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CURRENT LITERATURE

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

General and Historical

Zuiderhoek, B. The approach to the leprosy problem in the past in Indonesia—a historical review of leprosy control activities in Indonesia during the last centuries. Trop. Geogr. Med. **45** (1993) 2–5.

Due to the severe disabilities, leprosy has always been a disease which appealed to the imagination. In bygone centuries cause and treatment were unknown and the fear to be infected was enormous. In Indonesia medical officers struggled with the problem. As early as the 17th century the disease was described in detail by Ten Rhijne. In the 19th century leprosy was considered hereditary. After the discovery of the leprosy bacillus by Hansen in 1873, confusion continued as the bacillus could not be cultivated. Many therapies were tried, but with no result. At first patients were isolated in leprosaria, later on a more humane system of house-isolation was introduced. Since 1932 Indonesian medical officers have played a prominent part in research and the determination of the future approach to the problem. Seen against the background of our present knowledge about the disease, it is interesting to follow the struggle against leprosy in the past.—Authors' Abstract

Chemotherapy

Barrow, W. W., Wright, E. L., Goh, K. S. and Rastogi, N. Activities of fluoroquinolone, macrolide, and aminoglycoside drugs combined with inhibitors of glycosylation and fatty acid and peptide biosynthesis against *Mycobacterium avium.* Antimicrob. Agents Chemother. **37** (1993) 652–661.

Smooth-and rough-colony variants of *Mycobacterium avium* serovar 4 were treated with three classes of drugs. The drugs were chosen for their potential inhibitory effects on the biosynthesis of the cell envelope-associated serovar-specific glycopeptidolipid antigens. Growth was monitored radiometrically with a BACTEC 460-TB instrument, and MICs were determined for each drug. Both variants were then treated with inhibitory drugs in combination with antimicrobial agents that have demonstrated effectiveness against *M. avium.* No growth inhibition was observed with 6-fluoro-6-deoxy-D-glucose or avidin. Inhibitors of glycosylation, i.e., 2-deoxy-D-glucose, bacitracin, and ethambutol, were inhibitory to smooth-and rough-colony variants; whereas drugs that inhibit peptide synthesis, i.e., N-carbamyl-L-isoleucine and m-fluorophenylalanine, were more inhibitory for the rough-colony variant. Cerulenin, which affects fatty acid synthesis, was inhibitory for both variants, but it appeared to be more effective at inhibiting the growth of the smooth-colony variant at equivalent concentrations. Generally, when inhibitors of glycosylation were used with sparfloxacin and amikacin, a synergistic effect was observed for only the smooth variant. When drugs that affect peptide synthesis were used in combination with amikacin, a synergistic effect was observed for the rough variant, and when cerulenin was used in combination with sparfloxacin or amikacin, a synergistic effect was observed for both variants. Lipid analysis revealed that although the rough variant lacks the serovar-specific glycopeptidolipid antigens, it does possess

a group of phenylalanine-isoleucine-containing lipopeptides that may explain its different susceptibility patterns to m-fluorophenylalanine and N-carbamyl-L-isoleucine. The significance of these results is discussed with reference to various components in the cell envelope and their importance in cell wall permeability.—Authors' Abstract

Coleman, M. D. and Jacobus, D. P. Reduction of dapsone hydroxylamine to dapsone during methemoglobin formation in human erythrocytes *in vitro*. Biochem. Pharmacol. **45** (1993) 1027–1033.

The fate of the toxic metabolite of dapsone, dapsone hydroxylamine, has been studied in the human red cell. Twice-washed red cells were incubated at 37°C with dapsone hydroxylamine: at 3 and 5 min, 27.0 ± 2.2 and 33.2 ± 2.7% of the hemoglobin had been converted to methemoglobin, leading to a maximum at 45 min (45 ± 1.8%). HPLC analysis revealed that parent amine was produced from dapsone hydroxylamine during methemoglobin formation in the red cells. At 3 min, conversion of dapsone hydroxylamine to dapsone reached 7.0 ± 3.9% leading to a maximum at 30 min (18.1 ± 3.7%). There was a linear relationship between hydroxylamine-dependent methemoglobin formation and conversion of hydroxylamine to dapsone ($r = 0.97$). At 4°C, methemoglobin and dapsone formation was greatly retarded, and did not exceed 10%. Co-incubation of diethyl dithiocarbamate (DDC) with dapsone hydroxylamine and red cells led to a marked increase in methemoglobin formation (61.4 ± 3.4%) compared with hydroxylamine and red cells alone (45.0 ± 1.8%, $p < 0.001$) at 44 min, and conversion of dapsone hydroxylamine to dapsone was almost doubled at 45 min (35.7 ± 5.3%) compared with hydroxylamine and red cells (18.1 ± 2.5%). A linear relationship between methemoglobin formation and dapsone formation ($r = 0.96$) was also shown to occur in the presence of DDC. Incubation of red cells with DDC and dapsone hydroxylamine caused a significantly greater reduction in glutathione levels (98.3 ± 1.6%) compared with red cells and dapsone hydroxylamine alone (84.8 ± 2.7%) at 5 min ($p < 0.001$), although there was no significant difference between the groups at 15 min (96.9 ± 2.6 vs 98.1 ± 2.2%). Intraerythrocytic glutathione was then depleted by 75 ± 3.4%, by pretreatment with diethyl maleate (6 mM), and these cells in the presence of the hydroxylamine showed a significant fall in both methemoglobin generation (29.7 ± 1.2 vs 35.0 ± 1.7%) and parent amine formation (11.1 ± 0.2 vs 16.5 ± 1.1%) compared with untreated red cells at 45 min. It is possible that a cycle exists between hepatic oxidation of dapsone to its hydroxylamine and reduction to the amine within the red cell, which may lead to reoxidation by hepatic cytochrome P450. This process may contribute to the persistence of the drug *in vivo*.—Authors' Abstract

Dhople, A. M., Ibanez, M. A. and Gardner, G. D. *In vitro* synergistic activity between ofloxacin and ansamycin against *Mycobacterium leprae*. Arzneimittelforschung **43** (1993) 384–386.

The antimicrobial effects of ofloxacin, alone and in combination with either rifampin or rifabutin, were evaluated against *Mycobacterium leprae*, using an *in vitro* cell-free culture system. The minimum inhibitory concentration (MIC) of ofloxacin against *M. leprae* was 15 μg/ml, while MICs of rifampin and rifabutin were 0.4 and 0.2 μg/ml, respectively. Combination of 0.375 μg/ml ofloxacin and 0.05 μg/ml of rifabutin exhibited synergistic bactericidal activity while the effect of combination of 0.75 μg/ml ofloxacin and 0.2 μg/ml rifampin was additive bactericidal. Thus, combination of ofloxacin and rifabutin deserves further attention in multidrug therapy of leprosy.—Authors' Abstract

Gokhale, N. R., Sule, R. R. and Gharpure, M. B. Dapsone syndrome. Indian J. Dermatol. Venereol. Leprol. **58** (1992) 376–378.

Dapsone syndrome is a hypersensitivity reaction occurring within the first 6 weeks of starting treatment with dapsone. Out of 2014 leprosy cases receiving multidrug treatment (MDT) in Sassoon General Hospitals, Pune, India, 18 cases (0.89%) developed dapsone syndrome. Fever, skin le-

sions, and lymphadenopathy were present in all cases. Abnormal liver function tests were detected in 13 cases. Arthralgia was present in 12 cases while conjunctivitis was present only in 1 case. Combination of rifampin with dapsone as a part of MDT may be one of the most important factors in precipitating a rise in dapsone hypersensitivity.—Authors' Abstract

Krajewska, M. M. and Anderson, R. An *in vitro* comparison of the effects of the prooxidative riminophenazines clofazimine and B669 on neutrophil phospholipase-A2 activity and superoxide generation. J. Infect. Dis. **167** (1993) 899–904.

The effects of the antimycobacterial agent clofazimine and its experimental analog B669 on the phospholipase A2 activity of and superoxide generation by human blood neutrophils were investigated *in vitro*. Coincubation of neutrophils with clofazimine or B669 (1-10 μg/mL) was accompanied by a dose-related increase in the release of both [H-3]lysophosphatidylcholine (LPC) and [H-3]arachidonate (AA). Both agents (1 μg/mL) also increased the production of superoxide by resting and FMLP-stimulated neutrophils. Addition of reagent AA and especially LPC to neutrophils mimicked the effects of the riminophenazines, while alpha-tocopherol, a lysophospholipid complex-forming agent, neutralized the prooxidative interactions of clofazimine and B669 with these cells. These observations demonstrate that LPC, and to a lesser extent AA, generated during exposure of human neutrophils to clofazimine or B669 are the primary mediators of the prooxidative interactions of these riminophenazines with phagocytes.—Authors' Abstract

Mindermann, T., Landolt, H., Zimmerli, W., Rajacic, Z. and Gratzl, O. Penetration of rifampicin into the brain tissue and cerebral extracellular space of rats. J. Antimicrob. Chemother. **31** (1993) 731–737.

Rifampin is used to treat neurosurgical shunt infections because of its excellent *in vitro* activity against staphylococci and its adequate penetration into the CSF. However, nothing is known about rifampin concentrations in the cerebral extracellular space (CES). We measured the penetration of rifampin into the CES of anesthetized rats by microdialysis using low-flow and equilibrium methods. Depending on the method, rifampin concentrations in the CES were 0.3–1% of the serum concentration or 3–8% of brain tissue concentration, respectively. These experimental data in animals suggest that the recommended dose of rifampin in man might be inadequate for treatment of some brain infections.—Authors' Abstract

Peláez, E., Rodríguez, J. C., Cigarrán, S. and Pereira, A. Acute renal failure caused by two single doses of rifampicin with a year of interval. (Letter) Nephron **64** (1993) 152.

Numerous side effects have been reported in rifampin-treated patients. Renal effects of such treatment are not common and may manifest themselves clinically in different ways, with acute renal failure (ARF) being the most frequent manifestation. In most instances, this complication is encountered in patients treated with an intermittent regimen or discontinuously (resumption of therapy after a medication-free period), but there are also cases associated with continuous daily therapy. When treatment is discontinuous, the medication-free period and dose involved tend to vary, but a prior prolonged treatment with rifampin for tuberculosis has been reported in every case. We describe a severe case of ARF caused by the ingestion of two minimal doses of rifampin separated by the interval of an entire year, not related with antituberculous treatment.—From the Letter

Salafia, A. and Candida. Rifampicin induced flu-syndrome and toxic psychosis. Indian J. Lepr. **64** (1992) 537–539.

A report of a case in a young man with leprosy who had repeated episodes of rifampin-related adverse symptoms. Toxic psychosis induced by rifampin is known to occur during treatment of tuberculosis, as also is the flu-syndrome (which has been reported previously in other leprosy patients treated with this drug).—C. A. Brown (Trop. Dis. Bull.)

Vandeputte, D., Jacob, W., Van Grieken, R. and Boddingius, J. Study of intracellular deposition of the anti-leprosy drug clofazimine in mouse spleen using laser microprobe mass analysis. Biol. Mass Spectrom. **22** (1993) 221–225.

Laser microprobe mass analysis (LAMMA) was used to study the composition of the brick-red crystalline material which had accumulated in the spleen of mice that had received the antileprosy drug clofazimine in their diet for several months. The crystalline deposits light-microscopically resembled pure clofazimine crystals. The presence of the drug in the crystals was indicated by LAMMA by the appearance of the chloride mass peaks in the negative mass spectra. More specific information was obtained from the positive mass spectra. A mass signal for the protonated molecule was present.—Authors' Abstract

Xie, Z., Cheng, N., Ding, J., *et al.* [Therapeutic effect analysis of 2900 leprosy patients cured by MDT.] J. Clin. Dermatol. **22** (1993) 14–17. (in Chinese)

The therapeutic effects of MDT in 2900 cured leprosy patients in Jiangsu province were analyzed. The bacteria in skin smears were turned negative within 2 years in all PB patients and within 4 years in more than 85% of MB patients. Patients were followed up for 4.75 years on an average in the case of PB, and 2.75 years in MB; no relapse was found. There were no significant differences in therapeutic effects between 4 groups of patients: previously treated, newly diagnosed, relapsed and drug-resistant, nor between 3 methods: triple drug regimen "A," "B" and two drug regimen. The advantages of MDT included high effect, relative short therapy course, less drug resistance, and low rate of short-term relapse, etc.—Authors' English Abstract

Xie, Z. and Ding, J. [Evaluation of MDT retreatment in 13, 477 leprosy patients cured by DDS.] J. Clin. Dermatol. **22** (1993) 11–13. (in Chinese)

To control relapse of leprosy, MDT was used to retreat 13, 477 leprosy patients cured by DDS in Jiangsu province. Among them, 3475 patients belonged to MB and 10,002 patients to PB. A twelve-month course of triple drug regimen was given to 1280 patients, 6 months' course to 947, and two drug regimen to 11, 250 patients. It has been 5 years or more since MDT retreatment in 3532 patients. No relapse was reported in 13, 477 patients by the end of 1991. On the contrary, out of 22, 488 DDS-cured patients who did not receive MDT retreatment, 457 patients had a relapse, with a rate of 2.03%. Relapse rate was as high as 13.95% in MB.—Authors' English Abstract

Clinical Sciences

Sanchez, L. N. and Diaz, H. B. [Reactional episodes in leprosy.] Rev. Dom. Dermatol. **19** (1992) 9–19. (in Spanish)

The leprosy reactional episodes types 1 and 2 were reviewed. Pathogenesis, histological and clinical features as well as treatment were studied. The need to change the nomenclature in use for a simpler one was emphasized. More research to help clarify causes, precipitating factors, and immune aspects involved were also stressed.—Authors' English Summary

Immuno-Pathology

Demessias, I. J. T., Santamaria, J., Brenden, M., Reis, A and Mauff, G. Association of C4B deficiency (C4B•Q0) with erythema nodosum leprosum in leprosy. Clin. Exp. Immunol. **92** (1993) 284–287.

A considerable number of studies have postulated significant associations between susceptibility to the different clinical manifestations of leprosy and the MHC. In this investigation, the association between the

MHC class III complement proteins C2, BF, C4A and C4B and leprosy in a patient population of southern Brazil was studied. A total of 109 nonrelated leprosy patients was investigated; 73 presented with lepromatous leprosy (LL), 46 of them had the immunopathological reaction of erythema nodosum leprosum (ENL), the remaining 36 were tuberculoid, borderline and indeterminate leprosy (TIBL) patients. The control group included 172 healthy individuals matched with the patients according to their ethnic and geographical origin. C2, BF, C4A and C4B allotypes were determined by standard technologies including Western blots for C2 and C4 variant alleles with monoclonal and polyclonal antibodies. Nonexpressed ("silent") C4 alleles in hemizygously deficient individuals were estimated semiquantitatively on the basis of the C4A and C4B isotype ratio and by the MASC ("minimal chi-square") method. The results showed a significantly elevated presence of the nonexpressed C4B allele (C4B•Q0) in the LL and ENL patient groups in comparison with the controls. The most significant difference was observed in the ENL group when compared with the controls. In addition, all patients who were homozygously C4B-deficient had ENL, and most of them had the BF•F1 allele. The comparison between LL patients with and without ENL also showed a statistically significant difference in the presence of C4B•Q0, indicating that C4B deficiency itself is associated with ENL. The relative risk of LL patients with the C4B•Q0 allele suffering from ENL was 5.3 compared with LL patients without C4B•Q0. Since immune complexes (IC) are considered to be the pathogenic cause of ENL, our findings indicate that C4B deficiency may play an important role in the abnormal immune response against *Mycobacterium leprae* and in the lack of IC clearance, leading to ENL reactions. Individuals with this allele seem to be at a higher risk of developing pathological immune reactivity in lepromatous leprosy.—Authors' Abstract

Escobar-Gutiérrez, A., Amezcua, M. E., Pastén, S., Pallares, F., Cázares, J. V., Pulido, R. M., Flores, O., Castro, E. and Rodriguez, O. Comparative assessment of the leprosy antibody absorption test, *Mycobacterium leprae* extract enzyme-linked immunosorbent assay, and gelatin particle agglutination test for serodiagnosis of lepromatous leprosy. J. Clin. Microbiol. **31** (1993) 1329–1333.

A comparative assessment of three serological methods for leprosy diagnosis (the fluorescent leprosy antibody absorption [FLA-ABS] test, the *Mycobacterium leprae* soluble-extract enzyme-linked immunosorbent assay [ELISA], and the *M. leprae* particle agglutination [MLPA] test) was carried out. The objective was to identify their performance in clinical and epidemiological diagnosis of leprosy. The study group included 45 lepromatous leprosy patients under treatment. Specificity was >95% for all three assays, and sensitivity was 95%, 58%, and 74% for the FLA-ABS test, the MLPA test, and the ELISA, respectively. The only crossreactivity for *M. tuberculosis*-infected patients was with the soluble-extract ELISA. Although the FLA-ABS test displayed the highest specificity and sensitivity values, it can only be used in well-developed laboratories, and the patient's clinical and epidemiological background must be considered when results are interpreted because the test remains positive after therapeutic success and could be positive for some household contacts. The MLPA test is easier to perform and interpret, and it is adequate for small laboratories and epidemiological studies intended to detect active untreated or irregularly treated leprosy cases. Therefore, the FLA-ABS and MLPA tests are complementary, and both should be used for serodiagnosis of leprosy.—Authors' Abstract

Ford, A. L., Britton, W. J. and Armati, P. J. Schwann cells are able to present exogenous mycobacterial hsp70 to antigen-specific T lymphocytes. J. Neuroimmunol. **43** (1993) 151–159.

Peripheral nerves are frequently damaged during infection with *Mycobacterium leprae*. Although Schwann cells are host for this obligate intracellular parasite, the mechanisms of immunopathology are unresolved. This study examines the ability of Lewis rat Schwann cells to present an exogenous *M.*

leprae protein, the heat shock protein 70 (hsp70), to antigen-specific T lymphocytes isolated from the lymph nodes of immunized rats. Secondary reactivation of hsp70-specific T lymphocytes occurred producing an antigen-specific lymphoproliferative response. This was inhibited by monoclonal antibodies against rat major histocompatibility complex (MHC) class II molecules, but not antibodies against MHC class I molecules. Coculture of Schwann cells with the *M. leprae* hsp70-specific T lymphocytes and antigen (MLrp70) induced the expression of MHC class II molecules on the Schwann cell's surface. Although *M. leprae* hsp70 is immunodominant in the host response to the bacillus, there is a high degree of homology between human and *M. leprae* hsp70. The *M. leprae* hsp70-specific T lymphocytes also recognized human hsp70 presented by Schwann cells confirming that antigenic determinants are conserved between the proteins. The ability of Schwann cells to present protein antigens in an MHC class II-restricted manner, to antigen-specific T lymphocytes involved in surveillance of the peripheral nervous system, may play an important role in the activation of an immunological reaction associated with nerve damage seen in tuberculoid leprosy.—Authors' Abstract

Jacobs, J. M., Shetty, V. P. and Antia, N. H. A morphological study of nerve biopsies from cases of multibacillary leprosy given multidrug therapy. Acta Neuropathol. **85** (1993) 533–541.

Nerve biopsies were examined from 17 cases of lepromatous leprosy given WHO-recommended multidrug therapy (MDT) for 2 years. The pathological changes were assessed qualitatively and quantitatively to judge the effectiveness of MTD. The nerves varied very considerably in the severity of their lesions. Some regenerating fibers were seen in most of the nerves. In a few cases, the nerves were almost entirely populated by regenerated fibers, confirming that MDT was effective in halting the disease process within the nerve. *Mycobacterium leprae* showed morphological features of degenerate bacilli. Some pathological features of the lepromatous lesion are described.—Authors' Abstract

Moreira, A. L., Sampaio, E. P., Zmuidzinas, A., Frindt, P., Smith, K. A. and Kaplan, G. Thalidomide exerts its inhibitory action on tumor necrosis factor α by enhancing mRNA degradation. J. Exp. Med. **177** (1993) 1675–1680.

We have examined the mechanism of thalidomide inhibition of lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF-α) production and found that the drug enhances the degradation of TNF-α mRNA. Thus, the half-life of the molecule was reduced from ~30 to ~17 min in the presence of 50 μg/ml of thalidomide. Inhibition of TNF-α production was selective, as other LPS-induced monocyte cytokines were unaffected. Pentoxifylline and dexamethasone, two other inhibitors of TNF-α production, are known to exert their effects by means of different mechanisms, suggesting that the three agents inhibit TNF-α synthesis at distinct points of the cytokine biosynthetic pathway. These observations provide an explanation for the synergistic effects of these drugs. The selective inhibition of TNF-α production makes thalidomide an ideal candidate for the treatment of inflammatory conditions where TNF-α-induced toxicities are observed and where immunity must remain intact.—Authors' Summary

Mutis, T., Kraakman, E. M., Cornelisse, Y. E., Haanen, J. B. A. G., Spits, H., de Vries, R. R. P. and Ottenhoff, T. H. M. Analysis of cytokine production by mycobacterium-reactive T cells—failure to explain *Mycobacterium leprae*-specific nonresponsiveness of peripheral blood T cells from lepromatous leprosy patients. J. Immunol. **150** (1993) 4641–4651.

Recent analyses of antimycobacterial T-cell clones from a small number of individuals indicate that mycobacteria preferentially induce Th cells that produce high levels of IFN-gamma and no or little IL-4 in *Mycobacterium leprae*-resistant tuberculoid leprosy (TT) patients and healthy subjects; whereas in one study *M. leprae*-induced Ts clones from polar lepromatous leprosy (LL) patients showed a reciprocal cytokine secretion profile and mediated their suppressive activity via the release of high

levels of IL-4. We have evaluated these findings in peripheral blood T cells from a larger panel of TT and LL patients as well as healthy individuals. Mycobacterium-reactive T-cell lines generated from the PBMC of these individuals were tested for cytokine secretion and proliferative capacity in response to *M. leprae, M. tuberculosis,* and various individual mycobacterial Ag. The lepromatous pole of the leprosy spectrum was additionally investigated by analyzing the cytokine-secretion profile of *M. leprae*-induced (suppressor) T-cell clones as well as primary *ex vivo* PBMC. All T-cell lines from healthy individuals and TT patients responding to *M. leprae, M. tuberculosis,* or individual Ag, produced high levels of IFN-gamma and TNF-alpha but little or no IL-4 and IL-6. At the lepromatous pole, T-cell lines failed to proliferate upon stimulation with *M. leprae* but in some cases produced significant levels of IFN-gamma. No IL-4 or IL-6 secretion was observed in response to *M. leprae.* These lines displayed strong proliferation and Th1-like cytokine production upon stimulation with *M. tuberculosis.* Similarly, stimulation of primary PBMC from LL patients with *M. leprae* or *M. tuberculosis* resulted in the release of IFN-gamma but no detectable IL-4 production. Control tetanus toxoid-reactive T-cell lines from the same individuals instead produced large amounts of IL-4 and low levels of IFN-gamma. The analysis of *M. leprae*-induced T-cell clones, including those with known suppressive activity, revealed that all lepromatous T-cell clones produced large amounts of IFN-gamma. Most of these clones released no or little IL-4, but some clones produced higher levels of IL-4 in addition to IFN-gamma. Most clones tested produced IL-10 as well. The suppressor activity of suppressor T-cell clones could not be inhibited by a neutralizing anti-IL-4 antibody and only in one case by neutralizing anti-IL-10 antibody. Anti-IL-4 and anti-IL-10 could not overcome the *M. leprae*-specific unresponsiveness observed in primary PBMC from LL patients. These results show that mycobacteria preferentially induce Th1-like cells across the whole leprosy spectrum. Although some T cells from lepromatous leprosy patients secrete IFN-gamma as well as IL-4 and/or IL-10, neither IL-4 nor IL-10 seems to play a pivotal role in the *M. leprae*-specific T-cell unresponsiveness observed in the peripheral blood of lepromatous leprosy patients.—Authors' Abstract

Romain, F., Augier, J., Pescher, P. and Marchal, G. Isolation of a proline-rich mycobacterial protein eliciting delayed-type hypersensitivity reactions only in guinea pigs immunized with living mycobacteria. Proc. Natl. Acad. Sci. U.S.A. **90** (1993) 5322–5326.

Effective protection against a virulent challenge with *Mycobacterium tuberculosis* is induced only by a previous immunization with living attenuated mycobacteria, usually bacillus Calmette-Guérin (BCG). Living and killed bacteria share a number of common antigens. To identify and to purify molecules that are dominant antigens during immunization with living bacteria, a two-step selection procedure was used. Quantitative delayed-type hypersensitivity (DTH) reactions elicited in guinea pigs immunized either with living or with killed BCG were used to select or counterselect antigens present in BCG culture filtrates. Each major fraction eluted from a series of HPLC columns (gel filtration, DEAE, reverse-phase chromatography) was assayed and titrated on guinea pigs of each group. A protein with an unusual amino acid composition (40% proline, 12% threonine) was purified and N-terminally sequenced. To our knowledge, the sequence Thr-Pro-Pro-Xaa-Glu-Xaa-Pro-Pro-Pro-Pro-Gln-Xaa-Val-Xaa-Leu has not been previously reported. The protein was 100-fold more potent on guinea pigs immunized with living bacteria than on guinea pigs immunized with dead bacteria to elicit a DTH reaction.—Authors' Abstract

Schlesinger, L. S. Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. J. Immunol. **150** (1993) 2920–2930.

We have examined macrophage receptors that mediate phagocytosis of virulent strains

(Erdman and H37Rv) and an attenuated strain (H37Ra) of the intracellular pathogen, *Mycobacterium tuberculosis.* Adherence of the three strains to monocyte-derived macrophages (MDM) is markedly enhanced (>threefold) in the presence of low levels of fresh serum and requires heat-labile serum components because heat inactivation of serum reduces adherence by 65 ± 5 to 71 ± 2%. In the presence and absence of serum, adherence of the three strains to MDM is comparable. By electron microscopy, all bacteria are ingested and reside in phagosomes. C receptors (CR) play an important role in adherence of the three strains to MDM in the presence and absence of serum. mAb against CR1, CR3, and CR4 inhibit adherence of Erdman *M. tuberculosis* in fresh serum by 75 ± 3% and inhibit the low level of adherence of Erdman (71 ± 13%), H37Rv (72 ± 1%), and H37Ra (64 ± 14%) *M. tuberculosis* in the absence of serum. Mannose receptors (MR) play an important role in mediating macrophage adherence of the virulent strains but not the attenuated strain of *M. tuberculosis.* Preincubation of MDM with soluble mannan or mannose-BSA consistently and significantly inhibits adherence of Erdman and H37Rv (up to 60 ± 7%) but not H37Ra (0 ± 1 to 5 ± 5% enhancement of adherence) in the absence of serum. Down-modulation of macrophage MR on mannan substrates inhibits adherence of Erdman (52 ± 8%) and H37Rv (55 ± 6%) but not H37Ra (2 ± 2% enhancement of adherence). Preincubation of MDM with soluble N-acetylglucosamine-BSA also significantly inhibits adherence of the virulent strains (42 ± 3%). Preincubation of MDM with glucose-BSA minimally inhibits adherence of the three strains (2 ± 4 to 12 ± 5%). Anti-MR antibody inhibits adherence of Erdman (57 ± 2%) and H37Rv (44 ± 4%) but not H37Ra (4 ± 5% enhancement of adherence). Inhibition of adherence of zymosan was comparable with that seen with virulent strains of *M. tuberculosis* in these studies. Down-modulation of macrophage MR also inhibits adherence of Erdman (48 ± 9%) and H37Rv (20 ± 2%) in the presence of serum. Simultaneous blockade of MR and CR does not further inhibit adherence of the virulent *M. tuberculosis* strains over that seen with blocking CR alone. This study demonstrates that serum components such as C and CR on macrophages play important roles in mediating phagocytosis of both virulent and attenuated strains of *M. tuberculosis* but that MR also play an important role in phagocytosis of virulent strains. Differences in phagocytosis between virulent and attenuated *M. tuberculosis* strains will enhance our understanding of a pathway that may influence intracellular survival.—Author's Abstract

Yokota, S., Tsubaki, K., Kuriyama, T., Shimizu, H., Ibe, M., Mitsuda, T., Aihara, Y., Kosuge, K. and Nomaguchi, H. Presence in Kawasaki disease of antibodies to mycobacterial heat-shock protein HSP65 and autoantibodies to epitopes of human HSP65 cognate antigen. Clin. Immunol. Immunopathol. **67** (1993) 163–170.

The central features of Kawasaki disease are immune activation and cytokine-mediated generalized vasculitis. To identify the predisposing factors, we examined the antibody response to BCG antigens, since reactivation of a previous BCG inoculation site is an early, specific manifestation of this disease. BCG antigens were separated on SDS-PAGE, transferred to membrane, and incubated with acute-and convalescent-phase sera of 21 patients with Kawasaki disease. Sera were also examined for the presence of antibodies to mycobacterial 65-kDa heat-shock protein (HSP65), and to its human homolog P1 antigen using synthetic peptides of nonhomologous region. To demonstrate the HSP65-sensitized T cells, *in vitro* proliferation assay was performed. All convalescent, but not acute phase, sera showed a strong antibody reactivity against 65-kDa protein. The reactivity was directed to recombinant HSP65. Non-crossreactive sequences between rHSP65 and human HSP65 cognate were synthesized. The sera recognized these peptides of rHSP65 and autologous P1 antigen. Peripheral lymphocytes proliferated following the addition of rHSP65 (stimulation indices, 2.16-7.82; mean, 4.54). These findings suggest that HSP65 may be the most potent factor predisposing to Kawasaki disease, and that an

autoreactivity to the epitope of the human HSP65 homolog may be related to the susceptibility to the disease.—Authors' Abstract

Microbiology

Andersen, A. B. and Hansen, E. B. Cloning of the lysA gene from *Mycobacterium tuberculosis.* Gene **124** (1993) 105–109.

The lysA and proC genes of *Mycobacterium tuberculosis* were cloned by screening of a recombinant lambdagt11 *M. tuberculosis* DNA library for phages able to complement lysA and proC *Escherichia coli* mutants. The lysA gene encodes diaminopimelic acid decarboxylase which catalyzes the conversion of diaminopimelic acid (DAP) to lysine. The lysA gene from *M. tuberculosis* encodes a 44-kDa protein, as determined by maxicell experiments. The nucleotide sequence of the structural gene was established. The deduced amino acid sequence was found to exhibit significant homology (from 55% to 73% similarity, and from 27% to 53% identity) to DAP decarboxylase sequences from other bacterial species.—Authors' Abstract

Baer, H. H. The structure of an antigenic glycolipid (SL-IV) from *Mycobacterium tuberculosis.* Carbohydr. Res. **240** (1993) 1–22.

The structure of an antigenic glycolipid isolated recently from cell walls of various strains of *Mycobacterium tuberculosis* and believed to be a sulfolipid consisting chiefly of 2, 3-di-*O*-(hexadecanoyl/octadecanoyl)-alpha,alpha-trehalose 2'-sulfate (designated as SL-IV), was reinvestigated by mass spectrometry and nuclear magnetic resonance spectroscopy. The material proved to be a complex mixture of closely related components which are indeed 2, 3-di-*O*-acyltrehaloses. However, no sulfate group was found in the antigen, and revision of its designation as a sulfolipid is therefore required. The lipid substituents comprise an estimated 20% of C-14-C19 fatty acids, including tetradecanoic (myristic), 9-tetradecenoic (myristoleic), 9-hexadecenoic (palmitoleic), 9-octadecenoic (oleic), 10-methylhexadecanoic (tuberculopalmitic), and 10-methyloctadecanoic (tuberculostearic) acids in addition to hexadecanoic (palmitic) and octadecanoic (stearic) acids, and approximately 80% of higher, methyl-branched acids. Among the latter are, principally, 2,4-dimethyldocosanoic and 3-hydroxy-2,4,6-trimethyltetracosanoic acids, and a smaller proportion of 2,4,6-trimethyltetracos-2-enoic acid. Numerous further acids, related to those mentioned by methylene homology, are also present in small proportions.—Authors' Abstract

Belisle, J. T., Klaczkiewicz, K., Brennan, P. J., Jacobs, W. R., Jr. and Inamine, J. M. Rough morphological variants of *Mycobacterium avium*; characterization of genomic deletions resulting in the loss of glycopeptidolipid expression. J. Biol. Chem. **268** (1993) 10517–10523.

Previously, a gene cluster, termed *ser*2, which encodes for the synthesis of the specific oligosaccharide of the glycopeptidolipid antigen of *Mycobacterium avium* serovar 2 strain TMC 724, was defined. DNA probes from this cloned *ser*2 gene cluster have now been used to clone and characterize the *ser*2 region from a strain of *M. avium* which produces rough and smooth colony forms and to identify the genetic differences between these morphotypes. Interstrain differences were seen to exist between the *ser*2 gene cluster of *M. avium* strains TMC 724 and 2151. In addition, two distinct rough (Rg) genotypes of strain 2151 were defined by this analysis. The first of these, present in the *M. avium* Rg-0 and Rg-1 variants, was attributed to a deletion of approximately 28 kilobases from smooth variants, including the entire *ser*2 gene cluster. This particular deletion is thought to be mediated by recombination between repetitive sequences that flank both sides of the 28-kilobase excised region. The second genotype, seen in *M. avium* Rg-3 and Rg-4 variants, results from the deletion of an undefined amount of DNA from the right of the *ser*2 gene cluster. Reported separately (Belisle, J. T.,

McNeil, M. R., Chatterjee, D., Inamine, J. M., and Brennan, P. J. (1993) J. Biol. Chem. 268, 10510–10516) are the results of biochemical analyses of the glycopeptidolipid/lipopeptide population of the Rg genotypes which revealed that Rg-0 and Rg-1 possess lipopeptides devoid of all of the sugars of the glycopeptidolipids and are obviously biosynthetic precursors of the glycopeptidolipids. These studies help formulate a definition of the physiological effects of glycolipid expression, the biosynthetic and genetic mechanisms involved in their formation, and toward an understanding of the role of *M. avium* as a serious opportunistic pathogen.—Authors' Abstract

Belisle, J. T., McNeil, M. R., Chatterjee, D., Inamine, J. M. and Brennan, P. J. Expression of the core lipopeptide of the glycopeptidolipid surface antigens in rough mutants of *Mycobacterium avium.* J. Biol. Chem. **268** (1993) 10510–10516.

Toward studying the genetics, biosynthesis, and roles in the pathogenesis of the dominant surface glycopeptidolipid antigens of *Mycobacterium avium,* rough colony variants of *M. avium* serovar 2 were picked, cultured in quantity, and their lipid composition examined. Two of the rough (Rg) variants, Rg-3 and Rg-4, were devoid of glycopeptidolipids or any more elemental structures and thus were similar to those described previously. Two others, Rg-0 and Rg-1, each contained two novel lipopeptides, devoid of any of the carbohydrate substituents that confer serotypic activity on the glycopeptidolipids. The application of gas chromatography, fast atom bombardment-mass spectrometry and ^{1}H NMR to lipopeptide I established the structure of $C_{32:2}$-β-OH-fatty acyl-D-Phe-D-allo-Thr-D-Ala-L-alaninol. Lipopeptide II represented a minor variation of this structure: $C_{32:1}$-β-OH-fatty acyl-D-Phe-D-allo-Thr-D-Ala-L-alaninol. These newly discovered lipopeptides are identical to the fatty acyl-tripeptide-amino alcohol "core" component of the glycopeptidolipids of the *M. avium* complex, and thus the Rg-0 and Rg-1 variants represent a form of "deep rough" mutation in *M. avium.* Separately, we report that these rough variants of *M. avium* differ genetically from the smooth, virulent form by major deletions of portions of the genes responsible for glycopeptidolipid synthesis (Belisle, J. T., Klaczkiewicz, K., Brennan, P. J., Jacobs, W. R., Jr., and Inamine, J. M. (1993) J. Biol. Chem. 268, 10517–10523). The isolation of different sets of spontaneous mutants of *M. avium* differentially defective in the capacity to synthesize glycopeptidolipids provides the means to explore their biosynthesis and roles in opportunistic pathogenesis.—Authors' Abstract

Booth, R. J., Williams, D. L., Moudgil, K. D., Noonan, L. C., Grandison, P. M., McKee, J. J., Prestidge, R. L. and Watson, J. D. Homologs of *Mycobacterium leprae* 18-kilodalton and *Mycobacterium tuberculosis* 19-kilodalton antigens in other mycobacteria. Infect. Immun. **61** (1993) 1509–1515.

Most of the antigens of *Mycobacterium leprae* and *M. tuberculosis* that have been identified are members of stress protein families, which are highly conserved throughout many diverse species. Of the *M. leprae* and *M. tuberculosis* antigens identified by monoclonal antibodies, all except the 18-kDa *M. leprae* antigen and the 19-kDa *M. tuberculosis* antigen are strongly crossreactive between these two species and are coded within very similar genes. Studies of T-cell reactivity against mycobacterial antigens have indicated that *M. tuberculosis* bears epitopes that are crossreactive with the *M. leprae* 18-kDa antigen, but attempts to identify an 18-kDa antigen-like protein or protein coding, sequence in *M. tuberculosis* have been unsuccessful. We have used a combination of low-stringency DNA hybridization and polymerase chain reaction techniques to identify, isolate, and sequence genes from *M. avium* and *M. intracellulare* that are very similar to the 18-kDa antigen gene of *M. leprae* and others that are homologs of the 19-kDa antigen gene of *M. tuberculosis.* Unlike *M. leprae,* which contains a single 18-kDa antigen gene, *M. avium* and *M. intracellulare* each have two 18-kDa antigen coding sequences. Although the *M. leprae, M. avium,* and *M. intracellulare* 18-kDa antigen genes are all very similar to one another, as are the *M. tuberculosis, M. avium,* and *M. intracellulare* 19-kDa antigen genes, we have been unable to detect

any 18-kDa antigen-like coding sequences in DNA from *M. tuberculosis.*—Authors' Abstract

Cooksey, R. C., Crawford, J. T., Jacobs, W. R., Jr. and Shinnick, T. M. A rapid method for screening antimicrobial agents for activities against a strain of *Mycobacterium tuberculosis* expressing firefly luciferase. Antimicrob. Agents Chemother. **37** (1993) 1348–1352.

We developed a rapid method to screen the efficacy of antimicrobial agents against *Mycobacterium tuberculosis.* A restriction fragment carrying a promoterless firefly luciferase gene was cloned into a 4488-bp shuttle vector, pMV261, and luciferase was expressed under the control of a mycobacterial heat shock promoter. The resulting plasmid, pLUC10, was introduced by electroporation into the avirulent strain *M. tuberculosis* H_{37}Ra. Luciferase assays of sonic lysates of Triton X-100-treated cells of *M. tuberculosis* H_{37}Ra(pLUC10) yielded bioluminescence in excess of 1000 relative light units/$\sim 10^9$ tubercle bacilli, compared with 0.0025 for the same number of parental cells. A 48-hr microdilution antimicrobial agent-screening assay using this strain was developed.—Authors' Abstract

Crawford, J. T. Applications of molecular methods to epidemiology of tuberculosis. Res. Microbiol. **144** (1993) 111–116.

DNA fingerprinting of *Mycobacterium tuberculosis* isolates is proving to be a very powerful tool for epidemiology, providing reliable identification of specific strains. Its usefulness in tracing outbreaks has been established, and ongoing studies suggest that larger application in communities may provide useful information for tuberculosis control programs. The usefulness of the method on a larger scale will be dependent upon the application of standard methods and the development of computer analysis of fingerprint patterns. The technique will also find uses in answering questions about relapse versus reinfection, emergence and spread of drug resistance, and relative virulence of specific strains. The development of more rapid methods will facilitate use of this tool and further expand the applications.—Author's Conclusion

Folgueira, L., Delgado, R., Palenque, E. and Noriega, A. R. Detection of *Mycobacterium tuberculosis* DNA in clinical samples by using a simple lysis method and polymerase chain reaction. J. Clin. Microbiol. **31** (1993) 1019–1021.

We have evaluated the polymerase chain reaction for detection of *Mycobacterium tuberculosis* in clinical samples from patients with tuberculous infection. Two simple methods for mycobacterial DNA release have been compared: sonication and lysis with nonionic detergents and proteinase K. The more effective method was the enzymatic technique. By using this protocol with 75 specimens we detected *M. tuberculosis* DNA in all of the samples, whereas only 48 and 71 samples were positive by acid-fast staining and culture, respectively.—Authors' Abstract

Gan, H., Newman, G., McCarthy, P. L. and Remold, H. G. TNF-α response to human monocyte-derived macrophages to *Mycobacterium avium,* serovar 4, is of brief duration and protein kinase C dependent. J. Immunol. **150** (1993) 2892–2900.

Human monocyte-derived macrophages (Mφ) from the majority of normal donors respond to inoculation with *Mycobacterium avium,* serotype 4, (MAI) by elaboration of the inflammatory monokines TNF-α, IL-1β, and IL-6, which are of central importance for the protection against bacterial and parasitic infections. Peak TNF-α mRNA levels were of brief duration, being maximal at 1.5 hr and were only slightly higher than background levels at 4 hr. Increases of IL-1β and IL-6 mRNA levels, on the other hand, persisted for 48 to 72 hr. In contrast to LPS, MAI induced the production of only small amounts of TNF-α protein in the first 12 hr and of large amounts of IL-1β and IL-6 protein between 3 and 72 hr MAI-induced TNF-α transcripts, in contrast to LPS-induced TNF-α transcripts, were highly unstable. Their accumulation was blocked and their $t_{1/2}$ significantly decreased by the protein kinase C inhibitor staurosporine. In contrast, LPS-induced increases of TNF-α

mRNA levels and MAI-induced increases of IL-1β and IL-6 mRNA levels were PKC independent. The cAMP-and cGMP-dependent protein kinase inhibitors, KT5720 and KT5823, respectively, and the tyrosine kinase inhibitors herbimycin and erbstatin had no effect on the MAI-dependent mRNA accumulation of TNF-α, IL-1β, and IL-6. W7, a calmodulin-dependent protein kinase inhibitor, was inhibitory in all cases. Thus, MAI-induced TNF-α mRNA accumulation is of short duration and PKC dependent. MAI-induced TNF-α protein production is low, possibly resulting in a mitigated antimicrobial effect.—Authors' Abstract

Hamid, M. E., Fraser, J. L., Wallace, P. A., Besra, G. S., Goodfellow, M., Minnikin, D. E. and Ridell, M. Antigenic glycolipids of *Mycobacterium fortuitum* based on trehalose acylated with 2-methyloctadec-2-enoic acid. Lett. Appl. Microbiol. **16** (1993) 132–135.

Three characteristic glycolipids were identified in extracts of 10 representative strains of *Mycobacterium fortuitum*. The two most polar major lipids were shown to be antigens with strong reactions against homologous antiserum and sera raised against *M. tuberculosis* H37Rv. Both of these lipids were shown to be acyl trehaloses, containing substantial amounts of 2-methyloctadec-2-enoic acid in addition to straight-chain and monounsaturated acids. These lipids correspond to 'mycoside F, lipids previously identified by infra-red spectroscopy in extracts of *M. fortuitum*.—Authors' Abstract

Launois, P., Niang, M. N., Debruyn, J., Sarthou, J. L., Rivier, F., Drowart, A., Van Vooren, J. P., Millan, J. and Huygen, K. The major secreted antigen complex (Ag-85) from *Mycobacterium bovis* bacille Calmette-Guérin is associated with protective T cells in leprosy—a follow-up study of 45 household contacts. J. Infect. Dis. **167** (1993) 1160–1167.

T-cell proliferation and interferon (IFN)-gamma secretion were analyzed in 45 leprosy contacts stimulated with antigen 85 (Ag85), the major culture filtrate antigen from *Mycobacterium bovis* bacille Calmette-Guérin. All 14 Mitsuda reaction-positive contacts reacted to *M. leprae* and Ag85. Three Mitsuda reaction-negative contacts reacted weakly to *M. leprae* and Ag85. The other 28 Mitsuda reaction-negative contacts did not react to *M. leprae*, but 9 reacted to Ag85. Thirty-four contacts were retested 16 months later. Eleven contacts initially positive by the Mitsuda test remained lepromin positive and reactive to *M. leprae* and Ag85. Fourteen contacts initially negative by the Mitsuda test converted, and all reacted *in vitro* to *M. leprae* and Ag85. Finally, 9 contacts remained Mitsuda test-negative, and 7 were unreactive to Ag85. *In vitro* reactivity to Ag85 at baseline in Mitsuda test-negative contacts was associated with subsequent conversion to lepromin reactivity in 7 of 9 subjects. These data suggest that reactive T cells against Ag85 develop very early during *M. leprae* infection and that Ag85 is a potentially protective T-cell immunogen.—Authors' Abstract

Levin, M. E. and Hatfull, G. F. *Mycobacterium smegmatis* RNA polymerase: DNA supercoiling, action of rifampicin and mechanism of rifampicin resistance. Mol. Microbiol. **8** (1993) 277–285.

We have isolated RNA polymerase from *Mycobacterium smegmatis* and established conditions for specific transcription initiation *in vitro*. The *M. smegmatis* enzyme has a strong dependence on supercoiling of the DNA substrate for transcription from mycobacterial promoters. We also show that RNA polymerase is the target for rifampin, and that this antibiotic specifically inhibits the transition from synthesis of short oligoribonucleotides to full-length transcripts. RNA polymerase isolated from a rifampin-resistant mutant of *M. smegmatis* is less sensitive to rifampin *in vitro*, confirming that one mechanism of rifampin resistance in mycobacteria is through alteration of RNA polymerase. This *in vitro* transcription system provides a simple method for the characterization of gene expression in mycobacteria including the pathogens *M. tuberculosis*, *M. avium* and *M. leprae*. It also provides a system for evaluating potential antimycobacterial drugs.—Authors' Summary

Mahenthiralingam, E., Draper, P., Davis, E. O. and Colston, M. J. Cloning and sequencing of the gene which encodes the highly inducible acetamidase of *Mycobacterium smegmatis.* J. Gen. Microbiol. **139** (1993) 575–583.

The acetamidase of *Mycobacterium smegmatis* NCTC 8159 was purified, and the sequences of its amino-terminus and of two peptides obtained by proteolysis of the protein were obtained. A DNA fragment including the amidase structural gene was cloned in *Escherichia coli,* using oligonucleotide probes designed on the basis of the peptide sequences and a codon usage table calculated from published sequences of nine protein-antigen-encoding genes of the *M. tuberculosis* complex. Sequence analysis of the cloned DNA revealed that the amidase gene encoded 406 amino acid residues. The nucleotide sequence close to and upstream of the amidase gene contained a probable ribosome-binding site but no identifiable promoter sequences. Three additional potential open-reading frames were found upstream of and very close to the amidase gene, with consensus '-35' and '-10' promoter sites between the first and second of these. It is hoped that the highly inducible expression of the acetamidase gene can be exploited to allow regulated expression of other genes cloned in mycobacteria.—Authors' Abstract

Ohara, N., Matsuo, K., Yamaguchi, R., Yamazaki, A. Tasaka, H. and Yamada, T. Cloning and sequencing of the gene for alpha-antigen from *Mycobacterium avium* and mapping of B-cell epitopes. Infect. Immun. **61** (1993) 1173–1179.

The complete nucleotide sequence of alpha antigen secreted from *Mycobacterium avium* (A-alpha) was determined. The gene encodes 330 amino acids, including 40 amino acids for the signal peptide, followed by 290 amino acids for the mature protein with a molecular mass of 30,811 Da. This is the first sequence of A-alpha. Comparisons between A-alpha and alpha antigens of *M. leprae, M. bovis* BCG, and *M. kansasii* showed highly homologous regions which suggested a conserved functional domain and two less-homologous regions. Serological analysis of recombinant A-alpha, expressed by a series of deletion constructs, indicated the possibility that A-alpha carries at least six B-cell epitopes. The three antigenic determinants were common to *M. tuberculosis, M. kansasii,* and *M. avium.* The results also suggested the possibility that there are three species-specific epitopes.—Authors' Abstract

Palittapongarnpim, P., Chomyc, S., Fanning, A. and Kunimoto, D. DNA fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates by arbitrarily primed polymerase chain reaction. J. Infect. Dis. **167** (1993) 875–978.

Strain identification of *Mycobacterium tuberculosis* would prove whether transmission had occurred between individuals. A method to characterize strains of *M. tuberculosis* has been developed utilizing polymerase chain reaction (PCR). Purified chromosomal DNA of cultured clinical samples of *M. tuberculosis* were subjected to PCR using short (10-12 nucleotide) oligonucleotide primers. PCR products visualized after agarose gel electrophoresis and ethidium bromide staining demonstrated that different strains of *M. tuberculosis* give different banding patterns. This technique was used to confirm the relationship between cases of tuberculosis in several clusters, prove the lack of relationship between two isolates with the same antibiotic-resistance pattern, confirm a suspected mislabeling event, and suggest the source of infection in a case of tuberculous meningitis. This method is rapid and simple and does not require radioactive probes.—Authors' Abstract

Rambukkana, A., Burggraaf, J. D., Faber, W. R., Harboe, M., Teeling, P., Krieg, S. and Das, P. K. The mycobacterial secreted antigen-85 complex possesses epitopes that are differentially expressed in human leprosy lesions and *Mycobacterium leprae*-infected armadillo tissues. Infect. Immun. **61** (1993) 1835–1845.

The granulomatous skin lesions in leprosy are thought to be initiated by the immune response to certain antigens of the causative agent, *Mycobacterium leprae.* The antigen 85 complex is one of the major tar-

gets in the immune response to *M. leprae* infection. In the present study, a panel of previously characterized monoclonal antibodies (MAbs) (3A8, Rb2, A4g4, A2h11, Pe12, and A3c12) reacting with different epitopes of the 85 complex proteins of *M. tuberculosis* and *M. leprae* was employed in a comparative immunohistological analysis to demonstrate the *in situ* expression of 85 complex antigenic epitopes in leprosy lesions across the clinical spectrum and in *M. leprae*-infected armadillo liver tissues. These MAbs showed a heterogeneous staining pattern in a given leprosy lesion. In highly bacilliferous borderline and lepromatous leprosy lesions, MAbs Rb2, A4g4, A2h11, and Pe12 stained clear rod-shaped *M. leprae* bacilli within macrophages, and the degree of staining correlated with the bacillary index of the lesion. On the other hand, MAbs 3A8 and A3c12 staining was mostly seen as a diffuse staining pattern within interstitial spaces and on the membranes of the infiltrated cells but not the bacilli. In paucibacillary borderline and tuberculoid leprosy lesions, only 3A8, Rb2, and A3c12 showed distinct staining in association with infiltrates in the granuloma. None of these MAbs showed any detectable reaction with control nonleprosy skin lesions, while MAb A3c12 positively stained the granulomas of both leprosy and control specimens. *In situ* reactivity of these MAbs with *M. leprae*-infected armadillo liver tissues also showed a heterogeneous staining pattern. Interestingly, a clear difference in expression of these epitopes was observed between armadillo tissues and human leprosy lesions. By immunogold ultracytochemistry, we further showed the differential localization of these MAb-reactive epitopes on the cell surface, in the cytosol, and at the vicinity of *M. leprae* within Kupffer cells of armadillo liver tissues. Our results indicate that these antigenic epitopes of the antigen-85 complex are differentially expressed in leprosy lesions and infected armadillo tissues and that they could be target determinants in the immunopathological responses during *M. leprae* infection.—Authors' Abstract

Ratliff, T. L., McCarthy, R., Telle, W. B. and Brown, E. J. Purification of a mycobacterial adhesin for fibronectin. Infect. Immun. **61** (1993) 1889–1894.

Previous studies have demonstrated that mycobacteria attach to fibronectin (FN). The attachment of mycobacteria to FN is considered to be biologically important in *Mycobacterium bovis* BCG therapy for superficial bladder cancer, initiation of delayed hypersensitivity to mycobacterial antigens, and the phagocytosis of mycobacteria by epithelial cells. Therefore, we purified the mycobacterial receptor for FN. Culture supernatants from 3-week cultures of *M. vaccae,* which contained proteins that bound FN and inhibited the attachment of both *M. vaccae* and BCG to FN, were used as a source of receptor. Lyophilized *M. vaccae* supernatants were reconstituted in 0.02 M bis-Tris (pH 6.0) and applied sequentially to an ACA 54 gel filtration column and a DEAE-Sephacel anion-exchange column. A purified inhibitory protein of 55 kDa (p55) was obtained. The purified p55 protein was observed to bind to FN and to inhibit I-125-FN binding to viable BCG in a dose-dependent manner. Polyclonal and monoclonal antibodies to the protein were generated. The resulting polyclonal antiserum blotted a single protein band at 55 kDa in crude *M. vaccae* supernatants, crossreacted with a 55-kDa BCG protein by Western blot (immunoblot), and recognized a 55-kDa band that was associated with the BCG cell wall, which is consistent with its function as a FN receptor. A monoclonal immunoglobulin M(lambda) was isolated from mice immunized with purified *M. vaccae* p55 protein that was not functional in Western blots but inhibited the attachment of viable BCG to FN. These studies demonstrate that a protein or antigenically related proteins with M(r)s of 55,000 function as FN receptors for at least two distinct mycobacteria.—Authors' Abstract

Telenti, A., Imboden, P., Marchesi, F., Lowrie, D., Cole, S., Colston, M. J., Matter, L., Schopfer, K. and Bodmer, T. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis.* Lancet **341** (1993) 647–650.

Control of tuberculosis is threatened by widespread emergence of drug resistance in

Mycobacterium tuberculosis. Understanding the molecular basis of resistance might lead to development of novel rapid methods for diagnosing drug resistance. We set out to determine the molecular basis of resistance to rifampin, a major component of multidrug regimens used for treating tuberculosis. Resistance to rifampin involves alterations of RNA polymerase. The gene that encodes the RNA polymerase subunit beta (rpoB) was cloned. Sequence information from this gene was used to design primers for direct amplification and sequencing of a 411 bp rpoB fragment from 122 isolates of *M. tuberculosis*. Mutations involving 8 conserved aminoacids were identified in 64 of 66 rifampin-resistant isolates of diverse geographical origin, but in none of 56 sensitive isolates. All mutations were clustered within a region of 23 aminoacids. Thus, substitution of a limited number of highly conserved aminoacids encoded by the rpoB gene appears to be the molecular mechanism responsible for "single step" high-level resistance to rifampin in *M. tuberculosis*. This information was used to develop a strategy (polymerase chain reaction-single-strand conformation polymorphism) that allowed efficient detection of all known rifampin-resistant mutants. These findings provide the basis for rapid detection of rifampin resistance, a marker of multidrug-resistant tuberculosis.—Authors' Abstract

Thurman, P. F., Chai, W. G., Rosankiewicz, J. R., Rogers, H. J., Lawson, A. M. and Draper, P. Possible intermediates in the biosynthesis of mycoside-B by *Mycobacterium microti*. Eur. J. Biochem. **212** (1993) 705–711.

Two lipids were isolated from *Mycobacterium microti* which became labeled when the cells were grown in the presence of [2-C-14]propionate. They were purified by thin-layer chromatography and studied by chemical degradation and mass spectrometry. The lipids were identified as phenolphthiocerol dimycocerosate and phenolphthiodiolone dimycocerosate, the aglycosyl derivatives of mycoside B, the phenolic glycolipid produced by *M. microti*. Cell-free extracts of the organism were able to glycosylate the lipids to form mycoside B *in vitro*. It is probable that the lipids are intermediates in the biosynthesis of phenolic glycolipids by mycobacteria.—Authors' Abstract

Trias, J. Q. and Benz, R. Characterization of the channel formed by the mycobacterial porin in lipid bilayer membranes—demonstration of voltage gating and of negative point charges at the channel mouth. J. Biol. Chem. **268** (1993) 6234–6240.

We studied the channel formed by the mycobacterial porin from the cell wall of *Mycobacterium chelonae* by reconstituting the mycobacterial porin and cell wall extracts in lipid bilayer membranes. The channel exhibited two different states in lipid bilayer membranes at 10 mV of applied voltage. One was characterized by a steplike appearance while the other showed a fast, voltage-dependent, flickering behavior between a closed and an open state. The channel was voltage-gated, and starting at 40 mV of applied voltage the mycobacterial porin channel switched to a closed configuration in an asymmetric fashion. The channel was cation-selective and had 2.5-point negative charges at both sides of the channel. Identical channels were observed when membranes were reconstituted with cell wall extracts, suggesting that there is only one porin species in the mycobacterial cell wall.—Authors' Abstract

van der Vliet, G. M. E., de Wit, M. Y. L. and Klatser, P. R. A simple colorimetric assay for detection of amplified *Mycobacterium leprae* DNA. Mol. Cell. Probes **7** (1993) 61–66.

A colorimetric assay for the detection of PCR-products is described. The assay is based on amplification of DNA in the presence of digoxigenin-dUTP. After immobilization of the PCR products to a microtiter plate, amplified DNA could be detected colorimetrically. The sensitivity of this colorimetric assay was equal to gel-analysis allowing the detection of 100 fg of template DNA. Here, we show that it can be used to detect *Mycobacterium leprae* DNA in biopsy specimens from leprosy patients. The simplicity and the low degree of variation

make this assay an alternative to gel-analysis.—Authors' Abstract

Vega Lopez, F., Brooks, L. A., Dockrell, H. M., Desmet, K. A. L., Thompson, J. K., Hussain, R. and Stoker, N. G. Sequence and immunological characterization of a serine-rich antigen from *Mycobacterium leprae*. Infect. Immun. **61** (1993) 2145–2153.

Sera from lepromatous leprosy patients were used to screen a *Mycobacterium leprae* lambda gt11 library. Three positive plaques were picked, and lysogens were constructed. Immunoblot analysis showed that all of the lysogens expressed an apparently identical beta-galactosidase fusion protein which reacted strongly with the sera. The 1.7-kbp insert from one clone was subcloned into the lacZ gene in pUR290; sequence analysis of the end fused to lacZ revealed an open reading frame with no significant homology to previously published sequences. The insert was used to screen an *M. leprae* cosmid library, and five clones were isolated. The insert was also found to hybridize to clones expressing the *M. leprae* antigen which had previously been designated class III and 25L. A 1.8-kbp HindIII fragment was subcloned from one of the cosmids and sequenced. The sequence revealed a 1227-bp open reading frame, encoding a 408-amino-acid protein with a predicted molecular mass of 42,466 Da. The protein contains amino- and carboxy-terminal hydrophobic domains and a hydrophilic central domain; the amino-terminal domain shows some homology to a 51-kDa hypothetical antigen of *M. tuberculosis*, while the hydrophilic region contains a high proportion of serine residues, and we have therefore designated the protein serine-rich antigen (Sra). Some repeated motifs are present in the protein, but their significance is unknown. Seventy-eight percent of serum samples from multibacillary leprosy patients and 68% of serum samples from paucibacillary leprosy patients recognized the fusion protein, showing that this is a major *M. leprae* antigen. In contrast, all serum samples from endemic controls were negative, while 26% of serum samples from tuberculosis patients were weakly positive.—Authors' Abstract

Wheeler, P. R., Besra, G. S., Minnikin, D. E. and Ratledge, C. Stimulation of mycolic acid biosynthesis by incorporation of cis-tetracos-5-enoic acid in a cell-wall preparation from *Mycobacterium smegmatis*. Biochim. Biophys. Acta **1167** (1993) 182–188.

Mycolic acids are high molecular weight hydroxy fatty acids which are a covalently linked part of the cell-wall structure of all mycobacteria and their biosynthetic pathways offer potential drug targets. Three good candidates, cis-tetracos-5-enoic acid and R or S trans-6-methyl-tetracos-4-enoic acids, for the key initial intermediates where mycolic acid biosynthesis might diverge from other metabolic pathways, were tested as possible substrates. A cell-wall preparation from *Mycobacterium smegmatis,* capable of mycolic acid synthesis, was developed to investigate the possible incorporation of these, and other 16 to 24 carbon acids into mycolic acids. The wall preparations were extracted with hexane and suspended in hexane/water (7:1, v/v), and in this low-water assay, only one of these acids, cis-tetracos-5-enoic acid, stimulated the incorporation of radioactive label from [1-C-14]acetate into alpha- and alpha'-mycolic acids. The extraction method used did, however, abolish some enzyme activity and mycolic acid biosynthesis was not completely restored by cis-tetracos-5-enoate. The two methyl-branched acids did not enhance the amount of label in epoxymycolic acids. An initial key intermediate in the synthesis of alpha- and alpha'-mycolic acids has therefore been positively identified for the first time; intermediates in the initial stages of the biosynthesis of oxygenated mycolic acids such as epoxymycolates remain to be defined.—Authors' Abstract

Epidemiology and Prevention

Choudhury, A. M. Leprosy in Bangladesh, 1984–88. Jpn. J. Lepr. **60** (1991) 128–131.

A total of 2517 patients with leprosy were studied in Leprosy Control Institute and Hospital, Mohakhali, Dhaka [Bangladesh], during the period 1984–1988. Among the total cases, 1891 (75.1%) were male and 626 (24.9%) were female. The male to female ratio was 3:1. The age groups comprised 185 (7.4%) below 15 years and 2332 (92.6%) 15 years and over. Most of the cases were diagnosed by clinical examination and classified as indeterminate form (I), 52 (2.1%); tuberculoid form (T), 1326 (52.7%); borderline tuberculoid form (BT), 439 (17.4%); borderline lepromatous form (BL, 110 (4.4%); and lepromatous form (L), 590 (23.4%).—AS (Trop. Dis. Bull.)

Epidemiology of leprosy in relation to control. Lepr. Rev. **63** Suppl. 1 (1992) 1S–126S.

This supplement is devoted to papers presented at an international meeting held in Jakarta, Indonesia, on 17–21 June 1991.

The first section, on assessment of the leprosy problem, consists of four papers: "Needs and prospects of epidemiological tools in leprosy control" (P. Feenstra, p. 3s), "Issues involved in the rapid assessment of the leprosy problem" (T. K. Sundaresan, p. 11s), "Summary of 'Estimation of the leprosy problem through health services data' " (K. Jesudasan, p. 21s), "The epidemiology of disability in leprosy including risk factors" (W. C. S. Smith, p. 23s).

The section on prediction of future trends comprises "Epidemiometric modeling in leprosy based on Indian data" (M. F. Lechat, p. 31s), "Epidemiological modeling for tropical disease control" (J. H. F. Remme, p. 40s), "Toward the use of decision sciences in leprosy control" (J. D. F. Habbema, E. Jozefzoon and G. J. Van Oortmarssen, p. 48s).

Next comes a section on monitoring and evaluation. The five papers are entitled "Major issues involved in the evaluation of leprosy control programs through MDT" (C. K. Rao, p. 53s), "Defining a case of leprosy" (V. K. Pannikar, p. 61s), "Surveillance and monitoring of multidrug therapy using cohort analysis" (D. Daumerie, p. 66s), "Indicators for use in leprosy control programs" (Myo Thet Htoon, p. 73s), "OMSLEP as an evaluation tool" (E. Declercq, p. 77s).

The section on the impact of multidrug therapy consists of: "The measurement of the epidemiological impact of multidrug therapy" (C. Pirayavaraporn and S. Peerapakorn, p. 84s), "Treatment failures with multidrug therapy" (D. Lobo, p. 93s).

Finally two papers are grouped under the heading of miscellaneous: "The relevance of future leprosy vaccines to disease control" (M. D. Gupte, p. 99s), and "The role of health systems research in leprosy control' (I. Pathmanathan, p. 106s).

The supplement is completed by a report on the group discussions on the needs and prospects for the epidemiological tools in leprosy control, and a one-page summary of major conclusions and recommendations.—C. A. Brown (Trop. Dis. Bull.)

Foss, N. T., Callera, F. and Alberto, F. L. Anti-PGL-I levels in leprosy patients and their contacts. Braz. J. Med. Biol. Res. **26** (1993) 43–51.

We determined the anti-PGL-I levels of 402 individuals from the Ribeirao Preto region [of Brazil] since the PGL-I is a specific *Mycobacterium leprae* antigen. This group consisted of 47 leprosy patients (26 with the lepromatous form, 16 with the tuberculoid form and 5 with the borderline form), 12 tuberculosis patients, 19 leprosy contacts, and 324 healthy blood donors from the Hemocenter of the University Hospital, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo. Anti-PGL-I levels were detected by ELISA. Anti-PGL-I levels were normal in patients with tuberculoid and borderline leprosy, in tuberculosis patients, and in almost all of the healthy blood donors. Patients with untreated lepromatous leprosy had elevated anti-PGL-I levels while most patients under treatment (9/16) had normal anti-PGL-I levels. Only 3% of blood donors (10/324) had elevated anti-PGL-I levels, but when these individuals were sub-

mitted to clinical and bacilloscopic examination no signs of disease were found. To complete the clinical investigation, these 10 subjects were submitted to the Mitsuda reaction which was negative in 3 of them. All of these 10 subjects are being monitored, since they may be at risk to develop leprosy. On the basis of the present data, it seems that ELISA is a potentially important assay for the detection and chemotherapy of subclinical leprosy, permitting the control of epidemic centers of the disease.—Authors' Abstract

Myint, T., Htoon, M. T. and Shwe, T. Estimation of leprosy prevalence in Bago and Kawa Townships using 2-stage probability proportionate to size sampling technique. Int. J. Epidemiol. **21** (1993) 778–783.

Two surveys to estimate leprosy prevalence using two-stage probability proportionate to size sampling technique were conducted in Bago and Kawa townships [in Myanmar]. A total of 3519 and 3739 individuals were examined in each township. The two surveys were finished within 25 (Bago) and 30 (Kawa) working days at a cost of Kyats 10,000 (US$1500) for each survey. The estimated leprosy prevalence obtained in Bago was 9.95 per 1000 population [95% confidence interval (CI): 7.11–12.78] and in Kawa it was 12.04 per 1000 population [95% CI: 8.85–15.22]. A total of 30 (Bago) and 34 (Kawa) new leprosy cases were detected in the two surveys. Grade I disability was seen to be 20% in Bago and 18.78% in Kawa, whereas grade II disability was 17.14% in Bago and 15.56% in Kawa.—Authors' Abstract

Rehabilitation

Castellazzi, Z., Lora, M. and Canario, S. [Incapacity of leprosy patients; a ten-year analysis.] Rev. Dom. Dermatol. **19** (1992) 21–24. (in Spanish)

The incapacities produced by new cases of leprosy during a 10-year period were reviewed. Out of 3257 cases 585 (17.9%) showed incapacities; 9.4% of them had first-degree and 8.5% second-degree incapacities. The lepromatous group was more affected, with 35.9%. Patients older than 15 years were more affected, and males were more affected than females with 66.0% of the cases. Our results were compared to a previous review made by Martínez, *et al.* with no significant differences.—Authors' English Summary

Majoroh, T. O. Chronic plantar ulcer of a leprosy patient treated by fasciocutaneous instep flap transfer—preliminary result. Trop. Geogr. Med. **45** (1993) 39–40.

A case of chronic ulceration of the foot (chronicity about 15 years) was treated with fasciocutaneous instep flap transfer. The ulcer was on the heel. Healing was fast (about 40 days postoperatively. Duration for conventional method is about 6 months) and after 12 months of ambulation no recurrence was noticed. The female patient with only one lower limb (the other already amputated) now goes about smilingly selling her wares. However, the footwear for the patient had to be further modified.—Authors' Abstract

Zhang, G. C., Li, W. Z., Yan, L. B., *et al.* An epidemiological survey of deformities and disabilities among 14, 257 leprosy patients in 11 counties. Chin. Med. Sci. J. **7** (1992) 216–220.

Over half (56.97%) of 14,257 leprosy patients in 11 counties in Yangzhou Prefecture (China) were found to have permanent deformities and disabilities. The survey was conducted in 1988 by a trained team of medical and paramedical workers. Multibacillary leprosy cases had a higher disability rate than paucibacillary leprosy cases (81% compared with 53%). The data are analyzed by types of disease and by deformity and disability. The disability rate increased with age and with the duration of disease. Over half of paucibacillary and multibacillary cases had developed deformities and disabilities before starting anti-

leprosy treatment. Although up to a quarter of the 8122 disabled cases were thought to be suitable for reconstructive surgery, most (59%) refused surgical treatment. The need for education in self-care is stressed.—C. A. Brown (Trop. Dis. Bull.)

Other Mycobacterial Diseases and Related Entities

Bosne, S., Papa, F., Clavel-Seres, S. and Rastogi, N. A simple and reliable EDDA method for mycobactin production in mycobacteria—optimal conditions and use in mycobacterial speciation. Curr. Microbiol. **26** (1993) 353–358.

Ethylenediamino-di-(*O*-hydroxyphenylacetic acid) (EDDA) was found to be a suitable iron chelator for investigating mycobactin production in mycobacteria. The optimal conditions of EDDA concentration and time of exposure were established and were used to develop a two-step method for mycobactin production. Applied to representative strains of selected species, the method was found to yield reliable results useful for identification and speciation of mycobacteria.—Authors' Abstract

Brindle, R. J., Nunn, P. P., Githui, W., Allen, B. W., Gathua, S. and Waiyaki, P. Quantitative bacillary response to treatment in HIV-associated pulmonary tuberculosis. Am. Rev. Respir. Dis. **147** (1993) 958–961.

A group of 122 patients with culture-proven pulmonary tuberculosis was recruited to examine the concentrations of *Myobacterium tuberculosis* in sputum and the relationship to HIV-1 antibody status. They were followed for up to 28 days from the start of antituberculous chemotherapy to assess the early bacillary response to two chemotherapeutic regimens. Of 67 treated with streptomycin, thiacetazone, and isoniazid 17 were HIV positive, and subsequently 55, of whom 20 were HIV positive, were treated with streptomycin, rifampin, isoniazid, and pyrazinamide. The mean initial concentration of *M. tuberculosis* in the sputum of the HIV-negative patients was significantly higher than in HIV-positive patients (6.95 and 6.34 log colony forming units, respectively; $p = 0.019$). The HIV-positive patients had less radiologic evidence of disease and significantly fewer zones of lung affected with cavities. The response to treatment was similar, but with HIV-positive patients more likely to become culture negative by 28 days. The differences that exist between HIV-positive and HIV-negative patients are minor, and standard regimens are at least as effective in HIV-positive patients in the first month of treatment.—Authors' Abstract

Collins, D. M., Erasmuson, S. K., Stephens, D. M., Yates, G. R. and De Lisle, G. W. DNA fingerprinting of *Mycobacterium bovis* strains by restriction fragment analysis and hybridization with insertion elements IS*1081* and IS*6110*. J. Clin. Microbiol. **31** (1993) 1143–1147.

Strains of *Mycobacterium bovis*, the causative organism of bovine tuberculosis, can be clearly distinguished from each other by restriction fragment analysis. This method of DNA fingerprinting has been used for many epidemiological studies in New Zealand, but the technique presents practical difficulties that hinder its widespread use. The insertion element IS*6110* is being widely used as a DNA probe for distinguishing restriction fragment polymorphisms among strains of *M. tuberculosis*. Both this element and another recently sequenced element, IS*1081*, are also present in *M. bovis*. We assessed the usefulness of these two elements for distinguishing between 160 strains of *M. bovis*. These strains, most of which were isolated in New Zealand, were selected to be representative of the 95 different types that were identified among 530 strains that were previously typed by restriction fragment analysis. Fifteen IS*6110* types were identified, but more than half of the strains representing 46 restriction types had the same IS*6110* type. Virtually all *M. bovis*

strains as well as strains of *M. tuberculosis* and *M. africanum* had the same IS*1081* type. The results indicate that for *M. bovis,* IS*1081* cannot be used to type strains, IS*6110* can be used to distinguish strains into broad groups, but only restriction fragment analysis is sufficiently sensitive for detailed epidemiological studies. An investigation of the host range of IS*1081* revealed that, apart from its presence in species of the tuberculosis complex, it is also present in a strain of *M. xenopi.*—Authors' Abstract

Doi, T., Ando, M., Akaike, T., Suga, M., Sato, K. and Maeda, H. Resistance to nitric oxide in *Mycobacterium avium* complex and its implication in pathogenesis. Infect. Immun. **61** (1993) 1980–1989.

Susceptibility of three different strains of *Mycobacterium avium* complex (MAC), i.e., one strain of *M. avium* (Mino) and two strains of *M. intracellulare* (31F093T and KUMS 9007), to nitric oxide (NO) generated by rat alveolar macrophages (MPHI) or NO generated chemically by acidification of NO_2^- was examined *in vitro.* We also investigated the effects of NO on phagocytosis and superoxide anion (O_2^-) generation by MPHI. The intracellular growth of *M. avium* Mino was significantly suppressed by NO generated by gamma-interferon (IFN-γ)-stimulated MPHI; whereas that of two strains of *M. intracellulare* (31F093T and KUMS 9007) were not. *M. avium* Mino was also more susceptible to NO generated chemically by acidification of NO_2^- than the two *M. intracellulare* strains. In L-arginine (1 mM)-containing medium, NO release from the MPHI assessed by measuring NO_2^- increased as the concentration of IFN-γ increased. The enhancing potential of IFN-γ for NO release became more pronounced when MPHI were infected with 31F093T, an NO-resistant strain. A large amount of NO generated by IFN-γ-stimulated MPHI suppressed both phagocytosis and O_2^- generation by the MPHI, especially after infection of the MPHI with strain 31F093T. These results indicate that the intracellular growth of MAC is not always inhibited by NO generated by immunologically activated MPHI; rather, NO generation induced by infection with an NO-resistant MAC strain suppresses phagocytosis of the MPHI, which may allow extracellular spreading of such NO-resistant mycobacteria. Therefore, the pathogenic potential of MAC may be partly attributed to its resistance to NO.—Authors' Abstract

Emori, M., Saito, H., Sato, K., Tomioka, H., Setogawa, T. and Hidaka, T. Therapeutic efficacy of the benzoxazinorifamycin KRM-1648 against experimental *Mycobacterium avium* infections induced in rabbits. Antimicrob. Agents Chemother. **37** (1993) 722–728.

The therapeutic efficacy of the benzoxazinorifamycin KRM-1648 was studied in an experimental rabbit infection system with avian *Mycobacterium avium.* The infected rabbits died from Yersin-type infections, a peculiar type of experimental bovine tuberculosis characterized by a very rapid course, enlargement of the spleen and liver, and septic infection, 14 to 20 days after bacterial challenge, as evidenced by bacteremia and severe bacterial loads in the visceral organs. Histopathologic studies of the visceral organs of the infected rabbits revealed the development of numerous typical granulomatous lesions. This experimental rabbit infection system, features of which resemble certain features of disseminated *M. avium*-complex infections in AIDS patients, was used to evaluate the therapeutic efficacy of KRM-1648, a newly synthesized benzoxazinorifamycin. KRM-1648 given orally at 25 and 50 mg/kg of body weight reduced the incidence and degree of bacteremia in infected rabbits and protected against subsequent death. Moreover, the drug allowed almost complete recovery of infected rabbits by week 7. KRM-1648 cleared infections in the lungs, liver, spleen, and kidneys and restored histopathologic features of healthy tissue in the visceral organs. KRM-1648 exhibited a more potent therapeutic effect against *M. avium* infection than rifampin and clarithromycin.—Authors' Abstract

Fidler, H. M., Rook, G. A., Johnson, N. M. and McFadden, J. *Mycobacterium tuberculosis* DNA in tissue affected by sarcoidosis. Br. Med. J. **306** (1993) 546–549.

To investigate the prevalence of *Mycobacterium tuberculosis* DNA in granulo-

matous tissues from patients with sarcoidosis and from controls matched for age, sex, and tissue by using the polymerase chain reaction. The subjects were 16 patients with sarcoidosis who had undergone diagnostic biopsy of lung, skin, or lymph node and 16 patients with squamous cell carcinoma or Hodgkin's disease to act as controls. In addition, four lung specimens infected with *M. tuberculosis* were included as positive controls. *M. tuberculosis* DNA was present in sarcoid tissues containing granulomas from 7 of the 16 patients and 1 of the 16 matched controls. Two of the four specimens known to be infected with *M. tuberculosis* were positive in the controlled experiment. These figures suggest that *M. tuberculosis* DNA is detected as readily in patients with sarcoidosis as in patients with frankly tuberculous tissues and imply that *M. tuberculosis* may be linked to the cause of sarcoidosis.—Authors' Abstract

Frothingham, R. and Wilson, K. H. Sequence-based differentiation of strains in the *Mycobacterium avium* complex. J. Bacteriol. **175** (1993) 2818–2825.

The complete 16S–23S rDNa internal transcribed spacer (ITS) was sequenced in 35 reference strains of the *Mycobacterium avium* complex. Twelve distinct ITS sequences were obtained, each of which defined a "sequevar"; a sequevar consists of the strain or strains which have a particular sequence. ITS sequences were identified which corresponded to *M. avium* (16 strains, four ITS sequevars) and *M. intracellulare* (12 strains, one ITS sequevar). The other seven *M. avium*-complex strains had ITS sequences which varied greatly from those of *M. avium* and *M. intracellulare* and from each other. The 26S–23S rDNA ITS was much more variable than 16S rDNA, which is widely used for genus and species identification. Phylogenetic trees based on the ITS were compatible with those based on 16S rDNA but were more detailed and had longer branches. The results of ITS sequencing were consistent with the results of hybridization with *M. avium* and *M. intracellulare* probes (Gen-Probe) for 30 of 31 strains tested. Serologic testing correlated poorly with ITS sequencing. Strains with the same sequence were different serovars, and those of the same serovar had different sequences. Sequencing of the 16S-23S rDNA ITS should be useful for species and strain differentiation for a wide variety of bacteria and should be applicable to studies of epidemiology, diagnosis, virulence, and taxonomy.—Authors' Abstract

Fuursted, K. Comparison of growth and susceptibility testing of pyrazinamide in different BACTEC media using strains of the *M. tuberculosis* complex. APMIS **101** (1993) 154–159.

The aim of this study was to evaluate and compare growth and susceptibility testing of pyrazinamide (pza) in different BACTEC media using 85 strains of the *Mycobacterium tuberculosis* complex. Fifty-four were clinical isolates of *M. tuberculosis*, of which 34 strains were selected as they failed to grow in primary pza susceptibility testing. Fourteen were strains of *M. bovis*, 10 were different strains of *M. bovis* BCG, five were strains of *M. africanum*, and two were strains of *M. microti*. The following BACTEC media were evaluated: (1) BACTEC PZA test medium, (2) acidified BACTEC 12B medium with pH reduced to 5.6, (3) acidified 12B medium supplemented with fresh egg yolk, (4) acidified 12B medium supplemented with a commercial egg yolk enrichment. Demonstration of pyrazinamidase activity was included for comparison. All strains of *M. tuberculosis, M. microti* and *M. africanum* were inhibited by pza at 100 μg/ml, and all strains of *M. bovis* and *M. bovis* BCG were found to be resistant to pza at 100 μg/ml. BACTEC PZA test medium supported growth for all 85 strains tested. The two egg yolk-supplemented media supported growth in all strains except one strain of *M. microti*, and acidified but otherwise unsupplemented BACTEC 12B medium supported growth in 64 strains (75%). A pza sensitivity test result could be obtained within 1 week in >79% of strains. An overall correlation of 89% between susceptibility to pza and demonstration of pyrazinamidase activity was demonstrated.—Authors' Abstract

Gnaore, E., Sassanmorokro, M., Kassim, S., Ackah, A., Yesso, G., Adjorlolo, G., Digbeu, H., Coulibaly, D., Coulibaly, I. M.,

Doorly, R., Brattegaard, K. and Decock, K. M. A comparison of clinical features in tuberculosis associated with infection with human immunodeficiency virus-1 and virus-2. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 57–59.

Between July 1989 and December 1990, 4504 new adult patients with tuberculosis were screened for antibodies to human immunodeficiency viruses (HIV) 1 and 2 in Abidjan's two tuberculosis treatment centers. The prevalence levels of HIV-1 and HIV-2 infections were 30.2% and 4.2%, respectively, a further 9.3% of patients reacting serologically to both viruses. Patients in all three seropositive groups differed significantly from seronegatives in having a higher frequency of AIDS-related features such as wasting, chronic diarrhea, oral candidiasis and generalized lymphadenopathy. These data support earlier work showing an association between HIV-2 infection and similar opportunistic diseases which complicate HIV-1 infection, including tuberculosis. Despite the differences between seropositive and seronegative groups, symptoms and signs of tuberculosis may mimic those of AIDS. HIV testing should be more widely available for the clinical care of tuberculosis patients in Africa, as well as for epidemiological surveillance.—Authors' Abstract

Haas, W. H., Butler, W. R., Woodley, C. L. and Crawford, J. T. Mixed-linker polymerase chain reaction: a new method for rapid fingerprinting of isolates of the *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. **31** (1993) 1293–1298.

Rapid recognition of multidrug-resistant strains of *Mycobacterium tuberculosis* is a desirable goal for treatment of patients and protection of health care workers. DNA fingerprints produced with the insertion sequence IS*6110* generate restriction fragment length polymorphism (RFLP) patterns that reliably identify *M. tuberculosis*-complex strains. This report describes a rapid technique for RFLP typing using the polymerase chain reaction. The method uses one primer specific for IS*6110* and a second primer complementary to a linker ligated to the restricted genomic DNA. In one strand the linker contains uracil in place of thymidine, and specific amplification is obtained by elimination of this strand with uracil *N*-glycosylase. Mixed-linker fingerprinting clearly differentiated multidrug-resistant isolates from 12 outbreaks and unambiguously assigned them to 26 RFLP groups.—Authors' Abstract

Hernandez Frontera, E. and McMurray, D. N. Dietary vitamin-D affects cell-mediated hypersensitivity but not resistance to experimental pulmonary tuberculosis in guinea pigs. Infect. Immun. **61** (1993) 2116–2121.

Outbred, Hartley strain guinea pigs were fed purified diets varying only in their levels of vitamin D. The amounts of vitamin D in the diets were adjusted to represent 0, 25, 50, 100, or 200% of the recommended level (1180 IU/kg of body weight) for guinea pigs. In some experiments, half of the animals in each diet group were vaccinated with *Mycobacterium bovis* BCG vaccine at the time the diets were introduced. Six weeks later, all guinea pigs were infected by the respiratory route with a low dose of virulent *M. tuberculosis* H37Rv. Vitamin D-deficient animals exhibited marked reductions in levels of the major vitamin D metabolite, 25-hydroxyvitamin D3, in plasma. Altered vitamin D intake was accompanied by changes in antigen (purified protein derivative)-induced, cell-mediated immune responses both *in vivo* (tuberculin hypersensitivity) and *in vitro* (lymphoproliferation). Dermal tuberculin reactivity developed more slowly in vitamin D-deficient guinea pigs but eventually achieved normal levels. The proliferation of splenocytes cultured with purified protein derivative was suppressed by both deficiency and excess of dietary vitamin D. Vitamin D status did not affect the abilities of naive guinea pigs to control primary, pulmonary tuberculosis, nor did it influence the protective efficacy of BCG vaccination. We conclude that changes in dietary vitamin D are associated with alterations in some cellular immune functions but may not be an important determinant of disease outcome in pulmonary tuberculosis, as has been suggested previously.—Authors' Abstract

Hoel, T., Casals, J. B. and Eng, J. *In vitro* antimicrobial susceptibility testing of rapidly growing mycobacteria using the tablet diffusion method—resistance pattern of Norwegian *Mycobacterium fortuitum* and *Mycobacterium chelonae* isolates. APMIS **101** (1993) 27–32.

Thirty-one Norwegian clinical isolates of rapidly growing mycobacteria classified as Runyon's group IV, including 20 *Mycobacterium fortuitum* and 11 *M. chelonae* strains, were found resistant to a majority of tuberculostatic agents. Minimal inhibitory concentration (MIC) was determined for other antimicrobial agents: amikacin, tobramycin, streptomycin, cefoxitin, imipenem, norfloxacin, ciprofloxacin, doxycycline, erythromycin, fusidic acid, co-trimoxazole and capreomycin. The agar plate dilution method was employed and compared with the agar tablet diffusion method. Regression lines were established correlating MIC values and inhibition zones. The agar tablet diffusion method was found to be a simple and useful method for testing antimicrobial susceptibilities of *M. fortuitum* and *M. chelonae,* and a good correlation between MIC values and zone sizes with 12 antimicrobial agents was revealed. Correlation coefficients for most of these antimicrobial agents were around -0.90. *M. chelonae* was generally more resistant than *M. fortuitum.* Four antimicrobial agents, capreomycin, norfloxacin, ciprofloxacin and amikacin, showed differences between *M. fortuitum* and *M. chelonae* large enough to allow the zone diameter to be used diagnostically.—Authors' Abstract

Hoffner, S. E., Hjelm, U. and Källenius, G. Susceptibility of *Mycobacterium malmoense* to antibacterial drugs and drug combinations. Antimicrobial. Agents Chemother. **37** (1993) 1285–1288.

Mycobacterium malmoense is an opportunistic pathogen with increasingly recognized clinical importance. It is mainly isolated in northern Europe and Great Britain, most often from patients with pulmonary infections. Conventional therapy of *M. malmoense* infections with antituberculosis drugs is often of limited value, and there is thus a need for improved drug regimens. The potential efficacies of new alternative drugs, such as quinolones, macrolides, amikacin, and rifabutin, are still unknown, and so is the pathogen's *in vitro* susceptibility to most of these drugs. In this study, we used the BACTEC system for determining the pattern of resistance of clinical *M. malmoense* isolates to a number of antibacterial drugs as well as their possible synergistic interactions when each of them was combined with ethambutol. The majority of the strains were resistant or moderately resistant to the drug when it was tested alone at selected concentrations. However, pronounced *in vitro* synergism was demonstrated for combinations of ethambutol with ciprofloxacin, amikacin, and rifampin, rendering most isolates susceptible to the combined drugs. Thus, for *in vitro* susceptibility testing and rifampin, rendering most isolates susceptible to the combined drugs. Thus, for *in vitro* susceptibility testing of *M. malmoense,* examination of the possible synergistic effects of combined drugs also can be recommended.—Authors' Abstract

Honda, I., Kawajiri, K., Watanabe, M., Toida, I., Kawamata, K. and Minnikin, D. E. Evaluation of the use of 5-mycoloyl-beta-arabinofuranosyl-(1→2)-5-mycoloyl-alpha-arabinofuranyosyl-(1→1')-glycerol in serodiagnosis of *Mycobacterium avium-intracellulare* complex infection. Res. Microbiol. **144** (1993) 229–235.

5-Mycoloyl-beta-arabinofuranosyl-(1→2)-5-mycoloyl-alpha-arabinofuranosyl-(1→1')-glycerol, an antigenic glycolipid from the *Mycobacterium avium-intracellulare* complex (MAC) was examined for its applicability to the serodiagnosis of MAC infection by ELISA. Serum IgM antibody titers against this glycolipid in healthy controls, pulmonary tuberculosis patients and sputum-MAC-culture-negative MAC patients were generally below the cutoff point (ELISA-negative); whereas most of the MAC-culture-positive MAC patient sera were ELISA-positive (93.5%) and their titers were often very high. Thus, high serum IgM titers against this glycolipid may be said to imply that the MAC disease is in an active phase.—Authors' Abstract

Jacobs, W. R., Jr., Barletta, R. G., Udani, R., Chan, J., Kalkut, G., Sosne, G., Kieser, T., Sarkis, G. J., Hatfull, G. F. and Bloom, B. R. Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. Science **260** (1993) 819–822.

Effective chemotherapy of tuberculosis requires rapid assessment of drug sensitivity because of the emergence of multidrug-resistant *Mycobacterium tuberculosis.* Drug susceptibility was assessed by a simple method based on the efficient production of photons by viable mycobacteria infected with specific reporter phages expressing the firefly luciferase gene. Light production was dependent on phage infection, expression of the luciferase gene, and the level of cellular adenosine triphosphate. Signals could be detected within minutes after infection of virulent *M. tuberculosis* with reporter phages. Culture of conventional strains with antituberculosis drugs, including isoniazid or rifampin, resulted in extinction of light production. In contrast, light signals after luciferase reporter phage infection of drug-resistant strains continued to be produced. Luciferase reporter phages may help to reduce the time required for establishing antibiotic sensitivity of *M. tuberculosis* strains from weeks to days and to accelerate screening for new antituberculosis drugs.—Authors' Abstract

Klein, R., Wiebel, M., Engelhart, S. and Berg, P. A. Sera from patients with tuberculosis recognize the M2a-epitope (E2-subunit of pyruvate dehydrogenase) specific for primary biliary cirrhosis. Clin. Exp. Immunol. **92** (1993) 308–316.

Anti-M2 antibodies in primary biliary cirrhosis (PBC) have been shown to react with the alpha-ketoacid dehydrogenase complex of the inner mitochondrial membrane consisting of six epitopes [E2 subunit of the pyruvate dehydrogenase complex (PDC), 70 kD; protein X of the PDC, 56 kD; alpha-ketoglutarate dehydrogenase complex, 52 kD; branched-chain alpha-ketoacid dehydrogenase, 52 kD; E1alpha subunit of PDC, 45 kD; and E1beta-subunit of PDC, 36 kD]. These epitopes are also present in the M2 fraction which is a chloroform extract from beef heart mitochondria. The E2 subunit of the PDC at 70 kD (M2a), especially, is a major target epitope which is recognized by about 85% of all PBC sera. However, analyzing sera from 28 patients with active pulmonary tuberculosis it became evident that 12 (43%) also recognized the PDC-E2 subunit (M2a), as shown by Western blotting using the M2 fraction, the purified PDC, and the recombinant PDC-E2. In contrast, only two of 82 patients with other bacterial and viral infections including 25 patients with *Escherichia coli* infections reacted with the PBC-specific epitope at 70 kD. Naturally occurring mitochondrial antibodies (NOMA) were present in 54% of the patients with tuberculosis and in 50% of patients with other infectious disorders. They recognized either a determinant at 65 kD (epsilon) or at 60/55 kD (zeta/eta). None of the sera from 100 blood donors had anti-M2 but 14 had NOMA. Testing anti-M2 and NOMA-positive marker sera by Western blotting against membrane fractions derived from mycobacteria and *E. coli* it could be shown that—like mammalian mitochondria—they contain both the PBC-specific M2 antigen as well as the non-PBC-specific naturally occurring mitochondrial antigen system (NOMAg). The observation that PBC-specific antibodies were preferentially induced in patients suffering from a mycobacterial infection may provide some new clues to the still unknown etiology of PBC.—Authors' Abstract

Kong, T. H., Coates, A. R. M., Butcher, P. D., Hickman, C. J. and Shinnick, T. M. *Mycobacterium tuberculosis* expresses two chaperonin-60 homologs. Proc. Natl. Acad. Sci. USA. **90** (1993) 2608–2612.

A 65-kDa protein and a 10-kDa protein are two of the more strongly immunoreactive components of *Mycobacterium tuberculosis,* the causative agent of tuberculosis. The 65-kDa antigen has homology with members of the GroEL or chaperonin-60 (Cpn60) family of heat-shock proteins. The 10-kDa antigen has homology with the GroES or chaperonin-10 family of heat-shock proteins. These two proteins are encoded by separate genes in *M. tuberculosis.* The studies reported here reveal that *M. tuberculosis* contains a second Cpn60 ho-

molog located 98 bp downstream of the 10-kDa antigen gene. The second Cpn60 homolog (Cpn60-1) displays 61% amino acid sequence identity with the 65-Kda antigen (Cpn60-2) and 53% and 41% identity with the *Escherichia coli* GroEL protein and the human P60 protein, respectively. Primer-extension analysis revealed that transcription starts 29 bp upstream of the translation start of the Cpn60-1 homolog and protein purification studies indicate that the *cpn60-1* gene is expressed as an ~60-kDa polypeptide.—Authors' Abstract

Krajewska, M. M., Anderson, R. and O'Sullivan, J. F. Effects of clofazimine analogues and tumor necrosis factor-alpha individually and in combination on human polymorphonuclear leukocyte functions *in vitro*. Int. J. Immunopharmacol. **15** (1993) 99–111.

In the present study the individual and interactive effects of clofazimine, or three analogs of this agent (selected on the basis of similar or superior prooxidative properties: B669, B746 and B4021) and human recombinant TNF-alpha on the generation of antimicrobial oxidants by human polymorphonuclear leukocytes (PMNL), as well as release of granule enzymes from these cells, were investigated *in vitro*. All four riminophenazines at the concentrations tested (0.5 and 1.0 μg/ml) significantly increased myeloperoxidase (MPO)-mediated iodination, superoxide generation, oxygen consumption, and chemiluminescence as well as the release of both primary and secondary granule contents (measured as the release of MPO, lysozyme and vitamin B-12-binding protein) by stimulated PMNL. Similar, but less impressive effects were observed with TNF-alpha (0.4–50.0 ng/ml). When PMNL were preincubated with both TNF-alpha and clofazimine or its analogs, the observed stimulation of cellular oxidative metabolism and granule enzyme release was at least additive in many assays. These data demonstrate that the spectrum of effects of clofazimine and its analogous on PMNL closely resemble those of TNF-alpha. Furthermore, TNF-alpha potentiates the prooxidative effects of clofazimine and its analogs on PMNL. Among the riminophenazines tested, clofazimine and B669 appear to be the most potent prooxidative agents for PMNL.—Authors' Abstract

Li, H. Y., Ulstrup, J. C., Jonassen, T. O., Melby, K., Nagai, S. and Harboe, M. Evidence for absence of the MPB64 gene in some substrains of *Mycobacterium bovis* BCG. Infect. Immun. **61** (1993) 1730–1734.

Substrains of *Mycobacterium bovis* BCG have been divided in two major groups, high producers and low producers of the secreted proteins MPB64 and MPB70. Of these, *M. tuberculosis* secretes only the analog MPT64 during growth on Sauton medium. It has been confirmed that high-producer and low-producer substrains of BCG as well as *M. tuberculosis* contain the gene for the MPB/MPT70 protein. By contrast, polymerase chain reaction and hybridization experiments are reported here which indicate that the MPB64 gene is absent in the BCG substrains Copenhagen, Pasteur, Glaxo, and Tice, in which previous methods did not permit distinction between secretion of small amounts or absence of the protein in culture fluids.—Authors' Abstract

Mor, N. and Heifets, L. Inhibition of intracellular growth of *Mycobacterium avium* by one pulsed exposure of infected macrophages to clarithromycin. Antimicrob. Agents Chemother. **37** (1993) 1380–1382.

A single 2-hr pulsed exposure of either human monocyte-derived macrophages or J774 cells infected with *Mycobacterium avium* to clarithromycin at 3.0 μg/ml completely inhibited the intracellular bacterial growth during the first four days of observation, and then only a slight increase in the number of CFU per milliliter took place between the fourth and seventh days. These data suggest that *in vivo* the intracellular bacteria can be effectively inhibited after a short period when the concentration of the drug in blood reaches its maximum. On the basis of these data, the assumptions that the elimination of bacteremia observed in clarithromycin clinical trials is a result of the activity of the drug not only against bacteria in blood but in macrophages as well and that the peak concentration attainable in blood is essential for these effects can be made.—Authors' Abstract

North, R. J. and Izzo, A. A. Mycobacterial virulence. Virulent strains of *Mycobacterium tuberculosis* have faster *in vivo* doubling times and are better equipped to resist growth-inhibiting functions of macrophages in the presence and absence of specific immunity. J. Exp. Med. **177** (1993) 1723–1733.

The kinetics of growth of two virulent strains of mycobacteria (*Mycobacterium tuberculosis* Erdman and *M. tuberculosis* H37Rv) and two attenuated strains (*M. tuberculosis* H37Ra and *M. bovis* bacillus Calmette-Guérin [BCG]) were studied in the lungs, livers, spleens, and kidneys of severe combined immunodeficient (SCID) mice and of their coisogenic CB-17 immunocompetent counterparts. It was found, in keeping with the findings of earlier investigators that in immunocompetent mice, virulent organisms grew progressively only in the lungs, whereas the growth of attenuated organisms was controlled in all organs. In SCID mice, in contrast, virulent mycobacteria grew rapidly and progressively in all organs, as did BCG, although at a slower rate. However, H37Ra failed to grow progressively in any organs of SCID mice, unless the mice were treated with hydrocortisone. In fact, hydrocortisone treatment enabled virulent, as well as attenuated, organisms to grow strikingly more rapidly in all organs of SCID mice and in all organs of CB-17 mice. A histological study showed that in SCID mice, multiplication of mycobacteria in the liver occurs in the cytoplasm of macrophages in granulomas and presumably in macrophages in other organs. It is suggested, therefore, that the macrophages of SCID mice possess a glucocorticoid-sensitive mycobacterial mechanism that prevents virulent and avirulent mycobacteria from expressing their true minimal doubling times. In the absence of this mechanism in the lungs of hydrocortisone-treated SCID mice, the doubling times of Erdman, H37Rv, BCG, and H37Ra were 17.7, 17.4, 44.6, and 98.6 hr, respectively. The possible importance of a rapid multiplication rate for mycobacterial virulence is discussed.—Authors' Summary

Nyabenda, J., Turner, M., Waelbroeck, A., Duvivier, A. and Toppet, M. Interleukin-2 and soluble interleukin-2 receptor levels in children with active pulmonary tuberculosis and atypical mycobacterial adenitis. Eur. Cytokine Network **4** (1993) 43–49.

Interleukin-2 (IL-2) level was measured in sera and in supernatants of purified protein derivatives of tuberculin (PPD) stimulated peripheral blood mononuclear cells (PBMC) cultures from children with active primary pulmonary tuberculosis (TB), or adenitis caused by mycobacteria of the group *Mycobacterium avium, intracellulare, scrofulaceum* (MAIS). The control groups included BCG-vaccinated children (BCG) and children with negative skin test to PPD (NST). High mean IL-2 level was exclusively found in sera of mild TB patients (MTB), and not in sera of MAIS-infected or BCG-vaccinated children. The IL-2 level increased even more in MTB during treatment. In severe TB (STB) the IL-2 level was not elevated before treatment, but increased also during treatment. IL-2 production in supernatants of PPD-stimulated PBMC cultures was increased in MTB as well as in MAIS and BCG subjects. Further, soluble IL-2 receptor (sIL-2R) levels were measured in the different groups of children. With the exception of the STB group, there was otherwise no significant increase of the receptor in the sera levels between groups. During treatment the sIL-2R levels decreased in MTB as well as in STB. A slight but nonsignificant augmentation was found in the supernatants of PBMC cultures stimulated with PPD. This work suggests, along with other referable studies, that IL-2 and sIL-2R levels are inversely modulated by the disease. Indeed, the IL-2 seems to increase in MTB comparatively to NST children, and in treated TB comparatively to nontreated TB children. On the other hand, the sIL-2R level was found to decrease in TB under treatment. We conclude that the IL-2 and the sIL-2R in children with TB may serve as markers of disease activity.—Authors' Abstract

Pancholi, P., Mirza, A., Bhardwaj, N. and Steinman, R. M. Sequestration from immune CD4+ T cells of mycobacteria growing in human macrophages. Science **260** (1993) 984–986.

$CD4^+$ helper T cells mediate resistance to tuberculosis, presumably by enhancing the antimicrobial activity of macrophages within which the *Mycobacterium tuberculosis* organism grows. A first step in resistance should be the presentation of mycobacterial antigens by macrophages to $CD4^+$ T cells. However, when the antigenic stimulus is limited to organisms growing in human monocytes, the organisms become sequestered from immune $CD4^+$ T cells. This block in presentation is selective for growing mycobacteria and not for other stimuli. Sequestration would allow replicating organisms to persist in infected individuals and may contribute to virulence.—Authors' Abstract

Papa, F., Cruaud, P., Luquin, M., Thorel, M. F., Goh, K. S. and David, H. L. Isolation and characterization of serologically reactive lipooligosaccharides from *Mycobacterium tuberculosis.* Res. Microbiol. **144** (1993) 91–99.

Two major highly polar antigenic glycolipids were isolated from recent isolates of *Mycobacterium tuberculosis* from a wide range of geographical origins. The occurrence of these polar glycolipids was demonstrated by isolation, purification and chromatographic characterization and/or serological procedures in 12 strains. Based on their chromatographic properties, these polar glycolipids belong to the lipooligosaccharide family. Preliminary data on the use of these newly described antigens in the serodiagnosis of tuberculosis is presented.—Authors' Abstract

Rambukkana, A., Das, P. K., Kolk, A. H. J., Burggraaf, J. D., Kuijper, S. and Harboe, M. Identification of a novel 27-kDa protein from *Mycobacterium tuberculosis* culture fluid by a monoclonal antibody specific for the *Mycobacterium tuberculosis* complex. Scand. J. Immunol. **37** (1993) 471–478.

Mycobacterium tuberculosis antigens inducing species-specific immune responses are likely to be particularly important for serodiagnosis or for skin testing of tuberculosis. In the present study, we describe the characterization of two novel monoclonal antibodies (MoAbs) A3h4 (IgG2a) and B5g1 (IgM) that are directed to *M. tuberculosis* 27-kDa and 25-kDa proteins, respectively. Specificity analysis by immunoblotting using 20 different species of mycobacterial sonicates revealed that MoAb A3h4 was specific for *M. tuberculosis* complex alone while MoAb B5g1 showed a limited crossreactivity. Direct comparison with previously characterized MoAbs revealed that these MoAbs A3h4 and B5g1 defined new antigenic determinants of *M. tuberculosis.* By using *M. tuberculosis* complex-specific MoAb A3h4 we have identified a distinct 27-kDa protein in the *M. tuberculosis* H37Rv culture fluid. Since this MoAb did not bind to the previously characterized MPT44, MPT59, MPT45, MPT51 and MPT64 proteins as well as the 23-kDa superoxide dismutase (SOD) protein of *M. tuberculosis,* we conclude that MoAb A3h4 recognizes a novel protein in the *M. tuberculosis* H37Rv culture fluid. Studies of the subcellular distribution of these MoAb-reactive proteins indicate that the MoAb A3h4-reactive 27-kDa protein is present not only in the culture fluid but also in the cytosol and the cell wall of *M. tuberculosis.* By contrast, B5g1-reactive protein is mainly a cytosolic protein. When these MoAbs were tested in a previously established ELISA with intact mycobacteria derived from early cultures, only MoAb A3h4 showed the positive reactivity to mycobacteria belonging to the *M. tuberculosis* complex. In addition, during the present comparative studies of MoAbs we have also found that the previously described MoAb F116-5, which is known to recognize the mycobacterial 23-kDa SOD protein [17], crossreacted with the MPT44, MPT59, MPT45 and MPT51 secreted proteins but not with MPT64 and MPB70. These findings indicate that the family of four secreted proteins of *M. tuberculosis* share a common epitope with *M. tuberculosis* SOD protein.—Authors' Abstract

Rastogi, N., Labrousse, V. and DeSousa, J. P. C. Ethambutol potentiates extracellular and intracellular activities of clarithromycin, sparfloxacin, amikacin, and rifampin against *Mycobacterium avium.* Curr. Microbiol. **26** (1993) 191–196.

Intracellular bactericidal activities of the antituberculosis drugs rifampin and amikacin, as well as those of newly described drugs clarithromycin (a macrolide) and sparfloxacin (a difluoroquinolone), were assessed against three strains of the *Mycobacterium avium*-complex (MAC) growing in two different *in vitro* macrophage systems, namely, mouse bone-marrow-derived macrophages (BMMO) and human peripheral blood monocyte-derived macrophages (human MO). All of the infected macrophages were fed reported C(max) concentrations of the drugs, i.e., 15 μg/ml for rifampin, 20 μg/ml for amikacin, 4 μg/ml for clarithromycin, and 1.5 μg/ml for sparfloxacin. Further potentiation of drug activity in the presence of C(max) level of ethambutol (6 μg/ml) during 9 days of intracellular growth (measured by lysing the macrophages and making bacterial counts) was assessed. Our results showed that all four drugs were active against the strains used in this study and that the addition of ethambutol (which had no significant intracellular activity against the bacteria in this system) further potentiated the bactericidal effect of the drugs. When the same drug combinations were tested at their sublethal concentrations by BACTEC® radiometric methodology, a good correlation between the drug enhancement data in extracellular and intracellular systems was found. We conclude that ethambutol may serve as an essential component in effective anti-*M. avium* chemotherapy and that the effective drug combinations may be routinely screened by the BACTEC radiometric methodology.—Authors' Abstract

Shiratsuchi, H., Toossi, Z., Mettler, M. A. and Ellner, J. J. Colonial morphotype as a determinant of cytokine expression by human monocytes infected with *Mycobacterium avium*. J. Immunol. **150** (1993) 2945–2954.

Mycobacterium avium is an intracellular pathogen that causes disseminated infection in patients with AIDS. Colonial morphotype [smooth-transparent (SmT) vs smooth-domed (SmD)] is a key determinant of virulence in mice and the capacity for replication in human monocytes. Some cytokines (IL-1 and IL-6) promote, whereas others (IFN-gamma and TNF) inhibit intracellular *M. avium* growth. The specific factors that determine virulence of *M. avium*, however, are not clear. In this study, we examined cytokine expression by human monocytes stimulated with isogeneic cloned isolates of *M. avium*. Monocytes were prepared from healthy donors and cultured with or without isogeneic *M. avium* for up to 7 days. Cytokine levels (IL-1, IL-6, and TNF-alpha) in monocyte supernatants and cell lysates were measured by immunoassay using an ELISA. The expression of cytokine mRNA by monocytes infected with *M. avium* also was determined by extracting total RNA and subjecting it to Northern blot analysis. Optimal cytokine release occurred at 24 hr. SmD induced higher levels of the following cytokines in supernatants than SmT: IL-1alpha [140 ± 32 (mean ± S.E.) vs 47 ± 16 pg/ml, $p < 0.02$], IL-1beta (4.0 ± 0.9 vs 1.7 ± 0.5 ng/ml, $p < 0.01$), and TNF-alpha (2725 ± 546 vs 1464 ± 409 pg/ml, $p < 0.01$). IL-6 production was comparable for both strains. SmD and SmT isolates induced comparable levels of steady state mRNA for IL-1beta, TNF, and IL-6. Pulse-chase analysis indicated that differences in cytokine expression between SmT and SmD occurred in monocyte lysates at the earliest time point (immediately after pulse-labeling). The dissociation of the expression of specific mRNA from production of IL-1 and TNF suggests that translational capacity for the expression of certain cytokines was reduced by the more virulent SmT. Differential induction of cytokine may be a factor in the pathogenicity of *M. avium* strains isolated from patients with AIDS.—Authors' Abstract

Siddiqi, S. H., Laszlo, A., Butler, W. R. and Kilburn, J. O. Bacteriologic investigations of unusual mycobacteria isolated from immunocompromised patients. Diagn. Microbiol. Infect. Dis. **16** (1993) 321–323.

Mycobacterial isolates from blood and other extrapulmonary sites of six patients with AIDS were investigated because the isolates grew only in liquid media and failed to grow on solid culture media even on subculturing. Our investigations indicated that these mycobacteria possess common, but

unusual, characteristics and probably belong to an unrecognized species recently reported as "*Mycobacterium genavense.*"—Authors' Abstract

Small, P. M., Shafer, R. W., Hopewell, P. C., Singh, S. P., Murphy, M. J., Desmond, E., Sierra, M. F. and Schoolnik, G. K. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. N. Engl. J. Med. **328** (1993) 1137–1144.

In the United States there have been recent outbreaks of multidrug-resistant tuberculosis. These outbreaks have primarily involved persons infected with the human immunodeficiency virus (HIV). We collected clinical information on 17 patients seen at a New York hospital who had repeatedly positive cultures for *Mycobacterium tuberculosis.* Analysis of restriction-fragment-length polymorphisms (RFLPs) was performed on serial isolates of *M. tuberculosis* obtained from these patients. Six patients had isolates that remained drug-susceptible, and the RFLP patterns of these isolates did not change over time. Eleven patients had isolates that became resistant to antimicrobial agents. The RFLP patterns of the isolates from six of these patients remained essentially unchanged (two strains showed one additional band) despite the development of drug resistance. In five other patients, however, the RFLP patterns of the isolates changed dramatically at the time that drug resistance was detected. The change in the RFLP pattern of the isolate from one patient appeared to be the result of contamination during processing in the laboratory. In the remaining four patients, all of whom had advanced HIV disease, the clinical and microbiologic evidence was consistent with the presence of active tuberculosis caused by a new strain of *M. tuberculosis.* Resistance to antituberculous drugs can develop not only in the strain that caused the initial disease, but also as a result of reinfection with a new strain of *M. tuberculosis* that is drug-resistant. Exogenous reinfection with multidrug-resistant *M. tuberculosis* can occur either during therapy for the original infection or after therapy has been completed.—Authors' Abstract

Strath, M., Scott Finnigan, T., Gardner, M., Williamson, D. and Wilson, I. Antimalarial activity of rifampicin *in vitro* and in rodent models. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 211–216.

The antimalarial activity of rifampin, a specific inhibitor of bacterial ribonucleic acid (RNA) polymerase, was confirmed with *Plasmodium falciparum in vitro* and with *P. chabaudi in vivo.* The viability of ring forms of *P. falciparum,* measured by [H-3]hypoxanthine and [C-14]isoleucine uptake, was significantly reduced within 5 hr of exposure to 2.5 μM rifampin, the 50% inhibitory concentration. Streptolydigin and tagetitoxin, other specific inhibitors of bacterial RNA polymerase, were much less effective as antimalarials. A rifampin-tolerant subline of *P. falciparum* was selected *in vitro.* When released from drug pressure, the tolerant line showed appreciably greater rates of incorporation of precursors and growth than the parent line, but over a period of months these characteristics gradually reverted. Rifampin was effective against a chloroquine-resistant line of *P. falciparum* and the rifampin-tolerant line had increased chloroquine sensitivity. Treatment of patent parasitemias of *P. chabaudi* in mice with more than 100 mg/kg rifampin twice daily significantly reduced the parasitemia within 24 hr and parasites were barely detectable on blood films by the fourth day. Recrudescence occurred on release of drug pressure.—Authors' Abstract

Takahashi, M., Kazumi, Y., Fukasawa, Y., Hirano, K., Mori, T., Dale, J. W. and Abe, C. Restriction fragment length polymorphism analysis of epidemiologically related *Mycobacterium tuberculosis* isolates. Microbiol. Immunol. **37** (1993) 289–294.

Restriction fragment length polymorphism (RFLP) analysis of a large number of Japanese isolates of *Mycobacterium tuberculosis,* containing isolates from small outbreaks of *M. tuberculosis* infection, and clinical isolates of *M. bovis* BCG, was carried out using a DNA probe derived from the insertion sequence IS986. Clinical isolates of *M. tuberculosis* had a high degree of RFLP. The occurrences of the IS element

varied from 1 to 19, the majority of isolates having 8 to 15 copies. Very similar fingerprints, however, were seen among strains isolated in the Kanto district. In particular, 3 strains were of the same pattern with or without an additional band. Similarity of the banding patterns of strains isolated in the same district was observed in other areas. Six groups of strains, each group arising from a suspected common source of infection, were analyzed. Of these, 5 showed identical fingerprints within each group, but 1 showed different fingerprints. RFLP patterns of 3 strains isolated from individuals with lymphadenitis developed but 2 months after BCG vaccination, and 1 strain isolated from a bladder cancer patient with BCG instillation therapy were identical to those of BCG-Tokyo which had been used for the vaccination and therapy. These results confirm that RFLP analysis using IS986 is a suitable tool for epidemiology of tuberculosis.—Authors' Abstract

Thierry, D., Matsiota Bernard, P., Pitsouni, E., Costopoulos, C. and Guesdon, J. L. Use of the insertion element IS6110 for DNA fingerprinting of *Mycobacterium tuberculosis* isolates presenting various profiles of drug susceptibility. FEMS Immunol. Med. Microbiol. **6** (1993) 287–298.

IS6100 is an insertion sequence of the IS3 family and it is present in multiple copies in the chromosome of *Mycobacterium tuberculosis.* Four to 15 copies are present in various strains of *M. tuberculosis.* In this study, the value of IS6110 as an epidemiological marker of tuberculosis was examined. Unrelated clinical strains from Greek patients presented, in restriction fragment length polymorphism analysis, a high degree of polymorphism, whereas patterns of related clinical strains from familial outbreaks were identical. Since RFLP analysis with acetylaminofluorene labeled IS6110 as the probe gave satisfactory results, it is suggested that this nonradioactive probe can be used in hospitals and health centers for the epidemiological survey of *M. tuberculosis* infections.—Authors' Abstract

Thierry, D., Vincent, V., Clement, F. and Guesdon, J.-L. Isolation of specific DNA fragments of *Mycobacterium avium* and their possible use in diagnosis. J. Clin. Microbiol. **31** (1993) 1048–1054.

We cloned and sequenced two DNA fragments (DT1 and DT6) from *Mycobacterium avium* serotype 2 for use in the identification of members of the *M. avium-M. intracellulare* complex (MAC). Reference strains of MAC belonging to serovars 1 to 28 were examined by using these DNA fragments as probes. The study revealed that the DT6 probe hybridized with DNAs from *M. avium* strains (serovars 1 to 6, 8 to 11, and 21), while the DT1 probe hybridized with DNAs from serovars 2, 3, 7, 12 to 20, and 23 to 25. DT1-and DT6-derived oligonucleotides were selected for use as primers in a polymerase chain reaction test. Amplification of the DT1 and DT6 sequences may provide the basis for a rapid and reliable assay for the detection of mycobacteria belonging to MAC.—Authors' Abstract

Tomioka, H., Saito, H. and Sato, K. Comparative antimycobacterial activities of the newly synthesized quinolone AM-1155, sparfloxacin, and ofloxacin. Antimicrob. Agents Chemother. **37** (1993) 1259–1263.

AM-1155 is a newly synthesized 6-fluoro-8-methoxy quinolone. We assessed its *in vitro* antimycobacterial activity using sparfloxacin (SPFX) and ofloxacin (OFLX) as comparison drugs. The MICs of these agents for various mycobacterial strains were determined by the agar dilution method with 7H11 medium. AM-1155 had lower MICs for 50 and 90% of tested strains of *Mycobacterium kansasii, M. marinum,* and *M. fortuitum-M. chelonae* complex than SPFX and OFLX, and the values for *M. tuberculosis, M. scrofulaceum,* and the *M. avium-M. intracellulare* complex were similar to those of SPFX and considerably lower than those of OFLX. In addition, the antimicrobial activity of AM-1155 against *M. tuberculosis* and *M. intracellulare* phagocytosed into murine peritoneal macrophages was compared with that of OFLX. AM-1155 (1 μg/ml) inhibited the intracellular growth of both *M. tuberculosis* and *M. intracellulare,* whereas OFLX at the same concentration failed to show any such effect. Moreover,

AM-1155 (10 μg/ml) exhibited a steady bactericidal action against *M. tuberculosis,* whereas OFLX at the same concentration had only a weak effect. AM-1155 (10 μg/ml) also inhibited the growth of *M. intracellulare* more effectively than OFLX.—Authors' Abstract

Tsui, S. Y. T., Yew, W. W., Li, M. S. K., Chan, C. Y. and Cheng, A. F. B. Postantibiotic effects of amikacin and ofloxacin on *Mycobacterium fortuitum.* Antimicrob. Agents Chemother. **37** (1993) 1001–1003.

A study of postantibiotic effects (PAE) *in vitro* of amikacin and ofloxacin on *Mycobacterium fortuitum* isolates from sternotomy wounds by use of the dilution method for drug removal showed that both drugs exhibited good bactericidal activities, with the PAE of amikacin lasting from 13.5 to 27.6 hr and the PAE of ofloxacin lasting from 1.2 to 5.0 hr. These laboratory results concur with our experience of the efficacy of once-daily dosing with these drugs in the treatment of infections caused by *M. fortuitum.* These data may have therapeutic implications in guiding the scheduling of the administration of drugs in these infections, which require a long duration of therapy.—Authors' Abstract

Wilson, S. M., Nava, E., Morales, A., Godfrey Faussett, P., Gillespie, S. and Andersson, N. Simplification of the polymerase chain reaction for detection of *Mycobacterium tuberculosis* in the tropics. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 177–180.

It has been suggested that the technical complexities, the expense of equipment and consumables, and problems associated with contamination make the polymerase chain reaction (PCR) inappropriate for use in developing countries. These problems were addressed using a novel one-tube nested PCR, small reaction volumes and a "three room" system for the detection of *Mycobacterium tuberculosis.* The PCR of sputum samples dried on small filter-paper disks was also investigated. Using this strategy 5 smear-positive and 15 smear-negative specimens were correctly identified by PCR. This method of sample collection has the advantage that samples can be sent by post and stored in a minimum of space, and remain viable for PCR for at least 4 years after collection. These and future modifications to the PCR protocol will make the assay more suitable for use in the tropics.—Authors' Abstract

Yassin, A. F., Binder, C. and Schaal, K. P. Identification of mycobacterial isolates by thin-layer and capillary gas-liquid chromatography under diagnostic routine conditions. Zentralbl. Bakteriol. **278** (1993) 34–48.

The mycolic acid patterns of 75 strains of mycobacteria belonging to 46 different species were studied by thin-layer chromatography (TLC). Additionally, the mycolic acid pyrolytic cleavage products were determined by capillary gas-liquid chromatography (C-GLC). Eleven different patterns based on number, type and R(f) values of the detected lipid spots were identified. The mycolic acid methyl ester profiles of clinical isolates were compared with those of reference strains. In this way, it was possible to relate these clinical isolates to mycobacterial groups defined by their mycolic acid profiles. The 11 patterns and the methods used are described in detail.—Authors' Abstract

Yuen, K. Y., Chan, K. S., Chan, C. M., Ho, B. S. W., Dai, L. K., Chau, P. Y. and Ng, M. H. Use of PCR in routine diagnosis of treated and untreated pulmonary tuberculosis. J. Clin. Pathol. **46** (1993) 318–322.

To assess the routine use of a polymerase chain reaction (PCR) assay for the direct detection of *Mycobacterium tuberculosis* in expectorated sputum specimens, a pair of primers (20-mer) were designed to amplify the 38 kilodalton protein of *M. tuberculosis.* The specificity of the assay was evaluated in 31 *M. tuberculosis* strains, 15 atypical mycobacterium species, and several commensal bacteria of the upper respiratory tract. The assay was subsequently applied to 519 sputum specimens from 85 inpatients of a chest hospital in Hong Kong. An amplified product of 239 base pairs was

found in all *M. tuberculosis* strains, standard strains of *M. bovis,* and *M. africanum* but not in the other bacterial strains tested. For the 51 patients with pulmonary radiographic lesions, the diagnosis of pulmonary tuberculosis was subsequently confirmed by both culture and PCR in 41 of them. Five patients who were treated before admission were positive by PCR alone. All but one patient in the control group (patients with acute exacerbation of chronic obstructive airway diseases) or those with atypical mycobacterial diseases were PCR negative. The PCR remained positive after 4 weeks of antituberculosis treatment in 29 patients, 16 of whom had become culture negative. This PCR assay is a useful technique for the diagnosis of untreated and recently treated cases of pulmonary tuberculosis.—Authors' Abstract

Zhang, Y., Garbe, T. and Young, D. Transformation with *katG* restores isoniazid-sensitivity in *Mycobacterium tuberculosis* isolates resistant to a range of drug combinations. Mol. Microbiol. **8** (1993) 521–524.

Isoniazid-resistant isolates of *Mycobacterium tuberculosis* were transformed with a plasmic vector carrying the functional catalase-peroxidase (*katG*) gene. Expression of *katG* restored full drug susceptibility in isolates initially resistant to concentrations ranging from 3.2 to > 50 μg ml^{-1}. Transformation with the corresponding *katG* gene from *Escherichia coli* resulted in low-level expression of catalase and peroxidase activities and conferred partial isoniazid sensitivity.—Authors' Summary

Zhang, Y. H., Doerfler, M., Lee, T. C., Guillemin, B. and Rom, W. N. Mechanisms of stimulation of interleukin-1-beta and tumor necrosis factor-alpha by *Mycobacterium tuberculosis* components. J. Clin. Invest. **91** (1993) 2076–2084.

The granulomatous immune response in tuberculosis is characterized by delayed hypersensitivity and is mediated by various cytokines released by the stimulated mononuclear phagocytes, including tumor necrosis factor-alpha (TNFalpha) and Il-1beta. We have demonstrated that *Mycobacterium tuberculosis* cell-wall component lipoarabinomannan (LAM), mycobacterial heat shock protein-65 kDa, and *M. tuberculosis* culture filtrate, devoid of LPS as assessed by the amebocyte lysate assay, stimulate the production of TNFalpha and IL-1beta proteins and mRNA from mononuclear phagocytes (THP-1 cells). The effect of LAM on the release of these cytokines was specific, as only LAM stimulation was inhibited by anti-LAM monoclonal antibody. Interestingly, we found that LAM and gram-negative bacterial cell-wall-associated endotoxin LPS may share a similar mechanism in their stimulatory action as demonstrated by inhibition of TNFalpha and IL-1beta release by monoclonal antibodies to CD14. Anti-CD14 monoclonal antibody MY4 inhibited both TNFalpha and IL-1beta release with LAM and LPS but no effect was observed with other mycobacterial proteins. An isotype antibody control did not inhibit release of cytokines under the same experimental conditions. *M. tuberculosis* and its components upregulated IL-1beta and TNFalpha mRNAs in THP-1 cells. Nuclear run-on assay for IL-1beta demonstrated that LAM increased the transcription rate. The induction of IL-1beta was regulated at the transcriptional level, in which these stimuli acted through cis-acting element(s) on the 5′ flanking region of the IL-1beta genomic DNA. *M. tuberculosis* cell-wall component LAM acts similarly to LPS in activating mononuclear phagocyte cytokine TNFalpha and IL-1beta release through CD14 and synthesis at the transcriptional level; both cytokines are key participants in the host immune response to tuberculosis.—Authors' Abstract