CURRENT LITERATURE

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

General and Historical

Contreras Mas, A. and Rossello Vaquer, R. [The recognition of leprosy patients in late medieval Mallorca.] Rev. Leprol. Fontilles **19** (1993) 249–258. (in Spanish)

Four documents concerning analysis and determination of leprosy patients are analyzed. In them the presence of a *instigador de massells,* the equivalent of *mayor of the lepers* from castella in the beginning of the 15th century can be deducted and he would sentence after receiving advice from a doctor and surgeon. These documents permit us to observe how theoretical knowledge and medical practice were carried out in daily work. Also revealed are the diagnostic and prophylactic procedures of medieval Mallorca.—Authors' English Summary

Ulrich, M., Zulueta, A. M., Caceres-Dittmar, G., *et al.* Leprosy in women: characteristics and repercussions. Soc. Sci. Med. **37** (1993) 445–456.

Physiological, socioeconomic and cultural factors play important roles in the response of women to *Mycobacterium leprae* and in the impact of leprosy on their lives. They appear to develop stronger immunological responses to *M. leprae* than men, as suggested by lower incidence and less severe clinical forms of disease in most areas of the world, as well as stronger reactions of cell-mediated immunity after prophylactic vaccination. Genetic factors and physiological status including pregnancy, intercurrent infection and malnutrition might be among the factors which modulate this response. Women in leprosy-endemic areas of the world, with few exceptions, suffer from marked economic and social dependency and inferiority which can only be heightened by the social stigma associated with leprosy. Nevertheless, they bear an enormous responsibility for the health of their families, often as head of the household, and they often possess a unique capacity to influence community opinion. With the introduction of multidrug therapy, leprosy control throughout the world is no longer an unrealistic goal. Active vaccination may constitute the other factor necessary for eventual eradication of the disease. The incorporation of women at all levels into active roles in health care programs may constitute one of the decisive factors in the success or failure of leprosy control.—Authors' Abstract

Chemotherapy

Anderson, R. and Smit, M. J. Clofazimine and B669 inhibit the proliferative responses and Na+,K+-adenosine triphosphate activity of human lymphocytes by a lysophospholipid-dependent mechanism. Biochem. Pharmacol. **46** (1993) 2029–2038.

The relationship between the phospholipase-stimulating and immunosuppressive properties of the riminophenazine anti-mycobacterial agent clofazimine and its experimental analog, B669, has been investigated *in vitro.* At concentrations of 0.6 μM and upwards, both riminophenazines, particularly B669, caused dose-related inhibition of mitogen- and alloantigen-stimulated uptake of tritiated thymidine by human mononuclear leucocytes (MNL), while in short-term assays both agents increased the release of lysophosphatidylcholine (LPC) and arachidonic acid from these cells. Arachi-

donate per se at a concentration of 20 μM did not affect mitogen-activated lymphocyte proliferation, while cycloxygenase and 5'-lipoxygenase inhibitors, as well as water- and lipid-soluble oxidant-scavengers and anti-oxidant enzymes, failed to protect the cells against the anti-proliferative effects of clofazimine and B669. However, LPC caused dose-related inhibition of lymphocyte proliferation. Moreover, co-incubation of MNL with alpha-tocopherol (vitamin E), a lysophospholipid complex-forming agent, or with lysophospholipase, protected the cells against clofazimine and B669, as well as against LPC. Na+, K+-adenosine triphosphatase was identified as the primary target of riminophenazine/LPC-mediated inhibition of lymphocyte proliferation. Excessive release of anti-proliferative lysophospholipids during clofazimine or B669 treatment of mitogen- or antigen-activated lymphocytes is the probable biochemical mechanism of the immunosuppressive activity of these agents.—Authors' Abstract

Chan, G. P., Garcia Ignacio, B. Y., Chavez, V. E., Livelo, J. B., Jimenez, C. L., Parrilla, M. L. R. and Franzblau, S. G. Clinical trial of sparfloxacin for lepromatous leprosy. Antimicrob. Agents Chemother. **38** (1994) 61–65.

Nine previously untreated patients with lepromatous leprosy were treated with 200 mg of sparfloxacin daily for 12 weeks to determine whether this drug is bactericidal for *Mycobacterium leprae* in humans. The efficacy of therapy was monitored both clinically and by measuring changes in morphological index, mouse foot pad infectivity, and the radiorespirometric activity of *M. leprae* organisms obtained from serial biopsy specimens and also by determining titers of phenolic glycolipid-I in serum. Most patients showed clinical improvement within 2 weeks of treatment; this was accompanied by significant reductions in the morphological index, mouse foot pad infectivity, and bacillary radiorespirometric activity. After 4 weeks of treatment, all patients had a morphological index of zero and specimens from most patients were noninfectious for mice, while the median decrease in radiorespirometric activity was >99%. Overall results by the rapid radiorespirometric assay paralleled those of the mouse foot pad and morphological index assays. Sparfloxacin given at 200 mg once daily appears to be rapidly bactericidal in humans, with activity similar to that observed in a previous clinical trial with 400 mg of ofloxacin.—Authors' Abstract

Costa, H. C., Opromolla, D. V. A., Virmond, M. and Beiguelman, B. Influence of the rapid acetylator phenotype on the mergence of DDS resistant *Mycobacterium leprae*. Rev. Bras. Genet. **16** (1993) 1029–1034.

Twenty-one lepromatous cases with suspected diaminodiphenyl sulfone (DDS) resistance had their isoniazid acetylator phenotype determined. The resistance of *Mycobacterium leprae* to this sulfone was investigated by means of foot pad tests in BALB/c mice. Results indicate that the emergence of complete resistance to DDS in *M. leprae* is more probable in rapid than in slow acetylators. It appears that rapid acetylators require higher doses of DDS than slow acetylators since the bacillary concentration in the lesions of the patients with DDS sensitive *M. leprae* was more than 17 times higher among rapid than among slow acetylators.—Authors' Abstract

Garcia-Rodriguez, J. A. and Gomez Garcia, A. C. *In vitro* activities of quinolones against mycobacteria. J. Antimicrob. Chemother. **32** (1993) 707–808.

From the results of recently published *in-vitro* studies, we have reviewed the activities of novel quinolones which are currently available, either commercially or for investigative purposes, against *Mycobacterium tuberculosis*, the atypical mycobacteria (principally the *M. avium* complex, *M. chelonae, M. xenopi, M. marinum* and *M. fortuitum*) and *M. leprae*. We have also evaluated the effects of the various methods for determining the susceptibilities of the mycobacteria on the *in-vitro* activities of these agents. Sparfloxacin, Win-57273, ciprofloxacin and ofloxacin were the most active agents overall. The *in-vitro* activities, efficacies in animal models, tissue and cell penetration and results of preliminary clinical investigations suggest that some of the new-

er quinolones might be effective alternatives to standard antituberculous agents for the treatment of patients with infections caused by mycobacteria, particularly when there is resistance to the latter group of drugs.—Authors' Abstract

Habte-Mariam, H. S. and Guebre-Xabier, M. Loss of viability of *Mycobacterium leprae* isolated from nasal secretions of lepromatous leprosy patients following daily rifampicin and DDS therapy. Lepr. Rev. **64** (1993) 312–315.

Excreta from blowing their noses was collected from four previously untreated multibacillary (LL) patients in the ALERT hospital immediately before and during daily treatment with 600 mg rifampin and 100 mg dapsone (DDS). The *Mycobacterium leprae* recovered from the nasal secretions were enumerated and inoculated into the footpads of normal mice. Bacilli recovered from two of the patients failed to infect mice after 1 day's treatment, and all infectivity of the bacilli from the other two patients was lost after 2 days' treatment. These findings demonstrate the rapidity with which rifampin-containing multidrug treatment is likely to reduce a patient's level of infection to their contacts.—Authors' Summary

Ilett, K. F., Chiswell, G. M., Spargo, R. M., Platt, E. and Minchin, R. F. Acetylation phenotype and genotype in Aboriginal leprosy patients from the North-West Region of Western Australia. Pharmacogenetics **3** (1993) 264–269.

N-Acetyltransferases (NAT1, NAT2) play an important role in biotransformation of a number of drugs and carcinogens. A polymorphism in the metabolism of such compounds by NAT2 has been known for many years, but it is only recently that the underlying molecular genetics has been elucidated. In the present study, we have correlated acetylation phenotype and genotype in a group of 49 Australian Aborigines (26 males and 23 females; mean age = 50.5 yr) from the Derby region of Western Australia. Phenotype was determined using caffeine and genotype by an allele-specific polymerase chain reaction. The percentages of slow and rapid phenotypes were 36.7% and 63.3%, respectively, while the distribution of alleles for the NAT2 gene was 41% for the wildtype and 2%, 17% and 40% for the M1, M2 and M3 mutations, respectively. This is the highest proportion of M3 mutations reported for any ethnic population. The observed genotype proportions were not significantly different from those predicted by the Hardy-Weinberg Law ($chi^2 = 1.07$, $p > 0.05$). Phenotype was predictable from genotype in 100% of patients. At the time of study, 29 of the Aborigines were receiving acedapsone intramuscularly for control of leprosy. Plasma dapsone concentrations in these patients were similar for both slow (N = 11) and rapid (N = 18) acetylators, suggesting that phenotype is unlikely to influence treatment outcome. The data show that Aborigines have a similar phenotype distribution to that of some Asian populations, but that there are differences in the frequencies of the M1, M2 and M3 mutant alleles. We suggest that acetylation genotyping may be a useful tool for investigation of the anthropological links between population groups around the Asia-Pacific rim.—Authors' Abstract

Ji, B., Lounis, N., Truffot-Pernot, C. and Grosset, J. Selection of resistant mutants of *Mycobacterium avium* in beige mice by clarithromycin monotherapy. Antimicrob. Agents Chemother. **36** (1993) 2839–2840.

Beige mice were inoculated intravenously with $10^{7.90}$ CFU of *Mycobacterium avium* 101. Among the untreated control mice, when the mean CFU per spleen increased to a level greater than 10^8, small numbers of organisms resistant to clarithromycin (CLARI) were isolated from some of the spleens; the frequency of CLARI-resistant mutants was estimated to be between 10^{-8} and 10^{-9}. In mice treated with 200 mg of CLARI per kg of body weight six times weekly, however, CLARI-resistant organisms were isolated from the spleens of all mice examined after treatment for 8 weeks; the mean CFU per spleen and the frequency of resistant mutants were significantly greater than those of control mice and increased further after treatment for 16 weeks. The MICs of CLARI against the resistant organisms isolated from both control and

treated mice were ≥ 512 μg/ml.—Authors' Abstract

Ochonisky, S., Verroust, J., Bastuji-Garin, S., Gherardi, R. and Revuz, J. Thalidomide neuropathy incidence and clinico-electrophysiologic findings in 42 patients. Arch. Dermatol. **130** (1994) 66–69.

Thalidomide therapy was shown to be effective in numerous dermatologic diseases. As reliable methods of contraception are now available, neurotoxicity has become the most important side effect limiting the use of thalidomide. The incidence of this neuropathy and its relationship to thalidomide doses are still matters of debate. In a retrospective study, we reviewed the files of 42 patients who had received thalidomide between 1987 and 1992 for various dermatologic diseases. The incidence and the conditions of occurrence of the neuropathy were analyzed. Evidence of a thalidomide-induced neuropathy was present in nine patients (21%), who had both clinical and electrophysiologic typical abnormalities. Twelve other patients (28%), however, presented with isolated clinical or electrophysiologic signs. Thus, the diagnosis of thalidomide neuropathy could not be affirmed. The occurrence of the neuropathy did not appear to be related to the daily dose nor to the duration of treatment. The highest risk of developing a thalidomide neuropathy was found in female and elderly patients. Two monozygotic twin sisters, who received thalidomide for Behcet's disease, both developed a neuropathy. These data suggest that the incidence of thalidomide neuropathy may be between 21% and 50%. Individual susceptibilities with possible genetic predisposition seem to be more important than daily dose and duration of thalidomide therapy.—Authors' Abstract

Terencio de las Aguas, J. [Relapses in leprosy—personal experience.] Rev. Leprol. Fontilles **19** (1993) 277–286. (in Spanish)

Of a total of 413 patients treated with sulfone monotherapy we have observed 28 relapses, 14 of them the dimorphous type. The period of time between inactivity and relapse varies from 6–39 years.—Authors' English Abstract

Terencio de las Aguas, J., Albero, F., Gomez Echevarria, J. R., Arnedo, L. and Contreras Rubio, F. [Lepromatous leprosy with a fatal outcome.] Rev. Leprol. Fontilles **19** (1993) 269–275. (in Spanish)

A case of a 68-year-old LL patient is presented who was diagnosed as intensively positive 2 months ago. He arrived with necrotic ulcers that affected 50% of his integument, with bad general health and anemia. Although he received specific treatment and corticotherapy and hemotherapy, he died in 20 days.—Authors' English Abstract

Terencio de las Aguas, J., Gomez Echevarria, J. R. and Lopez Pla, J. [Side effects and toxicity of rifampin in the treatment of leprosy.] Rev. Leprol. Fontilles **19** (1993) 259–267. (in Spanish)

The toxicity and side effects of rifampin during the treatment of leprosy are analyzed. The experience has been observed in 131 patients with a generalized good tolerance, only isolated gastro-intestinal side effects. Only when it is used as monotherapy lepromatous reactions appeared in 60% of cases. When it is administered together with clofazimine the reactional episodes are less frequent. Tolerance is good when it is administered with Isoprodian. In intermittent administration (monthly or weekly) the "flu syndrome" appeared in 25% of the cases and only 3 cases presented with severe kidney insufficiency and toxic hepatitis.—Authors' English Summary

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

The susceptibilities of a range of gram-positive and gram-negative microbial pathogens to clofazimine and its analog B669 (0.1 to 32 μg/ml), as well as the effects of these agents on membrane phospholipid metabolism in *Staphylococcus aureus* and *Escherichia coli*, have been investigated *in vitro*. Gram-positive bacteria were found to be generally susceptible to these agents, whereas gram-negative organisms were uniformly resistant. Exposure of *S. aureus* to

both agents (1 to 5 μg/ml), especially B669, caused dose-related enhancement of the activity of phospholipase A_2, according to an increase in the release of ^{3}H-radiolabeled arachidonate and lysophosphatidylethanolamine ([^{3}H]LPE) from bacterial-membrane phospholipids. Treatment of *E. coli* with the riminophenazines also increased the release of [^{3}H]arachidonate and [^{3}H]LPE. Growth of gram-positive but not gram-negative bacteria was inhibited by LPE and lysophosphatidylcholine. Moreover, coincubation with α-tocopherol (vitamin E), a lysophospholipid complex-forming agent, or with lysophospholipase protected gram-positive bacteria against the riminophenazines as well as against lysophospholipids. The results from this study are consistent with a mechanism whereby lysophospholipids mediate the activities of the two drugs.—Authors' Abstract

Clinical Sciences

Bwire, R. and Kawuma, J. S. Hospital-based epidemiological study of reactions, Buluba Hospital, 1985–89. Lepr. Rev. **64** (1993) 325–329.

A retrospective study of 256 reactional episodes, both reversal reaction and erythema nodosum leprosum (ENL), seen in Buluba Hospital over a 5-year period (1985–1989) was made. Over 90% of these episodes were due to reversal reaction, with ENL being encountered infrequently. About 80% of reversal reactions occurred during chemotherapy but all the episodes of ENL occurred during this period. Over 70% of both reversal and ENL episodes presented with clinically apparent nerve and skin involvement. The need to assess the effect of multidrug therapy on the incidence of reactions and to develop more sensitive diagnostic tools to detect early neuritis is emphasized. It is also necessary to study those patients who develop recurrent reactional episodes.—Authors' Summary

Campbell, G. A. M., Patrus, O. A., Friedman, H. and Brant, P. C. [Hepatic involvement in reactions in Hansen's disease: a study of twenty patients.] An. Bras. Dermatol. **69** (1944) 7–14. (in Portuguese)

Background—Few are the studies in Brazilian medical literature that have evaluated hepatic damage in reactional states in Hansen's disease.

Objectives—To evaluate the hepatic involvement in leprosy patients LL and B in reactional states, through a study effected in the Hospital Universitário de Brasília, Brazil.

Materials and Methods—Twenty patients were evaluated (1BT, 2BB, 6BL and 11LL), all in reaction from the clinical, laboratorial and histopathological point of view.

Results—The results were: 25% of the symptoms were digestive, 25% of the patients had hepatomegaly, and 20% developed jaundice. The laboratory data revealed low serum albumin, increased globulin level and moderate increase of SGOT, SGPT, alkaline phosphatase and bilirubin. The histopathological changes in the liver showed 65% of specific lesions in the total and 72.7% among the V; there was reactional inflammation in 75%.

Conclusion—The evaluation of the hepatic damage in Hansen's disease in reactional state, must be global, emphasizing the clinical, laboratory, and specially histopathological data, if possible.—Authors' English Summary

Duncan, M. E. An historical and clinical review of the interaction of leprosy and pregnancy: a cycle to be broken. Soc. Sci. Med. **37** (1993) 457–472.

Pregnancy in women with leprosy is a hazardous undertaking. First appearance of leprosy, reactivation of the disease and relapse in "cured" patients is likely to occur particularly in the third trimester of pregnancy. Leprosy reactions caused by variation in cell-mediated and humoral immunity are triggered off by pregnancy: type 1

reaction (reversal reaction, RR) occurs post partum, while type 2 reaction (erythema nodosum leprosum, ENL) peaks in late pregnancy. Both types of reaction continue long into lactation. Neuritis with loss of both sensory and motor function is associated with relapse and reaction. Relapse, reaction and nerve damage, especially "silent neuritis," with subsequent deformity and disability, occur not only in women on apparently effective treatment but also in those who have received MDT and have been released from treatment (RFT). To prevent disability, research is urgently needed into the mechanisms of early and late reaction and neuritis. Pregnancy is not only a trigger factor for reaction but an ideal *in vivo* model for research.

Up to 20% of children born to mothers with leprosy may develop leprosy by puberty. While early leprosy in young children is self-healing, when marriage and childbearing take place at an early age the daughters of mothers with leprosy are likely to run the risk of experiencing the adverse effects of pregnancy on leprosy. Increased awareness and health education, as well as long-term surveillance of "cured" leprosy patients, are essential to break a potentially vicious cycle of leprosy and pregnancy. Women with cured leprosy could play an important role in screening for and detection of both early leprosy in children and late, post-MDT RFT, nerve damage in their mothers.—Author's Summary

Parkash, O., Girdhar, B. K. and Sengupta, U. Serum lactoferrin in lepromatous leprosy patients. Lepr. Rev. **64** (1993) 295–301.

The serum concentrations of lactoferrin were determined by competitive enzyme immunoassay in the sera of 38 lepromatous leprosy patients and 16 healthy volunteers. Of the 38 lepromatous patients, 25 were without any sign of reactions while 13 were suffering from ENL type of reactions. The lactoferrin levels, in both types of patients, were observed to be significantly higher ($p < 0.01$ and < 0.001, respectively) than in that of healthy volunteers. The rise in lactoferrin level in reactive patients was also higher ($p < 0.05$) when compared to those without reactions. The serum lactoferrin levels were also found to be associated with bacterial load ($r = 0.414$; $p < 0.01$) indicating that in lepromatous leprosy patients, lactoferrin may not be very effective in preventing the growth of *Mycobacterium leprae*. Further studies to improve the understanding of the role of elevated levels of lactoferrin in pathogenesis of lepromatous leprosy patients and in establishing its possible use in predicting the occurrence of ENL type of reactions would be worthwhile pursuing.—Authors' Summary

Sehgal, V. N., Bhattacharya, S. N., Shah, Y., Sharma, V. K. and Gupta, C. K. Reaction in leprosy: acute phase reactant response during and after remission. Int. J. Dermatol. **31** (1992) 632–634.

Sera from 25 patients with type 1 (lepra), upgrading and downgrading, and type 2 (erythema nodosum leprosum [ENL]) reactions were assayed, during the reaction and after its clinical remission, for changes in levels of alpha-1-antitrypsin (A1A) and C-reactive protein (CRP). The results were compared with those from normal healthy adults and patients of leprosy without history and/or clinical evidence of reaction. The A1A levels correlated better with changes in status of type 1 reaction; whereas CRP levels correlated well with alterations in type 2 reactions and were definitely superior to A1A in this situation for monitoring the course of these episodes.—Authors' Abstract

Immuno-Pathology

Bhatia, V. N., Chakraborty, S., Mukherjee, B., Panda, S. N. and Chakraborty, T. K. Application of filter paper method of collection of blood for MLPA test. Indian J. Lepr. **65** (1993) 207–210.

The MLPA (*Mycobacterium leprae* particle agglutination) test for the serodiagnosis of leprosy is based on the use of gelatin particles coated with synthetic antigens. The authors collected, on filter paper, blood from

finger pricks and tested the suitability of eluates from the bloodspots for use in a commercially available MLPA test kit compared with serum from the same leprosy patients (multibacillary and paucibacillary) and healthy volunteers. "Testing 64 patient samples at 1:32 dilution, 31 were negative by both serum and eluate, 20 were positive by both, 6 were positive only by serum and one was positive only by eluate. In six other cases eluate gave equivocal results while serum results was clearly positive. Some eluates negative at 1:32 dilution gave weak positive agglutination at 1:16 dilution." All of 20 control subjects were negative by both serum and eluate samples. The authors conclude that the filter-paper method of collecting blood gave comparatively less-sensitive results than serum unless lower dilutions of eluate were used. The MLPA test kits (imported from Japan) were noted to be too expensive for widespread use in India, but locally prepared particles were considered to be a possibility.—C. A. Brown (Trop. Dis. Bull.)

Bottasso, O., Besuschio, S., Merlin, V., Morini, J. C., Bernabo, J., Falcoff, R. and Falcoff, E. Lepromatous leprosy treated with recombinant interferon gamma: cutaneous histologic changes. Int. J. Dermatol. **31** (1992) 813–817.

We report on the histologic changes occurring in single cutaneous lesions, from six active lepromatous patients, 1 week following the administration of three daily intradermal injections, 35 μg each, of recombinant interferon gamma (rIFN-γ). Except for a strong induration at the injection site, rIFN-γ produced no adverse systemic reactions and was able to promote a remarkable influx of T lymphocytes, mononuclear phagocytes with large nuclei, nonvacuolated cytoplasm, and reduced lysozyme reactivity. Furthermore, despite no clear-cut reduction of mycobacterial dermal burden, bacilli showed a clear increase in the granular appearance. Present findings provide a basis for further elucidation of rIFN-γ as an additional tool for leprosy treatment.—Authors' Abstract

Fink, S., Delabarrera, S., Minnucci, F., Valdez, R., Balina, L. M. and Sasiain, M. C. IFN-gamma, IL-6 and IL-4 modulate *M. leprae*-specific or PPD-specific cytotoxic T cells in leprosy patients. Scand. J. Immunol. **38** (1993) 551–558.

Specific cytotoxic T cells against intracellular pathogens may be generated *in vitro*. On the other hand it is well known that cytokines can regulate almost every aspect of immune function. The aim of this study was to evaluate the effect of some cytokines on the generation of cytotoxic T cells with specificity for *Mycobacterium leprae*- or PPD-pulsed autologous macrophages from leprosy patients and normal controls. Peripheral blood mononuclear cells from *M. bovis* BCG-immunized controls or from leprosy patients were stimulated with antigen, in the presence or absence of cytokines, for 7 days. These were used as effector cells in a 4-hr [Cr-51]-release assay. Our results show that development of cytotoxic T cells may be enhanced by gamma-IFN, IL-6 or the combination of IL-6 and IL-2. Addition of IL-2 or TNF-alpha alone did not modify the generation of cytotoxic activity. IL-4 downregulated the cytotoxic response and gamma-IFN was able to counteract this effect. Hence, the generation of specific cytotoxic T cells can be modulated by cytokines. Whether this cytotoxic mechanism contributes to protection or tissue damage in *M. leprae* infection remains to be determined.—Authors' Abstract

Handzel, Z. H., Buchner, V., Leviatan, A., *et al.* Proliferative responses of leprosy patients, healthy contacts and BCG vaccinees to a major native 12-kDa protein of *Mycobacterium leprae*. Immunol. Infect. Dis. 2 (1992) 237–243.

The ability of MLP12α, a HPLC-purified native 12-kDa *Mycobacterium leprae* (ML) protein antigen, to stimulate peripheral blood mononuclear cells was investigated [in Israel] in leprosy patients, healthy leprosy contacts and some BCG-vaccinees. MLP12α showed structural homology at the amino terminal to the BCG-a protein of *M. tuberculosis/M. bovis*. Cells from 10 of 19 leprosy patients of all disease types, including 4 of 6 lepromatous ML nonresponders, as well as 4 of 5 healthy close family contacts recognized the antigen. The recognition of MLP12α by mononuclear cells of 3 of 4 BCG-vaccinees demonstrated for the first

time that the major 10/12-kDa proteins of *M. leprae, M. tuberculosis* and *M. bovis* are immunologically related. MLP12α appears to have a broad stimulatory capacity, with no difference noted among patients, contacts, and unrelated individuals. Highly crossreactive immunogens may play a role in the protective T-cell response against *M. leprae.*—Authors' Abstract

Hirsch, C. S., Ellner, J. J., Russell, D. G. and Rich, E. A. Complement receptor-mediated uptake and tumor necrosis factor-alpha-mediated growth inhibition of *Mycobacterium tuberculosis* by human alveolar macrophages. J. Immunol. **152** (1994) 743–753.

The relative phagocytosis and intracellular fate of *Mycobacterium tuberculosis* (MTB) (H37Ra) in human alveolar macrophages (AM) and their precursors blood monocytes (MN) was investigated. Uptake of MTB by MN and AM was confirmed by electron microscopy. At an infection ratio of 100:1 (MTB : target cell), the percentage of infected AM and the number of MTB per AM was > MN ($p < 0.001$, $p < 0.0001$, respectively). Uptake of MTB was increased by increasing concentrations of serum and decreased in the presence of heat-inactivated serum. Among complement receptors (CR) CR1, CR3, and CR4, the major CR mediating uptakes of MTB by MN were CR1 and CR3; whereas for AM, CR4 was the major CR. When MN and AM were infected with MTB and cultured for up to 7 days, AM limited intracellular growth of MTB more effectively than MN as determined by a CFU assay. MTB stimulated production of TNF-alpha by mononuclear phagocytes and by AM > MN ($p < 0.007$). Pentoxifylline inhibited TNF-alpha production by mononuclear phagocytes and concurrently increased MTB growth (AM > MN). A polyclonal neutralizing antibody to TNF-alpha also increased MTB growth in AM. Thus, AM are more efficient in phagocytosis of MTB than MN, and uptake is mediated through CR4 to a greater extent than CR1 or CR3. The slowed replication of MTB in AM is associated with an increase in TNF-alpha production, and intracellular growth is promoted by pentoxifylline and neutralizing antibody to TNF-alpha. These data suggest that AM may play a prominent and efficient role in the primary defense of the lung in tuberculosis through CR-mediated uptake, predominantly CR4, and TNF-alpha-mediated killing of MTB.—Authors' Abstract

Holoshitz, J., Romzek, N. C., Jia, Y. F., Wagner, L., Vila, L. M., Chen, S. J., Wilson, J. M. and Karp, D. R. MHC-independent presentation of mycobacteria to human gamma delta T cells. Int. Immunol. **5** (1993) 1437–1443.

The majority of human peripheral gamma/delta T cells express antigen receptors using the V(gamma)9 and V(delta)2 gene products. Cells of this subset have been previously shown to uniformly recognize mycobacteria regardless of their V-(D)-J junctional sequences in an MHC-unrestricted manner. This reactivity superficially resembles activation of alpha/beta cells by bacterial superantigens, which are thought to be presented by monomorphic regions of MHC class II molecules. It is not known whether presentation of the mycobacterial antigen to V(gamma)9/V(delta)2 T cells is also mediated by class II MHC molecules. In order to examine the similarity between presentation of bacterial superantigens to alpha/beta T cells and the presentation of mycobacteria to gamma/delta T cells we have studied the role of class II MHC molecules in presentation of the mycobacterial antigen AP-MT to V(gamma)9/V(delta)2 clones. Activation of gamma/delta T cells by AP-MT required direct contact with antigen-presenting cells, indicating that an interaction with cell-surface molecules on antigen-presenting cells is required. Class II MHC molecules were neither sufficient nor necessary for effective presentation of AP-MT to the gamma/delta T cells, as transfectants expressing class II MHC molecules were unable to present; whereas cell lines lacking expression of MHC class II molecules could present this mycobacterial antigen. Unlike presentation of staphylococcal enterotoxins to alpha/beta T cells, which could be mediated by class-II transfectants and was significantly augmented by co-expression of intercellular adhesion molecule (ICAM)-1, presentation of AP-MT to gamma/delta T cells could not be enhanced by

co-expression of class II and ICAM-1 mol ecules. Based on these results and our previous observation that presentation of AP-MT is independent of class-I MHC molecules, we conclude that presentation of mycobacteria to human V(gamma)9/V (delta)2 cells can be mediated by non-MHC cell surface molecules. These results indicate that despite apparent similarities, recognition of mycobacteria by V(gamma)9/V (delta)2 cells and activation of alpha/beta T cells by bacterial superantigens involve distinct presentation mechanisms.—Authors' Abstract

Kawatsu, K., Izumi, S., Yumi, M., Butt, K. I. and Wang, T. [Modification of Harada's method for rapid staining of mycobacteria.] Jpn. J. Lepr. **61** (1992) 175–181.

Harada employed periodic acid–carbol pararosaniline and periodic acid–methenamine silver stain for demonstrating chromophobic bacilli which do not get stained with conventional carbol fuchsin or counter stain. This staining method takes considerable time for complete oxidation with periodic acid. [The authors] have succeeded in reducing the oxidation time by using hydrogen peroxide treatment prior to periodic acid and with the use of acidified sodium hydrogen sulfite treatment before carbol pararosaniline stain. [The authors] also found that in methenamine silver stain, combined use of semi-carbazide and microwave treatment can shorten the whole staining time up to 4 hr without losing its sensitivity.—Authors' Abstract

Mustafa, A. S., Lundin, K. E. A. and Oftung, F. Human T cells recognize mycobacterial heat shock proteins in the context of multiple HLA-Dr molecules—studies with healthy subjects vaccinated with *Mycobacterium bovis* BCG and *Mycobacterium leprae*. Infect. Immun. **61** (1993) 5294–5301.

Heat-shock proteins (HSP) are considered to be important targets of the immune response to mycobacteria and, as such, relevant to subunit vaccine design. If HSP are major antigens in cell-mediated immunity, they should be recognized in the context of most of the HLA-DR molecules required for presentation of mycobacterial antigens to T cells. We tested peripheral blood mononuclear cells (PBMC) and T-cell lines from *Mycobacterium leprae*- and *M. bovis* BCG-vaccinated subjects for proliferation in response to the 18- and 65-kDa HSP of *M. leprae*, the 65-kDa HSP of *M. bovis* BCG, and the 70-kDa HSP of *M. tuberculosis*. Irrespective of HLA types, PBMC showing a strong response to *M. leprae* proliferated in response to mycobacterial HSP. HLA restriction analysis with T-cell lines showed that the *M. leprae* 18-kDa HSP was recognized in the context of HLA-DR4, HLA-Dw4, and HLA-DR1 molecules. The T-cell lines recognized the *M. leprae* 65-kDa HSP in the context of all of the HLA-DR molecules expressed by autologous antigen-presenting cells, i.e., HLA-DR1, HLA-DR2, HLA-DR5, HLA-DR7, and importantly HLA-DR4 (HLA-Dw4 and HLA-Dw14), which is relevant to autoimmunity. The *M. tuberculosis* 70-kDa antigen was also presented to the T-cell lines by HLA-DR1, HLA-DR2, HLA-DR5, and HLA-DR7 molecules. In addition, this HSP was recognized in the context of the HLA-DRw53 molecule, which is frequently expressed in many regions where leprosy is endemic. The T-cell lines proliferating in response to a given HSP lysed autologous monocytes-macrophages pulsed with that HSP. The results demonstrate that PBMC from individuals immunized with *M. leprae* respond to mycobacterial HSP and that these HSP are presented to T cells by multiple HLA-DR molecules, a prerequisite for their application in the next generation of subunit vaccines.—Authors' Abstract

Schlesinger, L. S. and Horwitz, M. A. A role for natural antibody in the pathogenesis of leprosy—antibody in nonimmune serum mediates C3 fixation to the *Mycobacterium leprae* surface and hence phagocytosis by human mononuclear phagocytes. Infect. Immun. **62** (1994) 280–289.

We have previously determined that complement receptors on human mononuclear phagocytes and complement component C3 in nonimmune serum mediate phagocytosis of the intracellular bacterial pathogen *Mycobacterium leprae*, the agent

of leprosy. We have also determined that C3 fixes selectively to the major surface glycolipid of *M. leprae,* phenolic glycolipid I (PGL-I). In this study, we have explored the role of natural antibody in nonimmune serum in C3 fixation and Clq binding to *M. leprae* and PGL-I. At serum concentrations within the range at which phagocytosis of *M. leprae* is maximal, C3 fixation was mediated by both the classical and the alternative complement pathways. At the low end of this serum concentration range (2.5%), C3 fixation was mediated predominantly by the classical pathway. Consistent with a role for both pathways, C3 fixation to *M. leprae* was enhanced by the addition of either pure Clq to Clq-depleted serum or pure factor B to factor B-depleted serum. C3 fixation to *M. leprae* was strictly antibody dependent regardless of the serum concentration used. C3 fixation to *M. leprae* occurred in nonimmune serum but not in a gammaglobulinemic serum unless heat-inactivated nonimmune serum or small amounts of pure immunoglobulin G (IgG) or IgM were added. C3 fixation by both the alternative and the classical complement pathways was mediated by antibody, and the antigen-binding portion of the antibody molecule was required. C3, IgG, IgM, and Clq were readily detected on the surface of *M. leprae.* Consistent with the previously demonstrated exclusive role of the classical complement pathway in C3 fixation to PGL-I, Clq bound to PGL-I in a dose-dependent fashion; Clq binding was evident in >1.25% nonimmune serum. Clq binding to PGL-I was strictly antibody dependent. When PGL-I was incubated with pure Clq, little or no Clq bound to PGL-I unless heat-inactivated nonimmune serum or pure IgG or IgM was added. When PGL-I was incubated in nonimmune serum, C3 bound directly to PGL-I and not to anti-PGL-I antibody, since the amount of C3 bound to PGL-I was not reduced by acid elution of the antibody. However, the amount of C3 bound to PGL-I was markedly reduced by hydroxylamine treatment, providing evidence for C3 fixation via a covalent ester bond. Nonimmune serum contained antibody to all four major *M. leprae* surface carbohydrates. Relative to PGL-I, nonimmune serum contained more antibody to the other surface carbohydrates. This study demonstrates that natural antibody in the serum of nonimmune hosts mediates C3 fixation to *M. leprae* and Clq binding to PGL-I. Taken together with previous findings, this study indicates that natural antibody mediates complement receptor-dependent phagocytosis of *M. leprae* by host cells and therefore potentially plays an important role in the pathogenesis of leprosy.—Authors' Abstract

Singh, S., Narayanan, N. P. S., Jenner, P. J., Ramu, G., Colston, M. J., Prasad, H. K. and Nath, I. Sera of leprosy patients with type 2 reactions recognize selective sequences in *Mycobacterium leprae* recombinant LSR protein. Infect. Immun. **62** (1994) 86–90.

Type 2 reactions (erythema nodosum leprosum [ENL]) are episodic, reactional states causing significant morbidity in lepromatous leprosy patients. With a view to defining the immunological differences between the stable and reactional forms of lepromatous leprosy, we determined antibody responses to LSR, a recombinant protein of *Mycobacterium leprae* previously described by us (S. Laal, Y. D. Sharma, H. K. Prasad, A. Murtaza, S. Singh, S. Tangri, R. S. Mishra, and I. Nath, Proc. Natl. Acad. Sci. USA 88:1054–1058, 1991), as well as to 10- to 15-mer overlapping peptides synthesized on the basis of the LSR amino-acid sequence. We report here the selective recognition of B-cell epitopes by sera from patients with ENL as compared with a control group with nonreactional lepromatous leprosy. Peptides 2 and 3, with the sequences GVTYEIDLTNKNAA and IDLTNKNAAKLRGD, respectively, were recognized by >95% of sera from patients with active ENL. Peptide 3 in addition showed reactivity with sera taken from 91.6% of lepromatous leprosy patients who were apparently stable but who were recorded to have had ENL several weeks before or after the sample collection. The core sequence IDLTNKNAA common to both these peptides may be a major target of humoral responses in ENL. In addition, the RGD motif at the C terminus appeared to influence the antigenicity of peptide 3 in enzyme-linked immunosorbent assay. It would appear that humoral responses dur-

ing ENL are directed to selective antigenic determinants of the leprosy bacillus. The use of such serological markers to identify lepromatous leprosy patients with a high risk for developing ENL would be of clinical and predictive value.—Authors' Abstract

Wang, D., *et al.* [Anti-idiotype antibody used as antigen in serological study of leprosy.] China Lepr. J. **9** (1993) 142–145. (in Chinese)

A series of leprosy patients were examined with indirect ELISA, using monoclonal antiidiotype antibody (MAb2) with inner image of terminal trisaccharide of PGL-I molecule as antigen made by the authors themselves, and compared it with the result of NT-O-BSA ELISA. Of 50 leprosy patients examined, all were positive to MAb2 (100%) but only 42 were positive to NT-O-BSA (84%). For 20 normal sera in an endemic area, the results of the two tests were all the same, i.e., 1 was positive and 19 negative, respectively. The person with serological positivity might be infected with *M. leprae.* For eight patients with positivity to MAb2 and negativity to NT-O-BSA, inhibitory ELISA were all positive. The authors believe that MAb2 may be used as an antigen in the serological diagnosis of leprosy and it has some advantages, e.g., homogeneous, safe, economical and easy to obtain.—Authors' English Abstract

Wang, X. H., Golkar, L., Uyemura, K., Ohmen, J. D., Villahermosa, L. G., Fajardo, T. T., Cellona, R. V., Walsh, G. P. and Modlin, R. L. T-cells bearing V beta 6 T-cell receptors in the cell-mediated immune response to *Mycobacterium leprae.* J. Immunol. **151** (1993) 7105–7116.

The skin lesions of leprosy provide a window into the immunoregulatory events involved in the human immune response to infection. T cells are thought to play a vital role in the pathogenesis of different forms of the disease. To identify predominant specific T-cell subpopulations in leprosy lesions, the TCR-beta chain repertoire was simultaneously studied in skin biopsy specimens and PBMC from both immunologically resistant tuberculoid leprosy and susceptible lepromatous leprosy patients. This was accomplished by obtaining RNA from lesions and PBMC, synthesizing cDNA, and performing the polymerase chain reaction. We found that TCR gene subfamilies Vbeta6.1 through Vbeta6.4 (Vbeta6.1–4) were strikingly overrepresented in lesions versus PBMC of 7 of 9 tuberculoid patients but only 1 of 9 lepromatous patients. Similarly, Vbeta6.5/6.8/6.9 subfamilies were predominant in 4 of 9 tuberculoid patients, but 0 of the 9 lepromatous patients. To explore the influence of the complementarity-determining region 3 (CDR3) in selection of T cells expressing Vbeta6 TCR, we sequenced the Vbeta6.1–4-Cbeta polymerase chain reaction products derived from the lesions and PBMC of two tuberculoid patients. From the analysis of deduced amino-acid sequences, we found conserved amino-acid residues and amino-acid motifs in the CDR3 region of the lesion-derived sequences from each patient. Our data suggest that the nominal Ag select T cells bearing Vbeta6 TCR in the cell-mediated immune response to *Mycobacterium leprae.*—Authors' Abstract

Weng, X., *et al.* [Monitoring leprosy relapse with immunologic tests.] China Lepr. J. **9** (1993) 133–138. (in Chinese)

To monitor leprosy relapse after completing MDT is the chief way of evaluating treatment and controlling the transmission of the disease. For studying predictability of leprosy relapse, 1658 persons cured of leprosy have been examined by using a serological test and lepromin reaction in eight counties, Weifang prefecture, Shandong province. Positivity of antibody to PGL plus negative reaction to lepromin is regarded as a hazardous factor. The hazard rate was 12.7% (211/1685). During the 3 years of the monitoring 27 relapsed cases were detected, including 23 in the hazardous group and 4 in the controls, and the relapse rates were 11% and 0.28%, respectively. The relative hazard degree was 39.5. The results showed that the antibody has been disappearing and CMI building up gradually in the cures as time goes on. The majority of the relapsed cases had a higher level of IgM antibody to PGL, 1–1½ years before relapse. The authors suggest that those who had gone

through longer time after cured with higher IgM level should be the focal point in monitoring.—Authors' English Abstract

Wu, Q., *et al.* [Potential of gelatin agglutination test in the study of leprosy.] China Lepr. J. **9** (1993) 138–141. (in Chinese)

In order to evaluate the gelatin particle agglutination test (MA) and NT-P-BSA ELISA (NE), which are two methods for detecting infection with *M. leprae*, we have systematically compared them among 158 leprosy patients (LL 58, BL 55, BT 20, TT 20) in 155 household contacts in 149 a randomly selected population and in 40 healthy controls. The results showed that when the serum dilution was at 1:16–91% while the PR of NE1 (OD = 0.01) and NE2 (OD = 0.20) were 98% and 91%, respectively. The individual agreement (IA) was more than 90% between MA 1:32 and NE. In MB leprosy patients the IA was 96–100% between MA 1:16 and NE, and 83–96% between MA 1:32 and NE. Quantitative data proved the results which suggested that NE could not be replaced with MA and MA is suitable only to detecting MB leprosy. The comparison of the sera with dried serum and dried blood on filter paper indicated that the best of them in effect was the serum in MA, and there was no significant difference between sera and dried blood in MA and NE ($p > 0.05$). An identical result was obtained in dried blood whose amount has been reconstituted by means of calculating its serum content. If the dried blood is not converted into its serum content the PR and IA all will decrease (<10%). There was no significant difference between the test tubes with U- and V-bottom ($p > 0.99$, IA = 93.3%), but when using V-bottom tubes the result is more easily judged.—Authors' English Abstract

Microbiology

Adams, E., Britton, W. J., Morgan, A., Godsall, A. L. and Basten, A. Identification of human T cell epitopes in the *Mycobacterium leprae* heat shock protein 70-kD antigen. Clin. Exp. Immunol. **94** (1993) 500–506.

In a number of pathogens, heat-shock proteins (hsp) stimulate humoral- and cellular-immune responses despite significant sequence identity with host hsp. The 70-kDa hsp of *Mycobacterium leprae*, which shares 47% identity with human hsp70 at the protein level, elicited a T-cell response in most *M. bovis* (bacille Calmette-Guérin (BCG)) vaccinees as well as leprosy and tuberculosis patients and their contacts. In order to locate T-cell epitopes, DNA fragments encoding portions of the 70-kDa hsp were expressed in the vector pGEX-2T and tested for T-cell reactivity in an *in vitro* proliferative assay. Cultures of peripheral blood mononuclear cells (PBMC) from BCG vaccinees indicated that the C-terminal half of the molecule contained multiple T-cell epitopes since the T cells from a majority of *M. leprae* hsp70-reactive individuals responded to C-344. Lower proportions of patients with paucibacillary leprosy (36%) and tuberculosis patients (16%) responded to C-344. The smaller C-142 fragment which includes the terminal 70 residues unique to *M. leprae* and is the target for the human antibody response, elicited a cellular response in few patients and no vaccinees. In order to map T-cell epitopes, two series of synthetic peptides encompassing the region 278-502 were prepared. Using overlapping 12mer and 20mer peptides, this region of the molecule was found to contain several potential T-cell epitopes. The longer peptides gave a clearer indication of reactive sequences including regions of the molecule which were not identified with the 12mer peptides. Fine mapping of reactive peptide pools using the 12mer peptides identified two T-cell epitopes. Although both were located in regions of the molecule shared with *M. tuberculosis*, one appeared to be cross-reactive with the equivalent human sequence, and thus has the potential to initiate autoimmune responses.—Authors' Abstract

Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Kyung, S., Wilson, T., Collins, D., de Lisle, G. and Jacobs, W. R., Jr. *inhA*, a gene encoding a target for isonazid and ethionamide in *Mycobacterium tuberculosis*. Science **263** (1994) 227–228.

Isoniazid (isonicotinic acid hydrazide, INH) is one of the most widely used antituberculosis drugs, yet its precise target of action on *Mycobacterium tuberculosis* is unknown. A missense mutation within the mycobacterial *inhA* gene was shown to confer resistance to both INH and ethionamide (ETH) in *M. smegmatis* and in *M. bovis*. The wild-type *inhA* gene also conferred INH and ETH resistance when transferred on a multicopy plasmid vector to *M. smegmatis* and *M. bovis* BCG. The InhA protein shows significant sequence conservation with the *Escherichia coli* enzyme EnvM, and cell-free assays indicate that it may be involved in mycolic acid biosynthesis. These results suggest that InhA is likely a primary target of action for INH and ETH.—Authors' Abstract

Besra, G. S., Minnikin, D. E., Wheeler, P. R. and Ratledge, C. Synthesis of methyl (Z)-tetracos-5-enoate and both enantiomers of ethyl (E)-6-methyltetracos-4-enoate—possible intermediates in the biosynthesis of mycolic acids in mycobacteria. Chem. Phys. Lipids **66** (1993) 23–24.

The high molecular weight 2-alkyl-3-hydroxy mycolic acids are key structural components of the cell envelope of pathogenic mycobacteria, such as *Mycobacterium tuberculosis*. A prime target for drug action would be the initial stages where the biosynthetic pathways diverge from those of ordinary fatty acids. It has been postulated that the pathway for the alpha-mycolates, without oxygen functions in addition to the hydroxy-acid unit, appears to diverge from (Z)-tetracos-5-enoic acid. The biosynthesis of oxygenated mycolic acids is considered to possibly diverge from (E)-6-(R)-methyltetracos-4-enoic and (E)-6-(S)-methyltetracos-4-enoic acids. This communication describes the synthesis of esters of these acids in order to test their potential role in the biosynthesis of mycolic acids.—Authors' Abstract

Byrd, S. R., Gelber, R. and Bermudez, L. E. Roles of soluble fibronectin and β_1 integrin receptors in the binding of *Mycobacterium leprae* to nasal epithelial cells. Clin. Immunol. Immunopathol. **69** (1993) 266–271.

The mechanisms by which *Mycobacterium leprae* invades the human host are presently unknown. We investigated the ability of *M. leprae* to bind to human RPMI 2650 cells, a human nasal septal epithelial cell line, using both microscopic observation and an ELISA technique. The results demonstrated that *M. leprae* adheres to nasal cells after binding to soluble fibronectin. Furthermore, it was observed that *M. leprae* could bind to the β_1 chain of the integrins in the absence of serum or mucus. These results demonstrated that *M. leprae* uses fibronectin and fibronectin receptors on the surface of epithelial cells to bind and possibly invade the nasal epithelial cells.—Authors' Abstract

Dellagostin, O. A., Wall, S., Norman, E., O'Shaughnessy, T., Dale, J. W. and McFadden, J. Construction and use of integrative vectors to express foreign genes in mycobacteria. Mol. Microbiol. **10** (1993) 983–993.

We have constructed a mycobacterial integrative vector by placing two copies of the insertion sequence IS900 flanking a kanamycin-resistance gene into a "suicide" vector unable to replicate in mycobacteria. The *Mycobacterium leprae* gene encoding the *M. leprae* 18-kDa protein was cloned between the two copies of IS900 to provide expression signals. Constructs were introduced into *Mycobacterium* species *smegmatis, vaccae* and *bovis* BCG by electroporation and selection for kanamycin resistance. The expression of the 18-kDa gene was analyzed by Western blotting. Integration of the vector into the *M. smegmatis* chromosome was analyzed by Southern blotting. One to five copies of the vector were detected in each

transformant. The SIV gag p27 gene and the foot-and-mouth disease virus VP1 140-160 epitope were successfully cloned into the 18-kDa gene and expression in *M. smegmatis* was obtained.—Authors' Abstract

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

By screening a *Mycobacterium leprae* λgt11 expression library with a serum from an Ethiopian lepromatous leprosy (LL) patient a clone was isolated (LL4) belonging to hybridization group III of a panel of previously isolated *M. leprae* clones. Members of this hybridization group encode a serologically recognized 45-kDa protein. The complete DNA sequences of the partially overlapping clones LL4 and L1 (hybridization group III) are presented, and these revealed the presence of an open reading frame (ORF) predicting a protein with a molecular size of 42, 448 Da. Southern hybridizations on total genomic DNA of *M. leprae, M. tuberculosis* and eight atypical mycobacteria showed that the LL4 DNA fragment is specific for *M. leprae* DNA even under low-stringency conditions. The *M. leprae* specificity of LL4 DNA was further confirmed by the polymerase chain reaction using four different sets of primers. Western blotting analyses showed that the *M. leprae* 45-kDa protein is frequently recognized by antibodies from leprosy patients and that this recognition is specific since no antibodies could be detected in sera of tuberculosis patients. T-cell proliferation assays also demonstrated T-cell recognition by leprosy patients and healthy contacts of the *M. leprae* 45-kDa protein. The specificity of the LL4 DNA region and the 45-kDa antigen that is encoded by hybridization group III could provide unique tools for the development of *M. leprae*-specific immunological and DNA reagents.—Authors' Abstract

Dunne, W. M., Mason, E. O. and Kaplan, S. L. Diffusion of rifampin and vancomycin through a *Staphylococcus epidermidis* biofilm. Antimicrob. Agents Chemother. **37** (1993) 2522–2526.

Using an equilibrium dialysis chamber, we evaluated the penetration of vancomycin, rifampin, or both through a staphylococcal biofilm to simulate treatment of an infected biomedical implant. A biofilm of ATCC 35984 (slime-positive *Staphylococcus epidermidis*; vancomycin MIC and MBC, 1 and 2 μg/ml, respectively; rifampin MIC and MBC, 0.00003 and 0.00025 μg/ml, respectively) was established on the inner aspect of the dialysis membrane (molecular mass exclusion, 6000 kDa). Serum containing vancomycin (40 μg/ml), rifampin (20 μg/ml), or a combination of both was introduced into the inner chamber of the dialysis unit (in direct contact with the biofilm), and serum alone was added to the outer chamber. Rifampin and vancomycin concentrations in both chambers were determined over a 72-hr period. In the absence of rifampin, the concentration of vancomycin in the outer chamber exceeded the MBC for the organism after 24 hr, and the MBC increased to nearly 8.8 μg/ml by 72 hr, demonstrating that therapeutic levels of vancomycin can penetrate a staphylococcal biofilm. However, viable bacteria were recovered from the biofilm after 72 hr of treatment with no apparent increase in the MIC or MBC of vancomycin. Similarly, concentrations of rifampin exceeding the MBC were detected in the outer chamber after 24 hr of treatment, but viable organisms were recovered from the biofilm after 72 hr of treatment. In this case, the rifampin MBCs for surviving organisms increased from 0.00025 to > 128 μg/ml. The combination of agents prevented the development of resistance to rifampin, improved the perfusion of vancomycin through the biofilm, and decreased the penetration of rifampin but did not sterilize the membrane. These observations provide evidence that bactericidal levels of vancomycin, rifampin, or both can be attained at the surface of an infected implant. Despite this, sterilization of the biofilm was not accomplished after 72 hr of treatment.—Authors' Abstract

Filley, E., Thole, J. E. R., Rook, G. A. W., Nagai, S., Waters, M., Drijfhout, J. W., Dewit, T. F. R., de Vries, R. R. P. and Abou Zeid, C. Identification of an antigenic domain on *Mycobacterium leprae* protein antigen 85B, which is specifically

recognized by antibodies from patients with leprosy. J. Infect. Dis. **169** (1994) 162–169.

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

Garbe, T. R., Barathi, J., Barnini, S., Zhang, Y., Abou-Zeid, C., Tang, D., Mukherjee, R. and Young, D. B. Transformation of mycobacterial species using hygromycin resistance as selectable marker. Microbiology **140** (1994) 133–138.

Electroporation with shuttle plasmids carrying a kanamycin resistance gene as a selectable marker failed to generate transformants in two mycobacterial species currently being used in human vaccine trials (*Mycobacterium w* and *M. vaccae*). In contrast, efficient transformation [10^3–10^5 transformants (μg DNA)$^{-1}$] was obtained using novel vectors with selection based on expression of resistance to hygromycin. The hygromycin resistance vector was also found to be more efficient than kanamycin resistance vectors for transformation of *M. smegmatis* and *M. bovis* BCG. The hygromycin resistance vector was used to overexpress superoxide dismutase of *M. tuberculosis* in *M. vaccae* in a form suitable for detailed structural analysis. The potential use of this approach for generation of novel recombinant mycobacterial vaccines is discussed.—Authors' Abstract

Godard, C. M. *Mycobacterium leprae*—behaviour in fat cells undergoing adipose differentiation *in vitro*. C. R. Acad. Sci. [III] **316** (1993) 1355–1362.

We have investigated the behavior of *M. leprae* in murine preadipocyte cells (clone Ob17) undergoing the adipose cell conversion process *in vitro*. Actively differentiating Ob17 cells were infected with *M. leprae*. The morphological index (MI) of the acid-fast bacteria (AFB) present at day 12 and day 25 after infection was compared to the MI of the AFB inoculated. An increase of the MI was consistently observed. This increase is suppressed by rifampin. Due to important cell loss, an increase of the number of the AFB per culture could not be obtained in monolayer tissue cultures. In order to prevent cell loss, we used a three-dimensional culture system. This cell culture system is an *in vitro* reconstitution of the human dermis, a main target organ for the leprosy bacillus. Adipocytes infected with *M. leprae* are incorporated in a condensed collagen lattice together with skin fibroblasts. Under such conditions, both an increase of the MI and an increase of the number of the AFB are obtained. This suggests that cellular functions related to the adipose cell differentiation process might complement the defective bacterial genome, leading to transient multiplication *in vitro*.—Author's Abstract

Guilhot, C., Otal, I., Van Rompaey, I., Martin, C. and Gicquel, B. Efficient transposition in mycobacteria—construction of *Mycobacterium smegmatis* insertional mutant libraries. J. Bacteriol. **176** (1994) 535–539.

The Tn611 transposon was inserted into pCG63, a temperature-sensitive plasmid isolated from an *Escherichia coli*-mycobacterial shuttle vector which contains the pAL5000 and pUC18 replicons. The resulting plasmid, pCG79, was used to generate a large number of insertional mutations in *M. smegmatis*. These are the first mycobacterial insertional mutant libraries to be constructed by transposition directly

into a mycobacterium. No highly preferential insertion sites were detected by Southern blot analysis of the chromosomal DNAs isolated from the insertion mutants. Auxotrophic mutants with various phenotypes were isolated at a frequency ranging from 0.1% to 0.4%, suggesting that the libraries are representative. The pCG79 system thus seems to be a useful tool for the study of *M. smegmatis* genetics and may be applicable to other mycobacteria, such as the *M. tuberculosis* complex.—Authors' Abstract

Hughes, A. L. Contrasting evolutionary rates in the duplicate chaperonin genes of *Mycobacterium tuberculosis* and *M. leprae.* Mol. Biol. Evol. **10** (1993) 1343–1359.

A phylogenetic analysis of chaperonin (heat-shock protein 60) sequences from prokaryotes and eukaryotes indicated that a single gene duplication event in the common ancestor of *Mycobacterium tuberculosis, M. leprae,* and *Streptomyces albus* gave rise to the duplicate chaperonin genes found in these species (designated HSP65 and GroEL in the mycobacterial species). Comparison of rates of synonymous and nonsynonymous nucleotide substitution in different gene regions suggested that the 5′ end of the HSP65 gene was homogenized by an ancient recombination event between *M. tuberculosis* and *M. leprae.* In *S. albus,* the two duplicated chaperonin genes have evolved at essentially the same rate. In both *M. tuberculosis* and *M. leprae,* however, the GroEL gene has evolved considerably more rapidly at nonsynonymous nucleotide sites than has the HSP65 gene. Because this difference is not seen at synonymous sites, it must be due to a difference in selective constraint on the proteins encoded by the two genes, rather than to a difference in mutation rate. The difference between GroEL and HSP65 is striking in regions containing epitopes recognized by T cells of the vertebrate host; in certain crossreactive epitopes conserved across all organisms, nonsynonymous sites in GroEL have evolved twice as fast as those in HSP65. It is suggested that these differences are correlated with differences in the way in which the duplicate chaperonins of *M. tuberculosis* and *M. leprae* interact with the host immune system.—Author's Abstract

Mori, T. and Aishima, T. [Specific odor component produced by *Mycobacterium lepraemurium* on Ogawa yolk medium.] Jpn. J. Lepr. **61** (1992) 153–155.

A characteristic odor is produced by *Mycobacterium lepraemurium* grown on 1% Ogawa yolk medium, but not by other, easily cultivable acid-fast bacilli. The key component of this odor has been identified as phenylacetic acid. The addition of phenylacetic acid inhibited the initial isolation culture on Ogawa yolk medium of *M. lepraemurium* from mouse tissue.—C. A. Brown (Trop. Dis. Bull.)

Qin, M. H., Taniguchi, H. and Mizuguchi, Y. Analysis of the replication region of a mycobacterial plasmid, pMSC262. J. Bacteriol. **176** (1994) 419–425.

We determined the nucleotide sequence of a DNA fragment which contains the replication region of pMSC262, a *Mycobacterium scrofulaceum* plasmid used to construct the *Mycobacterium-Escherichia coli* shuttle vector. The complete sequence of the fragment contained 2504 bp with an overall G + C content of 69.8%. By deletion analysis, we found that the minimum length required for plasmid replication in *M. bovis* BCG was about 1.6 kb. Within this region, several open reading frames (ORFs) and a putative replication origin (ori) were identified by computer analysis. One of the ORFs, ORF2, which encodes a putative 28.9-kDa basic protein with characteristics of DNA-binding proteins, appeared to be involved in replication of the plasmid in BCG. By separation of ORF2 and the putative ori region, it was revealed that the relative locations of ORF2 and the putative ori region are likely important for replication in BCG. No DNA or amino acid homologies were found between this replication region and that of pAL5000, another mycobacterial plasmid used for vector plasmid construction. In addition, we found that this replicon did not lead to replication in *E. coli* and was compatible in BCG with pAL5000-derived vector plasmid pYUB75 (R. G. Barletta, D. D. Kim, S. B. Snapper, B. R. Bloom and W. R. Jacobs, J., J. Gen. Microbiol. 138:23–30, 1992).—Authors' Abstract

Shannon, E. J., Harris, E. B., Haile-Mariam, H. S., Guebre-Xavier, M. and Frommel, D. Competency of human-derived *Mycobacterium leprae* to use palmitic acid in the synthesis of phenolic glycolipid-I and phthiocerol dimycocerosate and to release CO_2 in axenic culture. Lepr. Rev. **63** (1993) 101–107.

Suspensions of human-derived *Mycobacterium leprae* were able simultaneously to oxidize ^{14}C-labeled palmitic acid to $^{14}CO_2$ and incorporate the label into phenolic glycolipid-I and phthiocerol dimycocerosate which is essentially the lipid moiety of the phenolic glycolipid. These are catabolic and anabolic reactions, respectively.

Although it may appear surprising that catabolic and anabolic activities occur simultaneously, it is predictable if palmitate, a fatty acid, is the major or only carbon source available. Genuinely surprising is the finding that the pH optimum for the catabolic activity is at acidic pH 4.8; whereas the optimum for the anabolic activities is pH 6.8, near neutral, for human-derived *M. leprae*. In previous studies, using armadillo- or mouse-derived *M. leprae*, the anabolic activity had the more acidic optimal pH. Optimal pH should be a fixed property of a given enzyme, or uptake system, so it may be that the pH optima vary with *M. leprae* because both activities require a complex series of different activities to function. Further experiments using human-derived *M. leprae* are needed to verify these results.—P. Wheeler (Trop. Dis. Bull.)

Wieles, B., Vanagterveld, M., Janson, A., Clark-Curtiss, J., Dewit, T. R., Harboe, M. and Thole, J. Characterization of a *Mycobacterium leprae* antigen related to the secreted *Mycobacterium tuberculosis* protein Mpt32. Infect. Immun. **62** (1994) 252–258.

Secreted proteins may serve as major targets in the immune response to mycobacteria. To identify potentially secreted *Mycobacterium leprae* antigens, antisera specific for culture filtrate proteins of *M. tuberculosis* were used to screen a panel of recombinant antigens selected previously by leprosy patient sera. Four potentially secreted antigens were identified by this approach, and one was recognized by antibodies specific for MPT32, a secreted *M. tuberculosis* protein. The DNA coding for the *M. leprae* antigen, which we have designated 43L, was isolated and characterized and found to encode a 25.5-kDa protein that is preceded by a consensus signal peptide of 39 amino acids. The N-terminal amino-acid sequence of 43L shows 50% homology with the 20 known N-terminal amino acids of MPT32, and 47% homology was found with the N terminus of a 45/47-kDa antigen complex identified in *M. bovis* BCG. These findings indicate that 43L represents an antigen related to MPT32 and the *M. bovis* BCG 45/47-kDa complex and that 43L is likely to be a protein secreted by *M. leprae*. Purified recombinant 43L protein is recognized by antibodies and T cells from healthy contacts and leprosy patients, illustrating that secreted proteins are of importance in the immune response to *M. leprae*.—Authors' Abstract

Experimental Infections

Kohli, M., Kaur, S., Ganguly, N. K., Sharma, V. K. and Chugh, K. S. Transport of amino-acids across renal brush border membrane vesicles in *Mycobacterium leprae* infected Swiss albino mice—effect of Convit vaccine. Lepr. Rev. **64** (1993) 316–324.

Brush border membrane vesicles prepared from kidneys of *Mycobacterium leprae* infected (nonvaccinated) and vaccinated-infected Swiss albino mice were used to assess the effect of Convit's combined vaccine (BCG + *M. leprae*) on amino acid transport activity across the tubular basement membrane. The protective effect of Convit's vaccine was more pronounced with respect to the uptake of L-alanine than L-aspartate. Uptake of L-lysine showed no significant difference in the different groups. Foot pad counts followed characteristic

growth curves in the nonvaccinated infected group but showed a lag in the development of peak levels in the vaccinated group. Further, Convit's vaccine appeared to have a protective effect on renal impairment in the mouse model of leprosy in the initial stages of infection only, as indicated by the transient reversal of amino acid uptake and a diminution in the foot pad counts induced by *M. leprae* infection. No significant ($p > 0.05$) protective effect of the vaccine was found in the advanced disease state.—Authors' Summary

Epidemiology and Prevention

Centers for Disease Control. Recommendations of the International Task Force for Disease Eradication. MMWR **42** (1993) 1–38.

This report summarizes the conclusions of the International Task Force for Disease Eradication (ITFDE), a group of scientists who were convened by a secretariat at the Carter Center of Emory University six times during 1989–1992. The purpose of the ITFDE was to establish criteria and apply them systematically to evaluate the potential eradicability of other diseases in the aftermath of the Smallpox Eradication Program. The ITFDE defined eradication as "reduction of the worldwide incidence of a disease to zero as a result of deliberate efforts, obviating the necessity for further control measures."

The names of the members of the ITFDE, the criteria they developed and used, and summaries of the papers that were presented to the ITFDE by various experts are included in this report, as well as a brief history of the concept of disease eradication since the late 19th century. The ITFDE considered more than 90 diseases and reviewed 30 of these in depth, including one noninfectious disease. It concluded that six diseases—dracunculiasis, poliomyelitis, mumps, rubella, lymphatic filariasis, and cysticercosis—could probably be eradicated by using current technology. It also concluded that manifestations of seven other diseases could be "eliminated," and it noted critical research needs that, if realized, might permit other diseases to be eradicated eventually. The successful eradication of smallpox in 1977 and the ongoing campaigns to eradicate dracunculiasis by 1995 and poliomyelitis by 2000 should ensure that eradication of selected diseases will continue to be used as a powerful tool of international public health.

. . . Leprosy (Hansen's Disease)

This chronic infectious disease caused by *Mycobacterium leprae* affects an estimated 11–12 million persons worldwide. Leprosy is usually nonfatal but may be severely disfiguring and disabling, and affected persons are often ostracized. Prolonged contact with an infected person is required for transmission. Wild infected armadillos shed the bacteria into the soil and may transmit the disease from animal to animal.

The introduction of sulfones for chemotherapy in the 1940s was a major breakthrough, although many years of therapy were required for cure. Combination therapy with two to three drugs has had a major impact on the severity of the disease over the past decade. The new drug regimens are shorter but still require 6–24 months of therapy. Resistance of leprosy bacilli to chemotherapeutic drugs is an increasing problem. China, Japan, and South Korea have rapidly reduced the incidence and prevalence of this disease in recent years. India and China established national programs with goals of halting transmission of leprosy by 2000. In 1991, WHO set the goal of eliminating leprosy (defined as incidence $< 1/10{,}000$ population) worldwide by 2000. This disease is not now eradicable. Impediments include absence of a fast, simple diagnostic test; persistence of organisms, even in treated persons; cost and side effects of drugs; duration of chemotherapy; patient compliance; and the social stigma associated with the disease.—From the Report

Krishnan, B. K. and Gokarn, A. Study of leprosy among slum dwellers in Pune. Part I. Prevalence. Indian J. Pub. Health **36** (1992) 78–86.

An intensive house-to-house survey (involving clinical examination by daylight) in Ghorpuri slum of Pune, India, revealed a point prevalence of leprosy of 9.16/1000. The population surveyed was 4915 (> 95% coverage). The data are analyzed by age, gender, religion, marital status, occupational type, literacy status, socioeconomic status, type of family, and family size.

In Part II (pp. 87–92) Krishnan and Gokarn record that only 7 (15.6%) of the 45 cases of leprosy detected in the survey in Ghorpuri slum had some disability: 2 with insensitivity only and 5 with insensitivity and deformity.—C. A. Brown (Trop. Dis. Bull.)

Mittal, B. N. National strategy for elimination of leprosy in India. Indian J. Lepr. **64** (1992) 513–520.

The author gives a comprehensive and definitive account of the current and future strategies for the elimination of leprosy in India. The different approaches for areas of high, medium and low endemicity are described, as also are the procedures to be followed in those districts which have completed 8 years of multiple drug therapy (MDT). Strategies for disability and ulcer-care management, health education and manpower development are also described. It is acknowledged that disability and ulcer-care services "have been weak under the present implementation of the National Leprosy Eradication Programme" and proposals for reorganization of the relevant services are outlined. The modified MDT approach ["Guidelines for modified MDT scheme in selected districts," Leprosy Division, Directorate General of Health Services, Nirman Bhawan, New Delhi 110 011, India, 1990], launched in 1991 for 66 endemic districts with inadequate infrastructure for regular MDT implementation, has proved unsatisfactory and is to be replaced by a return to routine MDT, using experienced personnel and with financial backing from the World Bank. It is expected that there will be no more than 20,000 cases of leprosy on record in India by the year 2000, with 50,000–70,000 cases arising annually, declining through the years. The account ends with a reminder of the large number of treated, but disabled patients who will require help for many years to come.—A. C. McDougall (Trop. Dis. Bull.)

Ponnighaus, J. M., Fine, P. E. M., Bliss, L., Gruer, P. J. K., Kapira-Mwamondwe, B., Msosa, E., Rees, R. J. W., Clayton, D., Pike, M. C., Sterne, J. A. C. and Oxborrow, S. M. The Karonga Prevention Trial: a leprosy and tuberculosis vaccine trial in northern Malawi. I. Methods of the vaccination phase. Lepr. Rev. **64** (1993) 338–356.

In this report the methods of the Karonga Prevention Trial, a double-blind leprosy and tuberculosis vaccine trial in Karonga District, northern Malawi, are described in detail. During a total population house-to-house survey, which lasted from November 1985 until August 1989, 121,008 people (57,892 males and 63,116 females) were vaccinated. A further 5835 people refused vaccination and 5757 were ineligible for vaccination, 2652 of them because they had a history or signs of leprosy, or because they were suspected to have early leprosy. A total of 66, 145 individuals, without evidence of prior BCG vaccination, received one of the following: BCG, BCG + 5×10^7 killed *Mycobacterium leprae*, or BCG + 6×10^8 killed *M. leprae*; 54, 863 individuals found with a typical or a doubtful BCG scar received either placebo or BCG, or (from mid-1987 onward) BCG + 6×10^8 killed *M. leprae*. Side effects were not looked for systematically, but 4 individuals self-reported with glandular abscesses, 9 with large post-vaccination ulcers (> 25 mm in diameter) and 2 with ulcers which persisted for more than 1 year). BCG vials collected from paraffin refrigerators in the field showed satisfactory concentrations of viable BCG throughout the trial. Post-vaccination skin test (RT23 and *M. leprae* soluble antigen) results and post-vaccination ulcer rates indicate that few mistakes were made in the field when recording the vaccine codes.—Authors' Summary

Walia, R., Sarathchandra, K. G., Pandey, R. M., Parida, S. K., Zaheer, S. A., Kar, H. K., Mukherjee, A., Mukherjee, R. and Talwar, G. P. Field trials on the use of *Mycobacterium w* vaccine in conjunction with multidrug therapy in leprosy patients for immunotherapeutic and immunoprophylactic purposes. Lepr. Rev. **64** (1993) 302–311.

A double-blind field trial was started with a candidate antileprosy vaccine, *Mycobacterium w*, as an immunotherapeutic and immunoprophylactic agent against leprosy in a highly endemic region with a prevalence rate of over 18 per 1000 population. By 31 August 1992, 224 villages have been surveyed, covering a population of 307, 981 (1981 census). A total of 979 MB patients and 2801 PB patients have been registered. A total of 19,453 household contacts of leprosy patients have been examined for clinical signs of disease, of which 16,519 have received the initial dose while 10,434 have also received the booster dose of vaccine/placebo. The aims and objectives, study design of the trial, present status as well as the socio-cultural aspect involved are highlighted in this paper.—Authors' Summary

Zhao, D., *et al.* [Correlation analysis of economic development to incidence of leprosy.] China Lepr. J. **9** (1993) 145–147. (in Chinese)

The correlation analysis of leprosy incidence with economic development in 1959 to 1989 in Guangzhou city showed that there is a significant and negative correlation between them. Therefore, economic indices may be used in establishing a model for the calculation of the incidence rate of leprosy. And because economic indices may be regarded as a comprehensive index of the environmental level of life, the significant correlation of economic indices with leprosy incidence suggested that the environmental factor might be a key to influence leprosy incidence.—Authors' English Abstract

Rehabilitation

Miko, T. L., Gschmeissner, S. E., le Maitre, C., Kinfu, Y., Kazen, R. and Pereira, J. H. Regeneration at the predilective damage sites of nerve trunks in treated leprosy. Lepr. Rev. **64** (1993) 330–337.

Superficially located large and medium-sized, mixed peripheral limb nerves in active leprosy have previously been shown to have well-recognized fusiform swellings. It is generally agreed that these are the sites of predilective nerve involvement where the severest degeneration and fibrosis occur. A semiquantitative histopathological study on one of these sites, the flexor retinaculum region of the posterior tibial nerve, has been carried out on 14 treated leprosy patients who suffered from total sensory loss to the foot for between 2 and 40 years. The following observations were made: (1) large-scale nerve regeneration was present as characterized by numerous Schwann cells and unmyelinated axons which formed regeneration clusters; (2) thick myelinated axons were either absent or present only in very low numbers; (3) the intraneurial fibrosis was usually not severe; (4) the presence of active inflammation probably interfered with nerve regeneration; (5) it appeared that this regeneration started shortly after the onset of therapy and persisted for decades; (6) lepromatous cases were characterized by evenly distributed pathology, whereas borderline tuberculoid cases had an unevenly distributed pathology; (7) the massive nerve regeneration observed was functionally ineffective—these findings indicate that the total nerve damage may affect the more peripheral nerve branches.—Authors' Summary

Siddalingaswamy, M. K. and Rao, K. S. Nerve abscess in leprosy: a retrospective study. Lepr. Rev. **64** (1993) 357–361.

Nerve abscesses occur in both tuberculoid and lepromatous leprosy. We studied 20 patients who had undergone surgery for nerve abscess in mixed peripheral and cutaneous nerves. Details of these cases and the controversial question as to how long the PB regimen should be continued are discussed.—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Andersen, A. B., Thybo, S., Godfrey-Faussett, P. and Stoker, N. G. Polymerase chain reaction for detection of *Mycobacterium tuberculosis* in sputum. Eur. J. Clin. Microbiol. Infect. Dis. **12** (1993) 922–927.

The polymerase chain reaction (PCR) was evaluated in a trial which, with respect to the positive-to-negative ratio, approximated the situation of a diagnostic laboratory in a tuberculosis-endemic area. Three-hundred sputum samples were included in the study, of which one third were known to contain mycobacteria as judged by direct microscopy. The repetitive insertion sequence IS6110/IS986 of *Mycobacterium tuberculosis* was used as a target. The samples were spiked with DNA from a modified IS6110/IS986 sequence, which gives rise to PCR products easily distinguished from PCR products amplified from chromosomal *M. tuberculosis* DNA. This allowed identification of samples that contained substances inhibitory to the Taq polymerase. The detection limit of the assay was 0.05 pg to 0.5 pg of purified *M. tuberculosis* DNA, corresponding to 10 to 100 organisms. The sensitivity and specificity of the PCR was compared with that of conventional microscopy and culture. It was concluded that this method is fast and sensitive, but that culture currently is crucial for assessing viability and thus infectivity.—Authors' Abstract

Barrow, W. W., DeSousa, J. P. C., Davis, T. L., Wright, E. L., Bachelet, M. and Rastogi, N. Innumomodulation of human peripheral blood mononuclear cell functions by defined lipid fractions of *Mycobacterium avium*. Infect. Immun. **61** (1993) 5286–5293.

Mycobacterial fractions, some of which are associated with the cell envelope of *Mycobacterium avium* serovar 4, were assessed for their ability to affect various immunological functions of human peripheral blood mononuclear cells (PBM). Treatment of PBM with a total lipid fraction derived from *M. avium* serovar 4 resulted in a significant suppression of lymphoproliferative responsiveness to phytohemagglutinin stimulation at concentrations not affecting cell viability. Although a similar suppression was not observed when PBM were treated with purified serovar 4-specific glycopeptidolipids (GPL), treatment with the beta-lipid fragment derived from the GPL did result in a significant suppression of phytohemagglutinin responsiveness. Further studies revealed that the total lipid fraction and the beta-lipid fragment were effective at significantly reducing the ability of human macrophages to restrict the intracellular growth of mycobacteria and at stimulating PBM to secrete prostaglandin E_2. These same effects were not observed when purified GPL or the reduced oligosaccharide fragment of the GPL was used. Other studies revealed that the total lipid and purified GPL fractions were effective at stimulating tumor necrosis factor-alpha release from human PBM; whereas the beta-lipid fragment was not. These results indicate that mycobacterial lipids have various immunomodulatory capabilities, depending upon their chemical nature and ability to interact with certain host cells.—Authors' Abstract

Cave, M. D., Eisenach, K. D., Templeton, G., Salfinger, M., Mazurek, G., Bates, J. H. and Crawford, J. T. Stability of DNA fingerprint pattern produced with IS6110 in strains of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **32** (1994) 262–266.

To assess the stability of IS6110 restriction fragment length polymorphism pat-

terns, DNA fingerprints of 6 *Mycobacterium bovis* isolates from 1 patient and of 41 *M. tuberculosis* isolates from 18 patients were compared. The fingerprint pattern for a given patient remained identical or nearly identical despite recovery of the isolates during intervals which ranged from 8 months to 4.5 years. Changes in drug resistance profile did not alter a strain's fingerprint pattern.—Authors' Abstract

Chang, Z., Choudhary, A., Lathigra, R. and Quiocho, F. A. The immunodomiant 38-kDa lipoprotein antigen of *Mycobacterium tuberculosis* is a phosphate-binding protein. J. Biol. Chem. **269** (1994) 1956–1958.

Several antigens of *Mycobacterium tuberculosis* have been identified by monoclonal antibodies and are being exploited in the development of improved vaccines and diagnostic reagents, but none has been linked to a specific function. Herein we report that the 38-kDa extracellular lipoprotein antigen, the most potent immunogen of the mycobacteria, is a phosphate-binding protein with features very similar to those of the well-characterized periplasmic phosphate-binding protein of *Escherichia coli* which serves as an initial receptor for active transport. This is also the first report definitively linking a function of a binding protein anchored to a membrane and found in other than gram-negative bacteria.—Authors' Abstract

Cousins, D. V., Williams, S. N., Ross, B. C. and Ellis, T. M. Use of a repetitive element isolated from *Mycobacterium tuberculosis* in hybridization studies with *Mycobacterium bovis*—a new tool for epidemiological studies of bovine tuberculosis. Vet. Microbiol. **37** (1993) 1–17.

Typing of *M. bovis* isolates for epidemiological purposes is possible using restriction endonuclease analysis (REA). However, the DNA fragment patterns obtained are complex and difficult to analyze due to the large number of bands produced. In an attempt to develop a less-complicated typing scheme two DNA probes were used in hybridization studies to detect restriction fragment length polymorphisms (RFLP) in *M. bovis*. An oligonucleotide probe which matches part of the insertion sequence IS*6110* produced few bands and failed to discriminate between bovine isolates of *M. bovis*. A probe prepared from a highly repeated DNA sequence, cloned from *M. tuberculosis* when used on Southern blots of AluI digested *M. bovis* DNA, resulted in a discriminating typing scheme which was easier to perform and analyze than the REA. The RFLP typing scheme identified 27 different strains from a total of 36 isolates of *M. bovis* and 7 reference strains from the *M. tuberculosis* complex. Using REA, 24 types were identified using Bc/I and PvuII digests and 23 different types using BstEII digests. When results of all three enzyme digests were combined, the REA identified 27 types from the same strains. Ten isolates of *M. bovis* from five properties involved in an outbreak of bovine tuberculosis were all identified as the same type with both techniques.—Authors' Abstract

Durand, J. M., Lefevre, P., Kaplanski, G., Cretel, E., Mongin, M. and Soubeyrand, J. Correction of thrombocytopenia with dapsone in the primary antiphospholipid syndrome. J. Rheumatol. **20** (1993) 1777–1778.

A 25-year-old woman with the antiphospholipid syndrome developed severe thrombocytopenia (18×10^9/l), which was promptly corrected by treatment with dapsone. The mechanisms for the apparent therapeutic effect of dapsone are discussed.—Authors' Abstract

Elghazali, G. E. B., Paulie, S., Andersson, G., Hansson, Y., Holmquist, G., Sun, J. B., Olsson, T., Ekre, H. P. and Troye-Blomberg, M. Number of interleukin-4- and interferon-gamma-secreting human T cells reactive with tetanus toxoid and the mycobacterial antigen PPD or phytohemagglutinin—distinct response profiles depending on the type of antigen used for activation. Eur. J. Immunol. **23** (1993) 2740–2745.

The enzyme-linked immunospot (ELISPOT) assay has been proven to be an efficient and sensitive method for the enumeration of single cells secreting antibodies or

cytokines. Here we have used this method to determine the number of interleukin-4 (IL-4)-and interferon-gamma (IFN-γ)-producing cells in *in-vitro* secondary responses to tetanus toxoid (TT) and the mycobacterial antigen (purified protein derivative; PPD) or the mitogen phytohemagglutinin (PHA). PHA-induced IL-4 and IFN-γ secretion was well correlated, suggesting polyclonal activation of cells. This was not the case with the specific antigens, where PPD preferentially induced IFN-γ- and very few IL-4-producing cells, while TT induced both IL-4 and IFN-γ. These differences are probably a reflection of the types of immunity the two antigens induce, mycobacteria preferentially inducing a cell-mediated T-helper type 1 (Th 1) type of immunity, while immunity to tetanus is an antibody-dependent, Th 2 type of response. In individuals recently boosted with TT, a significant increase in both IL-4- and IFN-γ-producing cells in response to TT was seen at day 7 after boost, followed by decline. This was in contrast to what was seen in response to PPD where an increase of IFN-γ-producing cells after the TT boost at day 7 persisted for at least 14 days. These results suggest that after an *in vivo* boost both antigen-specific and nonspecific T cells are activated and that antigen-specific cells home to other organs and, therefore, may be difficult to demonstrate in the circulation. Our data show that the ELISPOT assay is a powerful tool for determining the frequency of cells secreting cytokines. The assay has several advantages over other assays since it is sensitive, measures the number of actually secreting cells, and avoids the problems of binding of cytokines to their cell-bound or soluble receptors.—Authors' Abstract

Flesch, I. E. A. and Kaufmann, S. H. E. Role of cytokines in tuberculosis. Immunobiology **189** (1993) 316–339.

Mycobacterium tuberculosis and *M. bovis* are facultative intracellular pathogens which preferentially utilize the macrophage as their host cell. Acquired resistance against mycobacteria depends on T cells which activate antimicrobial macrophage functions via the release of cytokines. The data summarized below suggest an important role for interferon-gamma (IFN-γ) as well as the B-cell-stimulatory factors interleukin-4 (IL-4) and IL-6 in the induction of tuberculostatic macrophage functions. Growth inhibition of mycobacteria by cytokine-stimulated macrophages is mediated by reactive nitrogen intermediates (RNI) derived from L-arginine. Tumor necrosis factor-alpha (TNF-α) and IL-10 act as autocrine regulators in the induction of the enzyme NO-synthase. Both cytokines are produced by macrophages stimulated with IFN-γ and infected with *M. bovis*. While TNF-α mediates activation of the NO-synthase and production of RNI, IL-10 suppresses this enzyme activity. The outcome of mycobacterial infection is probably regulated by a complex network between stimulatory and inhibitory cytokines.—Authors' Abstract

Forsgren, A. Antibiotic susceptibility of *Mycobacterium marinum*. Scand. J. Infect. Dis. **25** (1993) 779–782.

A radiometric respirometric technique (BACTEC) which is highly standardized for *Mycobacterium tuberculosis* was used for antibiotic susceptibility testing of clinical isolates of *M. marinum*. Ciprofloxacin, clarithromycin, rifampin and trimethoprim/sulfamethoxazole were effective at clinically relevant concentrations. Doxycycline, erythromycin and roxithromycin were ineffective. These *in vitro* results are discussed in relation to documented clinical experience.—Author's Abstract

Gangadharam, P. R. J. and Parikh, K. *In vitro* activity of streptomycin and clofazimine against established infections of *Mycobacterium avium* complex in beige mice. J. Antimicrob. Chemother. **30** (1992) 833–838.

Beige mice were challenged with 10^6–10^7 cfu of *Mycobacterium avium intracellulare* strain 101 and 22 days later treated with streptomycin 150 mg/kg/day alone, clofazimine 20 mg/kg/day alone, streptomycin 150 mg/kg/day plus clofazimine 20 mg/kg/day, or no antimicrobial agent (untreated controls). Both single-drug therapies partially reduced the cfu counts in spleen, liver and lungs compared with the controls; however the combination was significantly more

effective and completely eliminated the pathogen from the spleen and lungs of some animals after 8 weeks treatment.—Authors' Abstract

Glickman, S. E., Kilburn, J. O., Butler, W. R. and Ramos, L. S. Rapid identification of mycolic acid patterns of mycobacteria by high-performance liquid chromatography using pattern recognition software and a *Mycobacterium* library. J. Clin. Microbiol. **32** (1994) 740–745.

Current methods for identifying mycobacteria by high-performance liquid chromatography (HPLC) require a visual assessment of the generated chromatographic data, which often involves time-consuming hand calculations and the use of flow charts. Our laboratory has developed a personal computer-based file containing patterns of mycolic acids detected in 45 species of *Mycobacterium,* including both slowly and rapidly growing species, as well as *Tsukamurella paurometabolum* and members of the genera *Corynebacterium, Nocardia, Rhodococcus,* and *Gordona.* The library was designed to be used in conjunction with a commercially available pattern recognition software package, Pirouette (Infometrix, Seattle, Washington, U.S.A.). Pirouette uses the *K*-nearest neighbor algorithm, a similarity-based classification method, to categorize unknown samples on the basis of their multivariate proximities to samples of a preassigned category. Multivariate proximity is calculated from peak height data, while peak heights are named by retention time matching. The system was tested for accuracy by using 24 species of *Mycobacterium.* Of the 1333 strains evaluated, ≥ 97% were correctly identified. Identification of *M. tuberculosis* (N = 649) was 99.85% accurate, and identification of the *M. avium* complex (N = 211) was ≥ 98% accurate; ≥ 95% of strains of both double-cluster and single-cluster *M. gordonae* (N = 47) were correctly identified. This system provides a rapid, highly reliable assessment of HPLC-generated chromatographic data for the identification of mycobacteria.—Authors' Abstract

Groenen, P. M. A., Bunschoten, A. E., van Soolingen, D. and van Embden, J. D. A. Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*; application for strain differentiation by a novel typing method. Mol. Microbiol. **10** (1993) 1057–1065.

Mycobacterium tuberculosis complex strains contain a unique chromosomal region, which consists of multiple 36bp direct repeats (DRs), which are interspersed by unique spacers 35 to 41 bp in length. In this study we investigated the nature of the DNA polymorphism of this DR cluster by sequencing part of this region in a large number of *M. tuberculosis* complex strains. Two types of genetic rearrangements were observed. One type consists of the variation in one or a few discrete, contiguous DRs plus spacer sequences. This variation is probably driven by homologous recombination between adjacent or distant DRs. The other type of polymorphism is probably driven by transpositional events of the insertion sequence, IS*6110,* which is almost invariably present in the DR cluster of *M. tuberculosis* complex strains. Based on the nature of the DNA polymorphism in the DR cluster, we developed a novel method of strain differentiation, direct variable repeat polymer chain reaction (DVR-PCR), which enables typing of individual *M. tuberculosis* strains in a single PCR. The method allows an excellent differentiation of epidemiologically unrelated isolates and, in principle, the DVR-PCR allows the detection of *M. tuberculosis* and strain differentiation at the same time.—Authors' Summary

Helm, C. J., Holland, G. N., Lin, R., Berlin, O. G. W. and Bruckner, D. A. Comparison of topical antibiotics for treating *Mycobacterium fortuitum* keratitis in an animal model. Am. J. Ophthal. **116** (1993) 700–707.

The efficacy of three topical antibiotic treatments for *Mycobacterium fortuitum* (strain ATCC-6841) keratitis were compared in rabbits. Rabbits were treated with ciprofloxacin (3 mg/ml) or clarithromycin (20 mg/ml) or a combination of amikacin (100 mg/ml) and vancomycin (50 mg/ml). All three treatments significantly reduced the number of organisms in treated eyes

compared to untreated, control eyes (all p values < 0.001). No significant difference in treatment efficacy was found between the three treatment groups (all p values ≥ 0.48), although ciprofloxacin (3 mg/ml) was more effective than clarithromycin (20 mg/ml) after excluding outliers ($p = 0.01$). All treatments stabilized or reduced the size of stromal infiltrates after 4 days of therapy; whereas infiltrates continued to enlarge in untreated eyes. These results suggest that topical clarithromycin, topical ciprofloxacin, and combined amikacin and vancomycin may all be clinically useful for treating *M. fortuitum* keratitis. Both clarithromycin and ciprofloxacin were better tolerated than combined amikacin and vancomycin. This study supports the further development of clarithromycin, a new macrolide antibiotic, as a topical drug for treatment of *M. fortuitum* keratitis.—Authors' Abstract

Kaushik, N. K., Sharma, P., Shah, A., Venkitasubramanian, T. A. Serodiagnostic efficiency of phospholipid associated protein of *Mycobacterium tuberculosis* H37Rv. Med. Microbiol. Immunol. **182** (1993) 317–327.

The phospholipid-associated protein (55–67 kDa) fraction of *Mycobacterium tuberculosis* H(37)Rv was purified as the DE-V protein fraction. This DE-V fraction was used for diagnosis of tuberculosis by enzyme-linked immunosorbent assay (ELISA), detecting IgG antibody in sera collected from different categories of tuberculosis patients, i.e., with acid-fast bacilli (AFB) culture-positive pulmonary tuberculosis, with AFB culture-negative, but radiologically suspected, pulmonary tuberculosis, extrapulmonary tuberculosis, and control groups of patients suffering from diseases other than tuberculosis (asthma and/or rhinitis, lepromatous leprosy) as well as from healthy volunteers. Encouraging operational ELISA validity could be: achieved with 93% sensitivity, 100% specificity, 97% efficiency, 100% positive predictivity and 95% negative predictability even among the extrapulmonary and suspected pulmonary tuberculosis patients. The above assay was insensitive but with 100% specificity among control group of patients suffering from diseases other than tuberculosis. The DE-V protein fraction was associated with phosphatidyl inositol and phosphatidyl inositol mannosides. The dissociation of phospholipid-protein complex decreased ELISA specificity. ELISA reactivity of the DE-V fraction appeared to be thermostable; thus, it may have serodiagnostic utility in developing countries.—Authors' Abstract

Klemens, S. P., Grossi, M. A. and Cynamon, M. H. Comparative *in vivo* activities of rifabutin and rifapentine against *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **38** (1994) 234–237.

The dose-response activity of rifabutin and the comparative activities of rifabutin and rifapentine were evaluated in the beige mouse model of disseminated *Mycobacterium avium* complex (MAC) infection. In the dose-response study, mice were infected intravenously with approximately 10^7 viable *M. avium* ATCC 49601. Treatment with rifabutin at 10, 20, or 40 mg/kg of body weight was started 7 days postinfection and was administered daily for 10 days. The mice were sacrificed 3 to 5 days after the last dose. Spleens, livers, and lungs were homogenized, and viable cell counts were determined by serial dilution and plating onto Middlebrook 7H10 agar. A dose-related reduction in MAC cell counts in the organs was noted for this MAC isolate. The comparative activities of rifabutin and rifapentine were determined against a total of five MAC isolates in the beige mouse model. Rifabutin or rifapentine (20 mg/kg each) was administered to infected mice for 10 days. Groups of treated mice were compared with untreated control animals. Despite favorable *in vitro* susceptibility results, rifabutin and rifapentine had activities in the spleens against only two of the five MAC isolates. For these two MAC isolates, rifabutin was more active than rifapentine. These agents had activities in the lungs against three of five isolates. Further study of rifabutin or rifapentine against a broader range of clinical isolates in a murine infection model may be useful as part of the continuing development of newer rifamycins as anti-MAC agents.—Authors' Abstract

Kox, L. F. F., Rhienthong, D., Medo Miranda, A., Udomsantisuk, N., Ellis, K.,

van Leeuwen, J., van Heusden, S., Kuijper, S. and Kolk, A. H. J. A more reliable PCR for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Microbiol. **32** (1994) 672–678.

Diagnostic techniques based on PCR have two major problems: false-positive reactions due to contamination with DNA fragments from previous PCRs (amplicons) and false-negative reactions caused by inhibitors that interfere with the PCR. We have improved our previously reported PCR based on the amplification of a fragment of the *Mycobacterium tuberculosis* complex-specific insertion element IS*6110* with respect to both problems. False-positive reactions caused by amplicon contamination were prevented by the use of uracil-*N*-glycosylase and dUTP instead of dTTP. We selected a new set of primers outside the region spanned by the formerly used primers to avoid false-positive reactions caused by dTTP-containing amplicons still present in the laboratory. With this new primer set, 16 copies of the IS*6110* insertion element, the equivalent of two bacteria, could be amplified 10^{10} times in 40 cycles, resulting in a mean efficiency of 77% per cycle. To detect the presence of inhibitors of the *Taq* polymerase, which may cause false-negative reactions, part of each sample was spiked with *M. tuberculosis* DNA. The DNA purification method using guanidinium thiocyanate and diatoms effectively removed most or all inhibitors of the PCR. However, this was not suitable for blood samples, for which we developed a proteinase K treatment followed by phenol-chloroform extraction. This method permitted detection of 20 *M. tuberculosis* bacteria per ml of whole blood. Various laboratory procedures were introduced to reduce failure or inhibition of PCR and avoid DNA cross contamination. We have tested 218 different clinical specimens obtained from patients suspected of having tuberculosis. The samples included sputum (N = 145), tissue biopsy samples (N = 25), cerebrospinal fluid (N = 15), blood (N = 14), pleural fluid (N = 9), feces (N = 7), fluid from fistulae (N = 2), and pus from a wound (N = 1). The results obtained by PCR were consistent with those obtained with culture, which is the "gold standard." We demonstrate that PCR is a useful technique for the rapid diagnosis of tuberculosis at various sites.—Authors' Abstract

Lai, C. K. W., Wong, K. C., Chan, C. H. S., Ho, S. S., Chung, S. Y., Haskard, D. O. and Lai, K. N. Circulating adhesion molecules in tuberculosis. Clin. Exp. Immunol. **94** (1993) 522–526.

Leukocyte-endothelial adhesion molecules have been implicated in the pathogenesis of inflammatory diseases. To evaluate their role as markers of disease activity in tuberculosis, we have used an antigen capture ELISA to measure the serum concentrations of circulating intercellular adhesion molecule-1 (cICAM-1), E-selectin (cE-selectin) and vascular cell adhesion molecule-1 (cVCAM-1) in 34 patients with active tuberculosis (27 with pulmonary disease and 7 with lymph node disease) before the commencement of standard chemotherapy, 15 subjects who had previously completed treatment for pulmonary tuberculosis, and 27 healthy volunteers. Circulating ICAM-1 and E-selectin levels were significantly elevated in patients with active tuberculosis when compared to those with treated disease ($p < 0.01$), and healthy controls ($p < 0.02$). Circulating VCAM-1 was raised in patients with active or old pulmonary tuberculosis ($p < 0.02$ versus healthy controls) but not in those with tuberculous lymphadenitis. Significant correlations were observed between the levels of cICAM-1 and cE-selectin ($p = 0.63$, $p = 0.0001$), and between cICAM-1 and cVCAM-1 ($p = 0.28$, $p = 0.016$). Taking the mean + 2 S.D. of the serum level in healthy controls as the upper limit of normal range, circulating ICAM-1 had the best discriminative power in identifying active tuberculosis, being elevated in about 80% of patients but was raised in only 6.7% of subjects with treated disease and in 3.7% of normal subjects. Our data support the possibility that three adhesion molecules may be involved in the pathogenesis of tuberculosis, and cICAM-1 may be a useful marker of disease activity.—Authors' Abstract

Lazard, T., Perronne, C., Grosset, J., Vilde, J.-L. and Pocidalo, J.-J. Clarithromycin, minocycline, and rifabutin treatments before and after infection of C57BL/6 mice

with *Mycobacterium avium*. Antimicrob. Agents Chemother. **37** (1993) 1690–1692.

C57BL/6 mice were pretreated with rifabutin or clarithromycin alone or combined with minocycline 3 days before intravenous challenge (day 0) with *Mycobacterium avium*. Treatment was continued until sacrifice at days 1, 8, 15, and 21. Rifabutin or clarithromycin decreased the level of infection in both the lungs and the spleen. Rifabutin was as effective as clarithromycin in the lungs but was more effective in the spleen. The clarithromycin-minocycline combination was as effective as clarithromycin alone.—Authors' Abstract

Llatjos, M., Romeu, J., Clotet, B., Sirera, G., Manterola, J. M., Pedrobotet, M. L., Raventos, A. and Foz, M. A distinctive cytologic pattern for diagnosing tuberculous lymphadenitis in AIDS. J. AIDS **6** (1993) 1335–1338.

Tuberculous lymphadenitis (TL) is a very common infection in human immunodeficiency virus (HIV)-infected patients. We performed fine-needle aspiration biopsy (FNAB) of enlarged lymph nodes in 57 HIV-infected patients to evaluate its usefulness in this population. We observed three cytologic patterns in 21 patients diagnosed as having TL: granulomatous lymphadenitis (GL) in 4 FNABs, necrotizing granulomatous lymphadenitis (NGL) in 7 FNABs, and necrotizing lymphadenitis (NL) in 12 FNABs. GL and NGL are already well known and considered to be highly suggestive of TL. Our results support the idea that NL should have the same diagnostic value as GL or NGL. In the group of 12 patients with NL, TL was confirmed in 11 by microbiologic methods (7 by a positive Ziehl-Neelsen stain and 4 by a positive Lowenstein culture) and in the remaining patient by a biopsy that showed NGL with acid-fast bacilli. We conclude that FNAB is a useful, inexpensive, and safe technique for diagnosing TL in HIV-infected patients. The finding of a NL pattern is suggestive enough of TL to start antituberculous treatment.—Authors' Abstract

Mehta, R. T., Keyhani, A., McQueen, T. J., Rosenbaum, B., Rolston, K. V. and Tarrand, J. J. *In vitro* activities of free and liposomal drugs against *Mycobacterium avium–M. intracellulare* complex and *M. tuberculosis*. Antimicrob. Agents Chemother. **37** (1993) 2584–2587.

We compared MICs and MBCs of various free and liposome-incorporated antimicrobial agents against several patient isolates of *Mycobacterium avium–M. intracellulare* complex and certain American Type Culture Collection strains of *M. avium, M. intracellulare,* and *M. tuberculosis*. Seven of 19 agents were selected for incorporation into liposomes. The MICs of these agents for 50% and 90% of isolates tested (MIC(50)s and MIC(90)s, respectively) ranged from 0.5 to 62 μg/ml. Members of the *M. avium–M. intracellulare* complex were resistant to killing by most of the other agents tested in the free form. However, clofazimine, resorcinomycin A, and PD 117558 showed complete killing of bacteria at concentrations ranging from 8 to 31 μg/ml, represented as MBC(90)s. Among the liposome-incorporated agents, clofazimine anti-resorcinomycin A had the highest killing effects (MBC(90)s, 8 and 16 μg/ml, respectively). Furthermore, both free and liposome-incorporated clofazimine had equivalent growth-inhibitory and killing effects on all American Type Culture Collection strains of *M. avium, M. intracellulare,* and *M. tuberculosis* tested. These results show that the antibacterial activities of certain drugs, particularly those of clofazimine and resorcinomycin, were maintained after the drugs were incorporated into liposomes.—Authors' Abstract

Meier, A., Kirschner, P., Bange, F.-C., Vogel, U. and Bottger, E. C. Genetic alterations in streptomycin-resistant *Mycobacterium tuberculosis*: mapping of mutations conferring resistance. Antimicrob. Agents Chemother. **38** (1994) 228–233.

We report on the identification of mutations associated with streptomycin resistance in *Mycobacterium tuberculosis*. Two isolates (3656 and 3976) showed a wild-type ribosomal protein, S12, but exhibited a single point mutation at 16S rRNA position 491 (C→T) or 512 (C→T), respectively. Se-

quence analysis of a third isolate (2438) revealed a single base change at 16S rRNA position 904 (A→G). This position is equivalent to invariant position 913 of the *Escherichia coli* 16S rRNA gene, an A→G transition of which has been shown previously to impair streptomycin binding and streptomycin-induced misreading *in vitro*. Surprisingly, strain 2438 harbors an additional mutation in the ribosomal protein S12 (Lys-88→Gln).—Authors' Abstract

Miller, N., Hernandez, S. G. and Cleary, T. J. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Microbiol. **32** (1994) 393–397.

The Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test (AMTD) is a direct specimen assay for the identification of *Mycobacterium tuberculosis* from respiratory samples. rRNA is amplified, and the product is detected with a specific chemiluminescent probe. We performed a retrospective evaluation of three separate respiratory specimens from each of 250 patients by using the AMTD and compared the results with those of microscopy, culturing, and a patient chart review. From the latter results, 198 patients (594 specimens) were found negative for *M. tuberculosis* by culturing and clinical criteria. The overall specificity of the AMTD after discrepancy resolution was 98.5% (585 of 594). There were 52 patients with culture-proven and/or clinically diagnosed tuberculosis. Of these 156 specimens, the organism was cultured from 142 (91%), and acid-fast microscopy was positive for 105 (67.3%). The AMTD was positive for 142 (91%) specimens from these patients. Tuberculosis patient samples were tested by a PCR assay which uses primers for amplification of the IS*6110* insertion sequence of the *M. tuberculosis* complex. The PCR assay detected 144 of the 156 (92.3%) specimens. Overall, when three specimens per patient were examined, the AMTD found all 52 patients positive for tuberculosis, while the PCR assay found 51 patients positive by agarose gel analysis and all 52 patients positive by Southern blot hybridization.—Authors' Abstract

Noordhoek, G. T., Kolk, A. H. J., Bjune, G., Catty, D., Dale, J. W., Fine, P. E. M., Godfrey-Faussett, P., Cho, S.-N., Shinnick, T., Svenson, S. B., Wilson, S. and van Embden, J. D. A. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. J. Clin. Microbiol. **32** (1994) 277–284.

PCR is, in principle, a simple and rapid test for use in the detection of *Mycobacterium tuberculosis*. However, virtually no data are available on the reliability and reproducibility of the method. In order to assess the validity of PCR for the detection of mycobacteria in clinical samples, seven laboratories participated in a blinded study of 200 sputum, saliva, and water samples containing either known numbers of *M. bovis* BCG cells or no added organisms. Each laboratory used its own protocol for pretreatment, DNA extraction, and detection of the amplification product. Insertion sequence IS*6110* was the target for DNA amplification. Several participating laboratories reported high levels of false-positive PCR results, with rates ranging from 3% to 20% and with one extreme value of 77%. The levels of sensitivity also ranged widely among the different participants. A positive PCR result was reported for 2% to 90% of the samples with 10^3 mycobacteria. Although most participants did include control tests to check the sensitivity and specificity of the PCR, the sequence of operations from sample pretreatment to purification of DNA from bacteria was not always monitored adequately. During these procedures cross-contaminating DNA was introduced and/or bacterial DNA was lost. The results of the study show that the implementation of an effective system for monitoring sensitivity and specificity is required before the PCR can be used reliably in the diagnosis of tuberculosis.—Authors' Abstract

Pancholi, P., Mirza, A., Schauf, V., Steinman, R. M. and Bhardwaj, N. Presentation of mycobacterial antigens by human dendritic cells—lack of transfer from infected macrophages. Infect. Immun. **61** (1993) 5326–5332.

When exposed to a challenge of 10 *Mycobacterium bovis* BCG cells per antigen-

presenting cell, most human monocytes engulf several organisms. In contrast, blood dendritic cells which are potent antigen-presenting cells for several antigens are not detectably phagocytic for mycobacteria. We investigated the possibility that infected macrophages might regurgitate antigens for presentation by populations of human blood dendritic cells. Macrophages were infected with *M. bovis* BCG, mixed with uninfected dendritic cells, and added to immune T cells, either bulk T cells or cloned populations from BCG vaccinees or patients recovering from tuberculosis. The macrophages were from donors who were mismatched to the T cells so that transfer of antigen to major histocompatibility complex-matched dendritic cells could be evaluated. As we describe, there was no evidence for the transfer of mycobacterial antigens from macrophages to dendritic cells in a form that was stimulatory for the T cells.—Authors' Abstract

Perosio, P. M. and Frank, T. S. Detection and species identification of mycobacteria in paraffin sections of lung biopsy specimens by the polymerase chain reaction. Am. J. Clin. Pathol. **100** (1993) 643–647.

The authors analyzed 25 paraffin-embedded lung biopsy specimens for mycobacterial DNA by the polymerase chain reaction (PCR) from patients with pulmonary mycobacterial infection demonstrated by acid-fast stain, culture, or both. DNA was extracted from 4 μM unstained paraffin sections by proteinase K digestion followed by freeze-fracturing and amplified by nested PCR with primers for the mycobacterial 65-kDa antigen gene. Mycobacterial DNA was detected in 7 of 7 wedge and 9 of 18 transbronchial biopsy specimens by PCR. Nested PCR with direct visualization on an agarose gel was as sensitive as Southern blot hybridization. Serial dilution studies demonstrated that nested PCR could detect DNA amplified from 4–8 acid-fast organisms from a paraffin section. Restriction enzyme digestion of the amplified PCR product differentiated *Mycobacterium tuberculosis* from *M. avium-intracellulare*. PCR can detect low numbers of acid-fast organisms in paraffin sections and confirm and presumptively speciate mycobacterial infection when cultures are negative or not obtained.—Authors' Abstract

Pilkington, C., Costello, A. M. D., Rook, G. A. W and Stanford, J. L. Development of IgG responses to mycobacterial antigens. Arch. Dis. Childhood **69** (1993) 644–649.

Recent studies link mycobacterial and human heat-shock protein antigens with autoimmune diseases. Little is known about the development of antibody responses to these antigens in children. IgG responses to mycobacterial antigens were studied in children living in the U.K. (an environment low in mycobacteria) who had not received BCG vaccination. Age curves of IgG response to sonicates from different species of mycobacteria were similar, suggesting that the greater part of the developing IgG response is to the common antigens shared by all mycobacteria. The major part of the IgG response was to carbohydrate antigens: lipoarabinomannan is a mycobacterial cell-wall carbohydrate and was confirmed as a major immunodominant antigen. Infants showed a marked early response to the mycobacterial 65-kilodalton (kDa) and 70-kDa heat-shock proteins, but not to the human 65-kDa heat-shock protein. The early IgG response to heat-shock proteins may reflect crossreactivity to proteins released by a wide variety of bacteria (possibly from breakdown in the gut) or recognition of other immunodominant antigens with high levels of crossreactivity to self.—Authors' Abstract

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

Radiometric MICs of clarithromycin, a new macrolide drug, were determined against five mycobactin-dependent strains of *Mycobacterium paratuberculosis* (including two Crohn's disease clinical isolates) and compared with those of other drugs which included rifampin, ethambutol, amikacin, ofloxacin, ciprofloxacin, and sparfloxacin.

Among the drugs screened, clarithromycin was the drug for which MICs were lowest against the five strains tested. As MICs were significantly below the reported C_{max} levels (about 4 μg/ml), the intracellular activity of clarithromycin against the type strain of *M. paratuberculosis* maintained in cultured macrophages was screened. Clarithromycin was able to kill the initial inoculum by more than 1 log within 7 days, and this activity was further potentiated by ethambutol. Extracellular drug combination screened by using sublethal concentrations of the drugs showed that ethambutol was able to enhance clarithromycin activity in three out of four *M. paratuberculosis* strains instead of only one out of four strains (or none in the case of ofloxacin) when associated with other drugs. These results suggest that clarithromycin may be fruitful to treat human disease in which *M. paratuberculosis* may be etiologically involved.—Authors' Abstract

Rastogi, N., Goh, K. S. and Labrousse, V. Activity of subinhibitory concentrations of dapsone alone and in combination with cell-wall inhibitors against *Mycobacterium avium* complex organisms. Eur. J. Clin. Microbiol. **12** (1993) 954–958.

MICs of dapsone (p-p'-diaminodiphenylsulfone) were determined radiometrically for ten strains each of the *Mycobacterium avium* complex (MAC) and *M. tuberculosis*. MICs ranged from 50 to 250 μg/ml for *M. tuberculosis* and from 2 to 100 μg/ml for MAC. However, at a concentration as low as 1.5 μg/ml dapsone significantly inhibited growth of MAC bacteria when used in combination with other drugs specifically acting at the mycobacterial cell-wall level. The latter drugs were used in subinhibitory concentrations, and included m-fluorophenylalanine (an inhibitor of mycoside-C biosynthesis), ethambutol (an inhibitor of arabino-galactan biosynthesis), and ethionamide (an inhibitor of mycolic acid biosynthesis). Using a radiometric method (BACTEC 460-TB), the activity of dapsone was found to be enhanced for 2/10 strains in the presence of m-fluorophenylalanine, for 3/10 strains in the presence of ethambutol and for 5/10 strains in the presence of ethionamide. A satisfactory correlation between the radiometric data and bacterial viable counts was established.—Authors' Abstract

Rook, G. A. W., Onyebujoh, P., Wilkins, E., Ly, H. M., Al-Attiyah, R,. Bahr, G., Corrah, T., Hernandez, H. and Stanford, J. L. A longitudinal study of percent agalactosyl IgG in tuberculosis patients receiving chemotherapy, with or without immunotherapy. Immunology **81** (1994) 149–154.

An increased percentage of circulating IgG molecules that lack galactose from the oligosaccharides on the C(H)2 domain correlates with disease severity in tuberculosis, rheumatoid arthritis and Crohn's disease. We have recently observed that a single injection of 10^9 autoclaved *Mycobacterium vaccae* given to tuberculosis patients 7 days after the initiation of chemotherapy causes accelerated clinical improvement, and clearance of bacilli from the sputum. We now show that this immunotherapy also causes rapid loss of agalactosyl IgG, detectable within 14–21 days; whereas chemotherapy alone causes agalactosyl IgG to rise further for up to 2 months. There is simultaneous inhibition of the antibody response to lipoarabinomannan, and transient enhancement of the tuberculin skin-test response. These findings are compatible with a shift from antibody production toward increased cell-mediated immunity. The ideal treatment for tuberculosis would supplement a truncated course of chemotherapy with an immunotherapeutic preparation able to down-regulate the Koch phenomenon and replace it with an efficiently bactericidal mechanism. We tentatively postulate that a fall in per cent agalactosyl IgG[%Gal(0)] in tuberculosis patients may be a marker of such a change.—Authors' Abstract

Saint Marc, T., Marneff, E. and Touraine, J. L. *Mycobacterium avium intracellulare*—treatment with the clarithromycin-clofazimine combination—18 cases. Presse Med. **22** (1993) 1903–1907.

Infections caused by *Mycobacterium avium intracellulare* have become worrying because of their higher frequency, their new tendency to diffuse in all tissues (notably the

blood) and the lack of curative treatment. The mortality rate remains high and the survival of patients after AIDS is diagnosed is estimated at 7.4 months. The effectiveness of new antimycobacterial drugs, observed in experiments on beige mice, has not yet been confirmed. In 18 patients suffering from this infection, either disseminated (88%) or localized in the lung (12%), a 12-week treatment with the clarithromycin-clofazimine combination has succeeded in sterilizing the pathological samples. Most patients reported a distinct improvement in their general condition, with fall of temperature, disappearance of most other symptoms, weight gain and better quality of life. Treatment was interrupted in 1 patient owing to liver toxicity. In this study the median survival of the patients after the *Mycobacterium avium* complex has been estimated at 11.4 months after the diagnosis of AIDS at 28.9 months.—Authors' Abstract

Sepkowitz, K. A. Tuberculosis and the health care worker—a historical perspective. Ann. Intern. Med. **120** (1994) 71–79.

Many hospital outbreaks of tuberculosis have occurred in recent years in the United States, resulting in tuberculosis infection and disease among health care workers and patients. Several hospital workers have died of nosocomially acquired multidrug-resistant tuberculosis. Assuring the safety of the health care worker with respect to tuberculosis has become an urgent priority. A review of the medical literature of the past 100 years reveals that our current view of tuberculosis care as an occupational hazard emerged only in the 1950s, after a fierce and extensive debate. Many authorities had felt that care of the tuberculous patient conferred a health advantage to the care provider. This paper reviews this debate and considers steps taken decades ago, before our current environmental interventions were available to ensure the safety of the health care worker.—Authors' Abstract

Silve, G., Valero-Guillen, P., Quemard, A., Dupont, M.-A., Daffe, M. and Laneelle, G. Ethambutol inhibition of glucose metabolism in mycobacterai: a possible target of the drug. Antimicrob. Agents Chemother. **37** (1993) 1536–1538.

The addition of D-arabinose, D-galactose, D-glucosamine, or D-mannose to the growth medium of *Mycobacterium smegmatis* suppressed the inhibitory effects of ethambutol both on acetate labeling of cell-wall-linked mycolic acids and on the increase in the delipidated cell dry weight. The addition of D-glucose or D-fructose had no effect. It is proposed that ethambutol inhibits an early step of glucose conversion into the monosaccharides used for the biosynthesis of structurally and biologically important cell-wall polysaccharides: arabinogalactan, arabinomannan, and peptidoglycan.—Authors' Abstract

Sirgel, F. A., Botha, F. J. H., Parkin, D. P., Van De Wal, B. W., Donald, P. R., Clark, P. K. and Mitchison, D. A. The early bactericidal activity of rifabutin in patients with pulmonary tuberculosis measured by sputum viable counts: a new method of drug assessment. J. Antimicrob. Chemother. **32** (1993) 867–875.

The activity of rifabutin and rifampin against rapidly growing, extra-cellular *Mycobacterium tuberculosis* in cavity walls was measured by counting colony-forming units (cfu) in the sputum of 74 patients with newly diagnosed, severe pulmonary tuberculosis during the first 2 days of daily chemotherapy. The fall in counts, ($\log_{10}$ cfu/ml sputum/day), was termed the early bactericidal activity (EBA). The EBA, a highly reproducible measure within groups of 10–13 patients, was −0.015 for a low EBA reference group (who received no chemotherapy) and 0.495 for a high EBA reference group (who received 300 mg isoniazid daily). The EBAs in patients receiving 300 and 600 mg rifabutin were 0.014 and 0.075, and for those taking 150, 300 and 600 mg rifampin 0.021, 0.150 and 0.204, respectively. Weight-for-weight, the ratio rifabutin to rifampin producing the same EBA was estimated to be 2.73 (95% confidence limits 1.96–3.78). Determination of the EBA is a rapid and economical method of comparing the potency in human lesions of drugs of the same type before embarking on a conventional clinical trial.—Authors' Abstract

Stokes, R. W., Haidl, I. D., Jefferies, W. A. and Speert, D. P. Mycobacteria–macro-

phage interactions—macrophage phenotype determines the nonopsonic binding of *Mycobacterium tuberculosis* to murine macrophages. J. Immunol. **151** (1993) 7067–7076.

During tuberculosis, host defenses may be determined, in part, by the capacity of resident, elicited, and activated macrophages to bind and ingest *Mycobacterium tuberculosis*. We have investigated the mechanism by which macrophages bind *M. tuberculosis* and other mycobacteria in a serum-free system. The extent of binding of *M. tuberculosis* to macrophages was dependent on the phenotype of the macrophage; thioglycollate-elicited and immune-activated macrophages bound mycobacteria poorly; whereas resident macrophages bound mycobacteria efficiently. Within "freshly" explanted macrophage populations (from 2 to 24 hr *in vitro*) poor binding of mycobacteria correlated with poor binding of C3bi-coated particles, but not with variations in the level of complement receptor 3 (CR3) expression. Induction of C3bi-coated particle binding in thioglycollate-elicited macrophages by PMA was not accompanied by enhanced *M. tuberculosis* binding. Inhibition of *M. tuberculosis* binding by resident macrophages could only be achieved using a mAb recognizing an epitope within CR3 distinct from that which recognizes C3bi. Our results suggest that nonopsonic binding of *M. tuberculosis* is mediated by a site within CR3, which is distinct from the C3bi binding site. In addition, we show a variation in the capacity of different macrophage phenotypes to bind mycobacteria nonopsonically. These data suggest that heterogeneity in macrophage-mediated clearance of *M. tuberculosis* may be a significant factor in the progression of tuberculosis.—Authors' Abstract

Straus, W. L., Ostroff, S. M., Jernigan, D. B., Kiehn, T. E., Sordillo, E. M., Armstrong, D., Boone, N., Schneider, N., Kilburn, J. O., Silcox, V. A., Labombardi, V. and Good, R. C. Clinical and epidemiologic characteristics of *Mycobacterium haemophilum*, an emerging pathogen in immunocompromised patients. Ann. Intern. Med. **120** (1994) 118–125.

The objective was to describe 13 infections caused by *Mycobacterium haemophilum*. The design was the identification of patients by microbiologic record review, followed by medical record review and a case-control study involving seven metropolitan hospitals in New York.

All patients with *M. haemophilum* infections diagnosed between January 1989 and September 1991 and followed through September 1992 were identified and the surviving patients were enrolled in the case-control study.

Infection with *M. haemophilum* causes disseminated cutaneous lesions, bacteremia, and diseases of the bones, joints, lymphatics, and the lungs. Improper culture techniques may delay laboratory diagnosis, and isolates may be identified incorrectly as other mycobacterial species. Persons with profound deficits in cell-mediated immunity have an increased risk for infection. These include persons with human immunodeficiency virus infection or lymphoma and those receiving medication to treat immunosuppression after organ transplant. Various antimycobacterial regimens have been used with apparent success to treat *M. haemophilum* infection. However, standards for defining antimicrobial susceptibility to the organism do not exist.

Clinicians should consider this pathogen when evaluating an immunocompromised patient with cutaneous ulcerating lesions, joint effusions, or osteomyelitis. Microbiologists must be familiar with the fastidious growth requirements of this organism and screen appropriate specimens for mycobacteria using an acid-fast stain. If acid-fast bacilli are seen, *M. haemophilum* should be considered as the infecting organism as well as other mycobacteria, and appropriate media and incubation conditions should be used.—Authors' Abstract

Sturgill-Koszycki, S., Schlesinger, P. H., Chakraborty, P., Haddix, P. L., Collins, H. L., Fok, A. K., Allen, R. D., Gluck, S. L., Heuser, J. and Russell, D. G. Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. Science **263** (1994) 678–679.

The success of *Mycobacterium* species as pathogens depends on their ability to maintain an infection inside the phagocytic vacuole of the macrophage. Although the bacteria are reported to modulate maturation of their intracellular vacuoles, the nature of such modifications is unknown. In this study, vacuoles formed around *Mycobacterium avium* failed to acidify below pH 6.3 to 6.5. Immunoelectron microscopy of infected macrophages and immunoblotting of isolated phagosomes showed that *Mycobacterium* vacuoles acquire the lysosomal membrane protein LAMP-1, but not the vesicular proton–adenosine triphosphatase (ATPase) responsible for phagosomal acidification. This suggests either a selective inhibition of fusion with proton-ATPase–containing vesicles or a rapid removal of the complex from *Mycobacterium* phagosomes.—Authors' Abstract

Tabet, S. R., Goldbaum, G. M., Hooton, T. M., Eisenach, K. D., Cave, M. D. and Nolan, C. M. Restriction fragment length polymorphism analysis detecting a community-based tuberculosis outbreak among persons infected with human immunodeficiency virus. J. Infect. Dis. **169** (1994) 189–192.

Analysis of restriction fragment length polymorphisms (RFLP) was used to investigate an increase in tuberculosis (TB) among noninstitutionalized human immunodeficiency virus (HIV)-infected persons in King County, Washington. Using the IS6110 insertion sequence, RFLP analysis was done on *Mycobacterium tuberculosis* isolates from 18 HIV-infected patients and 10 randomly selected patients without HIV risk factors. Six HIV-infected patients with the same *M. tuberculosis* strain had contact at one or more of three bars as their only common exposure. Two other HIV-infected persons, a patient and a health care worker who had close contact, had matching strains. Isolates from the 10 remaining HIV-infected patients and the 10 patients without HIV risk factors had different DNA patterns. Analysis of RFLP patterns revealed a community outbreak of TB among HIV-infected persons who had not been previously linked following conventional investigation by the health department. This technique deserves further evaluation as an epidemiologic tool in the investigation of TB.—Authors' Abstract

Von Reyn, C. F., Barber, T. W., Arbeit, R. D., Sox, C. H., O'Connor, G. T., Brindle, R. J., Gilks, C. F., Hakkarainen, K., Ranki, A., Bartholomew, C., Edwards, J., Tosteson, A. N. A. and Magnusson, M. Evidence of previous infection with *Mycobacterium avium-Mycobacterum intracellulare* complex among healthy subjects—an international study of dominant mycobacterial skin test reactions. J. Infect. Dis. **168** (1993) 1553–1558.

Skin tests with 0.1 ml of intermediate-strength *Mycobacterium tuberculosis* purified protein derivative (PPD) and 0.1 ml of *M. avium* sensitin were conducted on 484 healthy subjects from diverse geographic sites. Reactions of ≥ 5 mm to one antigen that exceeded the reaction to the other by ≥ 3 mm were considered *M. avium*- or PPD-dominant. PPD-dominant reactions were more frequent at sites where routine bacille Calmette-Guérin immunization is done or where there are high rates of tuberculosis: New Hampshire, 2%; Boston, 7%; Finland, 14%; Trinidad, 26%; and Kenya, 28%. However, rates of *M. avium*-dominant reactions ranged from 7% to 12% at all sites. Analysis of dominant reactions based on a more stringent 10-mm minimum reaction size showed similar trends. These data suggest that exposure to MAC is similar in developed and developing countries but that broad mycobacterial immunity is greater in developing countries and may contribute to the lower rates of disseminated MAC infections in AIDS in these areas.—Authors' Abstract

Wasilauskas, B. and Morrell, R., Jr. Inhibitory effect of the isolator blood culture system on growth of *Mycobacterium avium–M. intracellulare* in BACTEC 12B bottles. J. Clin. Microbiol. **32** (1994) 654–657.

The examination of 6938 clinical specimens collected during the period January 1991 through December 1992 suggested that the Isolator blood culture system (Wam-

pole) inhibited growth of *Mycobacterium avium–M. intracellulare* complex (MAC) in BACTEC 12B medium. Of 162 MAC blood culture isolates, 94% were recovered from Lowenstein-Jensen (LJ) medium, while only 50% were recovered from 12B medium. The time to detection with LJ medium was 18 days, while that with 12B medium was 24 days. In contrast, 62% of the 305 MAC non-blood culture isolates were recovered from the LJ medium, while 87% were found in the 12B medium. The time to detection for these cultures was also reversed, i.e., 28 days for LJ medium versus 15 days for 12B medium. Dilution studies using the lysis-anticoagulant reagent from Isolator tubes demonstrated inhibition of both clinical and American Type Culture Collection strains of MAC, even at low concentrations of lysis-anticoagulant reagent. Washing the Isolator blood sediment prior to inoculating the 12B bottles eliminated any growth inhibition. Clinical and experimental data suggest that the use of the Isolator blood culture tube with the BACTEC 12B medium is contraindicated for mycobacterial blood cultures.—Authors' Abstract

Zambardi, G., Roure, C., Boujaafar, N., Fouque, B., Freney, J. and Fleurette, J. Comparison of three primer sets for the detection of *Mycobacterium tuberculosis* in clinical samples by polymer chain reaction. Ann. Biol. Clin. **51** (1993) 893–897.

A number of studies have underlined the interest of the polymerase chain reaction (PCR) in the detection of *Mycobacterium tuberculosis* in clinical samples. Among the different parameters to be carefully studied, the choice of target gene and primers is essential. The amplification of nucleotidic sequences localized on three different target genes (groEL, IS6110, Pab) was examined in 196 clinical samples from patients with suspected tuberculosis or receiving antituberculous therapy. The results obtained after hybridization with non-radioactive labeled probes were compared with the culture data. None of the primer sets studied showed a satisfactory sensitivity (79% to 84%) suitable for it to be used alone. The false-negative specimens with the PCR tests usually corresponded to those that contained few mycobacteria. With the methods described in this study, the use of two or three primer sets located on different target genes allowed to improve the positivity rate compared to the culture and sensitivity of the test (90%–98%), particularly for paucibacillary samples. On the other hand, the interpretation was easier when concordant results were obtained.—Authors' Abstract