CURRENT LITERATURE

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

General and Historical

Bhore, P. D., Bhore, C. P., Powar, S., Nade, A. L., Kartikeyan, S. and Chaturvedi, R. M. Child-to-parent education: a pilot study. Indian J. Lepr. **64** (1992) 51–57.

A controlled study carried out in the hilly Konkan region on the west coast of India showed that school children have the potential for transmitting their newly acquired knowledge to their parents. Although the results indicate that acquisition of knowledge does not mean a change in attitude concerning leprosy, child-to-parent education may show promising results in leprosy education in developing countries where most parents of school children are illiterate and are not easily reached by conventional methods of health education.—Authors' Abstract

Chemotherapy

Booth, S. A., Moody, C. E., Dahl, M. V., Herron, M. J. and Nelson, R. D. Dapsone suppresses integrin-mediated neutrophil adherence function. J. Invest. Dermatol. **98** (1992) 135–140.

The antiinflammatory influence of dapsone may involve suppression of neutrophil chemotaxis to selected attractants, but other actions of the drug are likely also involved. We have discovered that dapsone may suppress migration of neutrophils to extravascular sites through inhibition of adherence functions required for neutrophil recruitment. Neutrophil adherence mediated by integrins (CD11/CD18 or Mac-1 family receptors) was measured *in vitro* in terms of binding of stimulated cells to albumin-coated wells of microtiter plates, using phorbol myristate acetate (PMA) and N-formyl-methionyl-leucyl-phenylalanine (FMLP) as stimuli. Adherence was assessed by staining attached cells with crystal violet dye and measuring the dye concentration at OD_{590} using an automated plate reader. The role of integrins in this assay was confirmed by the ability of anti-integrin antibody to suppress stimulated neutrophil adherence. The OD_{590} value for cells adhering to albumin in the absence of stimulus and dapsone averaged 0.2 ± 0.04 (S.E.M.) over five experiments. In the presence of 0.1 μM PMA or 10^{-6} M FMLP, the OD_{590} values averaged 0.88 ± 0.1 and 0.75 ± 0.12, respectively. Dapsone did not affect unstimulated neutrophil adherence but, when present with stimulus, produced a dose-related inhibitory effect on adherence. Fifty percent inhibitory doses were approximately 150 μg/ml dapsone for both stimuli. Sulfapyridine reproduced the inhibitory effect of dapsone, but two structurally related compounds, hydrochlorothiazide and furosamide, did not. The observed ability of dapsone to inhibit neutrophil chemotaxis under agarose to FMLP and interleukin-8 may also be explained by interference with integrin-mediated adherence required for motility in this assay system. To consider if dapsone might have a similar inhibitory influence on neutrophil adherence *in vivo,* we tested the stimulated adherence function of neutrophils isolated from three individuals on dapsone therapy for dermatitis herpetiformis. Stimulated adherence of patients' cells averaged less than 40% of the control value. Suppression of leukocyte integrin function may therefore also contribute to the ability

of dapsone to inhibit neutrophil infiltration in neutrophilic dermatoses.—Authors' Abstract

Borcherding, S. M., Baciewicz, A. M. and Self, T. H. Update on rifampin drug interactions. 2. Arch. Intern. Med. **152** (1992) 711–716.

Rifampin is a potent inducer of hepatic P450 oxidative enzymes. Clinically important drug interactions have been documented between rifampin and numerous other drugs, such as oral anticoagulants, oral contraceptives, cyclosporine, digitalis, and ketoconazole. New, potentially clinically significant rifampin drug interactions have been reported for haloperidol, several antiarrhythmics, fluconazole, diltiazem, and select benzodiazepines. Further research has been conducted for previously reported drug interactions with rifampin involving such drugs as glucocorticoids, cyclosporine, verapamil, and oral anticoagulants. Proper management of these interactions is essential to avoid therapeutic failures on initiating rifampin therapy and potential toxic reactions after discontinuing rifampin. New rifampin drug interactions will continue to be identified with future investigations.—Authors' Abstract

Coleman, M. D., Russell, R. M., Tingle, M. D. and Park, B. K. Inhibition of dapsone-induced methaemoglobinaemia by cimetidine in the presence of thrimethoprim in the rat. J. Pharm. Pharmacol. **44** (1992) 114–118.

Administration of dapsone in combination with trimethoprim and cimetidine to male rats resulted in a marked decrease ($p < 0.05$) in measured methemoglobin levels (46.2 ± 24.4% Met Hb hr) compared with administration of dapsone alone (124.5 ± 24.4% Met Hb hr). The elimination half-life of dapsone (814 ± 351 min) was more than doubled in the presence of trimethoprim and cimetidine compared with control (355 ± 160 min, $p < 0.05$). However, there were no significant differences in AUC and clearance when dapsone was administered in combination with trimethoprim and cimetidine compared with dapsone alone. Co-administration of trimethoprim with dapsone in the absence of cimetidine did not affect either methemoglobin formation, AUCs, half-lives, or clearance values of dapsone compared with control. There was a threefold increase in the AUC of trimethoprim (6296 ± 2249 μg min mL^{-1}) in the presence of dapsone compared with trimethoprim alone (2122 ± 552 μg min mL^{-1}). There was also a corresponding decrease in the clearance of trimethoprim in the presence of dapsone compared with control (19.1 ± 6.9 vs 60.8 ± 21.0 mL min^{-1}). However, there was no change in the elimination half-life of trimethoprim between the two experimental groups (273 ± 120 vs 292 ± 54 min). The AUC of trimethoprim increased more than threefold in the presence of cimetidine (7100 ± 1501 μg min mL^{-1}) compared with trimethoprim alone (2122 ± 552 μg min mL^{-1}). There was also a corresponding reduction in the clearance of trimethoprim in the presence of cimetidine (61.2 ± 21.2 vs 17.8 ± 9.3 mL min^{-1}) compared with control. However, there was no significant change in the elimination half-life of trimethoprim after the administration of cimetidine (273 ± 136 vs 215 ± 109 min). Administration of either trimethoprim or cimetidine alone did not cause methemoglobin levels to exceed control values. The administration of trimethoprim with dapsone and cimetidine resulted in a significant increase in AUC (2122 ± 552 vs 5744 ± 3289 μg min mL^{-1}), a fall in clearance (17.8 ± 9.3 vs 60.8 ± 21 mL min^{-1}), but no change in half-life (252 ± 134 vs 273 ± 136 hr) of trimethoprim. The co-administration of cimetidine significantly reduced dapsone-mediated methemoglobin formation in the presence of trimethoprim, while the AUC of trimethoprim was significantly increased in the presence of both cimetidine and dapsone.—Authors' Abstract

Coleman, M. D. and Tingle, M. D. Use of a metabolic inhibitor to reduce dapsone-dependent haemotological toxicity. Drug Dev. Res. **25** (1992) 1–16.

Aside from its established use as an antileprotic and antiinflammatory drug, dapsone is also effective in the therapy of *Pneumocystis carinii* pneumonia. Unfortunately, its use is often limited by its dose-dependent

toxicity, such as methemoglobinemia and hemolysis; the latter condition occurs most frequently in glucose-6-dehydrogenase-deficient individuals. It is also responsible for occasional life-threatening disorders such as agranulocytosis. Dapsone may undergo acetylation, but its toxicity is due to the product of its oxidative metabolism, dapsone hydroxylamine. This is generated in man by the constitutive hepatic cytochrome P450 enzyme IIIA4. Studies in the rat revealed that dapsone-dependent methemoglobinemia could be greatly diminished by the co-administration of metabolic inhibitors. In the isolated perfused rat liver, dapsone hydroxylamine levels and, hence, methemoglobin formation fell significantly in the presence of cimetidine. In addition, the clearance of the parent drug was retarded, and perfusate concentrations of monoacetyl dapsone increased. The protective effect of cimetidine also reduced methemoglobin formation in the whole rat during the chronic administration of dapsone. Incubation of dapsone in a two-compartment *in vitro* system using human tissues in the presence of cimetidine or ketoconazole resulted in a decrease in methemoglobin formation in all the human livers tested, although cimetidine was only effective if incubated with microsomes and NADPH prior to the addition of dapsone. Administration of cimetidine (3 × 400 mg daily) to volunteers 3 days prior to and 4 days postadministration of a single dose of 100 mg dapsone caused drug concentrations to increase by almost 30%. There was a marked fall in peak methemoglobin levels and the percentage of the dose excreted in urine as dapsone hydroxylamine N-glucuronide was reduced by almost one third. During high-dose dapsone therapy it may be possible that the co-administration of cimetidine might reduce toxicity while maintaining drug efficacy.—Authors' Abstract

Franzblau, S. G., Biswas, A. N. and Harris, E. B. Fusidic acid is highly active against extracellular and intracellular *Mycobacterium leprae.* Antimicrob. Agents Chemother. **36** (1992) 92–94.

The activity of fusidic acid against *Mycobacterium leprae* was studied in axenic medium and in bacilli residing within mouse peritoneal macrophages. Activity was assessed by subsequent quantitation of bacillary radio-respirometric activity. Significant inhibition in both systems was observed at 0.156 μg/ml, and an approximately 50% reduction in activity occurred after exposure to 1.25 to 2.5 μg/ml. The excellent human pharmacokinetics and *in vitro* activity of fusidic acid against the leprosy bacillus warrant a clinical trial of this drug for leprosy.—Authors' Abstract

Hamada, K., Hiyoshi, T., Kobayashi, S., Ishida, S., Yagi, K. and Seino, M. Anticonvulsive effect of dapsone (4,4′-diaminodiphenyl sulfone) on amygdala-kindled seizures in rats and cats. Epilepsy Res. **10** (1991) 93–102.

Dapsone (4,4′-diaminodiphenyl sulfone; DDS), an established antileprosy drug, showed anticonvulsive effects in the amygdaloid kindling model of epilepsy. Single doses of the drug in rats (6.25–12.5 mg/kg, i.p.) suppressed the kindled seizures in a dose-dependent manner without overt behavioral toxicity. With repeated oral administration in cats, relatively higher initial doses (13–23 mg/kg) were required to obtain seizure suppression, and neurotoxic signs occurred within a few days with serum drug levels of approximately 20 μg/ml. Although dapsone showed anticonvulsive effects in both animal species, the effective serum levels overlapped the toxic levels reported in the clinical treatment of leprosy. In the majority of the cats, however, seizure suppression was maintained even after the discontinuation of dapsone with lower serum levels than those observed at the beginning of the seizure suppression. Therefore, dapsone would be useful as an antiepileptic drug only when long-term anticonvulsive efficacy is demonstrated using smaller doses comparable to those used in the treatment of leprosy.—Authors' Abstract

Lou, H.-Y., *et al.* [Observation on two-year therapeutic effect of leprosy MDT of 1482 cases in Jiangsu province.] Chin. J. Clin. Dermatol. **20** (1991) 296–298 (in Chinese).

This article analyzes the 2-year therapeutic effect of leprosy MDT on 1482 cases in

Jiangsu province, which include LL 418, BL 498, BB 132, BT 291, TT 136 and I 7. There were 1002 cases in which *Mycobacterium leprae* were positive. Results showed that clinical improvement was hastened by MDT. The bacterial index of the skin smears for *M. leprae* has decreased by 0.84 every year, and the decreasing rate during the 2 years was 69.25%. The bacteriologically negative rate is 50.19%. Leprosy reaction is reduced and obviously ameliorated. The chief side effect was red-coloring. Impairment of liver function was temporary; it did not affect therapies. Analyzing the data, the authors conclude that plan B of triple-drug regimen of leprosy therapies in Jiangsu province has superiority in on-the-spot implementation of multibacillary leprosy.—Authors' English Abstract

Prussick, R., Ali, M. A. M., Rosenthal, D. and Guyatt, G. The protective effect of vitamin E on the hemolysis associated with dapsone treatment in patients with dermatitis herpetiformis. Arch. Dermatol. **128** (1992) 210–213.

This study looked at whether oral vitamin C and vitamin E would protect the erythrocyte from oxidant damage caused by dapsone in patients with dermatitis herpetiformis. Fifteen consecutive patients with dermatitis herpetiformis taking dapsone therapy received, in addition, 800 U/d of vitamin E for 4 weeks; then 1000 mg of vitamin C per day for 4 weeks, and, finally, combined vitamin E and vitamin C therapy for 4 weeks. Hemolysis indexes were assessed at baseline and after each 4-week period. Statistical analysis of the results suggests that oral administration of 800 units of vitamin E daily for 4 weeks confers partial protective effect against dapsone-induced hemolysis in patients with dermatitis herpetiformis. Partial protection against dapsone-induced hemolysis by orally administered vitamin E, if confirmed as being clinically relevant by further trials, may allow clinicians to continue dapsone therapy orally in patients who develop significant hemolysis. Prophylactic vitamin E to minimize potential hemolysis at the initiation of dapsone therapy may also be appropriate.—From the article

Sandler, E. D., Ng, V. L. and Hadley, W. K. Clofazimine crystals in alveolar macrophages from a patient with the acquired immunodeficiency syndrome. Arch. Pathol. Lab. Med. **116** (1992) 541–543.

An induced sputum specimen from a 35-year-old patient with the acquired immunodeficiency syndrome (AIDS) contained numerous bright orange-red needle-shaped crystal inclusions in his alveolar macrophages. Careful questioning revealed that he recently had been treated for 7 months with clofazimine (200 mg/d) for persistent *Mycobacterium avium*-complex bacteremia. The striking cytologic finding observed is diagnosed easily if the characteristic morphologic appearance of the crystals and their location within the cytoplasm of macrophages and cells of the reticuloendothelial system is appreciated. Although this is the first observation at San Francisco (California, U.S.A.) General Hospital of clofazimine crystals in a respiratory specimen from a patient with AIDS, the potential of more widespread therapy with clofazimine in patients with AIDS who are infected with *M. avium*-complex makes it imperative that the microscopic appearance of these crystals be recognized.—Authors' Abstract

Tomioka, H., Saito, H., Sato, K., Yamane, T., Yamashita, K., Hosoe, K., Fujii, K. and Hidaka, T. Chemotherapeutic efficacy of a newly synthesized benzoxazinorifamycin, KRM-1648, against *Mycobacterium avium* complex infection induced in mice. Antimicrob. Agents Chemother. **36** (1992) 387–393.

Newly synthesized benzoxazinorifamycin, KRM-1648, was studied for its *in vivo* anti-*Mycobacterium avium*-complex (MAC) activities. When the MICs were determined by the agar dilution method with Middlebrook 7H11 agar medium, KRM-1648 exhibited similarly potent *in vitro* antimicrobial activities against the MAC isolated from AIDS and non-AIDS patients, indicating possible usefulness of KRM-1648 against AIDS-associated MAC infections. KRM-1648 exhibited potent therapeutic activity against experimental murine infections induced by *M. intracellulare* N-260 (virulent strain) and N-478, which has much weaker virulence. Similarly, KRM-1648 exhibited

an excellent therapeutic efficacy against *M. intracellulare* infection induced in NK-cell-deficient beige mice (as a plausible model for AIDS-associated MAC infection), in which a much more progressed state of gross lesions and bacterial loads at the sites of infection were observed. When the infected beige mice were killed at weeks 4 and 8, obvious therapeutic efficacy was seen on the basis of reduction in the incidence and degree of lung lesions and bacterial loads in the lungs and spleen with infections due to *M. intracellulare* N-241, N-256, and N-260. In this case, the efficacy was the highest in N-260 infection, followed by strain N-241. When mice were observed until infection-induced death, survival time of the infected beige mice was found to be prolonged by KRM treatment. However, KRM-1648 was not efficacious in suppressing the progression of pulmonary lesions and the increase in bacterial loads at the sites of infection, including lungs and spleen, at the late phase of infection. This may imply some difficulty with chemotherapy for AIDS-associated MAC infection, even with KRM-1648 treatment, which has excellent *in vitro* and *in vivo* anti-MAC activities, as shown in the present study.—Authors' Abstract

Zhang, Y., Steingrube, V. A. and Wallace, R. J., Jr. Beta-Lactamase inhibitors and the inducibility of the beta-lactamase of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. **145** (1992) 657–660.

Ten clinical isolates and the type strain (H37Rv) of *Mycobacterium tuberculosis* were shown to produce an intracellular β-lactamase. Crude enzyme preparations were extracted from acetone cell powders by grinding with zirconium beads in 0.133 M glycine with 1.0% Triton X-100. The enzymes had identical patterns on isoelectric focusing, with two major bands at isoelectric points of 4.9 and 5.1. The β-lactamase was highly susceptible to the new β-lactamase inhibitor BRL 42715, with an I_{50} of 0.0001 μg/ml. The enzyme was also susceptible to clavulanic acid with an I_{50} (0.05 μg/ml), which was similar to the value for the common bacterial β-lactamase TEM-1 (0.01 μg/ml). The latter result is consistent with previous MIC studies with *M. tuberculosis*, which have shown synergy between clavulanic acid and amoxicillin. BRL 42715 and clavulanic acid were more active than sulbactam, tazobactam, and cloxacillin. These studies support the potential value of penicillin/clavulanic acid and penicillin/BRL 42715 combinations in the treatment of tuberculosis.—Authors' Summary

Clinical Sciences

Ahaley, S. K., Sardeshmukh, A. S., Suryakar, A. N. and Samson, P. D. Correlation of serum lipids and lipoproteins in leprosy. Indian J. Lepr. **64** (1992) 91–98.

Serum lipids and lipoproteins were assessed in 60 leprosy patients and 40 age- and sex-matched healthy controls. The study subjects included cases of LL with reactions, LL without reactions, BL with reactions, BL without reactions, BT and TT types of leprosy. The levels of serum phospholipids, triglycerides, total cholesterol, LDL and VLDL fractions were significantly decreased in leprosy patients compared to control subjects. The levels of serum HDL cholesterol and HDL fraction were significantly elevated in leprosy patients. Maximum elevations in serum HDL cholesterol level and HDL fraction and maximum reduction in the levels of serum phospholipids, triglycerides, total cholesterol and LDL and VLDL fractions were observed in lepromatous leprosy (LL) patients with reactions.—Authors' Abstract

Alvardo, M. and Saúl, A. [A leprosy reaction involving the esophagus and the larynx.] Rev. Leprol. Fontilles **18** (1991) 227–228. (in Spanish)

A 52-year-old man with lepromatous leprosy of 20 years duration is presented. He had several reactional outbreaks. In the last one, dysphagia and dysphony were present,

which improved with treatment with thalidomide. It is supposed that such symptomatology was due to the involvement of the IX and X cranial nerves, a very rare feature in leprosy.—Authors' English Summary

Elissen, M. C. C. A. Beliefs of leprosy patients about their illness—a study in the province of South Sulawesi, Indonesia. Trop. Geogr. Med. **43** (1991) 379–382.

In Bone district, Province of South Sulawesi, Indonesia, a total of 50 randomly selected leprosy patients were interviewed about their beliefs about their illness with the help of a questionnaire. It became evident that their knowledge about leprosy was generally satisfactory, but only few patients adopted the bacterial theory as cause of their disease. Besides, it was found that leprosy patients tend to discriminate themselves, while more tolerance was found in their healthy contacts. Traditional beliefs and religious ideas played an important role. To overcome the stigma, more health education based on a multidisciplinary approach is required. Besides the modern medical theory, cultural beliefs and religious views have to be taken into consideration.—Author's Abstract

Furuta, M., Obara, A., Harada, N., Sokai, K. and Hiroshi, I. *Cryptococcus neoformans* can be misidentified as a microsporidian: studies of lung lesions in leprosy patients. J. Protozool. **38** (1991) 95S–96S.

Pulmonary lesions seen in autopsies of leprosy patients were initially thought to involve microsporidial infection. After immunohistochemical studies, it was concluded that the infectious microorganism was *Cryptococcus neoformans.*—Authors' Summary

Krishna Murthy, P., Subramanian, M., Reddy, B. N., Rao, P. S. and Neelan, P. N. Time lag between case registration and commencement of treatment in a leprosy control unit. Indian J. Lepr. **64** (1992) 8–13.

An analysis of client-based data as a part of computerized management information system in a government leprosy control unit in Tamil Nadu reveals that there was delay in initiating treatment of leprosy patients. The mean and standard deviation of the period of delay for cases registered before, within 6 months and after 6 months of start of MDT in the unit were 6.80 ± 6.40, 1.97 ± 3.60 and 0.90 ± 2.21 months, respectively. Further, the delay was longer in PB, female and child cases. Giving priority to therapy for backlog cases and an effective monitoring system with specific indicator for time lag in starting treatment is indicated.—Authors' Abstract

Ramirez, M. M. [Leprosy reaction in Lucio's phenomenon; treatment with griseofulvin.] Rev. Med. San Luis Potosi **1** (1991) 7–12. (in Spanish)

The particular form of reaction of diffuse lepromatous leprosy, called "Lucio's phenomenon," is reviewed. We have studied a patient with this kind of reaction from the clinical, histopathological and immunological point of view. We have utilized griseofulvin as a therapy with complete success, as far as we know, for the first time.—Author's English Summary

Immuno-Pathology

Baganha, M. F., Motapinto, A., Pego, M. A., Marques, M. A. T., Rosa, M. A. S. and Cordeiro, A. J. A. R. Neopterin in tuberculous and neoplastic pleural fluids. Lung **170** (1992) 155–161.

Neopterin is derived from guanosine-triphosphate, produced by stimulated macrophages under the influence of gamma-interferon of lymphocyte origin. It has been suggested as an excellent marker for acti-

vation of the monocyte/macrophage axis in some clinical situations. We evaluated its concentration in the pleural effusions of 25 individuals (10 tuberculous and 15 neoplastic) as well as in the blood of 22 of them (8 tuberculous and 14 neoplastic), comparing these levels with those of a control group in 99 normal individuals. The concentration of neopterin was determined by radioimmunologic assay. This showed a significant increase ($p < 0.001$) of neopterin levels in the tuberculous pleural fluid, compared to the neoplastic group (42 ± 23, 17 ± 9 nmol/L). In the blood, values were nearly identical to the pleural fluid (41.3 ± 25, 15.8 ± 6.9 nmol/L), although with significant differences between them and in relation to the control group ($p < 0.001$), which had a normal serum value (5.11 ± 1.92 nmol/L). We emphasize the influence of the neopterin levels in the pleural fluid on the diagnosis of causes of pleurisy and its importance as a marker of immunologic cellular activity.—Authors' Abstract

Barnes, P. F., Chatterjee, D., Brennan, P. J., Rea, T. H. and Modlin, R. L. Tumor necrosis factor production in patients with leprosy. Infect. Immun. **60** (1992) 1441–1446.

The spectrum of host responses to *Mycobacterium leprae* provides a model for investigating the role of cytokines in the pathogenesis of mycobacterial disease. Of particular interest is tumor necrosis factor (TNF), a cytokine which may have both antimycobacterial and immunopathologic effects. To evaluate the potential role of TNF in leprosy, we measured TNF production in response to *M. leprae* and its defined constituents by peripheral blood mononuclear cells (PBMC) from patients across the spectrum of disease. The levels of TNF induced through the stimulation of cells with *M. leprae* or its dominant "lipopolysaccharide," lipoarabinomannan, were higher in patients with the tuberculoid form of the disease than in those with the lepromatous form. In patients with erythema nodosum leprosum (ENL), a reactional state of lepromatous leprosy, the levels of TNF released by PBMCs were higher than in any other form of the disease. Treatment of ENL patients with thalidomide reduced TNF secretion by more than 90%. The mycolylarabinogalactanpeptidoglycan complex of *Mycobacterium* species, the protein-peptidoglycan complex, and muramyl dipeptide all elicited significant TNF release. Therefore, TNF release appears to be triggered by at least two major cell-wall structural constituents of *M. leprae*, lipoarabinomannan and segments of the cell-wall skeleton. The prominent TNF release in patients with the paucibacillary tuberculoid form of the disease compared with that in patients with the multibacillary lepromatous form suggests that this cytokine contributes to a resistant immune response to mycobacterial infection. However, the marked TNF release in patients with ENL indicates that TNF may also mediate immunopathologic effects, such as fever and tissue damage.—Authors' Abstract

Bloom, B. R., Modlin, R. L. and Salgame, P. Stigma variations—observations on suppressor T-cells and leprosy. Annu. Rev. Immunol. **10** (1992) 453–488.

Few areas of immunology have been so controversial as that of suppressor T cells. Studies of T-cell clones derived from patients with infectious diseases, including leprosy, and allergies have allowed the delineation of functional human T-cell subsets. Both CD4 and CD8 cells can be discriminated into subsets that are differentiated by their functions and patterns of lymphokines. Type 1 CD4 cells reactive with lepromin and PPD produce IFN-gamma and IL-2 predominantly, while Type 2 CD4 clones, specific for tetanus toxoid, produce IL-4 and IL-5. Type 1 CD8 cytotoxic T lymphocytes produce predominantly IFN-gamma and IL-2. T-suppressor clones derived from immunologically unresponsive lepromatous leprosy patients are antigen-specific, CD8 cells, HLA-DQ restricted, and produce predominantly IL-4, and were designated Type 2 CD8 cells. Several models for peripheral tolerance based on distinct functional T-cell subsets are discussed. Previous models of T-cell suppression in the mouse and the reciprocal relationship between humoral and cell-mediated immunity in general are reinterpreted in light of such T-cell subset interactions.—Authors' Abstract

Chiplunkar, S. V., Kudalkar, J. L., Butlin, R., Samson, P. D., Deo, M. G. and Gangal, S. G. Major proteins of mycobacterial strain ICRC and *Mycobacterium leprae*, identified by antibodies in sera from leprosy patients and their contacts. J. Clin. Microbiol. **30** (1992) 336–341.

Sera from leprosy patients across the clinical spectrum, healthy contacts, tuberculosis patients, and healthy donors were tested for their reactivity with antigens of mycobacterial strain ICRC (a cultivable mycobacterium) and *Mycobacterium leprae* by immunoprecipitation technique. Using *M. leprae* antigens, it was not possible to distinguish between reactivities of sera from lepromatous, borderline lepromatous, borderline tuberculoid, and tuberculoid leprosy patients. All these sera identified *M. leprae* antigens with molecular masses of 47, 36, 21, and 14 kDa. When the same sera were tested for their reactivities with antigens of mycobacterial strain ICRC, several differences were observed. The 21-kDa antigen of mycobacterial strain ICRC was exclusively precipitated by sera from all lepromatous leprosy patients and from those undergoing erythema nodosum leprosum reaction. Sera from all the other donors tested failed to identify the 21-kDa antigen of mycobacterial strain ICRC. The 14-kDa protein of mycobacterial strain ICRC was identified by sera from a few lepromatous leprosy patients (5 of 26) and all their contacts. Our studies indicate that antigens present on cultivable mycobacteria rather than species-specific antigens may prove to be useful in the serodiagnosis of leprosy.—Authors' Abstract

Chujor, C. S. N., Kuhn, B., Schwerer, B., Bernheimer, H., Levis, W. R. and Bevec, D. Specific inhibition of mRNA accumulation for lymphokines in human T cell line Jurkat by mycobacterial lipoarabinomannan antigen. Clin. Exp. Immunol. **87** (1992) 398–403.

The immunomodulatory effect of *Mycobacterium tuberculosis*-derived lipoarabinomannan (LAM) on mitogen/antigen-induced expression of mRNAs for a number of cytokines in human monocytic cell line Mono-Mac-6 and in T-cell line Jurkat was investigated. Interestingly, LAM exhibited a down-regulatory effect on the accumulation of mRNAs for IL-2, IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-2 receptor alpha (IL-2Rα) in T cells co-stimulated with phytohemagglutinin-P (PHA) and 4β-phorbol-12-myristyl-13-acetate (PMA). In human Mono-Mac-6 cells, LAM has a weak inhibitory effect on the lipopolysaccharide (LPS)-induced mRNA accumulation for IL-1β, a slight stimulatory effect on mRNAs accumulation for IL-8 and tumor necrosis factor-alpha (TNF-α), but clearly no effect on mRNA accumulation for intercellular adhesion molecule-1 (ICAM-1). These findings imply that LAM may contribute to the immunologic defects associated with a number of mycobacterial infections by modulating these mediators.—Authors' Summary

Convit, J., Sampson, C., Zúñiga, M., Smith, P. G., Plata, J., Silva, J., Molina, J., Pinardi, M. E., Bloom, B. R. and Salgado, A. Immunoprophylactic trial with combined *Mycobacterium leprae*/BCG vaccine against leprosy: preliminary results. Lancet **1** (1992) 446–450.

In an attempt to find a vaccine that gives greater and more consistent protection against leprosy than BCG vaccine, we compared BCG with and without killed *Mycobacterium leprae* in Venezuela. Close contacts of prevalent leprosy cases were selected as the trial population since they are at greatest risk of leprosy.

Since 1983, 29,113 contacts have been randomly allocated vaccination with BCG alone or BCG plus 6×10^8 irradiated, autoclaved *M. leprae* purified from the tissues of infected armadillos. We excluded contacts with signs of leprosy at screening and a proportion of those whose skin-test responses to *M. leprae* soluble antigen (MLSA) were 10 mm or more (positive reactions). By July 1991, 59 postvaccination cases of leprosy had been confirmed in 150,026 person-years of follow up through annual clinical examinations of the trial population (31 BCG, 28 BCG/*M. leprae*). In the subgroup for which we thought an effect of vaccination was most likely (onset more than a year after vaccination, negative MLSA skin-test response before vaccination), leprosy de-

veloped in 11 BCG recipients and 9 BCG/*M. leprae* recipients; there were 18% fewer cases (upper 95% confidence limit [CL] 70%) in the BCG/*M. leprae* than in the BCG alone group. For all cases with onset more than a year after vaccination irrespective of MLSA reaction the relative efficacy was 0% (upper 95% CL 54%; 15 cases in each vaccine group). Retrospective analysis of data on the number of BCG scars found on each contact screened suggested that BCG alone confers substantial protection against leprosy (vaccine efficacy 56%, 95% CL 27%–74%) and there was a suggestion that several doses of BCG offered additional protection. There is no evidence in the first 5 years of follow-up of this trial that BCG plus *M. leprae* offers substantially better protection against leprosy than does BCG alone, but the confidence interval on the relative efficacy estimate is wide.—Authors' Abstract

Davenport, M. P., McKenzie, K. R., Basten, A. and Britton, W. J. The variable C-terminal region of the *Mycobacterium leprae* 70-kilodalton heat shock protein is the target for humoral immune responses. Infect. Immun. **60** (1992) 1170–1177.

The 70-kDa heat-shock protein of *Mycobacterium leprae* has a high degree of homology with the human hsp70 protein, yet it still elicits T-lymphocyte responses in subjects infected with *M. leprae* or vaccinated with the related *M. bovis* BCG. We examined the serological responses to this protein by using recombinant protein fragments expressed from mutants with deletions of the *M. leprae* p70 gene. Monoclonal antibodies raised against either *M. bovis* or *M. leprae* p70 reacted with the C-terminal fragments but not the N-terminal fragments in a solid-phase enzyme-linked immunosorbent assay and an immunoblot assay. Inhibition enzyme-linked immunosorbent assays confirmed that two separate epitopes were defined by these monoclonal antibodies. Murine polyclonal sera also showed stronger binding to the C-terminal fragments. Sera from 33 and 48% of lepromatous leprosy patients reacted with *M. leprae* and *M. bovis* p70. This reactivity was mycobacterium specific, since few sera from control subjects in the same leprosy-endemic region were seropositive. The levels of antimycobacterial hsp70 antibodies were higher in patients with lepromatous leprosy than in those with tuberculoid leprosy or tuberculosis. The reactivity of sera from patients with leprosy was maximal with the C-terminal fragments. Therefore the C-terminal portion of *M. leprae* hsp70, which includes the region of maximum divergence from human hsp70, is the major target for the humoral immune response to the protein.—Authors' Abstract

Denis, M. Mouse T cell clones against *Mycobacterium avium*: identification of clones that modify resistance against atypical mycobacteria infection. J. Leukoc. Biol. **51** (1992) 7–12.

Mouse T-cell clones against live *Mycobacterium avium* were generated from the spleens of BALB/c mice infected with *M. avium* TMC 702. Eight clones were of the $L3T4^+$ subset; whereas two were of $Lyt2^+$ subset. Six of the $L3T4^+$ T-cell clones were of the TH_1 subset; whereas two were of the TH_2 subset, judged on the profile of cytokine release. One of the $Lyt2^+$ clones exhibited significant cytoxicity against *M. avium*-infected mouse macrophages. Transfer of clones to nude BALB/c mice infected with *M. avium* was associated with insignificant changes in resistance for seven clones. One clone, of the $L3T4^+/TH_2$ subset, transferred significant resistance to the infection, also associated with infusion of supernatants from the clone, which was fully inhibited by neutralizing with anti-interleukin 4. By contrast, infusion of one TH_1 clone and the cytolytic $Lyt2^+$ led to increased microbial growth in the spleens and livers of infected mice, which was not apparent on infusion with supernatants. Application of clones' supernatants on infected macrophages had marginal effects on *M. avium* growth and was not correlated with protective or suppressive activity. Overall, these results suggest that T cells may influence *M. avium* growth *in vivo* in a bidirectional manner and also suggest that interleukin 4 may be an important factor in host resistance to *M. avium*.—Author's Abstract

Dhandayuthapani, S., Izumi, S., Anandan, D. and Bhatia, V. N. Specificity of IgG subclass antibodies in different clinical

manifestations of leprosy. Clin. Exp. Immunol. **88** (1992) 253–257.

We analyzed specific IgG subclasses levels to *Mycobacterium leprae* sonicate extract (MSE), lipoarabinomannan B (LAM) and phenolic glycolipid-I (PGL-I) in the sera of leprosy patients with different clinical manifestations. IgG2 was found to be the predominant antibody to MSE regardless of clinical manifestations, and IgG1 response was mostly seen in lepromatous patients. IgG3 reacted only rarely but IgG4 reacted relatively more in certain clinical groups such as borderline lepromatous and lepromatous with erythema nodosum leprosum (ENL) reaction. Most of the IgG subclass responses to MSE could be accounted for reactivity with LAM, suggesting that LAM is the major immunogen involved in the pathogenesis of leprosy. In contrast to LAM, PGL-I antigen showed considerably lower reactivities for IgG subclasses. An association between IgG subclass responses and clinical manifestations of leprosy was also seen. Whereas borderline lepromatous patients were found to have significantly higher levels of IgG2 and IgG4 to MSE, lepromatous patients had elevated levels of IgG1 and lower levels of IgG2. An interesting observation, however, was the significantly higher levels of IgG2 to LAM in the pure neuritic leprosy patients.—Authors' Abstract

Ehrenstein, M. and Isenberg, D. Autoimmunity associated with infection: leprosy, acute rheumatic fever and Lyme disease. Curr. Opin. Immunol. **3** (1991) 930–935.

This review examines the links between autoimmunity and three common infectious diseases. These disorders are associated with a variety of clinical and serological autoimmune phenomena. In addition they might conceivably trigger autoimmune diseases themselves. Mechanisms that may be responsible for these links, including molecular mimicry, are explored.—Authors' Abstract

Espitia, C., Sciutto, E., Bottasso, O., González-Amaro, R., Hernández-Pando, R. and Mancilla, R. High antibody levels to the mycobacterial fibronectin-binding antigen of 30–31 kD in tuberculosis and lepromatous leprosy. Clin. Exp. Immunol. **87** (1992) 362–367.

Immunoblot assays showed that mycobacterial fibronectin-binding antigens are important targets of the humoral immune response in tuberculosis and leprosy. Using culture filtrate antigens of *Mycobacterium tuberculosis,* strong reactivity with the fibronectin-binding of 30–31 kDa (Fn 30–31) was demonstrated in 55.9% of tuberculosis sera and in 56.5% of lepromatous leprosy sera. Sera from patients with tuberculoid leprosy and control sera gave very weak binding. Reactivity of tuberculosis and lepromatous leprosy sera with the fibronectin-binding antigen of 58–60 kDa (Fn 58–60) was less conspicuous. The ability to react with fibronectin of the antigens of 58–60 and 30–31 kDa was demonstrated by parallel labeling with a fibronectin-biotin conjugate. Fn 30–31 was purified to homogeneity by a two-step procedure and used for ELISA. Positive titers were found in 63% out of 65 tuberculosis sera and in 60.5% out of 43 lepromatous leprosy sera. Antibody titers in lepromatous leprosy sera were higher than in tuberculosis sera. Our observations indicate indirectly that *M. leprae* possess a highly immunogenic molecule homologous to *M. tuberculosis* Fn 30–31, which elicits a high antibody response in lepromatous leprosy but not in tuberculoid leprosy. In this investigation, direct evidence for the presence of this antigen in *M. leprae* was obtained by immunochemistry of lepromatous leprosy lesions with a monospecific antibody raised against *M. tuberculosis* Fn 30–31.—Authors' Summary

Gelber, R. H., Murray, L., Siu, P. and Tsang, M. Vaccination of mice with a soluble protein fraction of *Mycobacterium leprae* provides consistent and long-term protection against *M. leprae* infection. Infect. Immun. **60** (1992) 1840–1844.

Groups of BALB/c mice were vaccinated intradermally with either Freund's incomplete adjuvant (FIA) alone, 10^7 heat-killed *Mycobacterium leprae* organisms in FIA, or a number of fractions of *M. leprae* containing soluble and/or cell wall components. At 1, 3, 6, 9, and 12 months later, vaccinated

mice were challenged in the right hind foot pad with 5000 live *M. leprae* organisms, and vaccine protection was assessed 6 to 8 months later, at the peak of *M. leprae* multiplication in the negative control (FIA alone), by the two-sample rank-sum test. In these studies, a cell-wall fraction rich in peptidoglycan was consistently ineffective. Both heat-killed *M. leprae* and a fraction containing cell-wall and fixed proteins generally protected when the interval between vaccination and challenge was 1 or 3 months but not subsequently. On the other hand, soluble proteins of *M. leprae* alone or in combination (with cell-wall fractions) consistently (14 of 14 instances) afforded highly significant protection ($p \leq 0.01$) at all challenge intervals up to 1 year after vaccination. These results suggest that the soluble protein fraction of *M. leprae* offers promise for a vaccine against leprosy.—Authors' Abstract

Geluk, A., Bloemhoff, W., de Vries, R. R. P. and Ottenhoff, T. H. M. Binding of a major T-cell epitope of mycobacteria to a specific pocket within HLA-DRw17 (DR3) molecules. Eur. J. Immunol. **22** (1992) 107–113.

$CD4^+$ T cells recognize antigenic peptides bound to the polymorphic peptide-binding site of major histocompatibility complex (MHC) class II molecules. The polymorphism of this site is thought to dictate which peptides can be bound and thus presented to the T-cell receptor. The mycobacterial 65-kDa heat-shock protein (hsp65) peptide 3-13 is an important T-cell epitope: it is immunodominant in the mycobacterium-specific T-cell response of $HLA\text{-}DR3^+$ individuals but, interestingly, cannot be recognized in the context of any other HLA-DR molecules. We, therefore, have tested whether the hsp65 epitope p3-13 is selected for T-cell recognition in the context of only HLA-DR3 molecules by an unique binding specificity for HLA-DR3. Using biotinylated peptides and EBV-transformed BLCL comprising all known HLA class II specificities, we find that p3-13 binds to HLA-DRw17(DR3) but not to any other HLA-DR molecule. Conversely, a control peptide p307-319 influenza hemagglutinin binds to all known HLA-DR molecules but only weakly to HLA-DRw17 and HLA-DR9. Peptide binding could be inhibited by excess unbiotinylated competitor analog as well as by anti-DR monoclonal antibodies but not by anti-class I-, anti-DP-, or anti-DQ-monoclonal antibodies.

The amino acid sequence of DRw17 molecules differs uniquely at five positions from the other DRβ1 sequences. Three of these five residues (positions 26, 71 and 74) are potential peptide contacting residues. These residues map closely together in the hypothetical three-dimensional model of the DR molecule and, thus, most probably form a positively charged pocket, critical for the binding of p3-13. Interestingly, p3-13 does not bind to a DR3 variant, the DRw18 molecule. The DRw18β1 chain differs from DRw17 at two major positions, close to or within the DRw17-specific pocket. These substitutions drastically change the structure and charge of the pocket, and thus presumably abrogate its ability to bind p3-13.—Authors' Abstract

Harboe, M., Wiker, H. G. and Nagai, S. Protein antigens of mycobacteria studied by quantitative immunologic techniques. Clin. Infect. Dis. **14** (1992) 313–319.

Crossed immunoelectrophoresis has great resolving power in the demonstration of immunogenic constituents of mycobacteria. The pattern with multiple precipitate lines is highly reproducible and allows precise identification of components. After the isolation of individual proteins, immunologic specificity combined with molecular weight determination and *N*-terminal amino acid sequencing should be used to ensure consistent identification in different laboratories. Simultaneous quantification of individual proteins in sonicates of washed bacilli and culture fluids permits the determination of a localization index, which indicates whether the proteins are cytoplasmic constituents or actively secreted. Several "new," actively secreted proteins have recently been defined, and the role of these proteins in the interaction between the bacilli and the infected host is discussed.—Authors' Abstract

Kanchana, M. V., Lakshminarayana, C. S., Sengupta, U., Sinha, S. and Ramu, G. An appraisal of enzyme linked immunosorbent assay (ELISA) and serum antibody

competition test (SACT) in leprosy. Indian J. Lepr. **64** (1992) 42–50.

Seventy-eight untreated leprosy patients, 104 treated patients and 105 healthy contacts were tested using two serological tests, SACT (serum antibody competition test based on competitive inhibition of monoclonal antibody binding to the MY2a determinant of *Mycobacterium leprae*) and ELISA (measurement of IgM antibodies to the neoglycoproteins D-BSA and ND-BSA representing the phenolic glycolipid antigen of *M. leprae*). The controls included normal healthy individuals, patients with sputum-positive pulmonary tuberculosis, and active cases of rheumatoid arthritis from the department of rheumatology. The specificity of SACT was found to be very high. ELISA was found to be positive in two patients with rheumatoid arthritis, one each for D-BSA and ND-BSA ELISA. Both tests had a high sensitivity in BL and lepromatous patients. The sensitivity to both tests was considerably lower in tuberculoid and BT patients, i.e., below 40%. Therefore, the diagnostic value of a negative test in suspected cases of leprosy was very low employing either of the two tests. A proportion of patients with paucibacillary tuberculoid and BT leprosy were positive after 6 months or longer after therapy. Similarly a large number of BL and lepromatous patients were positive after considerably longer periods of treatment. The use of either tests for determining the duration of therapy is therefore limited. SACT appears to be more sensitive than ELISA with ND-BSA in detecting subclinical infection. The cumulative positivity of the two tests may be used as a measure of the infectivity of the disease in the community and for evaluating disease control methods.—Authors' Abstract

Kumar, V., Narayanan, R. B. and Malaviya, G. N. An ultrastructural study of Schwann cells in peripheral nerves of leprosy patients. Indian J. Lepr. **64** (1992) 81–87.

An ultrastructural study of peripheral nerves in leprosy patients was carried out to ascertain the changes in Schwann cells containing myelinated and nonmyelinated axons. Axonal multiplication was noticed in nonmyelinated axons in specimens from both tuberculoid and lepromatous leprosy. The Schwann cells in tuberculoid nerves were devoid of *Mycobacterium leprae* in contrast to those in lepromatous nerves in which large numbers of bacilli were seen. These observations suggest that the Schwann cells containing nonmyelinated axons may be affected more frequently in either type of leprosy.—Authors' Abstract

Launois, P., Niang, M., Dieye, A. and Sarthou, J.-L. TNF-α failed to reverse the *M. leprae*-induced defective chemiluminescence response of human mononuclear cells. FEMS Microbiol. Immunol. **89** (1991) 91–96.

The effect of phagocyte activation by TNF-α on the ability to trigger a chemiluminescence (CL) response associated with the release of oxidizing species was evaluated in healthy human mononuclear cells in the presence of *Mycobacterium leprae*. Recombinant TNF-α (r-TNF-α) increased the CL response of unstimulated *M. bovis* BCG- and PMA-stimulated cells but did not reverse the *M. leprae*-defective activation of the human phagocyte oxidative burst. *M. leprae* was less well phagocytosed than *M. bovis* BCG but phagocytosis of mycobacteria was not altered by addition of r-TNF-α. The failure of activation of oxygen-free radical production might have some relevance to the pathogenesis of leprosy.—Authors' Summary

Mehra, V., Bloom, B. R., Bajardi, A. C., Grisso, C. L., Sieling, P. A., Alland, D., Convit, J., Fan, X.-D., Hunter, S. W., Brennan, P. J., Rea, T. H. and Modlin, R. L. A major T cell antigen of *Mycobacterium leprae* is a 10-kD heat-shock cognate protein. J. Exp. Med. **175** (1992) 275–284.

Several mycobacterial antigens, identified by monoclonal antibodies and patient sera, have been found to be homologous to stress or heat-shock proteins (hsp) defined in *Escherichia coli* and yeast. A major antigen recognized by most *Mycobacterium leprae*-reactive human T-cell lines and cell-wall-reactive T-cell clones is a 10-kDa protein that has now been cloned and sequenced. The predicted amino acid sequence of this protein is 44% homologous to the hsp 10 (GroES) of *E. coli*. The purified

native and recombinant 10-kDa protein was found to be a stronger stimulator of peripheral blood T-cell proliferation than other native and recombinant *M. leprae* proteins tested. The degree of reactivity paralleled the response to intact *M. leprae* throughout the spectrum of leprosy. Limiting-dilution analysis of peripheral blood lymphocytes from a patient contact and a tuberculoid patient indicated that approximately one third of *M. leprae*-reactive T-cell precursors responded to the 10-kDa antigen. T-cell lines derived from lepromin skin tests were strongly responsive to the 10-kDa protein. T-cell clones reactive to both the purified native and recombinant 10-kDa antigens recognized *M. leprae*-specific epitopes as well as epitopes crossreactive with the cognate antigen of *M. tuberculosis*. Further, the purified hsp 10 elicited strong delayed-type hypersensitivity reactions in guinea pigs sensitized to *M. leprae*. The strong T-cell responses against the *M. leprae* 10-kDa protein suggest a role for this heat-shock cognate protein in the protective/resistant responses to infection.—Authors' Summary

Near, K. A. and Lefford, M. J. Use of serum antibody and lysozyme levels for diagnosis of leprosy and tuberculosis. J. Clin. Microbiol. **30** (1992) 1105–1110.

Active tuberculosis (TB) and leprosy are difficult to diagnose early because there are few organisms to detect and the specific immune response does not distinguish between active and inactive disease. We developed an immunoassay for lysozyme to see whether serum lysozyme levels could be used to identify individuals with clinical leprosy or tuberculosis (TB). The immunoassay for lysozyme proved superior to standard enzyme assays that were less sensitive and reliable. The lysozyme assay was compared with assays for antibodies to *Mycobacterium tuberculosis* lipoarabinomannan (LAM) and *M. leprae* phenolic glycolipid-I. The sera tested were from Ethiopian leprosy (paucibacillary and multibacillary) and TB patients and from healthy Ethiopian and U.S. controls. The lysozyme assay was able to detect more of the individuals with TB (sensitivity, 100% for 19 patients) or leprosy (sensitivity, 86% for 36 patients) than either antibody assay. In particular, lysozyme levels were raised in a higher proportion of the paucibacillary leprosy patients (83% of 17), for whom the antibody assays were less sensitive; the LAM IgG and the phenolic glycolipid-I IgM levels were raised in only 62% and 44% of 16 patients, respectively. The data suggest that lysozyme measurements may be useful in the diagnosis of mycobacterial infections and other chronic infectious granulomatoses.—Authors' Abstract

Parida, S. K., Grau, G. E., Zaheer, S. A. and Mukherjee, R. Serum tumor necrosis factor and interleukin 1 in leprosy and during lepra reactions. Clin. Immunol. Immunopathol. **63** (1992) 23–27.

Tumor necrosis factor-α (TNF), one of the mediators of septic shock, has a role in the immunopathological complications of several infections. However, its role in leprosy is yet unclear. In this study, serum TNF and IL-1 levels in 64 patients spread over the spectrum of leprosy [lepromatous leprosy (LL), 30; borderline lepromatous, 12; borderline borderline, 8; and borderline tuberculoid-tuberculoid leprosy, 14] were measured at the time of admission. Elevated levels of TNF ranging from 15 to 4500 pg/ml were detected in lepromatous leprosy cases (399 $\pm$ 189) and low levels ranging from 15 to 160 pg/ml were detected in the tuberculoid form of leprosy. Patients undergoing type 1 and type 2 lepra reactions also exhibited high TNF levels of 15–2100 pg/ml. Of the 14 clinically healthy individuals studied, 3 showed TNF levels of 15, 50, and 58 pg/ml. Interleukin 1-β (IL-1) levels were found to be significantly higher in LL cases (70–5000 pg/ml) (328 $\pm$ 184) in comparison to other groups or normal controls (9 $\pm$ 3). The coefficient of correlation between TNF and IL-1 levels was statistically significant in LL and reaction cases ($r = 0.96$, $p < 0.001$). These patients were followed up as outpatients for a period of 1 year. It was observed that 4 out of 8 patients with TNF levels > 100 pg/ml went into lepra reactions between 2 and 6 months after entry into the study; whereas only 5 out of 56 with < 100 pg/ml went into mild lepra reactions ($\chi^2 = 9.7$, $p < 0.01$). Determination of TNF and IL-1 levels thus seems to have a prognostic significance in terms of

lepra reaction in patients.—Authors' Abstract

Park, J. Y., Cho, S. N., Youn, J. K., Kim, D. I., Cellona, R. V., Fajardo, T. T., Jr., Walsh, G. P. and Kim, J. D. Detection of antibodies to human nerve antigens in sera from leprosy patients by ELISA. Clin. Exp. Immunol. **87** (1992) 368–372.

Antineural antibodies have been implicated to play a role in the pathogenesis of nerve damage in leprosy patients. To find the relationship between antineural antibodies and clinical findings, we attempted to detect antibodies against neurofilament-enriched proteins by ELISA in sera from leprosy patients. Of 289 sera from leprosy patients, 74 (25.6%) had significant antineural antibodies; in contrast, 1 (5.0%) of 20 tuberculosis patients and 11 (7.1%) of 154 controls were seroreactive to nerve antigen. When clinical types were considered, a significant level of antineural IgG antibodies was detectable in 53 (30.1%) of 176 sera from lepromatous patients compared with 21 (18.6%) of 113 sera from tuberculoid patients, indicating that lepromatous patients were more likely to be seropositive to nerve antigens in ELISA. Some of the ELISA-reactive sera showed antibody reactivity with 38-kDa, 40-kDa and 43-kDa nerve antigens in Western blotting analysis. There was no apparent correlation between seroreactivity to nerve antigens and bacterial load in leprosy patients. Although there was no statistical significance, antineural antibodies were detectable more often among the patients on chemotherapy than the untreated and among the patients with erythema nodosum leprosum than without. The results, therefore, suggest that antineural antibodies are elicited during the course of leprosy and may be associated with the extensiveness of nerve involvement in the patients.—Authors' Summary

Pfeffer, K., Schoel, B., Plesnila, N., Lipford, G. B., Kromer, S., Deusch, K. and Wagner, H. A lectin-binding, protease-resistant mycobacterial ligand specifically activates $V\gamma9^+$ human $\gamma\delta$ T cells. J. Immunol. **148** (1992) 575–583.

Bacterial (exogeneous) superantigens have been defined as bifunctional proteinaceous molecules. They bind to class II MHC molecules of presenting cells and engage with particular TCR-$V\beta$ gene elements, thereby activating $\alpha\beta$ T cells in $V\beta$-oriented fashion. In previous studies we have elucidated that $\gamma\delta$ T cells exhibit a propensity to vigorously respond toward mycobacterial antigens. Intrigued by this finding we now analyzed whether mycobacteria express a superantigen for a subset of human $\gamma\delta$ T cells definable by the selective use of TCR-V gene elements. Here we describe that a protease-resistant, low molecular weight (1 to 3 kDa) component of mycobacteria selectively activates $\gamma\delta$ T cells expressing TCR-$V\gamma9$ gene segments. Contained in mycobacterial lysates it stimulates TCR-$V\gamma9$-positive $\gamma\delta$ T cells at a frequency of 1/6. Stimulation is critically dependent on the presence of class II MHC-positive presenting cells, the important structure being HLA-DR molecules. The fine specificity of the $V\gamma9$ seeking mycobacterial ligand differs from the $\gamma\delta$ T-cell-stimulating structures expressed by Daudi cells. In addition, the mycobacterial, $V\gamma9$-seeking ligand is bound selectively to lectins such as UEAI, SBA, and DBA. We conclude that mycobacteria contain a component that acts as a superantigen for human $\gamma\delta$ T cells, and we believe it is this property that explains the vigorous participation of $\gamma\delta$ T cells in mycobacterial infections.—Authors' Abstract

Ponninghaus, J. M., Fine, P. E. M., Sterne, J. A. C., Wilson, R. J., Msosa, E., Gruer, P. J. K., Jenkins, P. A., Lucas, S. B., Liomba, N. G. and Bliss, L. Efficacy of BCG vaccine against leprosy and tuberculosis in Malawi. Lancet **1** (1992) 636–639.

Protection afforded by BCG (bacillus Calmette-Guérin) vaccines against tuberculosis and leprosy varies widely between different populations. In the only controlled trial which assessed protective efficacy of BCG (Danish and Pasteur strains) against both diseases, there was slightly more protection against leprosy than against tuberculosis. We have studied the protective efficacy of BCG (Glaxo, freeze dried) vaccine against these two diseases in Karonga District, northern

Malawi. BCG vaccination was introduced into this population in 1974.

Prior information about BCG scar status was available for 83,455 individuals followed up between 1979 and 1989; 414 new cases of leprosy and 180 new cases of tuberculosis were found in this population over that period. Protection was estimated at 50% or greater against leprosy, and there was no evidence for lower protection against multibacillary (84%; 95% confidence interval 26% to 97%) than against paucibacillary (51%; 30% to 66%) disease. There was no statistically significant protection by BCG against tuberculosis in this population. These findings add to the evidence that BCG vaccines afford greater protection against leprosy than against tuberculosis.—Authors' Abstract

Rani, R., Zaheer, S. A., Suresh, N. R., Walia, R., Parida, S. K., Mukherjee, A., Mukherjee, R. and Talwar, G. P. Association of HLA antigens with differential responsiveness to *Mycobacterium w* vaccine in multibacillary leprosy patients. J. Clin. Immunol. **12** (1992) 50–55.

Leprosy patients undergoing phase II trials in two hospitals of New Delhi, India, were HLA typed to see the association of HLA with differential responsiveness to *Mycobacterium w* vaccine. The vaccine comprises an atypical, nonpathogenic mycobacterium, *M. w*, which has crossreactive antigens with *M. leprae*. Multibacillary patients who are lepromin negative are vaccinated at an interval of 3 months. Considerable improvement is evident in the patients in terms of a decline in bacterial indices and histopathological and immunological upgrading. But all the patients do not respond to the vaccine in the same manner; some are slow responders, while others are good responders. HLA-A28 and DQw3 (DQw8 + 9) were found to be associated with slow responsiveness; while DQw1 and DQw7 were found to be associated with a more rapid responsiveness to the *M. w* vaccine. However, these associations were not significant after p correction for the number of antigens tested for each locus except for HLA-DQw3 (DQw8 and DQw9) and DQw7. DQw7, a new defined split of HLA-DQw3, seems to be associated with the responsiveness to *M. w* vaccine.—Authors' Abstract

Rasouli, I. and Mehta, L. N. Reaction of peripheral nerves to vascular and bacterial injuries. Indian J. Lepr. **64** (1992) 14–27.

Mouse sciatic nerves were subjected to devascularization, *Mycobacterium leprae* inoculation, and combined insult of devascularization plus foot pad inoculation (FPI). Changes were seen in FPI nerves only after 8 months, but in cases of combined insult, changes were evident in hours. Both the groups showed initial loss of small myelinated fibers. No proliferation of Schwann cells was in FPI nerves, but in combined insult it was maximum after 2 weeks. The presence of *M. leprae* seemed to be arresting Schwann cell activity after 2 weeks. Blood vessels showed increased endothelial cell cytoplasm, basement membrane proliferation and villi formation. These changes seem to be specific for endoneurial blood vessels of leprosy nerves. Increased numbers of mast cells seem to be specific for devascularized and FPI nerves. Increased numbers of macrophages expressed low immunity of devascularized nerves. Eosinophils migrated to the endoneurium as a result of leakage of axoplasm.—Authors' Abstract

Rastogi, N., McFadden, J., Ottenhoff, T. H. M. and van Eden, W. First International Conference on the Pathogenesis of Mycobacterial Infections: a summary. Clin. Infect. Dis. **14** (1992) 308–312.

Two-hundred scientists representing 25 countries assembled for the First International Conference on the Pathogenesis of Mycobacterial Infections, which was held in Stockholm on 27–29 June 1990. A total of 40 speeches and 80 poster presentations covered nearly all of the recent progress made in the field of mycobacterial pathogenicity. The present report is intended as a brief summary of the research results presented during the conference and focuses on findings of broad scientific interest. However, the omission of other presented findings from this report does not constitute any judgment of their scientific quality.—Authors' Abstract

Verbon, A., Hartskeerl, R. A., Schuitema, A., Kolk, A. H. J., Young, D. B. and Lathigra, R. The 14,000-molecular-weight antigen of *Mycobacterium tuberculosis* is related to the alpha-crystallin family of low-molecular-weight heat shock proteins. J. Bacteriol. **174** (1992) 1352–1359.

Eight monoclonal antibodies (MAbs) directed against the 14,000-molecular-weight (14K) antigen of *Mycobacterium tuberculosis* reacted specifically with mycobacteria of the *M. tuberculosis*-complex. The nucleotide sequence of the gene encoding the 14K antigen was determined by using recombinant DNA clones isolated from λgt11 and cosmid libraries of the *M. tuberculosis* genome. The DNA sequence of the 14K protein gene coded for a polypeptide of 144 amino acids with a calculated molecular mass of 16,277 Da. The 14K antigen has a marked homology with proteins belonging to the alpha-crystallin family of low-molecular-weight heat-shock proteins, which includes the 18K antigen of *M. leprae*. The eight MAbs recognized at least four distinct epitopes localized within the following three regions of the 14K protein: amino acids 10 to 92 (MAbs F67-8 and F67-16), amino acids 41 to 92 (F159-1 and F159-11), and amino acids 41 to 144 (F23-41, F24-2, F23-49, and TB68).—Authors' Abstract

Walker, K. B., Butler, R. and Colston, M. J. Role of Th-1 lymphocytes in the development of protective immunity against *Mycobacterium leprae*; analysis of lymphocyte function by polymerase chain reaction detection of cytokine messenger RNA. J. Immunol. **148** (1992) 1885–1889.

The patterns of lymphokine mRNA expression during the development of protective immunity to *Mycobacterium leprae* after intradermal vaccination of mice with killed *M. leprae* were studied. Using a polymerase-chain-reaction-based technique for detecting mRNA expression in small numbers of cells, we observed changes in the mRNA expression of a number of cytokine genes in the lymph nodes draining the site of vaccination. In particular, IL-1 (-α and -β), IL-2, TNF (-α and -β), and IFN-γ mRNA were readily detected; whereas IL-3, IL-4, IL-5, IL-6, IL-7, and granulocyte-macrophage colony-stimulating factor mRNA were not detected, or were detectable only at very low levels. This is consistent with the selective activation of Th-1 T_h cells. The effect of *in vitro* exposure of these cells to the immunizing antigen was also investigated; again, IL-1, IL-2, TNF, and IFN-γ mRNA were abundant but, in addition, IL-3, IL-6, and granulocyte-macrophage colony-stimulating factor mRNA were greatly increased, suggesting an important role in the recall response.—Authors' Abstract

Microbiology

Barletta, R. G., Kim, D. D., Snapper, S. B., Bloom, B. R. and Jacobs, W. R., Jr. Identification of expression signals of the mycobacteriophages Bxb1, L1 and TM4 using the *Escherichia-Mycobacterium* shuttle plasmids pYUB75 and pYUB76 designed to create translational fusions to the *lacZ* gene. J. Gen. Microbiol. **138** (1992) 23–30.

Mycobacterial expression signals were cloned using specially constructed gene fusion shuttle plasmid probes carrying a truncated *Escherichia coli lacZ* (β-galactosidase) gene which lacked a promoter, a ribosome binding site, and an ATG start codon. Libraries of mycobacteriophage Bxb1, L1 and TM4 DNAs were constructed, and introduced by electroporation into *Mycobacterium smegmatis* and the "bacille Calmette-Guérin" (BCG). Clones carrying mycobacterial expression sequences were detected by their blue color or characteristic fluorescence when plated on media containing chromogenic or fluorogenic substrates. Varying degrees of β-galactosidase expression were observed, and one Bxb1 expression signal was identified where

β-galactosidase expression is repressed in phage lysogens.—Authors' Abstract

Bhatia, V. N. and Rathinavel, L. Isolation of a DOPA positive rapid growing mycobacterium from blood of a leprosy patient. Indian J. Lepr. **64** (1992) 88–90.

A rapid-growing acid-fast organism was isolated from the blood of a borderline leprosy patient. The isolate appeared to be close to the *Mycobacterium cheloni* group of organisms but showed globi, cigar-shaped bundles and was positive for DOPA-oxidase. Catalase, iron uptake, sodium chloride tolerance, tellurite reduction, Tween 80 hydrolysis and pyridine extraction tests were also positive. The 3-day arylsulfatase test and nitrate reduction test were negative.—Authors' Abstract

Chan, J., Xing, Y., Magliozzo, R. S. and Bloom, B. R. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. J. Exp. Med. **175** (1992) 1111–1122.

Tuberculosis remains one of the major infectious causes of morbidity and mortality in the world, yet the mechanisms by which macrophages defend against *Mycobacterium tuberculosis* have remained obscure. Results from this study show that murine macrophages, activated by interferon γ, and lipopolysaccharide or tumor necrosis factor α, both growth inhibit and kill *M. tuberculosis.* This antimycobacterial effect, demonstrable both in murine macrophage cell lines and in peritoneal macrophages of BALB/c mice, is independent of the macrophage capacity to generate reactive oxygen intermediates (ROI). Both the ROI-deficient murine macrophage cell line D9, and its ROI-generating, parental line J774.16, expressed comparable antimycobacterial activity upon activation. In addition, the oxygen radical scavengers superoxide dismutase (SOD), catalase, mannitol, and diazabicyclooctane had no effect on the antimycobacterial activity of macrophages. These findings, together with the results showing the relative resistance of *M. tuberculosis* to enzymatically generated H_2O_2, suggest that ROI are unlikely to be significantly involved in killing *M. tuberculosis.* In contrast, the antimycobacterial activity of these macrophages strongly correlates with the induction of the L-arginine-dependent generation of reactive nitrogen intermediates (RNI). The effector molecule(s) that could participate in mediating this antimycobacterial function are toxic RNI, including NO, NO_2, and HNO_2, as demonstrated by the micobacteriocidal effect of acidified NO_2. The oxygen radical scavenger SOD adventitiously perturbs RNI production, and cannot be used to discriminate between cytocidal mechanisms involving ROI and RNI. Overall, our results provide support for the view that the L-arginine-dependent production of RNI is the principal effector mechanism in activated murine macrophages responsible for killing and growth inhibiting virulent *M. tuberculosis.*—Authors' Summary

Daffé, M., Varnerot, A., and Lévy-Frébault, V. V. The phenolic mycoside of *Mycobacterium ulcerans*: structure and taxonomic implications. J. Gen. Microbiol. **138** (1992) 131–137.

Mycobacterium ulcerans and some pathogenic mycobacterial species elaborate wax A consisting of related long-chain β-diol components (phthiocerol and related compounds) esterified by multimethyl-branched fatty acids. With the exception of *M. ulcerans,* wax A-containing mycobacteria also synthesize glycosylated phenol phthiocerol diester and related compounds: the so-called phenolic mycosides. In a deliberate effort to characterize this latter class of compounds in *M. ulcerans,* 20 strains were examined. Phenolic mycosides were found in two strains. Application of chemical analyses, including one- and two-dimensional NMR spectroscopy, allowed the structural elucidation of glycolipids identified as 3-*O*-methyl-α-L-rhamnosyl phenol phthiocerol diphthioceranate and related compounds which correspond to mycoside G, previously characterized in *M. marinum* by other investigators. As phenolic mycosides are highly species-specific molecules, this finding stresses the close phylogenetic link between *M. marinum* and *M. ulcerans.* Incidentally, a survey of the mycolate content of *M. ulcerans* showed that methoxy-

mycolate could not be detected in three strains.—Authors' Abstract

Denis, M. Interleukin-6 is used as a growth factor by virulent *Mycobacterium avium*: presence of specific receptors. Cell. Immunol. **141** (1992) 182–188.

In this paper, we examined the contribution of the lymphokine interleukin-6 (IL-6) to the growth of four virulent strains of *Mycobacterium avium* and the nature of the binding moieties on the mycobacteria. First, we showed that human or mouse recombinant IL-6 are potent growth factors for four strains of virulent *M. avium*. This was shown to occur in tissue culture medium, which does not support maximal growth of *M. avium*. Bioactive IL-6 was required, inasmuch as heat-activating IL-6 or adding an antibody against IL-6 blocked this growth-enhancing ability. The rapid uptake of IL-6 by *M. avium* was indicated by the fact that the incubation of IL-6 with the four *M. avium* strains led to a rapid removal of the bioactivity from the culture medium and a rapid removal of radiolabeled IL-6. Scatchard analysis of receptor interaction showed that the *M. avium* strains had a single receptor species with a K_d of 50 n*M* and the number of receptor sites was approximately 15,000/bacterium. Blocking experiments showed that the binding of radiolabeled IL-6 was fully displaceable with cold IL-6, but not with other lymphokines. These data suggest that IL-6 may play an important role in the pathogenesis of *M. avium* infections, notably by promoting growth of *M. avium*, and that some virulent *M. avium* strains bind IL-6 in a specific manner.—Author's Abstract

Hinshelwood, S. and Stoker, N. G. An *Escherichia coli-Mycobacterium* shuttle cosmid vector, pMSC1. Gene **110** (1992) 115–118.

A shuttle cosmid vector, pMSC1, has been constructed which replicates in *Escherichia coli* and *Mycobacterium smegmatis*. The vector was mainly derived from the λ *ori* cosmid, Lawrist4, and the *M. fortuitum* cryptic plasmid, pAL5000, which replicates in *M. smegmatis* and *M. bovis* BCG. The vector contains two *cos* sites which facilitates library construction, unique *Bam*HI and *Hin*dIII sites for cloning, and a kanamycin-resistance-encoding gene for selection in mycobacteria. After packaging, the vector sequences comprise 10.3 kb, so that the theoretical size limits for inserts are 30–42 kb. A genomic library from *M. smegmatis* was constructed in *E. coli*; clones from this library were transferred into *M. smegmatis* by electroporation, and back again to *E. coli*, without any apparent rearrangements. This vector will be useful in cloning genes encoding complex pathways in mycobacteria.—Authors' Summary

Koníček, J., Koníčková-Radochová, M. and Šlosárek, M. Gene manipulation in mycobacteria. Folia Microbiol. **36** (1991) 411–422.

Gene manipulation in mycobacteria developed in two phases. In the first phase genes of mycobacteria were transferred into cells of *Escherichia coli* and *Streptomyces lividans*. In the second phase, heterologous genes were transferred into mycobacteria either with a shuttle phasmid or hybrid plasmids. A prerequisite for successful gene manipulation in mycobacteria was a thorough understanding of plasmids in mycobacteria. Construction of recombinant DNA molecules contributed not only to the fact that mycobacteria did not remain outside the mainstream of modern genetic research but also to their present practical importance.—Authors' Abstract

Pain, S., Bera, A., Das, M., Das, B. N. and Banerjee, A. On structural aspects of peptidoglycan of bacterial cell wall with special attention on mycobacteria by computer modeling. Indian J. Lepr. **64** (1992) 28–41.

The cell-wall components of mycobacteria are said to be vitally linked with their pathogenicity. Peptidoglycan, one of the major cell-wall components in most of the bacteria are multilayered in gram-positive bacteria, and it is diverse in nature for the gram-positive strain rather than gram-negative. The cell walls of bacteria are primary targets for many drugs and antibiotics, and conformation of the major cell-wall components provides invaluable information

and understanding at a molecular level to medicinal chemists and drug designers. Mycobacterial peptidoglycan has been studied critically by computer modelling on various aspects. A plausible structure and conformation has been identified and glycan chain is found to have a pseudo twofold symmetry taking the disaccharide unit as a monomer with Knox and Murthy H-bond scheme. This paper attempts to clarify the understanding of the organization and possible interaction mode of peptidoglycan in the complex mycobacterial cell-wall structure.—Authors' Abstract

Thole, J. E. R., Schöningh, R., Janson, A. A. M., Garbe, T., Cornelisse, Y. E., Clark-Curtiss, J. E., Kolk, A. H. J., Ottenhoff, T. H. M., de Vries, R. R. P. and Abou-Zeid, C. Molecular and immunological analysis of a fibronectin-binding protein antigen secreted by *Mycobacterium leprae*. Mol. Microbiol. **6** (1992) 153–163.

By screening a *Mycobacterium leprae* λgt11 genomic DNA library with leprosy-patient sera we have previously identified 50 recombinant clones that expressed novel *M. leprae* antigens. In this study, we show by DNA sequencing and immunoblot analysis that three of these clones express a *M. leprae* homolog of the fibronectin-binding antigen 85-complex of mycobacteria. The complete gene was characterized and it encodes a 327-amino-acid polypeptide, consisting of a consensus signal sequence of 38 amino acids followed by a mature protein of 289 amino acids. This is the first sequence of a member of the *M. leprae* antigen 85 complex, and Southern blotting analysis indicated the presence of multiple genes of the 85-complex in the genome of *M. leprae*. The amino acid sequence displays 75%–85% sequence identity with components of the antigen 85 complex from *M. tuberculosis*, *M. bovis* BCG and *M. kansasii*. Furthermore, antibodies to the antigen 85-complex of *M. tuberculosis* and *M. bovis* BCG reacted with two fusion proteins containing the amino acid regions 55–266 and 265–327 of the *M. leprae* protein. The *M. leprae* 30/31 kDa protein induces strong humoral and cellular responses, as judged by Western blot analysis with patient sera and proliferation of T cells derived from healthy individuals and leprosy patients. Amino acid regions 55–266 and 265–327 both were shown to bind to fibronectin, indicating the presence of at least two fibronectin-binding sites on the *M. leprae* protein. These data indicate that this 30/31 kDa protein is not only important in the immune response against *M. leprae*, but may also have a biological role in the interaction of this bacillus with the human host.—Authors' Summary

Wheeler, P. R., Bulmer, K., Ratledge, C., Dale, J. W. and Norman, E. Control of acyl-CoA carboxylase activity in mycobacteria. FEMS Microbiol. Lett. **90** (1992) 169–172.

Acyl-CoA carboxylase activity in four pathogenic mycobacteria and *Mycobacterium smegmatis* was shown with both acetyl-CoA and propionyl-CoA substrates. Only very low activity was detected in mycobacteria grown in host tissues or on egg-based media rich in lecithin and avidin. This appeared to be a result of severe depression of activity, as strains which could be grown both in host tissue and egg-based media, and in the relatively simple Dubos or Sauton's media showed 8- to 120-fold higher activity in the simpler media.—Authors' Summary

Young, D. B., Kaufmann, S. H. E., Hermans, P. W. M. and Thole, J. E. R. Mycobacterial protein antigens: a compilation. Mol. Microbiol. **6** (1992) 133–145.

In response to recommendations from the steering committees responsible for co-ordination of World Health Organization programs for research on the immunology of leprosy (IMMLEP) and tuberculosis (IMMTUB), a list was prepared summarizing the properties of mycobacterial proteins currently under investigation with respect to their immunological activities. After consultation with more than 40 laboratories world-wide this list was extended to form the compilation shown herein and is intended to provide a comprehensive and convenient reference for future studies in this field.—Authors' Summary

Experimental Infections

Xabier, M. G., Howe, R. C. and Frommel, D. [Infection and elimination of *Mycobacterium leprae* in the SCID mouse.] C. R. Acad. Sci. [III] **314** (1992) 99–103.

Previous studies documented that T-cell deficient nude mice failed to control *Mycobacterium leprae* infection. In the present investigation we monitored the growth of *M. leprae* for up to 15 months in the SCID C.B.-17 mouse, a host deficient in both T and B lymphocytes. At 8 months postinfection 10^8 organisms/foot pad were recovered from SCID mice vs 5×10^6 in normal BALB/c mice. Thereafter the number of bacilli decreased rapidly in mice infected with high-dose inoculum (10^7); however, at all doses SCID mice eventually cleared *M. leprae*. During infection both T and B cells as well as serum Ig remained as low as in uninfected mice; however, in the spleen MAC-1^+ cells which include macrophages and NK cells were substantially increased. These results suggest that MAC-1^+ cells are involved in the antimycobacteria-1 defense mechanisms adopted by SCID mice to compensate their deficiency in T and B cells.—Authors' English Abstract

Rehabilitation

Mahaisavariya, B., Jeeravipoolvarn, P., Vipulakorn, K. and Sirichativapee, V. Shrinkage of the below-knee stump in leprosy. Br. J. Surg. **79** (1992) 340–341.

The long-term progress of below-knee stumps was studied in 65 leprotic amputees. Thirty-seven patients underwent amputation with a long posterior flap (LP) and 28 patients with an equal anterior and posterior flap (EF) technique. Mean follow-up was for 6 and 7 years, respectively. Shrinkage of the soft tissue and retraction of the posterior calf muscles caused the posterior skin flap of the stump to rotate posteriorly in all cases, with a mean 26-degrees of rotation in LP stumps and 42-degrees of rotation in EF stumps. The LP stumps remained thicker, with soft tissue and padding at the bony ends, and had fewer stump complications than the EF stumps.—Authors' Abstract

Thappa, D. M., Sharma, V. K., Kaur, S. and Suri, S. Radiological changes in hands and feet in disabled leprosy patients: a clinico-radiological correlation. Indian J. Lepr. **64** (1992) 58–66.

Seventy-six consecutive leprosy patients with disabilities were subjected to radiological examination of hands and feet, and bone changes were found in 63 of them (82.9%). Specific, nonspecific and osteoporotic bone changes were observed in 22.4%, 78.9% and 28.9% of cases, respectively. Bone cysts (10.5%), subarticular erosions (10.5%) and enlargement of nutrient foramena (5.3%) were the common specific bone changes; whereas bone absorptive-changes (59.2%), soft tissue changes (39.5%) and concentric absorption (39.5%) were the most frequent nonspecific bone changes. Specific bone changes were more common in older patients (age 40 years) and nonspecific bone changes correlated with duration of disease, duration of deformity, and disability index. Osteoporotic bone changes were found to be affected by ageing and severity of disability of hands and feet.—Authors' Abstract

Other Mycobacterial Diseases and Related Entities

Appelberg, R. and Pedrosa, J. Induction and expression of protective T cells during *Mycobacterium avium* infections in mice. Clin. Exp. Immunol. **87** (1992) 379–385.

Mycobacterium avium is an opportunistic pathogen that infects individuals suffering from chronic lung disease or immunocompromized patients such as AIDS patients. Here we show that a highly virulent isolate of *M. avium* proliferated as extensively in T-cell-deficient as in immunocompetent mice. T-cell-deficient mice allowed a progressive growth of a less virulent AIDS-derived isolate of *M. avium* while immunocompetent mice arrested the growth of this isolate. Adoptive transfer of T-cell-enriched spleen cells between congenic strains of mice differing at the *Bcg/Ity/Lsh* locus showed that only naturally resistant BALB/c.*Bcg*^{r}(C.D2) mice infected with the highly virulent strain of *M. avium* or the naturally susceptible BALB/c mice infected with the lower virulence isolate developed protective T cells, and that these cells only mediated protection when transferred to naturally susceptible, but not to naturally resistant, mice. Both strains of *M. avium* proliferated in bone-marrow-derived macrophages cultured *in vitro*, and they were both susceptible to the bacteriostatic effects induced in the macrophages by crude lymphokines produced by concanavalin-A-stimulated spleen cells.—Authors' Summary

Ashbridge, K. R., Bäckström, B. T., Liu, H.-X., Vikerfors, T., Englebretsen, D. R., Harding, D. R. K. and Watson, J. D. Mapping of T helper cell epitopes by using peptides spanning the 19-kDa protein of *Mycobacterium tuberculosis*; evidence for unique and shared epitopes in the stimulation of antibody and delayed-type hypersensitivity. J. Immunol. **148** (1992) 2248–2255.

In vivo and *in vitro* T-cell responses to overlapping 20-mer peptides that span the entire 19-kDa protein of *Mycobacterium tuberculosis* have been compared in three different strains of mice. Immunization of the mice with peptides and analysis of specific antibody production is an *in vivo* assay of Th cell activity. Peptides 1-20 and 61-80 elicited strong IgG_1 responses in BALB/cJ, C57BL/10J, and B10.BR mice, indicating that these peptides could stimulate Th cells, possibly of a Th2 phenotype. T cells isolated from peptide-immunized mice were challenged *in vitro* with peptide, and their proliferative responses were analyzed. T cells from these three strains of mice immunized with peptides 1-20, 61-80, and 76-95 also responded to challenge with specific peptide *in vitro*. In addition, B10.BR mice and BALB/cJ mice showed antibody and T-cell proliferative responses to peptides 136-155 and 145-159, respectively. Thus, *in vitro* proliferating T cells were found to possess specificities for peptide epitopes that were almost identical to those of the antibody-producing cells. Delayed-type hypersensitivity (DTH) responses to these peptides were also examined in the three strains. Interestingly, the T cells responding to the DTH assay had antigen specificities that were quite different from those identified in the antibody and proliferation assays. These results suggested that DTH Th cells form a separate population from antibody Th and proliferative T cells and these populations of cells were differentially activated, in an antigen-specific manner.—Authors' Abstract

Atrat, P., Hösel, P., Richter, W., Meyer, H. W. and Hörhold, C. Interactions of *Mycobacterium fortuitum* with solid sterol substrate particles. J. Basic Microbiol. **31** (1991) 413–422.

Mycobacterium fortuitum NR RL B-8119 transforms sterols into 9 alpha-hydroxy-androsta-4-ene-3.17-dione (90H-AD) at high efficiency. Cells strongly aggregate to the surface of the sterol particles forming stable agglomerates ("substrate immobilized cells"). Substrate uptake and product formation were studied as function of the size of the sterol particles. Using particle sizes comparable with the size of the mycobacterial cells ($< 5\ \mu m$) highest rates were found for both the substrate uptake and the product formation. After mechanical reinforcement of the contact between mycobacterial cells and the sterol substrate by co-grinding of the components, a significantly increased product formation was observed. Associated cells and cell-sterol agglomerates were investigated by freeze-fracture electron microscopy. The micrographs obtained demonstrate that cells of *Mycobacterium fortuitum* are growing into the sterol microcrystallite. The uptake of the sterol substrate

is assumed to take place via direct contact between cells and the substrate particles. To understand the transport mechanism, a model is proposed that includes a flexible multicomponent mesophase (FMCM) which is placed between cells and particles and which mediates the sterol uptake. The putative FMCM is assumed to be composed of mycobacterial glycolipids, extracellular biolipids, synthetical detergents, sterol and water.—Authors' Abstract

Barnes, P. F., Mehra, V., Rivoire, B., Fong, S.-J., Brennan, P. J., Voegtline, M. S., Minden, P., Houghten, R. A., Bloom, B. R. and Modlin, R. L. Immunoreactivity of a 10-kDa antigen of *Mycobacterium tuberculosis*. J. Immunol. **148** (1992) 1835–1840.

Identification of antigen of *Mycobacterium tuberculosis* recognized by T cells is essential to understanding the pathogenesis of tuberculosis and mechanism(s) of resistance to infection. Previous studies evaluating the immunoreactivity of nitrocellulose transfers of *M. tuberculosis* antigen separated by SDS-PAGE indicated that a high proportion of *M. tuberculosis*-reactive T-cell lines proliferate in response to a 10-kDa antigen. We therefore purified this antigen from *M. tuberculosis* culture filtrates and evaluated its immunoreactivity in patients with tuberculous infection. Proliferative responses of peripheral blood mononuclear cells (PBMC) to the 10-kDa antigen were similar to those induced by whole *M. tuberculosis* and greater than those elicited by other proteins isolated from culture filtrate. Furthermore, in patients with tuberculous pleuritis, proliferative responses to the 10-kDa antigen were higher in pleural fluid mononuclear cells than in PBMC, indicating that T-cell reactivity to this antigen is enhanced at the site of disease. The first 15 amino acids of the 10-kDa antigen were identical to those defined previously for *Bacillus Calmette-Guérin*-a (BCG-a), and a T-cell clone recognized the 10-kDa antigen and a peptide of BCG-a, indicating that the 10-kDa antigen corresponds to BCG-a. This antigen elicited IFN-γ production by pleural fluid mononuclear cells and by PBMC from healthy tuberculin reactors, suggesting that the 10-kDa antigen can enhance macrophage activation and resistance to mycobacterial infection. Our findings indicate that the 10-kDa antigen of *M. tuberculosis* is highly immunoreactive and should be evaluated for its capacity to elicit protective immunity—Authors' Abstract

Barrett, M. S., Jones, R. N., Erwin, M. E. and Koontz, F. P. CI-960 (PD127391 or AM-1091), sparfloxacin, WIN57273, and isepamicin activity against clinical isolates of *Mycobacterium avium-intracellulare* complex, *M. chelonae*, and *M. fortuitum*. Diagn. Microbiol. Infect. Dis. **15** (1992) 169–171.

A 7H9 broth microdilution method against CI-960, sparfloxacin, WIN57273, ciprofloxacin, norfloxacin, isepamicin, amikacin, kanamycin, ethambutol, isoniazid, and rifampin was used to test 35 *Mycobacterium avium-intracellulare* complex (MAI) and five *M. chelonae-fortuitum* strains. The majority of MAI isolates were inhibited by all tested compounds, with sparfloxacin (MIC_{90}, 0.5 μg/ml) being the most active among the fluoroquinolones; isepamicin (MIC_{90}, 4 μg/ml), the most potent aminoglycoside; and isoniazid, rifampin, and ethambutol also demonstrating some degree of activity. *Mycobacterium chelonae* strains were resistant to all drugs except ciprofloxacin (MIC_{50}, 1 μg/ml). *Mycobacterium fortuitum* isolates were generally susceptible, especially to the newer fluoroquinolones.—Authors' Abstract

Bech-Nielsen, S., Berg Jorgensen, J., Ahrens, P. and Feld, N. C. Diagnostic accuracy of a *Mycobacterium phlei*-absorbed serum enzyme-linked immunosorbent assay for diagnosis of bovine paratuberculosis in dairy cows. J. Clin. Microbiol. **30** (1992) 613–618.

The purpose of this study was to describe the responses of sera from five groups of cattle to an enzyme-linked immunosorbent assay (ELISA) for paratuberculosis by using serum absorbed with *Mycobacterium phlei* at a single working dilution. The infection status of the cattle was determined by fecal culture. Cattle with different levels of exposure (high versus low prevalence and test negative) and disease manifestation (clini-

cally suspect infection versus subclinical infection) were examined, as follows: (i) two paratuberculosis-negative herds; (ii) a fecal culture-confirmed, clinically suspect cases of paratuberculosis; (iii) cows from a paratuberculosis-infected herd with a high infection rate, as determined by fecal culture, but with no clinical cases at the time of sampling; (iv) cows from three paratuberculosis-infected herds known to have paratuberculosis diagnosed on the farm (low infection rate determined by fecal culture); and (v) one fecal culture-negative herd with known serologically positive cattle. Results generally showed a decreased ELISA response when absorbed rather than nonabsorbed serum from each animal was used. The results of the fecal culture confirmed clinically suspect cases, which were analyzed in relation to the amount of colonies isolated from the animals on fecal culture (0, +, ++, +++, ++++, and above). There was a significant increase in the ELISA response for animals with heavy *M. paratuberculosis* shedding (++++ or above), when both unabsorbed and absorbed sera were used, compared with the response in animals that were fecal culture negative or that shed *M. paratuberculosis* at lower levels (less than ++++) ($p < 0.05$). The effects on sensitivity and specificity by using different cutoff points for the five groups of cattle with different levels of exposure is described, since sera were not discretely segregated into distinct groups of positive and negative samples. The specificity of the ELISA in the two fecal culture-negative herds was 100% at an ELISA cutoff of an optical density (OD) of 0.1 and above for absorbed serum. For unabsorbed serum the specificity was 62.9% at a similar cutoff value. Similarly, the specificity of the fecal culture-negative, serologically positive herd increased from 37.5 to 72.2 at an ELISA cutoff value of 0.1 to 0.2 (OD) by using absorbed versus unabsorbed serum and from 75.0 to 94.4 at an ELISA cutoff value of 0.2 to 0.3 (OD).—Authors' Abstract

Brown, B. A., Wallace, R. J., Jr., Onyi, G. O., de Rosas, V. and Wallace, R. J., III. Activities of four macrolides, including clarithromycin, against *Mycobacterium fortuitum, Mycobacterium chelonae,* and *M. chelonae*-like organisms. Antimicrob. Agents Chemother. **36** (1992) 180–184.

Susceptibilities to erythromycin by broth microdilution were compared with those to the newer macrolide clarithromycin for 223 isolates of rapidly growing mycobacteria belonging to seven taxonomic groups. Seventy-nine random isolates were also tested against azithromycin and roxithromycin. The MIC of clarithromycin for 90% of strains tested (MIC_{90}) was 0.25 μg/ml for isolates of *Mycobacterium chelonae* subsp. *chelonae* and 0.5 μg/ml for *M. chelonae* subsp. *abscessus,* with 100% of strains inhibited by ≤ 1 μg/ml. Clarithromycin was 10 to 50 times more active than erythromycin and four- to eightfold more active than the other newer macrolides against *M. chelonae.* MICs of clarithromycin frequently increased with prolonged incubation with isolates of *M. chelonae* subsp. *abscessus* but not *M. chelonae* subsp. *chelonae.* MICs of clarithromycin were much higher for *M. fortuitum* bv. *fortuitum* (MIC_{50}, 2.0 μg/ml; MIC_{90}, > 8.0 μg/ml). The three newer macrolides had comparable activity against *M. fortuitum* bv. *peregrinum* (MIC_{90}s of 0.5 to 2.0 μg/ml compared with erythromycin MIC_{90}s of > 8.0 μg/ml). Overall, clarithromycin was the most active agent, inhibiting all isolates of *M. chelonae* subsp. *chelonae, M. chelonae* subsp. *abscessus, M. fortuitum* bv. *peregrinum,* and the *M. chelonae*-like organisms and 35% of *M. fortuitum* bv. *fortuitum* at ≤ 1 μg/ml. Clinical trials of the newer macrolides, especially clarithromycin, against these environmental mycobacterial species appear to be warranted.—Authors' Abstract

Buck, G. E., O'Hara, L. C. and Summersgill, J. T. Rapid, simple method for treating clinical specimens containing *Mycobacterium tuberculosis* to remove DNA for polymerase chain reaction. J. Clin. Microbiol. **30** (1992) 1331–1334.

Several simplified methods for treating mycobacteria to release DNA for amplification by the polymerase chain reaction (PCR) were investigated. The most effective of the methods was sonication. Samples were placed in screw-capped microcentrifuge tubes that were then placed in a plastic rack.

The rack was floated in a dish of water next to the ultrasonic probe so that the ultrasonic energy was transmitted through the walls of the tubes. This allowed multiple samples to be processed safely and effectively. Forty-three clinical samples were processed by this procedure, and the crude preparations were analyzed for *Mycobacterium tuberculosis* by PCR. Twenty-six of these specimens contained *M. tuberculosis,* and 17 either had no growth or contained other species of mycobacteria. Twenty-four of the 26 (92%) positive specimens were correctly identified, and all of the negative specimens were correctly identified. This sonication procedure appears promising as a rapid, simple means of treating clinical specimens containing mycobacteria for PCR analysis.—Authors' Abstract

Bullington, R. H., Lanier, J. D. and Font, R. L. Nontuberculous mycobacterial keratitis—report of two cases and review of the literature. Arch. Ophthalmol. **110** (1992) 519–524.

We report two cases of nontuberculous mycobacterial keratitis. To our knowledge, case 1 is the first documented case of *Mycobacterium chelonei* sclerokeratitis and case 2 is the first report of *M. flavescens* keratitis. A total of 40 cases of nontuberculous mycobacterial keratitis involving at least five different species have been reported previously in the literature. Almost all of these opportunistic infections have occurred following either accidental or surgical ocular trauma, usually associated with the use of local corticosteroids. Encountered infrequently, these organisms can be incorrectly identified as other bacteria, including diphtheroids and *Nocardia* species. Histopathologic examination and special stains of infected tissues may be helpful in establishing the correct diagnosis. Cultures and sensitivity testing are mandatory in determining appropriate treatment.—Authors' Abstract

Chatterjee, D., Hunter, S. W., McNeil, M. and Brennan, P. J. Lipoarabinomannan; multiglycosylated form of the mycobacterial mannosylphophosphatidylinositols. J. Biol. Chem. **267** (1992) 6228–6233.

The lipopolysaccharides of mycobacteria, lipoarabinomannan (LAM) and lipomannan (LM), of key importance in host-pathogen interaction, were recently shown to contain a phosphatidylinositol "anchoring domain." We now have established that LAM and LM are based on the phosphatidylinositol mannosides, the characteristic glycophospholipids of mycobacteria. Digestion of the arabinose-free LM with an endo-$\alpha 1 \rightarrow 6$-mannosidase yielded evidence for the presence of the 1-(*sn*-glycerol-3-phospho)-D-*myo*-inositol-2, 6-bis-α-D-mannopyranoside unit, indistinguishable from that derived from phosphatidylinositol dimannoside. This same inositol substitution pattern was shown to be present in LAM by methylation analysis before and after dephosphorylation. Positions C-2 and C-6 of the inositol unit of LAM are occupied by mannosyl residues and C-1 by a phosphoryl group. Partial acid hydrolysis of per-*O*-methylated LAM and comparison by gas chromatography-mass spectrometry of the resulting derivatized oligosaccharides with like products from phosphatidylinositol hexamannoside demonstrated that the C-6 of inositol is the point of attachment of the mannan core of LAM, which consists of an $\alpha 1 \rightarrow 6$-linked backbone with considerable α-$1 \rightarrow 2$ side chains. Thus, a structural and presumably biosynthetic relationship is established between some of the membranous mannosylphosphatidylinositols described some 25 years ago and the newly emerging, biologically active lipopolysaccharides of mycobacteria.—Authors' Abstract

Chatterjee, D., Lowell, K., Rivoire, B., McNeil M. R. and Brennan, P. J. Lipoarabinomannan of *Mycobacterium tuberculosis*; capping with mannosyl residues in some strains. J. Biol. Chem. **267** (1992) 6234–6239.

Previously we had demonstrated that the termini of the arabinan component of mycobacterial cell-wall arabinogalactan, the site of mycolic acid location, consists mostly of clusters of a pentaarabinofuranoside, [β-D-Ara*f*-(1 $\rightarrow$ 2)-α-D-Ara*f*-(1 $\rightarrow$]$_2 \rightarrow$ (3 and 5)-α-D-Ara*f*. Subsequently, the same arrangement was shown to dominate the nonreducing ends of lipoarabinomannan

(LAM), a key component in the interaction of mycobacteria with host cell. Accordingly, we had proposed that mycobacteria universally elaborate the same Ara*f*-containing motifs in two settings for different pathophysiological purposes. However, we now report that the termini of LAM from the virulent, Erdman, strain of *Mycobacterium tuberculosis,* unlike those from the attenuated H37Ra strain, are extensively capped with mannosyl (Man*p*) residues, either a single α-D-Man*p*, a dimannoside (α-D-Man*p*-(1 → 2)-α-D-Man*p*), or a trimannoside (α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*). The use of monoclonal antibodies demonstrates a clear difference in the antigenicity of the basic and mannose-capped LAM. The possibility that these structures are a factor in the virulence of some strains of *M. tuberculosis* and represent an example of carbohydrate mimicry in mycobacterial infections is discussed.—Authors' Abstract

Chen, K. T. K. Mycobacterial spindle cell pseudotumor of lymph nodes. Am. J. Surg. Pathol. **16** (1992) 276–281.

Two cases of spindle-cell pseudotumor in the lymph nodes of patients with acquired immunodeficiency syndrome caused by mycobacterial infection are reported and the literature reviewed. The lesions mimicked neoplasms because they were composed predominantly of spindle cells arranged in a storiform pattern. Most of the spindle cells were phagocytic cells that contained large amounts of mycobacteria. It is important for the pathologist to recognize the lesion so that a prompt tissue diagnosis can be provided because specific therapy is available.—Author's Abstract

Coppes, M. J., Olivieri, N. F., Howes, M., Pusic, M., Gold, R. and Richardson S. E. Mycobacterial brain abscess possibly due to bacille Calmette-Guérin in an immunocompromised child. Clin. Infect. Dis. **14** (1992) 662–665.

Disseminated infection with bacille Calmette-Guérin (BCG) is rare, even in immunocompromised patients who receive BCG injections as immunotherapy or immunization. When such infection occurs, it is usually in patients with decreased cellular immunity. A 6-year-old Caucasian girl who was receiving maintenance chemotherapy for acute lymphoblastic leukemia presented with symptoms of meningitis. A temporal-lobe biopsy revealed acid-fast bacilli that were identified as *Mycobacterium bovis* BCG. Neither the patient nor any family members had been immunized previously. Appropriate therapy resulted in a complete recovery.—Authors' Abstract

Cox, N. L., Prowse, M. V., Maddison, M. C. and Maddison, P. J. Treatment of early rheumatoid arthritis with rifampicin. An. Rheum. Dis. **51** (1992) 32–34.

Following a report that 7 of 20 patients with rheumatoid arthritis (RA) had come into clinical and laboratory remission after treatment with rifampin, and that 6 of the 7 responders had a disease duration of less than 3 years, 21 patients with classical or definite RA of recent onset were treated with 600 mg rifampin and 300 mg isoniazid daily for 6 months. Fourteen of 21 patients completed 6 months' treatment, but there was no significant improvement in the mean values of the clinical and laboratory parameters measured. The improvement suggested by preliminary studies in patients with early RA is not seen in this larger group. In patients with a disease duration of less than 18 months, however, there was a significant decrease in the erythrocyte sedimentation rate and the serum concentrations of C-reactive protein after treatment for 6 months, although there was no significant clinical improvement. Future studies of this drug in patients with RA should concentrate on this group.—Authors' Abstract

Denis, M. and Ghadirian, E. Transforming growth factor beta (TGF-b_1) plays a detrimental role in the progression of experimental *Mycobacterium avium* infection; *in vivo* and *in vitro* evidence. Microb. Pathogen. **11** (1991) 367–372.

BALB/c mice were infected with 10^5 colony-forming units (cfu) of *Mycobacterium avium* TMC 702 i.v. and the growth of the inoculum followed in the spleens of control mice. Other infected mice given weekly dos-

es of 1 μg of TGF-b_1 or weekly doses of 2 mg of a rabbit antiserum against mouse TGF-b_1 were evaluated for their resistance to *M. avium* TMC 702. Growth of *M. avium* in the spleens of mice given repeated doses of TGF-b_1 (1 μg weekly) was significantly higher than in the spleens of control mice starting at day 40 of infection. Similarly, growth of *M. avium* was significantly diminished (0.7 log difference at 80 days) in mice given infusions of anti-TGF-b_1 (2 mg weekly). Macrophage activation status was similar in the three groups of mice, as seen by a comparable release of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) by peritoneal macrophages of infected mice. However, TGF-b_1-pulsed peritoneal macrophages were found to be more permissive for *M. avium* growth *in vitro* than control macrophage monolayers. Overall, these results suggest that TGF-b_1 plays a detrimental role in the progression of experimental *M. avium* infections by an unclear mechanism.—Authors' Abstract

Denner, J. C., Tsang, A. Y., Chatterjee, D. and Brennan, P. J. Comprehensive approach to identification of serovars of *Mycobacterium avium* complex. J. Clin. Microbiol. **30** (1992) 473–478.

Serotyping of nontuberculous mycobacteria, especially those of *Mycobacterium avium* complex, provides important epidemiological information, particularly in tracing origins of infections. Seroagglutination with whole cells and polyclonal rabbit antibodies was the original way of identifying serovars and is still commonly used. The discovery of the glycolipid nature of the typing antigens allows differentiation of serovars on the basis of thin-layer chromatography of whole antigens and gas chromatography-mass spectrometry of the characteristic sugars of the oligosaccharide haptens of these antigens. In particular, the generation of monoclonal antibodies to the glycolipid antigens allows facile differentiation of serovars through enzyme-linked immunosorbent assay. All of these protocols were applied in developing a comprehensive approach to the typing of members of the *M. avium* complex.—Authors' Abstract

Dhillon, J. and Mitchison, D. A. Activity *in vitro* of rifabutin, FCE 22807, rifapentine, and rifampin against *Mycobacterium microti* and *M. tuberculosis* and their penetration into mouse peritoneal macrophages. Am. Rev. Respir. Dis. **145** (1992) 212–214.

The activities of the rifamycins, rifabutin, FCE 22807, rifapentine, and rifampin, were studied within unstimulated peritoneal macrophages infected with *Mycobacterium microti* and in cultures of *M. microti* and *M. tuberculosis* in 7H-9 medium without Tween 80. In macrophage cultures, serial rifamycin concentrations were added after a 2.5-hr phagocytosis period, and viable counts were done after incubation for 5 to 6 days. To ensure comparability with the daily drug replacements in the macrophage experiments, the period of exposure to serial rifamycin concentrations in 7H-9 medium was kept to only 3 days. The MICs of *M. microti* and *M. tuberculosis* were similar. The MICs of rifabutin and FCE 22807 were 2.5 times lower and that of rifapentine 1.7 times lower than the MIC of rifampin. None of the rifamycins were concentrated in macrophages, the MICs being higher in the macrophages than *in vitro* by a factor of 2-fold for rifabutin, 6.7-fold for rifampin, 20-fold for FCE22807, and 26-fold for rifapentine.—Authors' Summary

Fifis, T., Costopoulos, C., Corner, L. A. and Wood, P. R. Serological reactivity to *Mycobacterium bovis* protein antigens in cattle. Vet. Microbiol. **30** (1992) 343–354.

The serological response to 12 purified *Mycobacterium bovis* antigens were examined in an ELISA. These antigens included the majority of *M. bovis* protein antigens described to date and in most cases they were very similar to the *M. tuberculosis* antigens of the same molecular mass. The purified antigens were tested against sera from *M. bovis*-infected cattle, *M. bovis* culture-negative cattle from infected herds, and animals infected with related microorganisms, mainly other mycobacterial species. All the antigens gave strong reactions with at least some sera from the *M. bovis*-infected group and showed crossreactivity with some of the

sera from the other two groups. The antigen with the highest specificity reacted strongly with only 60% of the *M. bovis*-infected sera. Antigens that reacted with most or all of the *M. bovis*-infected sera also gave the highest crossreactivity with sera from the other two groups. These results indicate that a serological test based on any one or a combination of these antigens, without removal of the crossreacting epitopes, would be unsatisfactory.—Authors' Abstract

Flory, C. M., Hubbard, R. D. and Collins, F. M. Effects of *in vivo* T lymphocyte subset depletion on mycobacterial infections in mice. J. Leukoc. Biol. **51** (1992) 225–229.

The relative importance of $CD4^+$ and $CD8^+$ T-cell subsets in the expression of acquired resistance to systemic infection by *Mycobacterium kansasii* was determined. T-cell subsets were depleted in thymectomized C57BL/6 mice by the intravenous administration of monoclonal antibodies directed against the relevant T-cell determinants. Depletion of the $CD4^+$ subset exacerbated the severity of the infection in intravenously challenged mice. This effect was apparent in the first 2 weeks of the infection and persisted throughout the 12 weeks of the study. On the other hand, depletion of the $CD8^+$ cells had no apparent effect on the growth curves. Infections by *M. tuberculosis* Erdman or bacille Calmette-Guérin (BCG) Pasteur were also substantially enhanced by CD4 depletion, but not by the depletion of $CD8^+$ cells. The effect of subset depletion on infections by *M. tuberculosis* and BCG was examined in both innately susceptible C57BL/6 mice and innately resistant B6D2 mice.—Authors' Abstract

Grosset, J., Truffot-Pernot, C., Lacroix, C. and Ji, B. Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice. Antimicrob. Agents Chemother. **36** (1992) 548–551.

Mice that had been inoculated intravenously with 6.30 $\log_{10}$ *Mycobacterium tuberculosis* H37Rv 14 days earlier were administered one of three combinations of drugs, i.e., isoniazid (INH)-rifampin (RMP)-pyrazinamide (PZA), INH-RMP, and RMP-PZA, during an initial 2-month period to mimic the initial phase of chemotherapy for human tuberculosis and during a later 4-month period to mimic the continuation phase of chemotherapy. At the end of the initial phase, all three combined regimens were found to have been highly effective in terms of the number of CFUs in the spleens of infected mice. The bactericidal activities of INH-RMP-PZA and INH-RMP were similar; whereas that of RMP-PZA was significantly greater. The spleens of all of the mice that had been treated initially with INH-RMP-PZA were culture negative by the end of 6 months of treatment, regardless of the regimen employed during the continuation phase. However, after an additional period of 6 months without treatment, the proportion of spleen culture positivity, or relapse rate, was significantly smaller in the subgroup treated with RMP-PZA during the continuation phase than in the subgroups treated with INH-RMP-PZA or INH-RMP; the relapse rate did not differ significantly between the latter two subgroups. These results suggest that antagonism occurs between INH and the combination RMP-PZA during both the initial and continuation phases of chemotherapy, compromising the benefit conferred by the addition of PZA to the combined regimen. The preliminary pharmacokinetic analysis suggested that the pharmacological interaction between INH and RMP was very likely to be involved in the mechanism of antagonism, since concomitant treatment with INH had significantly reduced the peak serum level and the area under the serum concentration-time curve of RMP in mice.—Authors' Abstract

Havlik, J. A., Jr., Horsburgh, C. R., Jr., Metchock, B., Williams, P. P., Fann, S. A. and Thompson, S. E., III. Disseminated *Mycobacterium avium* complex infection: clinical identification and epidemiologic trends. J. Infect. Dis. **165** (1992) 577–580.

To evaluate the incidence of disseminated *Mycobacterium avium*-complex infection (DMAC) and to define the association between signs and symptoms and development of DMAC in patients with human

immunodeficiency virus (HIV) infection, all cases of DMAC at Grady Memorial Hospital Infectious Disease Clinic (Atlanta, Georgia, U.S.A.) between 1985 and 1990 were reviewed, and a prospective study of the association of symptoms with DMAC was done. Between 1985 and 1990, DMAC occurred in 16% of patients with AIDS. Incidence increased from 5.7% in 1985–1988 to 23.3% in 1989–1990 ($p < 0.001$). Median time from AIDS diagnosis to diagnosis of DMAC increased from 4.5 months in 1985–1988 to 8 months in 1989–1990 ($p < 0.02$). In the prospective study, DMAC was seen only in persons with a $CD4^+$ count < 100 cells/mm^3 and was associated with fever ($p < 0.03$), anemia ($p < 0.001$), weight loss ($p < 0.01$), diarrhea ($p < 0.01$), and elevated alkaline phosphatase ($p < 0.01$). It is recommended that all such HIV-infected persons have mycobacterial blood cultures done.—Authors' Abstract

Hayman, J. Postulated epidemiology of *Mycobacterium ulcerans* infection. Int. J. Epidemiol. **20** (1991) 1093–1098.

Mycobacterium ulcerans infection occurs in closely defined areas throughout the world, mostly in the tropics. Wherever it occurs there is a relationship with rain forest and this relationship is apparent in Gippsland, Australia, which is not tropical but which contains isolated pockets of relict warm temperate rain forest. Human infection follows rain forest disturbance; it is postulated that the mycobacterium is carried into draining lacustrine systems where it multiplies over a period of months or years and is then disseminated in aerosol form to re-infect its ancestral home and incidently to infect man.—Author's Abstract

Houssaini-Iraqui, M., Lazraq, R., Clavel-Sérès, S., Rastogi, N. and David, H. L. Cloning and expression of *Mycobacterium aurum* carotenogenesis genes in *Mycobacterium smegmatis*. FEMS Microbiol. Lett. **90** (1992) 239–244.

This report describes the first successful transfer and complete expression of clustered mycobacterial genes controlling a biosynthetic pathway (carotenogenesis) in a homologous system. A genomic library of pigmented *Mycobacterium aurum* A^+ (yellow-orange) DNA was constructed in shuttle vector pHLD-69. The colorless mutant A_{11} and the brick-red mutant NgR_9 derived from *M. aurum* A^+ were electroporated with the plasmid library. Among the transformants, colonies different in color from the recipient mutants were detected, and were cloned. One of the clones from the transformed A_{11} mutant had a yellow-orange phenotype, and was designated $A_{11}T$; one of the clones from the NgR_9 (brick-red) mutant had a yellow-orange phenotype and was designated NgR_9T. The carotenoid pigments from the $A_{11}T$ and NgR_9T clones were analyzed and in both the end product of carotenogenesis in *M. aurum* (leprotene) was detected. $A_{11}T$ and NgR_9T harbored the same recombinant plasmid (pC1) containing an 11-kb *M. aurum* fragment. pC1 was used to transform the colorless *M. smegmatis* MC^2-155 strain. All the transformants were pigmented. A colony (MC^2-T) was arbitrarily chosen and leprotene was detected. It was therefore concluded that *M. aurum* genes involved in carotenogenesis had been cloned, and were expressed not only in *M. aurum* mutants, but also in *M. smegmatis*.—Authors' Summary

Hubbard, R. D., Flory, C. M. and Collins, F. M. Immunization of mice with mycobacterial culture filtrate proteins. Clin. Exp. Immunol. **87** (1992) 94–98.

Culture filtrate proteins were obtained from *Mycobacterium tuberculosis* cultures after 7 days' growth in Proskauer and Beck medium. The protein yield increased substantially to peak about the time the number of viable organisms reached its maximum level (day 8). Examination of the protein concentrate by SDS-PAGE revealed the presence of at least 12 separate protein bands varying from 10 to 90 kDa. Mice were injected subcutaneously with 20 μg of *M. tuberculosis* culture filtrate (MTCF) protein suspended in saline or Freund's complete or incomplete adjuvant. The vaccinated mice were subjected to an aerogenic challenge with 10^3 colony-forming unit (CFU) *M. tuberculosis* Erdman, and a significant reduction in the number of viable organisms was observed in the spleens and lungs determined over a 21-day period compared

with age-matched normal controls. Mice immunized with the same culture filtrate proteins bound to nitrocellulose particles also showed some resistance to the virulent challenge, suggesting that individual antigens present in the culture filtrate were able to induce a protective T cell-mediated immune response in appropriately immunized mice.—Authors' Summary

Hubbard, R. D., Flory, C. M., Collins, F. M. and Cocito, C. Immunization of mice with the antigen A60 of *Mycobacterium bovis* BCG. Clin. Exp. Immunol. **88** (1992) 129–131.

Antigen 60 (A60) is a thermostable component of the cytoplasm of *Mycobacterium tuberculosis* and BCG which can be fractionated into at least 15 protein bands when analyzed by Western blot. Normal B6D2 mice were immunized subcutaneously with 20 μg of the A60 protein suspended in Freund's incomplete adjuvant (FIA) or in saline. Three weeks later the mice received a second dose of vaccine followed 2 weeks later by an aerogenic challenge with approximately 10^3 CFU of *M. tuberculosis* Erdman. The mice receiving the adjuvanted A60 showed a significant reduction ($p < 0.05$) in the number of viable organisms recovered from the lungs and the spleen 3 weeks after challenge. However, this response was less than that seen in BCG-vaccinated controls.—Authors' Abstract

Ip, M., Cheng, K. P. and Cheung, W. C. Disseminated intravascular coagulopathy associated with rifampicin. Tubercle **72** (1992) 291–293.

A case of subclinical disseminated intravascular coagulopathy due to antituberculosis drugs, probably rifampin, is described. The patient also developed marked leukocytosis, a flu-like illness, intravascular hemolysis, and acute renal failure as part of the drug reaction.—Authors' Summary

Ishaque, M. [Energy production in *Mycobacterium lepraemurium* cultivated *in vitro*.] Res. Microbiol. **142** (1992) 1013–1018.

Cell-free extracts prepared from *in vitro* cultured *Mycobacterium lepraemurium* catalysed phosphorylation coupled to the oxidation of NADH and succinate, yielding P/O ratios of 0.52 and 0.34, respectively. No ATP synthesis occurred during oxidation of ascorbate. Oxidative phosphorylation was uncoupled by dinitrophenol and dibromophenol. Oxidation of NADH and coupled phosphorylation was markedly inhibited by rotenone, whereas this inhibitor had no effect on succinate oxidation and associated ATP synthesis. Oxidative phosphorylations and coupled oxidations of NADH and succinate were strongly inhibited by antimycin A and cyanide.—Author's English Summary

Ishida, S., Hamada, K., Yagi, S., and Seino, M. Comparing the anticonvulsive effects of dapsone on amygdala-kindled seizures and hippocampal-kindled seizures in rats. Acta Neurol. Scand. **85** (1992) 132–135.

Dapsone, an antileprosy drug, was administered to rats with amygdala (AM)-kindled seizures or hippocampal (HIPP)-kindled seizures to elucidate its anticonvulsive efficacy. Adult male Wistar rats were subjected to kindling stimulations 2 weeks after electrode implantation. The subjects were tested once a day for 7 successive days after inducing three generalized (stage 5) seizures to study the effects of dapsone. Dapsone had an inhibitory effect on stage 5 seizures at 12.50 mg/kg in the AM-kindled rats and at 6.25 mg/kg and 9.375 mg/kg in the HIPP-kindled rats. Thus, there was a distinct difference in the effective dose for generalized seizures between the AM-kindled rats and the HIPP-kindled rats. The inhibitory action of dapsone on stage 5 seizures may be due mainly to the elevation of the afterdischarge-triggering threshold at the stimulation site of the AM or HIPP. Such inhibitory action appears prominently at serum concentrations of about 13 μg/ml in AM-kindled rats and about 6 μg/ml in HIPP-kindled rats. The level of 6 μg/ml almost equals the therapeutic serum concentration of dapsone used in the treatment of leprosy.—Authors' Abstract

Jereb, J. A., Kelly, G. D., Dooley, S. W., Jr., Cauthen, G. M. and Snider, D. E., Jr. Tuberculosis morbidity in the United States: final data, 1990.

The number of tuberculosis cases reported to the Centers for Disease Control, U.S.A., has been increasing since 1988, after a long historic decline. In 1990, 25,701 cases were reported, an increase of 9.4% over the 1989 figure and the largest annual increase since 1953. From 1985 to 1990, reported cases increased by 15.8%. Disproportionately greater increases in reported cases occurred among Hispanics, non-Hispanic blacks, and Asians/Pacific Islanders. In contrast, decreases were observed among non-Hispanic whites and American Indians/Alaskan Natives. By age, the largest increase in reported cases occurred in the 25- to 44-year age group; this increase may be largely attributable to rising numbers of tuberculosis cases among persons with human immunodeficiency virus infection or acquired immunodeficiency syndrome. Notable increases also occurred among children. The proportion of cases among foreign-born persons has risen steadily, from 21.6% in 1986 to 24.4% in 1990.—Authors' Summary

Kirschner, R. A., Jr., Parker, B. C. and Falkinham, J. O., III. Epidemiology of infection by nontuberculosis mycobacteria; *Mycobacterium avium, Mycobacterium intracellulare,* and *Mycobacterium scrofulaceum* in acid, brown-water swamps of the Southeastern United States and their association with environmental variables. Am. Rev. Respir. Dis. **145** (1992) 271–275.

Mycobacterium avium, M. intracellulare, and *M. scrofulaceum* (MAIS) organisms were isolated and identified from waters, soils, aerosols, and droplets ejected from water collected from four geographically separate aquatic environments (Okefenokee Swamp, Georgia; Dismal Swamp, Virginia; Claytor Lake, Virginia; and Cranberry Glades, West Virginia, U.S.A.) during several seasons. Recovery of MAIS was significantly higher from waters, soils, and aerosols collected from the two acid, brown-water swamps located in the southeastern coastal plain. High MAIS numbers correlated with warmer temperature, low pH, low dissolved oxygen, high soluble zinc, high humic acid, and high fulvic acid. This research, in relation to previous findings for the geographic distribution and physiologic ecology of MAIS, supports the conclusion that waters, soils, and aerosols of the acid, brown-water swamps of the southeastern United States coastal plain represent major environmental sources likely connected with the higher incidence of human infection in this region.—Authors' Summary

Knoring, B. E., Pavlova, M. V. and Ivanova, L. A. [Influence of tuberculin therapy on the immunologic parameters in adolescents with pulmonary tuberculosis.] Probl. Tuberk. **12** (1991) 31–34. (in Russian)

Study of a combination of immunologic parameters in 29 adolescents with pulmonary tuberculosis who were subject to a combined treatment with the use of tuberculin demonstrated a decline in heightened specific cellular sensitivity, enhancement of antituberculous antibodies synthesis, normalization of the content and functional activity of T and B lymphocytes and rise of the level of class G and M immunoglobulins. It has been concluded that tuberculin has a desensitizing action combined with simultaneous stimulation of humoral response and the normalizing action on the T-lymphocyte system.—Authors' English Abstract

Lagranderie, M., Frehel, C., de Chastellier, C. and Gheorghiu, M. Cellular oxidative responses and mycobacterial growth inhibition in aerosol and intradermal BCG-immunized guinea-pigs. Biologicals **19** (1991) 335–345.

Although the dissemination of tuberculosis is aerogenic, less than 10% of infected subjects develop the active disease. Local immunity plays a major role in systemic cell-mediated immunity against this disease. BCG immunization may be more effective if administered via aerosol rather than intradermally. In this study, the immune responses seen in guinea pigs vaccinated with a BCG aerosol were compared with those seen following intradermal vaccination. At regular intervals after each vaccination, the activation of alveolar macrophage was determined by their capacity to produce superoxides, phagosome-lysosome fusion and the inhibition of *in vitro* BCG

growth. Concurrently, BCG multiplication or growth inhibition in the target organs was also determined. This study demonstrates that the alveolar route of BCG administration activated broncho-alveolar macrophages more effectively than the intradermal route. Superoxide production correlated with *in vitro* and *in vivo* inhibition of BCG growth. The spread, by the BCG inoculum, to the draining lymph nodes and spleen was similar for both test routes of administration. However, the lung BCG counts were significantly lower following intradermal vaccination. In contrast, the activation of broncho-alveolar macrophage was higher following aerogenic, rather than intradermal, BCG immunization.—Authors' Abstract

Li, S.-G., Quayle, A. J., Shen, Y., Kjeldsen-Kragh, J., Oftung, F., Gupta, R. S., Natvig, J. B. and Kørre, O. T. Mycobacteria and human heat shock protein-specific cytotoxic T lymphocytes in rheumatoid synovial inflammation. Arthritis Rheum. **35** (1992) 270–281.

Joint inflammation and destruction might be partly attributable to a crossreaction of mycobacteria-induced cytotoxic T cells with self heat shock protein.—Authors' Conclusion

Lupatkin, H., Brau, N., Flomenberg, P. and Simberkoff, M. S. Tuberculous abscesses in patients with AIDS. Clin. Infect. Dis. **14** (1992) 1040–1044.

Five cases of large tuberculous abscesses in patients with AIDS were observed over a 2-year period at the New York Veterans Affairs Medical Center, U.S.A. These cases represent 11.6% of the 43 cases of tuberculosis diagnosed in patients with AIDS during that period. The abscesses were located in the liver, abdominal wall, psoas muscle, mediastinum, and peripancreatic area. All patients presented with localized pain or swelling, and 4 of 5 patients had fever. The diagnosis was made on the basis of detection of abscesses on computed tomography (CT) and the results of culture of abscess material obtained by CT-guided aspiration. CT-guided therapeutic drainage was performed in two cases. Despite administration of therapy, 2 of 5 patients died of tuberculous infection. Formation of tuberculous abscesses appears to be a common complication of tuberculosis in patients with AIDS. This diagnosis should be considered for patients with AIDS who have fever and localized pain or swelling.—Authors' Abstract

Martin-Casabona, N., Fuente, T. G., Papa, F., Urgell, J. R., Pla, R. V., Grau, G. C. and Camps, I. R. Time course of anti-SL-IV immunoglobulin-G antibodies in patients with tuberculosis and tuberculosis-associated AIDS. J. Clin. Microbiol. **30** (1992) 1089–1093.

Immunoglobulin G (IgG) and IgM antibodies against the SL-IV antigen of *Mycobacterium tuberculosis* in the sera of patients with tuberculosis with negative serology for human immunodeficiency virus (HIV) infection (TB group; N = 97), patients with tuberculosis with positive serology for HIV infection (TB-HIV group; N = 59), and healthy controls (N = 289) were determined by enzyme-linked immunosorbent assay. All sera were obtained at the onset of tuberculosis, i.e., when clinical symptoms appeared. Clinical specimens were collected and cultured for the isolation of *M. tuberculosis,* and treatment with antituberculous drugs was started. Sera were also obtained from patients in the TB group at fixed intervals during treatment; sera were available from 13 patients in the TB-HIV group before the onset of tuberculosis. The best specificity and positive predictive value were obtained with the IgG assays. In the IgG assays at specificities above 96.0%, the sensitivities of the tests were 45.3% and 72.8% for the TB and TB-HIV groups, respectively, and the sensitivity was 51.9% when data from both groups were combined for analysis. For the TB group, results of this study indicated that the levels of IgG antibodies remain high during treatment. Thus, repetitive serological assays may not be useful for treatment follow-up. In the TB-HIV group, 12 of 13 patients had IgG-specific antibodies against the SL-IV antigen between 1 and 30 months before the onset of tuberculosis, so we suggest that the IgG antibody assay against SL-IV may be helpful

for identifying tuberculosis in patients infected with HIV.—Authors' Abstract

Michelini-Norris, M. B., Blanchard, D. K., Pearson, C. A. and Djeu, J. Y. Differential release of interleukin (IL)-1α, IL-1β, and IL-6 from normal human monocytes stimulated with a virulent and an avirulent isogenic variant of *Mycobacterium avium-intracellulare* complex. J. Infect. Dis. **165** (1992) 702–709.

Members of the *Mycobacterium avium-intracellulare* complex (MAC) can exist in a transparent or opaque colonial morphology when cultured on synthetic medium. An opaque variant was developed from a transparent strain of a clinical MAC isolate. Comparison of the two variants showed a greater ability of the transparent colonial variant to infect normal human monocytes as measured by growth in monocyte-bacteria cocultures. Further analyses indicated diminished ability of the transparent variant to induce extracellular secretion of interleukin (IL)-1 and IL-6, as well as membrane-associated IL-1 when compared with the opaque isotype. At the molecular level, induction of specific IL-1α, IL-1β, and IL-6 mRNAs was consistent with the protein results. These results suggest that the virulent transparent MAC, as opposed to the avirulent opaque type, may escape host defenses by failing to induce IL-1 and IL-6, key factors in the initiation of a normal immune response.—Authors' Abstract

Mikolich, D. J. and Mates, S. M. Granulomatous prostatitis due to *Mycobacterium avium* complex. Clin. Infect. Dis. **14** (1992) 589–591.

Granulomatous infections of the genitourinary tract are rare, especially those caused by nontuberculous mycobacteria. A case of prostatitis due to *Mycobacterium avium* complex in an immunocompetent man is reported. The patient had sterile pyuria, and a Mantoux skin test, using 5 tuberculin units, was positive (induration, 10 mm in diameter). Pathologic examination of the prostate revealed necrotizing granulomata with acid-fast bacilli, and repeated performance of urine cultures before initiating therapy yielded *M. avium* complex.—Authors' Abstract

Mitchell, I. C., Turk, J. L. and Mitchell, D. N. Detection of mycobacterial rRNA in sarcoidosis with liquid-phase hybridisation. Lancet **1** (1992) 1015–1017.

Because sarcoidosis resembles tuberculosis clinically and histologically, it has been suggested that mycobacteria might have a role in the pathogenesis of the disorder. Mycobacteria have not been found in sarcoid tissues by conventional culture techniques, so we have used a liquid-phase hybridization method to see whether we could detect mycobacterial rRNA in such tissues. RNA was extracted from five sarcoid and five normal spleens. Extracts were assayed by liquid-phase DNA/RNA hybridization with a DNA probe specific for the rRNA of the *Mycobacterium tuberculosis* complex. Hybridization obtained with the sarcoid spleens, from which mycobacteria were neither seen on microscopy nor cultured with standard methods, was 4.8 times higher than that with normal spleens ($p < 0.001$). Our demonstration of mycobacterial nucleic-acid components in sarcoid splenic tissues supports the notion that mycobacteria play a part in the cause of sarcoidosis.—Authors' Abstract

Morrissey, A. B., Aisu, T. O., Falkinham, J. O., Eriki, P. P., Ellner, J. J. and Daniel, T. M. Absence of *Mycobacterium-avium* complex disease in patients with AIDS in Uganda. J. AIDS **5** (1992) 477–478.

The absence of disease due to *Mycobacterium avium* in Ugandan patients with AIDS, which we previously observed in a blood culture study, has been confirmed and our observations have been extended to 165 additional clinical isolates. Fourteen soil and water samples from the Ugandan environment have been cultured and revealed a high frequency of isolation of *M. avium*. The absence of *M. avium*-complex disease in Uganda remains unexplained.—Authors' Abstract

Orrell, J. M., Brett, S. J., Ivanyi, J., Coghill, G., Grant, A. and Beck, J. S. Morphometric analysis of *Mycobacterium tu-*

berculosis infection in mice suggests a genetic influence on the generation of the granulomatous inflammatory response. J. Pathol. **166** (1992) 77–82.

There is evidence in natural human disease and experimental infection in mice that host genetic factors influence susceptibility to infection with *Mycobacterium tuberculosis* and the progress of the disease. In mouse models, both H-2 and non-H-2 genes have been implicated. In this study, four inbred strains of mice (Balb/b, Balb/k, B10, B10.BR), selected for combinations of two different H-2 haplotypes on two different non-H2 backgrounds, were inoculated with *M. tuberculosis,* strain H37Rv, by intraperitoneal injection. The histological features of the granulomatous inflammatory response in the liver and lungs were investigated during the first 18 weeks of the infection. Granuloma fraction, mean granuloma area, bacillary load, and the density of acid-fast bacilli within granulomata were measured. Animals of all four strains showed the same general pattern of infection with an early, and later self-limiting, infection of the liver and delayed onset, but progressive, infection of the lung. The non-H-2 related genetic background appears to influence the morphology of the granulomatous inflammatory response. In comparison, H-2 differences appeared to be small and inconsistent.—Authors' Summary

Papa, F., Luquin, M. and David, H. L. Dot-ELISA for detection of phenolic glycolipid PGL-Tb1 and diacyl-trehalose antigens of *Mycobacterium tuberculosis.* Res. Microbiol. **143** (1992) 327–331.

A dot-ELISA method for detection of 2,3-diacyl-trehalose (DAT, previously referred to as SL-IV antigen) and triglycosyl phenol phthiocerol dimycocerosate (PGL-Tbl) antigens from *Mycobacterium tuberculosis* is described. The method enabled the detection of both antigens in 14 clinical isolates of *M. tuberculosis* from different geographic origins; the presence of the glycolipids was confirmed by chemical analysis. It was therefore concluded that the synthesis of both of these compounds is characteristic of the species.—Authors' Abstract

Saboor, S. A., Johnson, N. M. and McFadden, J. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet **1** (1992) 1012–1015.

The cause of sarcoidosis is unknown. However, the histological similarity between the disorder and tuberculosis suggests that mycobacteria might contribute to the pathogenesis of sarcoidosis. We have used the polymerase chain reaction (PCR) to detect mycobacterial DNA in clinical samples from patients with sarcoidosis. One-hundred-four patients were included in the study (62 referred for possible tuberculosis and 20 for possible sarcoidosis, and 22 control patients who had undergone bronchoscopy for other reasons). Bronchoalveolar lavage samples, bronchial washings, and tissue specimens (1 from each patient) underwent assay by PCR as well as bacteriological, histological, and cytological examination. We used two PCR reactions: in the first the complex-specific insertion sequence IS986/IS6110 was used to specifically detect DNA from *Mycobacterium tuberculosis* complex bacteria; in the second, conserved sequences of the mycobacterial groEL gene were used to detect DNA from mycobacteria other than *M. tuberculosis.* The PCR was more sensitive than culture for diagnosis of tuberculosis. However, the false-positive PCR rate for *M. tuberculosis* was 9%. *M. tuberculosis* DNA was found in half the sarcoidosis patients, and non-tuberculosis mycobacterial DNA in a further 20%. The findings that a significant proportion of the sarcoidosis patients in this study have mycobacteria in their lungs and that most of these mycobacteria belong to *M. tuberculosis* complex suggest an etiological role for mycobacteria in sarcoidosis.—Authors' Abstract

Schoel, B., Gulle, H. and Kaufman, S. H. E. Heterogeneity of the repertoire of T cells of tuberculosis patients and healthy contacts to *Mycobacterium tuberculosis* antigens separated by high-resolution techniques. Infect. Immun. **60** (1992) 1717–1720.

In this report, we describe studies to examine the repertoire of freshly isolated, hu-

man T lymphocytes to 400 distinct antigen fractions of *Mycobacterium tuberculosis* separated by a novel method involving two-dimensional gel electrophoresis. Separated antigens were probed with T cells from tuberculosis patients and purified protein derivative (PPD)-positive (PPD^+) and PPD-negative (PPD^-) contacts as well as normal healthy donors. T cells from all donors tested responded to separated antigens. Stimulation profiles for tuberculosis patients and PPD^+ contacts were extremely heterogeneous, formally demonstrating that an enormous number of different antigens serve as targets of the cellular immune response to *M. tuberculosis*. Stimulation profiles for tuberculosis patients and PPD^+ contacts were indistinguishable. However, stimulation profiles for tuberculosis patients and PPD^+ contacts were easily distinguishable from those for PPD^- contacts. Normal healthy donors showed T-cell responses similar to those of either PPD^+ or PPD^- contacts.—Authors' Abstract

Singh, N. and Yu, V. L. Successful treatment of pulmonary infection due to *Mycobacterium chelonae*; case report and review. Clin. Infect. Dis. **14** (1992) 156–161.

During the past decade, *Mycobacterium chelonae* has been recognized with increasing frequency as a pulmonary pathogen. A review of previously reported cases reveals that most patients with pulmonary infections due to *M. chelonae* are nonimmunosuppressed but have underlying chronic lung disease. The infection is notably absent among blacks. *M. chelonae* organisms are characterized by a high degree of *in vitro* resistance to antituberculous drugs, and attempts at eradicating the organism through chemotherapy have been largely unsuccessful. The case of a 63-year-old previously healthy woman with progressive bilateral pulmonary disease due to *M. chelonae* is reported; she was treated successfully with a combination of cefoxitin and orally administered ciprofloxacin. Our experience supports the use of quinolones in combination with other active agents for the treatment of pulmonary infection due to *M. chelonae*.—Authors' Abstract

Thompson, S. J., Butcher, P. D., Patel, V. K. R., Rook, G. A. W., Stanford, J., Van Der Zee, R. and Elson, C. J. Modulation of pristane-induced arthritis by mycobacterial antigens. Autoimmunity **11** (1991) 35–43.

Several prominent mycobacterial protein antigens involved in antibody and T-cell responses have been identified as members of highly conserved heat-shock protein families. In particular, immune responses to the mycobacterial 65-kDa heat-shock protein (hsp65) have been implicated in the pathogenesis of autoimmune diseases, both in experimental animal models and in man. Additionally, hsp65 has been shown to modulate the course of autoimmune disease in such experimental animal systems. In this report, we have examined the synthesis of heat-shock proteins by a fast-growing mycobacterial strain, *M. vaccae*, in heat-stressed cultures and used the pristane-induced arthritis model to investigate the immunoprophylactic and immunotherapeutic potential of heat-killed *M. vaccae*. Heat-shock of *M. vaccae* cultures at 48°C demonstrated a 43-fold increase in hsp65 over that expressed at 37°C. It is therefore suggested that heat-killed *M. vaccae* contains sufficient hsp that can be presented in the context of appropriate adjuvant properties for use as an effective immunomodulatory agent. Immunization experiments with *M. vaccae* revealed that protection or exacerbation of pristane induced arthritis was dependent on the dose (given in an oil or aqueous suspension), route and time of immunization. In addition, it was demonstrated that the development of arthritis correlated with high levels of agalactosyl IgG and that "protected" animals had significantly depressed levels.—Authors' Abstract

Thorel, M. F., Moreau, R., Charvin, M. and Ebiou, D. [Enzymatic release of mycobacteria from the environment.] C.R. Soc. Biol. **185** (1991) 331–337. (in French)

Polysaccharases release mycobacteria from the natural environment. The enzymatic activity works both on the microbial adherence polysaccharides and on the support surfaces (cellulose). The release of mycobacteria from the natural environment

increases both the number of isolates and the number of species of mycobacteria.—Authors' English Summary

Torlakovic, E., Clayton, F. and Ames, E. D. Refractile mycobacteria in Romanowsky-stained bone marrow smears; a comparison of acid-fast-stained tissue sections and Romanowsky-stained smears. Am. J. Clin. Pathol. **97** (1992) 318–321.

The appearance of mycobacteria was studied in Wright-stained bone-marrow preparations of human immunodeficiency virus-infected patients and compared with acid-fast-stained trephine biopsy sections and culture results. *Mycobacterium avium* complex in Romanowsky-stained preparations may be seen as extracellular and intracellular clear or red refractile beaded rods and nonrefractile "negative images." Refractile mycobacteria were seen in 17 of 20 culture-positive cases. Acid-fast stain of the trephine biopsy demonstrated organisms in only 11 of the 20 cases. Thus, six cases were culture positive and contained refractile rods but had no acid-fast organisms on the trephine biopsy. No false-positive results were seen with Romanowsky stain; the three false-negative results for refractility also were negative with acid-fast stain. Examination of Romanowsky-stained smears or imprints for refractile mycobacteria provides a reliable and sensitive method to identify mycobacteria in this population. Romanowsky-stained bone-marrow aspirate and imprint smears should be examined for refractile bacilli when mycobacterial infection is suspected.—Authors' Abstract

Tsang, A. Y., Denner, J. C., Brennan, P. J. and McClatchy, J. K. Clinical and epidemiological importance of typing of *Mycobacterium avium* complex isolates. J. Clin. Microbiol. **30** (1992) 479–484.

The results of the application of a range of typing procedures to the identification and classification of 6264 cultures of nontuberculous mycobacteria from human sources and the environment are reported. Seroagglutination, an enzyme-linked immunosorbent assay applied to whole bacteria or the glycolipid typing antigens and based on serovar-specific polyclonal or monoclonal antibodies, thin-layer chromatography of these antigens, and gas chromatography of their specific sugar determinants were used to arrive at identifications. As a result of this comprehensive approach, 4452 (71%) of all cultures and 88% of those of samples from patients with AIDS proved to be typeable. The rank order of frequency of occurrence of individual organisms within the entire group of isolates was *Mycobacterium avium*-complex serovar 4 > serovar 8 > serovar 1 > serovar 9 > serovar 6 > serovar 14 > serovar 2 > *M. fortuitum* > *M. kansasii* > *M. xenopi* > an apparent mixture of serovar 4 and *M. xenopi* > a mixture of serovar 4 and serovar 8. These results were similar but not identical to the pattern observed for isolates obtained from patients with AIDS; the order was *M. avium* complex serovar 4 > serovar 8 > serovar 1 > a mixture of serovar 4 and *M. xenopi*, a mixture of serovar 4 and serovar 8 > serovar 9 > serovar 2 > serovar 6. Serotyping was also used to demonstrate the possible clinical significance of nontuberculous mycobacteria recovered from different body sites. Other information on the distribution of *M. avium* serovars in patients from different geographical environments is provided.—Authors' Abstract

Valentin-Weigand, P. and Moriarty, K. M. *Mycobacterium paratuberculosis* binds fibronectin. Res. Microbiol. **143** (1992) 75–79.

Fibronectin, an adhesive glycoprotein which is present in plasma and on many host-cell surfaces of many host organisms, binds to certain bacterial pathogens. This study demonstrates the ability of *Mycobacterium paratuberculosis* (*M. ptb*) to interact with ^{125}I-labeled fibronectin purified from bovine and ovine plasma. Two *M. ptb* strains were tested: a clinical isolate and a commercially available vaccine strain. Both strains showed significant fibronectin-binding activities of 22% and 41%, respectively; whereas nonpathogenic *M. phlei* had almost no affinity for fibronectin. Binding activities were similar for ovine and bovine fibronectin. We found that fibronectin binding by *M. ptb* was (1) time-dependent, reaching saturation within 90 min, (2) specific, since it was inhibited by an excess of unlabeled

fibronectin but not by albumin, (3) saturable, with an apparent dissociation constant of 1.25×10^{-9} M and a maximal number of 1600 binding sites per bacterium, and (4) sensitive to detergents, proteases and heat treatments, indicating the protein nature of the responsible binding component(s). Scatchard plot analysis gave a straight line suggesting the presence of a single type of fibronectin receptor on *M. ptb.*—Authors' Summary

Vandergiessen, J. W. B., Eger, A., Haagsma, J., Haring, R. M., Gaastra, W. and Vanderzeijst, B. A. M. Amplification of 16S rRNA sequences to detect *Mycobacterium paratuberculosis.* J. Med. Microbiol. **36** (1992) 255–263.

A probe based on 16S ribosomal RNA (rRNA) sequences was developed to detect *Mycobacterium paratuberculosis,* the causative agent of Johne's disease in cattle. Three universal primers were used to sequence the amplified fragments of the 16S rRNA gene of various species of mycobacteria. When the nucleotide sequences were analyzed, a deletion was detected in the sequence of the fast-growing species. An oligonucleotide probe (P) directed to this region was synthesized and hybridized directly with total RNA of various mycobacterial strains in a dot-spot assay. The probe detected *M. paratuberculosis,* some other slow-growing mycobacteria of the *M. avium-intracellulare* (MAI) complex, and one atypical strain, *M. gordonae.* To increase the sensitivity of the probe, a 413-bp fragment of the 16S rRNA gene of *M. paratuberculosis* between P and a second oligonucleotide primer was amplified and hybridized with a *M. paratuberculosis/M. avium*-specific probe. When fecal samples of cattle were tested, all culture-positive samples were positive in the PCR assay.—Authors' Abstract

van Helden, P. D., du Toit, R., Jordaan, A., Taljaard, B., Pitout, J. and Victor, T. The use of the polymerase chain reaction test in the diagnosis of tuberculosis. S. Afr. Med. J. **80** (1991) 515–516.

Current techniques for laboratory diagnosis of tuberculosis have some serious limitations. These include the high cost and time required for the current assays. The development of a rapid, sensitive, specific and low-cost assay is therefore of considerable importance. We report here the development and laboratory testing of a polymerase chain reaction, DNA-based diagnostic test for the presence of *Mycobacterium tuberculosis* in sputum. The assay shows a high level of sensitivity and specificity and requires considerably less capital, consumables and time inputs than existing laboratory tests. We believe this technology is ready for large-scale evaluation and use, particularly in hospital-based laboratories.—Authors' Summary

van Scoy, R. E. and Wilkowske, C. J. Antituberculous agents. Mayo Clin. Proc. **67** (1992) 179–187.

Antituberculous agents have radically improved the prognosis of patients with active tuberculosis. Generally, 6-month and 9-month antituberculous regimens have been successful, and surgical therapy is rarely needed. Extrapulmonary tuberculosis should be managed with the same drug regimens as pulmonary tuberculosis. The major cause of therapeutic failure is poor compliance of the patient in taking the prescribed medication regularly. A second cause of failure of treatment is resistance of tubercle bacilli to antimicrobial agents used. When failure of treatment is apparent, careful reassessment by physicians experienced in the treatment of tuberculosis is indicated. A single drug should never be added to a failing regimen. Isoniazid administered prophylactically for 6 to 12 months is effective in most cases.—Authors' Abstract

Vasilyev, A. V. and Dovgalyuk, I. F. [Immunogenetic grounds for the development of local forms of primary tuberculosis in children.] Probl. Tuberk. **10** (1991) 61–62. (in Russian)

The clinicoimmunogenetic status of children with local forms of primary tuberculosis was analyzed. Examination included 99 children aged 4–14 years with pulmonary tuberculosis. The control group comprised 51 children who had negative tuberculin tests and no intercurrent diseases. The HLA composition was determined by the

A, B, C and DR loci. The HLA-DR2 representation is responsible for a high specific process risk and HLA-A11 and HLA-B15 can be characterized as antigens causing a resistance to tuberculosis infection.—Authors' English Abstract

Vogelsang, G. B., Farmer, E. R., Hess, A. D., Altamonte, V., Beschorner, W. E., Jabs, D. A., Corio, R. L., Levin, L. S., Colvin, O. M., Wingard, J. R. and Santos, G. W. Thalidomide for the treatment of chronic graft-versus-host disease. N. Engl. J. Med. **326** (1992) 1055–1058.

In this preliminary trial, thalidomide appeared to be safe and effective for the treatment of chronic graft-versus-host disease (GVHD). A trial comparing thalidomide with prednisone in patients with newly diagnosed chronic GVHD will be required to demonstrate its relative efficacy.—Authors' Conclusions

Wayne, L. G. and Sramek, H. A. Agents of newly recognized or infrequently encountered mycobacterial diseases. Clin. Microbiol. Rev. **5** (1992) 1–25.

Although potentially pathogenic environmental mycobacteria have been recognized as the cause of human disease for many decades, we are continuing to discover previously unrecognized or unappreciated mycobacterial pathogens. The recognition depends in part on advances that have been made in mycobacterial systematics; these advances have included the description of new species, the development of practical techniques for distinguishing between species that are phenotypically similar to one another, and improvement in methods for recovery of fastidious organisms. In part, however, the recognition of new disease entities associated with mycobacteria probably reflects increased incidence of infection with environmental organisms in individuals whose immunologic competence has been compromised by AIDS or by exposure to immunosuppressive agents used to treat other disease conditions. An awareness of the possibility of infection with some of these more obscure agents of disease and the application of appropriate primary culture techniques and an adequate panel of identification tests are essential if these infections are to be recognized and managed in an optimal manner.—Authors' Conclusions

Wheeler, P. R. and Ratledge, C. Control and location of acyl-hydrolysing phospholipase activity in pathogenic mycobacteria. J. Gen. Microbiol. **138** (1992) 825–830.

Phospholipase activities releasing fatty acyl moieties from phosphatidylcholine and phosphatidylethanolamine and lysophospholipase activity releasing fatty acid from lysophosphatidylcholine were detected in both *Mycobacterium microti* and *Mycobacterium avium*. Fatty acyl groups were released from both the 1- and 2-positions of phosphatidylcholine. Generally, phospholipase activities of *M. avium* were cryptic while phospholipase activities of *M. microti* were located on the bacterial surface. However, intact *M. microti* did not release fatty acids from phospholipids faster than *M. avium*. Neither mycobacterium secreted acyl-hydrolysing phospholipase activity. All phospholipase activities were stimulated by including phospholipids in growth media: generally, cell extracts contained 6- to 15-fold higher specific activities than extracts from mycobacteria grown in media without added phospholipid. However, not all phospholipase activities were stimulated to the same degree in any given set of conditions, suggesting the existence of more than one phospholipase gene in each mycobacterium.—Authors' Abstract

Yamaguchi, R., Matsuo, K., Yamazaki, A., Takahashi, M., Fukasawa, Y., Wada, M. and Abe, C. Cloning and expression of the gene for the Avi-3 antigen of *Mycobacterium avium* and mapping of its epitopes. Infect. Immun. **60** (1992) 1210–1216.

The Avi-3 antigen, which is found only in *Mycobacterium avium* culture sonic extracts, is species specific and results in strong skin-test activity in guinea pigs sensitized with heat-killed *M. avium*. Its gene was cloned by using a previously developed single-probe method and was sequenced. The gene encoded a 194-amino-acid polypeptide with a molecular weight of 21,500. A recombinant Avi-3 antigen expressed in

Escherichia coli reacted with monoclonal and polyclonal antibodies raised against the native Avi-3 antigen. To identify epitopes on this protein for immunodiagnostic purposes, various parts of the Avi-3 antigen were expressed as β-galactosidase fusion proteins, using pUR and pURS expression vectors. The clones screened by both antibody reactivity and T-cell proliferative activity defined fragments with coexisting B- and T-cell epitopes. A B-cell epitope (Asn-176 to Ala-186) and two T-cell epitopes (Glu-75 to Ile-86 and Arg-155 to Leu-164) were thus defined. The synthetic polymerized peptides of the T-cell epitopes were proven to elicit a delayed cutaneous hypersensitivity reaction in guinea pigs. This mapping method would be useful in the development of a subunit vaccine consisting of an immunodominant B-cell epitope linked to a T-cell epitope in the vicinity.—Authors' Abstract

Yamori, S., Ichiyama, S., Shimokata, K. and Tsukamura, M. Bacteriostatic and bactericidal activity of anti-tuberculosis drugs against *Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium intracellulare* complex and *Mycobacterium kansasii* in different growth phases. Microb. Immunol. **36** (1992) 361–368.

Bacteriostatic and bactericidal activities of rifampin, isoniazid, streptomycin, enviomycin and ethambutol against *Mycobacterium tuberculosis, M. avium-M. intracellulare* complex and *M. kansasii* were studied in different growth phases. Bacteriostatic activities of the drugs were similar in different growth phases, except isoniazid. *M. tuberculosis* was much less susceptible to isoniazid in the lag phase than in the log and the stationary phases. In contrast, bactericidal activity was influenced by the growth phase. *M. tuberculosis* was killed by isoniazid, streptomycin and rifampin. The bactericidal activity of isoniazid was strongest. The bactericidal activity of isoniazid and streptomycin was most marked in the log phase. *M. avium*-complex and *M. kansasii* resisted the bactericidal activity, but some strains of *M. avium*-complex were killed by streptomycin and enviomycin, and the activities of these two drugs were most marked in the lag phase.—Authors' Abstract

Yang, X.-D., Gasser, J. and Feige, U. Prevention of adjuvant arthritis in rats by a nonapeptide from the 65-kD mycobacterial heat shock protein: specificity and mechanism. Clin. Exp. Immunol. **87** (1992) 99–104.

In a previous study we have shown that Lewis rats were completely protected from adjuvant arthritis by pretreatment with a nonapeptide (residues 180–188) of the 65-kDa mycobacterial heat shock protein. Here we address questions of specificity and mechanism(s) of protection. We demonstrate that complete protection against adjuvant arthritis can only be achieved by preimmunization with the nonapeptide; while pretreatment with either the octapeptide (residues 181–188) of the 65-kDa heat-shock protein or unrelated immunogenic peptides failed to affect adjuvant arthritis. Interestingly, pretreatment with the nonapeptide of the 65-kDa heat-shock protein did not protect Lewis rats from type II collagen-induced arthritis. These results demonstrate that protection is both epitope and disease specific. Co-injection of the nonapeptide with mycobacterial antigen even at a weight ratio of 5:1 (nonapeptide : mycobacteria) failed to influence the disease, suggesting that the role of the nonapeptide is not as a "blocking peptide." T cells from rats immunized with nonapeptide respond to the nonapeptide as well as to mycobacteria *in vitro*, and adoptively transfer protection to naive recipients. The data indicate that the nonapeptide-induced protection may result from a T-cell-mediated specific suppression.—Authors' Summary

Zemskova, Z. S. and Dorozhkova, I. R. [Pathomorphological evaluation of therapeutic effect of mycobacteriophages in tuberculosis.] Probl. Tuberk. **11** (1991) 63–66. (in Russian)

The effect of DS_6A mucophage was studied in comparison with that of isoniazid on 30 guinea pigs with disseminated tuberculous infection in order to reveal the therapeutic effect of the mycobacteriophage and

tissue reactions caused by it. The mycophage was found to have therapeutic properties in disseminated tuberculosis in guinea pigs, but its action is less pronounced than in isoniazid monotherapy. Study of the special features of tissue reactions in mycophage monotherapy has demonstrated that with the mycophage phagocytosis remains incomplete and granulomatous processes that gradually lose morphological signs of tuberculous inflammation and acquire typical features of sarcoidosis develop in the animal organs.—Authors' English Abstract