoux negative. The ability of purified protein derivative (PPD) stimulated peripheral blood lymphocytes to lyse autologous, mycobacterial antigen bearing macrophages was examined by using a chromium 51 release assay. PPD-stimulated lymphocytes from normal, Mantoux-positive, BCG-vaccinated subjects produced high levels of PPD-specific cytolysis; whereas lymphocytes from unvaccinated, uninfected subjects caused little or no cytolysis. The generation of cytolytic T lymphocytes by patients with tuberculosis was related to their clinical state. Those with cavitating pulmonary disease or lymph node tuberculosis generated PPD-specific lymphocytes with cytotoxic ability similar to that of those from Mantoux-positive control subjects; whereas lymphocytes from patients with noncavitating pulmonary infiltrates showed poor antigen-specific cytolysis. After 7 days of stimulation with PPD *in vitro,* lymphoblasts contained both CD4+ and CD8+ cells. Mycobacterial antigen-specific cytolysis was restricted to the CD4+ cell population and was blocked by monoclonal antibodies directed against major histocompatibility class II (MHC) antigens. CD4+ cytolytic T cells can lyse autologous macrophages presenting mycobacterial antigen and were found in patients with cav-

itating pulmonary tuberculosis or tuberculous lymphadenitis and in normal, Mantoux-positive control subjects. The ability to generate these T-cell responses seems to be a marker for response to mycobacteria and may contribute to tissue damage in tuberculosis. These responses do not provide protective immunity against *Mycobacterium tuberculosis* but may help in disease localization.—Authors' Abstract

Plikaytis, B. D., Plikaytis, B. B. and Shinnick, T. M. Computer-assisted pattern recognition model for the identification of slowly growing mycobacteria including *Mycobacterium tuberculosis.* J. Gen. Microbiol. **138** (1992) 2265–2273.

We present a computerized pattern recognition model used to speciate mycobacteria based on their restriction fragment length polymorphism (RFLP) banding patterns. DNA fragment migration distances were normalized to minimize lane-to-lane variability of band location both within and among gels through the inclusion of two internal size standards in each sample. The computer model used a library of normalized RFLP patterns derived from samples of known origin to create a probability matrix which was then used to classify the RFLP patterns from samples of unknown origin. The probability matrix contained the proportion of bands that fell within defined migration distance windows for each species in the library of reference samples. These proportions were then used to compute the likelihood that the banding pattern of an unknown sample corresponded to that of each species represented in the probability matrix. As a test of this process, we developed an automated, computer-assisted model for the identification of *Mycobacterium* species based on their normalized RFLP banding patterns. The probability matrix contained values for the *M. tuberculosis* complex, *M. avium, M. intracellulare, M. kansasii* and *M. gordonae* species. Thirty-nine independent strains of known origin, not included in the probability matrix, were used to test the accuracy of the method in classifying unknowns: 37 of 39 (94.9%) were classified correctly. An additional set of 16 strains of known origin representing species not included in the model were tested to gauge the robustness of the probability matrix. Every sample was correctly identified as an outlier, i.e., a member of a species not included in the original matrix. This strategy may be readily adapted to other chromatographic and electrophoretic systems that generate peak profiles or banding patterns.—Authors' Abstract

Polozov, A. I., Dolzhansky, V. M., Vladimirsky, M. A., Abrikosova, T. N., Kuznetsov, A. A., Filippov, V. I. and Goncharov, L. A. [Use of highly dispersed ferroparticles for raising sensitivity of the luminescence microscopic method to determine tuberculosis mycobacteria.] Probl. Tuberk. **9** (1991) 61–63. (in Russian)

A highly dispersed ferromagnetic powder obtained by a plasmochemical method (particle size was 100–500 Å) was treated by means of an ultrasonic disperser: suspension was added to the trisodium phosphate homogenized and neutralized sputum (0.3 mg of the initial powder per 1 ml of sputum) of patients with various forms of pulmonary tuberculosis. The sputum was then incubated at slight stirring for 40 min and centrifuged; the precipitate was used to prepare smears which were stained with auramine; mycobacteria were detected by luminescence microscopy. The ferromagnetic suspension was found to increase luminescence microscopy sensitivity to 85.4%. The efficacy of the method was 30.8% more than that in the cultivation of infectious material in solid nutrient media.—Authors' English Abstract

Portaels, F., Dawson, D. J., Larsson, L. and Rigouts, L. Biochemical properties and fatty acid composition of *Mycobacterium haemophilum*—study of 16 isolates from Australian patients. J. Clin. Microbiol. **31** (1992) 26–30.

The biochemical properties and fatty acid compositions of 16 strains of *Mycobacterium haemophilum* from Australian patients were studied. The strains proved to be indistinguishable from each other but could readily be differentiated from other slowly growing mycobacteria with similar cultural features. Mycolic acid analyses re-

vealed the presence of alpha-, methoxy-, and ketomycolates. The fatty acid composition supports the validity of the fact that *M. haemophilum* is a distinct species. The fatty acid composition was consistent among the 16 strains, but it was unusual in that there was some resemblance to the fatty acid composition of *M. leprae*. The wide range of pHs (5.4 to 7.4) that supported growth of *M. haemophilum* on artificial medium is in keeping with suggestions that *M. haemophilum* exists in an environmental habitat.—Authors' Abstract

Rambukkana, A., Das, P. K., Krieg, S., Yong, S., Lepoole, I. C. and Bos, J. D. Mycobacterial 65,000 MW heat-shock protein shares a carboxy-terminal epitope with human epidermal cytokeratin-1/2. Immunology **77** (1992) 267–276.

Molecular mimicry between mycobacterial heat-shock protein (hsp) 65 and host tissue antigens has been implicated in the autoimmune pathogenesis of certain idiopathic diseases. Here, we demonstrated that two of our previously characterized monoclonal antibodies (mAb), Ne5 and Nd4 that were directed to a carboxy-terminal epitope on the mycobacterial hsp 65, specifically crossreacted with suprabasal cytokeratin of the normal human skin. These mAb also showed similar keratin staining of hair follicle epithelia and produced no reaction with other dermal components. Both mAb strongly stained the cytoplasm of the majority of freshly isolated epidermal keratinocytes from the normal human skin. None of these mAb showed staining with human HeLa cells and with human skin fibroblasts. Immunoblotting using total keratin extract prepared from isolated epidermal keratinocytes revealed that mAb Ne5 and Nd4 specifically reacted with a molecular size of 65,000–67,000 MW keratin protein(s) and such reactivity was not observed from cytoskeletal proteins extracted from HeLa cells and skin fibroblasts. Comparison of immunoblotting reactivity with conventional anti-cytokeratin mAb further revealed that mAb Ne5/Nd4 recognized a 65,000–67,000 MW molecular-sized protein corresponding to cytokeratin 1/2 from the same keratinocyte extract as anti-cytokeratin mAb. Preincubation of mAb Ne5/Nd4 with the purified mycobacterial hsp 65 abolished this keratin crossreactivity in both immunohistochemistry and immunoblotting. Moreover, these mAb showed no keratin staining in lesional psoriatic skin and also reacted weakly with cultured epidermal keratinocytes. Since mAb Ne5/Nd4 specifically recognized a 67,000–65,000 MW molecular-sized protein(s) derived from epidermal keratinocytes and the known characteristics of epidermal cytokeratin 1/2 appeared to be consistent with present results, we concluded that Ne5/Nd4 crossreactive protein(s) in the human epidermis is suprabasal cytokeratin 1/2. Comparison of the previously mapped Ne5/Nd4 epitope region of amino acid residues 525–540 of the mycobacterial hsp 65 with the entire sequence of human 65,000 MW keratin revealed that a stretch of nine amino acids of the Ne5/Nd4 epitope sequence resembled certain regions of the carboxy-terminus of the human 65,000 MW keratin. This similarity of the mycobacterial hsp 65 probably contributes to the cytokeratin crossreactive epitope. Our results presented here demonstrate direct evidence of immunological crossreactivity between mycobacterial hsp 65 and human epidermal cytokeratin 1/2. We speculate that Ne5/Nd4 crossreactive epitope of epidermal cytokeratins might be an important target for skin diseases.—Authors' Abstract

Rao, S. P., Gehlsen, K. R. and Catanzaro, A. Identification of a beta1-integrin on *Mycobacterium avium–Mycobacterium intracellulare*. Infect. Immun. **60** (1992) 3652–3657.

Mycobacterium avium–Mycobacterium intracellulare (MAI) is an opportunistic intracellular pathogen responsible for the highest incidence of disseminated bacterial infection in patients with AIDS. Treatment of the infection is extremely difficult and has shown limited efficacy. A critical event in the initiation of a variety of bacterial infections involves the adherence of bacteria to host cell surfaces. In the present study, we have shown that MAI organisms bind avidly to extracellular matrix proteins such as laminin, collagen I, and fibronectin in an *in vitro* attachment assay. Immunoblot analysis of a sonicate of MAI with polyclonal antibodies against different integrin recep-

tors indicated that the sonicate crossreacts with polyclonal antibodies against a human laminin-binding integrin, alpha-3-beta-1, and a human fibronectin-binding integrin, alpha-5-beta-1, although it is reactive with only the beta-1 subunit in the case of both antisera. Antibodies against the alpha-3-beta-1 and alpha-5-beta-1 integrins specifically inhibited the binding of MAI to laminin, collagen I, and fibronectin by 70% to 97%, depending on the ligand, suggesting that the attachment of MAI to these extracellular matrix proteins may be mediated by a beta-1 integrin. Furthermore, the attachment of MAI to laminin, collagen I, and fibronectin was found to be cation dependent. MAI may use this and other beta-1-containing integrins to adhere and penetrate through basement membrane structures that underlie host cell linings. An understanding of the mechanism of attachment and a definition of the adhesive molecules on the surface of MAI may open up new approaches to the prevention of serious infection caused by this organism.—Authors' Abstract

Ratcliffe, L. T., Mackenzie, C. R., Lukey, P. T. and Ress, S. R. Reduced natural killer cell activity in multi-drug resistant pulmonary tuberculosis. Scand. J. Immunol. **36** (1992) 167–170.

Cellular immune status in five patients with multidrug resistant pulmonary tuberculosis was investigated and compared with five matched controls with nonresistant tuberculosis. A significant reduction in fresh natural killer (NK)-cell activity was found in the resistant group ($p < 0.005$). There were no significant differences between the two groups in lymphocyte phenotype, proliferation or PPD-specific cytotoxicity. Reduced NK-cell function may play a role in the pathogenesis of multidrug resistant pulmonary tuberculosis.—Authors' Abstract

Rodrigues, L. C., Gill, O. N. and Smith, P. G. BCG vaccination in the first year of life protects children of Indian subcontinent ethnic origin against tuberculosis in England. J. Epidemiol. Comm. Health **45** (1991) 78–80.

The aim was to assess the protection conferred by BCG given during the first year of life against tuberculosis among children of Asian ethnic origin born in England. This was a matched case-control study. Cases were selected from notifications of tuberculosis and controls were selected from child health or school health records in 14 English health districts; 111 cases of childhood tuberculosis with Asian names were selected. For each case there were five controls with Asian names, matched for age, sex and district of birth. Child health or school health records were searched to determine the proportions of cases and controls who had been vaccinated with BCG. Overall, BCG vaccination given in the first year of life was estimated to confer 49% protection against tuberculosis with 95% confidence interval 14%–62%.

BCG vaccination in infancy was found to be associated with a lower protective efficacy than has been found for the secondary school age BCG program (80%) but nevertheless the protection is substantial and, in the United Kingdom, BCG vaccination of infants considered to be at relatively higher risk of tuberculosis is likely to reduce the incidence of childhood tuberculosis.—Authors' Abstract

Ruf, B., Schurmann, D., Mauch, H., Jautzke, G., Fehrenbach, F. J. and Pohle, H. D. Effectiveness of the macrolide clarithromycin in the treatment of *Mycobacterium avium* complex infection in HIV-infected patients. Infection **20** (1992) 267–272.

In a randomized double-blind study, nine mycobacteremic patients with AIDS-related disseminated *Mycobacterium avium* complex (MAC) infection received clarithromycin or placebo in addition to a basic regimen that included isoniazid, ethambutol and clofazimine. All four patients receiving clarithromycin showed blood culture conversion and clinical response. Of the five patients treated without clarithromycin, two showed resolution of mycobacteremia and clinical response, while another two died without having shown response. The remaining patient deteriorated until a switch from placebo to clarithromycin led to blood culture conversion and rapid clinical improvement. After finishing 6 weeks of intensive treatment, clarithromycin was given in an open maintenance phase to all

patients, initially in combination with rifabutin for 24 weeks and then alone. One patient had a relapse of MAC infection while receiving clarithromycin alone. The relapse was associated with acquired resistance to the drug. Clarithromycin appears to be a promising component of multidrug therapy for patients with MAC infection. Monotherapy can lead to drug resistance.—Authors' Abstract

Sada, E., Aguilar, D., Torres, M. and Herrera, T. Detection of lipoarabinomannan as a diagnostic test for tuberculosis. J. Clin. Microbiol. **30** (1992) 2415–2418.

A coagglutination technique was established for the detection of lipoarabinomannan of *Mycobacterium tuberculosis* in human serum samples and evaluated for its utility in the diagnosis of tuberculosis at the Instituto Nacional de Enfermedades Respiratorias in Mexico City. The test had a sensitivity of 88% in patients with sputum-smear-positive active pulmonary tuberculosis. The sensitivity in patients with active pulmonary tuberculosis negative for acid-fast bacilli in sputum was 67%. Less favorable results were obtained for patients with AIDS and tuberculosis, with a sensitivity of 57%. The specificity in control patients with lung diseases different from tuberculosis and in health subjects was 100%. The positive predictive value was 100%, and the negative predictive value for patients with sputum-positive active pulmonary tuberculosis was 97%. The results of this study suggest that the detection of lipoarabinomannan is an accurate test for the diagnosis of pulmonary tuberculosis.—Authors' Abstract

Savic, B., Sjobring, U., Alugupalli, S., Larsson, L. and Miorner, H. Evaluation of polymerase chain reactions, tuberculostearic acid analysis, and direct microscopy for the detection of *Mycobacterium tuberculosis* in sputum. J. Infect. Dis. **166** (1992) 1177–1180.

Tuberculosis remains a major global cause of morbidity and mortality. There is an urgent need for improved bacteriologic diagnosis of *Mycobacterium tuberculosis* infection. Three methods for rapid identification of *M. tuberculosis* in sputum samples (direct microscopy, gas chromatography–mass spectrometry [GC-MS], and polymerase chain reaction [PCR]), were compared with culture on Lowenstein-Jensen medium. Growth of *M. tuberculosis* was observed in 38 of 145 sputum samples. Detection of acid-fast bacilli by direct microscopy gave a sensitivity of 66% and a specificity of 100%. Detection of tuberculostearic acid by GC-MS gave a sensitivity of 55% and a specificity of 87%. Amplification by PCR of a fragment of the insertion sequence IS6110 gave a sensitivity of 95% and a specificity of 93% compared with culture and a corrected specificity of 99% compared with both culture and clinical data. This study indicates that PCR can be adapted for clinical use and is the method of choice for rapid diagnosis of pulmonary tuberculosis.—Authors' Abstract

Sbarbaro, J. A., Iseman, M. D. and Crowle, A. J. The combined effect of rifampin and pyrazinamide within the human macrophage. Am. Rev. Respir. Dis. **146** (1992) 1448–1451.

A recent study in the murine model suggested that a combination of rifampin and pyrazinamide used as preventive therapy might shorten the duration of treatment time. Clinical trials using this combination have been initiated, but significant results will not be available for many years. The *ex vivo* human macrophage model has been instructive in expanding our knowledge of the activity of chemotherapeutic agents against intracellular virulent tubercle bacilli. Prior studies have shown rifampin to have a bactericidal effect in this model while even at clinically unachievable levels, pyrazinamide had only a bacteriostatic impact.

This study finds an enhanced bacteriostatic effect when low, nonbactericidal levels of rifampin are combined with clinically achievable levels of pyrazinamide but not with higher bactericidal levels of rifampin. Adding pyrazinamide 2 days after the introduction of rifampin clearly enhanced the combined killing effect. However, reversing the order and adding rifampin 2 days after the introduction of pyrazinamide produced a result weaker than introducing the agents simultaneously. Our findings do not support the use of these agents as a potentially effective preventive therapy combination, but

they suggest that the timing of the administration of these chemotherapeutic agents could be an important factor in their effectiveness.—Authors' Abstract

Shawar, R. M., Elzaatari, F. A. K., Nataraj, A. and Clarridge, J. E. Detection of *Mycobacterium tuberculosis* in clinical samples by 2-step polymerase chain reaction and nonisotopic hybridization methods. J. Clin. Microbiol. **31** (1993) 61–65.

Detection of *Mycobacterium tuberculosis* in clinical specimens by the polymerase chain reaction (PCR) was compared with detection by culture. A 317-bp segment within the *M. tuberculosis*-specific insertion sequence IS6110 was amplified. The detection limit of the PCR assay for cultured mycobacteria was 50 cells per reaction by ethidium bromide-stained agarose gel electrophoresis and 5 cells per reaction by hybridization with an oligonucleotide probe conjugated with either digoxigenin or alkaline phosphatase (AP). This sensitivity was reduced fivefold in sputum specimens seeded with *M. tuberculosis.* Seventy-six clinical specimens were amplified and examined by the three detection methods. Both the digoxigenin and AP procedures were found to be more sensitive than agarose gel electrophoresis, but they were occasionally associated with a high background. An additional 308 specimens were examined only by agarose gel electrophoresis and the AP procedure. Of 71 specimens found to contain *M. tuberculosis,* amplified products were detected from 56 (79%) samples by agarose gel electrophoresis and/or the AP procedure. Of the additional 313 specimens that were culture negative for *M. tuberculosis,* 19 (6%) had amplified products detectable by agarose gel electrophoresis and/or the AP procedure. Compared with culture, PCR showed sensitivities and specificities of 55% and 98%, respectively, for agarose gel electrophoresis and 74% and 95%, respectively, for the AP procedure. Despite this low sensitivity, a rapid positive PCR result was accurate and clinically useful.—Authors' Abstract

Stelandre, M., Bosseloir, Y., DeBruyn, J., Maes, P. and Content, J. Cloning and sequence analysis of the gene encoding an NADP-dependent alcohol dehydrogenase in *Mycobacterium bovis* BCG. Gene **121** (1992) 79–86.

The nucleotide sequence of a 1619-bp fragment of *Mycobacterium bovis* BCG containing the gene that encodes an alcohol dehydrogenase (ADH) has been determined. The M(r) calculated from the deduced amino acid (aa) sequence, as well as the N terminus, are in good accordance with those determined for the ADH purified from *M. bovis* BCG extracts. The *M. bovis* BCG cloned adh gene was expressed in *Escherichia coli* by its own promoter and the synthesized product shows ADH activity in the butane-1-ol-NADP system. Based on comparison of the aa sequence, this enzyme belongs to the zinc-containing, long-chain alcohol/polyol dehydrogenase family, which has been primarily described in eukaryotes. Of the 22 strictly conserved residues in this group, 19 are also conserved in *M. bovis* BCG ADH (BCGADH).—Authors' Abstract

Styblo, K. The impact of HIV infection on the global epidemiology of tuberculosis. Bull. IUATLD **66** (1991) 27–32.

This paper draws together data on the levels of HIV and tuberculous infection in different countries and considers the impact of HIV infection on tuberculous infection and what strategies can be adopted to control tuberculosis (TB). HIV infection is the strongest risk factor for the development of tuberculous disease observed in the past 100 years. The impact of HIV infection on the epidemiology of TB will depend on the prevalence of HIV infection in the community, the prevalence of tuberculous infection, the breakdown rate from tuberculous infection to active TB, the average annual risk of tuberculous infection and the detection and cure rates of TB.

The author argues that in developed countries the incidence of TB is so low and declining that there HIV infection will not stop the elimination of TB. [This assertion should be viewed with caution for it assumes that tubercle bacilli will remain sensitive to the currently available anti-TB drugs.] In many developing countries there has been no change in the incidence of TB over the past 4 decades and it is estimated

that 50% of the adult population have been infected with tubercle bacilli. Thus there are substantial numbers of young adults exposed to the risk of primary tuberculous infection as well as others in whom HIV infection could lead to a reactivation of tuberculous infection. The absolute number of cases of TB in developing countries will increase in those countries where AIDS is a major problem.

Styblo then asks how can the predicted increase in TB cases associated with HIV infection be curbed. He postulates a country in sub-saharan Africa with 4 million of the population aged 15–44 years of whom 1.5 million have previously been infected with tubercle bacilli and an annual infection rate of 2% among the remaining 2.5 million. For a mass chemoprophylaxis program this 4 million would have to be tuberculin tested together with a seroprevalence survey of HIV infection. This is not a feasible proposition. Voluntary HIV and tuberculin testing followed by chemoprophylaxis for positive individuals would, at best, lead to a 25% overall reduction in TB cases associated with HIV infection. The author argues that the only way to curb the increase in tuberculous cases by HIV infection is by scrupulously maintaining high detection and cure rates and coping with the anticipated increase in TB cases until the HIV epidemic stabilizes in the population.—D. N. J. Lockwood (Trop. Dis. Bull.)

Tazi, A., Bouchonnet, F., Valeyre, D., Cadranel, J., Battesti, J. P. and Hance, A. J. Characterization of gamma/delta lymphocytes-T in the peripheral blood of patients with active tuberculosis—a comparison with normal subjects and patients in sarcoidosis. Am. Rev. Respir. Dis. **146** (1992) 1216–1221.

Studies in experimental animals have suggested that gamma/delta T cells play an important role in the immune response against mycobacteria, but evidence for the participation of these cells in the course of human tuberculosis remains fragmentary. We have evaluated the number and state of activation of gamma/delta T cells in the peripheral blood of patients with active tuberculosis using two-color cytofluorometry, and we have sought evidence that these cells might play a role in the impaired responses to recall antigens seen in some patients by comparing the proliferation of blood T lymphocytes before and after removing gamma/delta T cells by panning. Results were compared with those obtained for cells from normal subjects and from patients with sarcoidosis. The proportion and absolute number of circulating CD3+ gamma/delta T cells were not significantly different comparing blood from patients with tuberculosis and that from control subjects [54.6 ± 39.9 (N = 17) and 59.1 ± 30.2 cells/μl (N = 10), respectively, $p > 0.21$, and the proportion of cells expressing receptors using the Vdelta 1 variable region remained unchanged in patients with tuberculosis. Few gamma/delta T cells from patients with tuberculosis expressed surface antigens associated with activation (IL-2R, < 1%; HLA-DR, 2.6 ± 3.4%). Four of 15 patients with sarcoidosis had a proportion of gamma/delta T cells that was outside the range observed in normal subjects, but the absolute number of CD3+ gamma/delta T lymphocytes was not different comparing the two groups ($p > 0.2$). Removal of gamma/delta T cells did not significantly modify the PPD or candida-induced proliferative response of blood T lymphocytes from patients or control subjects and did not increase the proliferative response of patients who responded poorly to these antigens. Thus, tuberculous infection is not associated with an increase in number or state of activation of circulating gamma/delta T cells in humans. Alpha/beta T cells, not gamma/delta T cells, are the predominant population that proliferates in response to PPD, and no evidence for the participation of gamma/delta T cells in the anergy observed in some patients could be identified.—Authors' Summary

Thierry, D., Chureau, C., Aznar, C. and Guesdon, J.-L. The detection of *Mycobacterium tuberculosis* in uncultured clinical specimens using the polymerase chain reaction and a non-radioactive DNA probe. Mol. Cell. Probes **6** (1992) 181–191.

A *Sal* I–*Hin* dIII restriction fragment from *Mycobacterium tuberculosis* was found to hybridize specifically with genomic DNA from *M. tuberculosis.* Primers were de-

signed from the sequence of this fragment and used to amplify uniquely *M. tuberculosis*-group DNA in a polymerase chain reaction. It is suggested that a combination of these primers and an acetylaminofluorene-labeled probe will prove to be a useful tool for the early diagnosis of tuberculous infections.—Authors' Abstract

Thomas, T. J., Andrews, R. E. and Thoen, C. O. Molecular cloning and characterization of *Mycobacterium paratuberculosis* promoters in *Escherichia coli.* Vet. Microbiol. **32** (1991) 351–362.

DNA fragments from *Mycobacterium paratuberculosis* were cloned in the promoter probe plasmid pKO1. Of 957 recombinant DNA clones, 24 induced synthesis of galactokinase (the reporter gene) when these plasmids were transformed into an *Escherichia coli* strain deficient for the enzyme. A DNA insert from one putative promoter-containing plasmid, designated pAG5, was sequenced and shown to contain a characteristic RNA polymerase binding site, a probable ribosomal binding site and a putative open reading frame.—Authors' Abstract

Varnerot, A., Clement, F., Gheorghiu, M. and Lévy-Frèbault, V. V. Pulsed field gel electrophoresis of representatives of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG strains. FEMS Microbiol. Lett. **98** (1992) 155–160.

Using field inversion gel electrophoresis (FIGE), different *Mycobacterium tuberculosis* strains, such as phage prototypes, exhibit different DNA restriction patterns which are easy to compare. Virulent and avirulent variants of *M. tuberculosis* H37, as well as daughter strains of *M. bovis* BCG, display characteristic DNA profiles. BCG strains isolated from suppurative adenitis following vaccination of French patients showed patterns identical to the BCG Pasteur strain used for vaccination. These results demonstrate that FIGE of DNA restriction fragments generated by DraI represents a suitable technique for the analysis of mycobacteria at a genomic level. The DraI profiles allow the differentiation and precise identification of the BCG Pasteur, Glaxo, Russian and Japanese strains.—Authors' Abstract

Vazquez, J. A. and Sobel, J. D. A case of disseminated *Mycobacterium marinum* infection in an immunocompetent patient. Eur. J. Clin. Microbiol. Infect. Dis. **11** (1992) 908–911.

An unusual case of *Mycobacterium marinum* cutaneous infection is described. As a result of marked delay in the diagnosis, extensive local inflammation and destructive osteomyelitis occurred together with cutaneous dissemination in an immunocompetent host. Pathologic fractures in the infected bone necessitated amputation of the involved digit. The most striking feature of this case was the development of multiple widespread cutaneous lesions for several months following amputation of the infected digit and initiation of appropriate antimicrobial therapy. These new cutaneous lesions may reflect local immune and inflammatory reactions to previously disseminated microorganisms.—Authors' Abstract

Verbon, A., Hartskeerl, R. A., Moreno, C. and Kolk, A. H. J. Characterization of B cell epitopes on the 16K antigen of *Mycobacterium tuberculosis.* Clin. Exp. Immunol. **89** (1992) 395–401.

To characterize the antigenic parts of the 16K protein of *Mycobacterium tuberculosis,* overlapping peptides according to the amino acid sequence of the 16K protein were synthesized. In total, 14 peptides of 20 amino acids in length with an overlap of 10 amino acids and two additional decapeptides (amino acids 31–40 and 61–70) were tested with 8 anti-16K MoAbs and human sera. The common recognition site of MoAbs F67-8 and F67-16 was LRPTFDTRLM (amino acids 31–40) and of MoAbs F159-1 and F159-11 DPDKDVDIMV (amino acids 61–70). However, for binding of the MoAbs to these peptides additional amino acids were required at either the N- or C-terminus, suggesting that some kind of conformation is required. The recognition sites of the MoAbs F23-41, F23-49, F24-2 and TB68 could not be identified using the peptides, indicating that the MoAbs only

bound to conformational epitopes and not to peptides which may contain parts of these epitopes. The MoAbs bound to β-galactosidase fusion proteins comprising parts of the 16K protein, indicating that some kind of native conformation is present on the recombinant proteins. Sera from 14 of 19 patients with tuberculosis and from 0 of 19 controls reacted with the purified 16K protein. Sera from 4 of these 14 patients reacted with two overlapping peptides (amino acids 71–100). Apparently, antibodies in patients' sera against the 16K protein are predominantly directed against conformational epitopes.—Authors' Summary

Verbon, A., Kuijper, S., Jansen, H. M., Speelman, P. and Kolk, A. H. J. Antibodies against secreted and nonsecreted antigens in mice after infection with live *Mycobacterium tuberculosis.* Scand. J. Immunol. **36** (1992) 371–384.

Mice from four different inbred strains were infected with live *Mycobacterium tuberculosis* and the immune response to *M. tuberculosis* was followed for 24 weeks using Western blotting. Nearly all mice, irrespective of H-2 type, reacted with the 38-kDa protein band. Antibodies against this secreted 38-kDa protein were the first to appear 4 weeks after infection. Thereafter the secreted 19-kDa protein and nonsecreted antigens, such as the 65-kDa and 33-kDa proteins, were recognized. The immune response against the nonsecreted antigens was influenced by the mouse strain. However, the 33-kDa protein band was recognized by all mouse strains after a second injection with live *M. tuberculosis.* The specificity of the antibodies was analyzed in Western blot using sonicates of *M. tuberculosis, M. kansasii, M. avium, M. terrae, M. gordonae* and *Escherichia coli.* Antibodies against the 38-kDa and 33-kDa protein bands seemed to be specific for *M. tuberculosis,* while antibodies against the 19-kDa protein band showed limited crossreactivity. Antibodies against the 65-kDa protein were strongly crossreactive. These results suggest that the 38-kDa protein is secreted *in vivo* and, therefore, may be available to the humoral immune system at an early stage of infection. The nonsecreted 33-kDa protein is only recognized by all mouse strains after prolonged contact with *M. tuberculosis.*—Authors' Abstract

Veringa, E., Van Harsselaar, B. and Hermans, P. Polymerase chain reaction to detect *Mycobacterium tuberculosis* in a clinical microbiology laboratory. J. Microbiol. Methods **16** (1992) 139–147.

Direct detection of DNA from *Mycobacterium tuberculosis* in clinical specimens was performed by *in vitro* amplification of DNA using the polymerase chain reaction (PCR), followed by specific detection of the amplified product using DNA hybridization with a non-radioactive detection system. Primers based on a recently described insertion element IS986 were used to detect *M. tuberculosis.* The results were compared with results from traditional culture techniques. A total number of 107 specimens were examined. PCR and hybridization results were positive in 54 of 67 culture positive specimens. The number of PCR-positive specimens was increased to 63 by concentrating the samples by ethanol precipitation prior to amplification. This treatment, however, decreased specificity. The negative PCR reaction in the remaining 4 culture-positive samples could be explained by the presence of inhibitors. Of the 40 culture-negative samples 5 were PCR positive, one of which was false-positive. PCR and hybridization generated results significantly more rapidly than traditional culture techniques, but the former assay also appeared to be more expensive.—Authors' Abstract

Vordermeier, H. M., Harris, D. P., Friscia, G., Roman, E., Surcel, H. M., Moreno, C., Pasvol, G. and Ivanyi, J. T-cell repertoire in tuberculosis—selective anergy to an immunodominant epitope of the 38-kDa antigen in patients with active disease. Eur. J. Immunol. **22** (1992) 2631–2637.

It is generally accepted that both host protection and pathogenic reactions in tuberculosis are mediated by T lymphocytes. However, little is known about the structures and discrete functions of epitopes stimulating the immune response. In this study, proliferative responses of blood T lymphocytes to synthetic peptides derived

from the sequence of the 38-kDa antigen from *Mycobacterium tuberculosis* have been investigated in 41 healthy individuals and in 36 patients with active tuberculosis. Of the healthy purified protein derivative (PPD)-positive donors, 90% responded to a permissively recognized peptide, 38.G (residues 350–359), located at the carboxy terminus of the molecule. Four other permissively recognized epitopes of this molecule (38.A, 38.1, 38.E, 38.K) were stimulatory for more than 50% of healthy PPD-positive individuals. Patients with lymphatic tuberculosis responded to these peptides in a similar manner. In contrast, we observed a selective anergy to stimulation with peptide 38.G in the majority of patients with pulmonary (11% responders) and nonlymphatic extrapulmonary tuberculosis (25% responders). The lack of responsiveness to 38.G was epitope specific since the degree of responsiveness to the other four permissively recognized peptide epitopes was similar for patients and PPD-positive controls. Using the PEPSCAN technology and truncated peptides, the core epitope of 38.G was localized to a peptide 10 amino acids long (HFQPLPPAVV). This minimal structure was capable of inducing a proliferative response in all healthy 38.G responders tested. The mechanisms influencing this epitope-specific anergy in patients could give new insights into the immunopathogenesis of tuberculosis.—Authors' Abstract

Vorobyev, A. A., Baduksharova, N. M., Golovlev, I. R., Krasnoproshina, L. I., Khlebnikov, V. S., Fadeeva, N. I., Zimin, A. E. and Afanasyev, S. S. [Use of enzyme immunoassay and immunoblotting for the serological characterization of mycobacterial antigens.] Zh. Mikrobiol. Epidemiol. Immunobiol. **2** (1992) 65–68. (in Russian)

The enzyme immunoassay and immunoblotting were used for the study of the serological activity of different mycobacterial antigens and the spectrum of antibodies to them in patients with different forms of tuberculosis and healthy persons. Antibodies in patients' sera were shown to bind antigens with different molecular weights. The level and spectrum of antibodies to purified protein fraction I made it possible to differentiate between patients with various forms of tuberculosis and healthy persons.—Authors' English Abstract

Walker, D. A., Taylor, I. K., Mitchell, D. M. and Shaw, R. J. Comparison of polymerase chain reaction amplification of two mycobacterial DNA sequences, IS6110 and the 65-kDa antigen gene, in the diagnosis of tuberculosis. Thorax **47** (1992) 690–694.

Knowledge of the sequences of mycobacterial genes and the availability of DNA amplification techniques have raised the possibility that identification of mycobacterial DNA may offer a rapid and specific diagnostic test for tuberculosis. The correlation between the presence of *Mycobacterium tuberculosis* DNA and clinical tuberculosis, however, is not known. This study compared the results of polymerase chain reaction amplification of two *M. tuberculosis* DNA sequences, IS6110 and the gene encoding the 65kDa heat shock protein (65kDa Ag), from sputum, bronchoscopy washings, and bronchoalveolar lavage fluid and related these findings to the presence of active and past tuberculosis. Highly specific primers were used for amplification of IS6110 and 65kDa Ag DNA. Analysis was performed on one or more samples from 87 patients. IS6110 DNA was identified in samples from all 6 patients with active tuberculosis, from 15 to 18 patients with past tuberculosis, from 5 of 9 contacts of patients with tuberculosis, and from 9 of 54 patients with lung disease unrelated to tuberculosis. The 65kDa Ag DNA was identified in samples from all patients with active and past tuberculosis, from contacts of patients with tuberculosis, and from 14 of 42 patients with nontuberculous lung diseases. These data suggest that the presence of IS6110 DNA correlates more closely with a tuberculosis-related diagnosis than that of 65kDa Ag DNA, and that both DNAs are found in most subjects with past tuberculosis or contacts of patients with tuberculosis. This may limit the clinical usefulness of these tests.—Authors' Abstract

Whittier, P. S., Westfall, K., Setterquist, S. and Hopfer, R. L. Evaluation of the Septi-Chek AFB system in the recovery of my-

cobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **11** (1992) 915–918.

The performance of the Septi-Chek AFB System (Roche) in the isolation of mycobacteria was compared to that of culture on Lowenstein-Jensen (LJ) medium and the BACTEC radiometric system. The Septi-Chek AFB system detected a significantly higher number of positive specimens (62/66 versus 47/66 for BACTEC and 39/56 for LJ medium) and was more often the only medium in which an isolate was recovered. The average time for detection of isolates was very similar for the Septi-Chek AFB and BACTEC systems which were both significantly faster than LJ medium in the majority of isolates.—Authors' Abstract

Wiker, H. G. and Harboe, M. The antigen-85 complex—a major secretion product of *Mycobacterium tuberculosis*. Microbiol. Rev. **56** (1992) 648–661.

The large number of different proteins synthesized by the mycobacterial cell are currently classified and studied in terms of groups of proteins with certain common properties such as physical and chemical characteristics, function, and localization in the mycobacterial cell. Proteins that are actively secreted during culture on synthetic media represent a particular group of great current interest. At least eight proteins secreted by *Mycobacterium tuberculosis* have been isolated and characterized to various extents. The genes coding for five proteins secreted from *M. tuberculosis* and/or *M. bovis* BCG have been cloned and sequenced. All of them contain typical signal sequences. The proteins of the antigen 85 complex, which form the main subject of this review, are often the most common proteins in *M. tuberculosis* culture fluid. The constituents denoted 85A, 85B, and 85C are encoded by three genes located at different sites in the mycobacterial genome and show extensive crossreactivity as well as homology at amino acid and gene levels. The proteins differ slightly in molecular mass in the 30- to 31-kDa region, and all of them are fibronectin-binding proteins, but the significance of the latter observation and the role of these proteins in mycobacterial physiology and interaction with the infected host remain to be elucidated. The antigen 85 complex proteins are strongly immunogenic in natural and experimental mycobacterial infections in terms of both induction of antibody synthesis and T-cell-mediated reactions. The well-recognized difference in the efficacy of live and dead mycobacterial vaccines should be considered in relation to the group of secreted antigens. After inoculation, live bacteria in vaccines such as BCG multiply in the host, probably releasing several constituents belonging to the class of secreted proteins and hence resulting in more efficient stimulation of the immune system. Secreted mycobacterial antigens are expected to be of particular significance in induction of various immune responses that are responsible for development of protective immunity in some individuals and for clinical symptoms and complications of the ensuing disease in others.—Authors' Abstract

Wiker, H. G., Nagai, S., Harboe, M. and Ljungqvist, L. A family of cross-reacting proteins secreted by *Mycobacterium tuberculosis*. Scand. J. Immunol. **36** (1992) 307–319.

Crossreactions between five proteins actively secreted by *Mycobacterium tuberculosis* were studied by crossed immunoelectrophoresis, SDS-PAGE with immunoblotting, and ELISA using polyclonal rabbit antisera and mouse monoclonal antibodies to the purified proteins. The monoclonal antibody HBT4 was demonstrated to react with the MPT51 protein. The 85A, 85B and 85C constituents of the *M. tuberculosis* and *M. bovis* BCG antigen 85 complex crossreact extensively, each of the components containing component-specific as well as crossreacting epitopes. These components also crossreacted with MPT51 and MPT64. N-terminal sequence studies revealed striking homology at the amino acid level between 85A, 85B, 85C and MPT51. MPT64 showed less homology. In addition, striking homology was demonstrated between two different stretches within the 85B sequence and indicated between three stretches within the MPT64 molecule. Thus, a family of at least four secreted proteins with common structural features has been demonstrated in mycobacteria. MPT64 may

also belong to this family.—Authors' Abstract

Wilkinson, P. C. and Newman, I. Identification of IL-8 as a locomotor attractant for activated human lymphocytes in mononuclear cell cultures with anti-CD3 or purified protein derivative of *Mycobacterium tuberculosis.* J. Immunol. **149** (1992) 2689–2694.

On culture of human blood mononuclear cells for 24 to 48 hr with anti-CD3 (aCD3) or purified protein derivative of *Mycobacterium tuberculosis,* chemoattractants are released into the medium which induce polarization and locomotion of activated (G1) lymphocytes but not resting lymphocytes. Here we show that, during a period of up to 72 hr of culture, IL-8 is released in nanomolar quantities into the supernatant and that the lymphocyte chemoattractant activity of these supernatants is inhibited by incubation with anti-IL-8. Examination of the cultured mononuclear cells by immunofluorescence suggests that many monocytes, but almost no lymphocytes in aCD3 cultures contain IL-8 in cytoplasmic organelles, yet few monocytes direct from blood stained for IL-8. IL-8 is an attractant for only a small proportion (ca 10%) of lymphocytes direct from blood. The proportion of responding cells is increased after culture for 24 to 48 hr in aCD3 or purified protein derivative of *M. tuberculosis,* and these are a phenotypically distinct subpopulation consisting of large lymphocytes enriched for CD45RO. These cells respond to their own culture supernatants and to IL-8 in polarization assays and by invasion of collagen gels into which the attractants are incorporated. They also show orientation to a source of IL-8 in a chemotactic gradient. These responses are consistent with *in vivo* observations that the lymphocytes which migrate selectively into inflammatory sites are activated. The fact that many lymphocytes do not respond to IL-8 may reflect the diversity of migratory pathways shown by lymphocytes *in vivo,* the locomotion of small, recirculating, lymphocytes being regulated by other, unknown, locomotor stimuli.—Authors' Abstract

Ziegler, T. Synthesis of species-specific *Mycobacterium avium* trisaccharide (serovar-21) for the preparation of a neoglycoprotein for immunological studies. Angewandte Chemie (Int. edition in English) **31** (1992) 1358–1360.

A blockwise construction strategy was used to synthesize the complex pyruvylated oligosaccharide 1. The oligosaccharide can be easily attached to a carrier protein (R = (CH2)5-NH-BSA, BSA = bovine serum albumin) and might thus be used directly as a neoantigen for diagnostic purposes or for the preparation of specific antibodies. This is crucial for the early specific diagnosis of infections in AIDS patients, which was formerly possible only by time-consuming cultivation of isolated bacteria.—Authors' Abstract