CURRENT LITERATURE

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

General and Historical

Eidarous, A. H., Kamel, Z. and Ahmad, F. Divorce among Saudi female leprotic patients: an experience at Ibn Sina Hospital. (Letter) Lepr. Rev. **64** (1993) 166–173.

During the 8-year period 1984–1992, 1276 cases of leprosy were diagnosed and treated at Ibn Sina Hospital in Saudi Arabia; the patients included 149 female Saudi nationals and 115 female non-Saudi nationals, the remainder being male. Of the 139 married female Saudi patients aged 17–50 years (the other 10 were excluded from the study because they were single or older) 20 (14.9%) were divorced by their husbands mainly because of leprosy. During the same time period 14 women patients with leprosy became married (in 4 cases to leprosy-free men) as did 7 male patients (to leprosy-free women). The correspondents speculate that the divorce rate would be lower if the length of hospital stay were shortened.—C. A. Brown (Trop. Dis. Bull.)

Feenstra, P. Leprosy control through general health services and/or combined programmes. (Editorial) Lepr. Rev. **64** (1993) 89–96.

The editorialist discusses the concept and rationale of integrating leprosy control activities with the general health service, pointing out the limitations of vertical leprosy control programs. Various obstacles to integration are tabulated (under the headings, commitment, planning and evaluation, and implementation) as also are the advantages of combined vertical programs (for leprosy with tuberculosis or with dermatological services). Vital considerations for the planning and implementation of integration are explored. The conclusion reached is that full utilization of the existing general health service will have to be made to ensure that all leprosy patients in need of chemotherapy receive multidrug treatment as soon as possible. This objective, essential if the goal of the elimination of leprosy as a public health problem is to be reached by the year 2000, cannot (in the opinion of the editorialist) be achieved through vertical programs in most countries where the disease is endemic. However, specialized leprosy services must be maintained within the integrated program at central and intermediate levels.—C. A. Brown (Trop. Dis. Bull.)

Salo, W. L., Aufderheide, A. C., Buikstra, J. and Holcomb, T. A. Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. Proc. Natl. Acad. Sci. U.S.A. **91** (1994) 2091–2094.

The existence of tuberculosis in the pre-Columbian Americas is controversial because the morphology of the lesion is not specific, the organism is culturally nonviable in ancient tissues, and nonpathogenic soil mycobacteria can contaminate buried bodies. We report the recovery of DNA unique to *Mycobacterium tuberculosis* from a lung lesion of a spontaneously mummified, 1000-year-old, adult female body in southern Peru. This provides the most specific evidence possible for the pre-Columbian presence of human tuberculosis in the New World.—Authors' Abstract

Chemotherapy

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 clarithromycin for lepromatous leprosy. Antimicrob. Agents Chemother. **38** (1994) 515–517.

Clarithromycin was administered to nine previously untreated lepromatous leprosy patients. Patients received two 1500-mg doses on the first day, followed by 7 days of no treatment, in order to evaluate the potential efficacy of intermittent therapy. Patients then received 1000 mg daily for 2 weeks followed by 500 mg daily for 9 weeks. The efficacy of therapy was monitored clinically, by changes in morphological index, mouse foot pad infectivity, and radiorespirometric activity of *Mycobacterium leprae* obtained from serial biopsies and by serum levels of phenolic glycolipid-I. Clarithromycin was well tolerated, with only minor side effects noted in two patients. Most patients showed reductions in morphological index and radiorespirometry 1 week after the first two doses. Within 3 weeks of starting treatment (total of 17 g of clarithromycin), biopsy-derived *M. leprae* specimens from all patients had a morphological index of zero, were noninfectious for mice, and had less than 1% of the radiorespirometric activity of pretreatment specimens. Reductions in serum phenolic glycolipid-I levels were observed for most patients at 3 weeks. Significant clinical improvement was evident after 4 weeks of treatment. All analyses indicate that clarithromycin is rapidly bactericidal for *M. leprae* in humans.—Authors' Abstract

Gordan, P. A., Grion, C. M. C., de Sousa, V., de Carvalho, V. P., Delfino, V. D. A., Mendes, M. F., Matini, A. M. and Mocelin, A. J. [Acute renal insufficiency with multidrug therapy for HD.] Hansen. Int. **17** (1992) 21–26. (in Portuguese)

From August 1989 to November 1991, 283 leprosy patients were on multiple drug therapy (MDT), which consisted of monthly supervised administration of rifampin, clofazimine, dapsone and/or thalidomide. Seven of them developed acute renal failure. Four men and three women were admitted to two general hospitals (Hospital Universitário Regional do Norte do Paraná and Hospital Evangélico de Londrina) in Londrina- PR.- Brazil, usually just after the second time they took their drugs. The patients complaints were abdominal cramps, nausea and "flu-like" syndrome (malaise, fever, myalgia and bone pain). They also passed dark brown urine and became oliguric. Three had evidence of liver damage with choluria and icterus. One patient in whom the treatment has not been, unintentionally, interrupted had two episodes of severe acute renal failure. All patients fully recovered, but dialysis therapy was needed in four. Three patients had a renal biopsy that showed normal glomeruli and tubulointerstitial nephritis with an inflammatory monuclear cell infiltrate, eosinophilis and edema in interstitium. The acute renal failure in these leprosy patients is attributed to the intermittent use of rifampin. All patients on that schedule should be carefully monitored for allergic reactions and signs and symptoms described above, and special attention should be paid to renal function. Immediate withdrawal of the drugs and fluids and electrolytes administration should be properly done in order to prevent oliguric renal failure.—Authors' English Abstract

Guerrero, C., Stockman, L., Marchesi, F., Bodmer, T., Roberts, G. D. and Telenti, A. Evaluation of the *rpoB* gene in rifampipin-susceptible and -resistant *Mycobacterium avium* and *Mycobacterium intracellulare*. (Letter) J. Antimicrob. Chemother. **33** (1994) 661–663.

Our data confirm at the molecular level that the most frequent mechanism of resistance to rifampin among clinical isolates of *Mycobacterium avium* and *M. intracellulare* does not involve alterations of the RNA polymerase subunit β. Thus, alternative mechanisms of resistance are responsible for the intrinsic resistance to rifampin in MAC. The frequency with which these isolates exhibit resistance to multiple structurally unrelated antimicrobial agents, and the existence of intermediate and high-level

resistance phenotypes are most consistent with changes in drug uptake or with efflux mechanisms. A significant permeability barrier to rifampin, that could be reduced with Tween, has been described in a type strain of *M. intracellulare* and *M. smegmatis* shown to possess a rifampin-susceptible RNA polymerase. The genetic determinants of permeability, and the possibility for additional mechanisms of resistance to antimicrobial agents in MAC, including the acquisition of exogenous genetic elements encoding for drug resistance, have not yet been established. This information will be important in the development of more active drugs against MAC.—From the Letter

Ji, B., Perani, E. G., Petinon, C., N'Deli, L., and Grosset, J.-H. Clinical trial of ofloxacin alone and in combination with dapsone plus clofazimine for treatment of lepromatous leprosy. Antimicrob. Agents Chemother. **38** (1994) 662–667.

Twenty-four patients with newly diagnosed lepromatous leprosy were allocated randomly to three groups and treated for 56 days with 400 mg of ofloxacin daily, 800 mg of ofloxacin daily, or 400 mg of ofloxacin, 100 mg of dapsone, and 50 mg of clofazimine daily plus 300 mg of clofazimine once every 28 days. The patients in all three groups demonstrated remarkable clinical improvements, accompanied by rapid decline of the morphological index in skin smears during treatment. More than 99% > 99.99%, and > 99.99% of the viable *Mycobacterium leprae* organisms had been killed by 14, 28, and 56 days of treatment, respectively, as measured by inoculation into the foot pads of immunocompetent and nude mice of organisms recovered from skin biopsy specimens obtained before and during treatment. Mild-to-moderate elevations of the serum glutamic pyruvic transaminase level were observed in four patients, all after 28 days of treatment, which returned to normal after the trial had been completed. Clinical improvement, bactericidal activity, and hepatotoxicity did not differ significantly among the three groups. Ofloxacin displayed powerful bactericidal activity against *M. leprae* in leprosy patients and may be an important component of new multidrug regimens for the treatment of leprosy. Its optimal dosage appears to be 400 mg daily, and combination with dapsone and clofazimine does not enhance its activity.—Authors' Abstract

Kailasam, S., Wise, D. L. and Gangadharam, P. R. J. Bioavailability and chemotherapeutic activity of clofazimine against *Mycobacterium avium* complex infections in beige mice following a single implant of a biodegradable polymer. J. Antimicrob. Chemother. **33** (1994) 273–279.

We have studied the bioavailability of clofazimine following administration of a single dose of the drug in the biodegradable polymer polylactic-co-glycolic acid (PLGA). We compared the levels of clofazimine achieved in the liver with single implants with those obtained with daily oral treatment. Even though the levels achieved with implants were much lower than those obtained after daily oral treatment, they were higher than the MIC of clofazimine for *Mycobacterium leprae, M. tuberculosis* and *M. avium* complex (MAC). Experimental studies in beige mice after infection with MAC strain 101 showed similar reductions in cfu counts, after both single dose polymer and daily oral treatment. Macroscopically, hyperpigmentation giving an orange-yellow color to all visceral organs, was seen in animals after daily oral treatment but not in those animals that received polymer implants.—Authors' Abstract

Lim, J. T. E. and Tan, T. Efficacy and safety of multidrug therapy in paucibacillary leprosy in Singapore. Lepr. Rev. **64** (1993) 136–142.

A total of 49 patients with paucibacillary leprosy (PB) who completed multidrug therapy (MDT) between 1985 and 1990 were analyzed retrospectively for efficacy and complications; 20 (40.8%) patients had borderline-tuberculoid (BT), 13 (26.5%) had tuberculoid (TT), 1 (2.1%) had indeterminate (I) and 15 (30.0%) had pure neural (N) leprosy; 26 patients (76.5% of 34 non-neural leprosy) were skin biopsied for histological cure before MDT was stopped. Of these 26 patients, 19 had histological clearance at 6 months while the remaining 7 cleared beyond 1 year (18–36 months). The remain-

ing 8 non-neural patients who refused rebiopsy had MDT for 6–8 months and the MDT was stopped when there was clinical clearance. Of the 15 neural leprosy patients, 11 were given MDT for 6 months while the rest had 12–18 months of treatment; 1 patient with neural leprosy, who was treated for 6 months, relapsed with BT leprosy 18 months post treatment.

There were few complications among the 49 patients—4 (8.2%) patients developed reaction to dapsone, 1 (2.0%) had the dapsone syndrome, 2 (4.1%) had hemolytic anemia and 1 (2.0%) had dapsone hepatitis; 7 (14.3%) patients had type 1 reaction.—AS (Trop. Dis. Bull.)

Ochonisky, S. and Revuz, J. Thalidomide use in dermatology. Eur. J. Dermatol. **4** (1994) 9–15.

Since the discovery of the dramatic efficacy of thalidomide in erythema nodosum leprosum, many works have demonstrated antiinflammatory and immunosuppressive properties of this drug. During the past two decades, thalidomide has been shown to be effective in several dermatological diseases such as actinic prurigo, discoid lupus erythematosus, prurigo nodularis, recurrent severe aphtosis and Jessner's lymphocytic infiltration of the skin. Extra-dermatological indications are now being tested, such as graft-versus-host disease, rheumatoid arthritis or systemic lupus erythematosus. The precise way of thalidomide action remains unknown. The pharmacokinetics of the drug could explain in part its particular efficacy in muco-cutaneous disorders. Since the teratogenic effect of thalidomide can now be controlled, the neurotoxicity of the drug is now the principal factor limiting its use.—Authors' Abstract

Pan, Y., *et al.* [A survey of relapse in 20,091 persons whose leprosy has been cured.] China Lepr. J. **9** (1993) 202–205. (in Chinese)

A retrospective analysis with the life-table method on the relapse in 20,091 cured cases of leprosy from 1955 to 1990 in Shandong Province is presented. The result showed that the relapse was relevant to the drugs used in the treatment. The relapse rates in MB and PB were 6.84 and 4.29/1000 person years, respectively. In MB patients treated with dapsone (DDS) and DDS plus rifampin (RFP) they were 7.92% (446/5628) and 1.07% (15/1405), respectively, and in PB treated with thiacetazone, DDS and WHO-MDT 12.6% (96/763), 5.4% (589/10,903) and 0.18% (2/1101), respectively, being significantly lower in the group of WHO-MDT ($p < 0.001$). Most important was that there was no relapse in 256 MB and 35 PB cases treated with WHO-MDT; 96.3% of the relapses in MB and 90.1% in PB occurred within 15 years after cured and the drug used in them mainly was DDS in monotherapy but DDS still is effective in retreatment for them. The authors suggest that the follow-up period should not be less than 15 years for those cured with DDS monotherapy and some short-term MDT would be given to them in order to prevent the relapse.—Authors' English Abstract

Premkumar, R. and Dave, S. L. Impact of multidrug therapy on health personnel in their level of job satisfaction. Indian J. Lepr. **65** (1993) 429–438.

This study examines the "service" factors of the health professionals working in the National Leprosy Eradication Program (NLEP) resulting from the introduction of multidrug therapy (MDT) technology, and their impact on their job satisfaction. The findings show that both among physicians and paramedicals, the significant chemotherapeutic dissatisfaction observed before the introduction of MDT has been replaced by a moderately positive satisfaction. This was much higher than the other incentives like pay, promotional prospect and job significance within NLEP and the community. It was also consistent over 5 years which was not the case with hydnocarpus and monotherapy technologies. Intercorrelation matrix test revealed three positive intercorrelations. First, personnel associated technology with personal progress which provided a sense of accomplishment while also satisfying their economic needs; second, they saw it as a mode of developing relationships with their clients; and third, it improved their self-image in the community. However, this satisfaction may not be static when there is a reduction of work load or the lep-

rosy program is integrated into general health services. Therefore, while planning these changes care must be taken that the present level of technological satisfaction is maintained or further improved.—Authors' Abstract

Saito, H., Tomioka, H. and Hidaka, T. Therapeutic efficacy of a newly synthesized benzoxazinorifamycin KRM-1648, combined with other antimicrobials against *Mycobacterium leprae* infection induced in nude mice. (Abstract) Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 81–82.

Multidrug therapy using rifampin (RFP), clofazimine (CFZ), and diaminodiphenylsulfone (DDS) is considered to be the most effective treatment for patients with leprosy. However, it takes at least 6 months and up to 4 years or more to achieve appreciable results in the control of multibacillary leprosy patients, even using this multidrug regimen. Prolongation of drug administration tends to generate drug-resistant *M. leprae*. The development of new protocols for multidrug therapy which enable more rapid therapy for leprosy patients and the use of other types of antileprosy drugs is therefore considered urgent. A newly developed benzoxazinorifamycin, KRM-1648, is known to have excellent *in vitro* and *in vivo* antimycobacterial activities. Its activity is much more potent than RFP. In this study, we evaluated the therapeutic efficacy of KRM-1648 against *M. leprae* infection induced in athymic nude mice. Moreover, we evaluated *in vivo* anti-*M. leprae* activity of KRM-1648 in combination with other agents. Furthermore, combined effect of either RFP or DDS with ofloxacin (OFLX), which we previously found to have appreciable antileprosy activity, was examined. BALB/c nude mice infected s.c. with 1×10^6 of *M. leprae* Thai-53 strain were given test drugs finely emulsified or dissolved in gum arabic-Tween 80 solution, by gavage, once daily, six times per week, for up to 50 days from day 31 to day 80, and the animals were observed for the growth of organisms at the hindfoot pad during the 12 months following infection. KRM-1648 markedly reduced the growth of leprosy bacilli at the site of infection in a dose-dependent manner (0.001 to 0.01 mg/mouse/day), and its therapeutic efficacy was much greater than that of RFP. Therapeutic efficacy of KRM-1648 was considerably improved by feeding mice with the drug-containing feed. *In vivo* anti-*M. leprae* activity of KRM-1648 (0.001 mg/mouse) was also intensified by combined use of other agents, such as DDS (0.2 mg/mouse) and CFZ (0.1 mg/mouse), as compared to the efficacy of each drug alone. Similarly, *in vivo* antileprosy activity of OFLX (3 mg/mouse) was much improved by combination with either RFP (0.01 mg/mouse) or DDS (0.2 mg/mouse), as compared to the efficacy of each drug alone.

From these findings, it seems preferable to use KRM-1648 instead of RFP in the multidrug regimens for clinical control of leprosy patients, if recommended that KRM-1648 has similar or more improved level of toxicity, pharmacokinetics, and cost, as compared to RFP. Furthermore, some new quinolones having appreciable *in vivo* anti-*M. leprae* activity, such as OFLX and sparfloxacin, may be used in the multidrug regimens for leprosy patients. Further studies are underway to evaluate the therapeutic efficacy of KRM-1648 combined with other agents when they are given to mice in the same dosages and protocols as to leprosy patients.

Clinical Sciences

Awofeso, N. Inventory of skin smear practices in 6 leprosy control programmes in Nigeria. Lepr. Rev. **64** (1993) 150–156.

The author describes a study to determine the quality of smearing, the quality of reagents used, the accuracy of microscope reading, and other factors relevant to the improvement of the quality of slit-skin smear results. This study was carried out in leprosy programs in each of Nigeria's 4 Primary Health Care Zones and included: (1)

a brief questionnaire about skin smear practices, (2) a sample of a smear from the laboratory of the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa Ethiopia, to serve as a test reading for laboratory technicians in the above areas, and (3) a request that a sample of slides, together with the (local) reading of the bacteriological index (BI), be sent to ALERT in Addis Ababa for quality control, comment and advice.

The study revealed that only about 50% of the leprosy hospitals in Nigeria have facilities and manpower for skin-smear examination in leprosy. Most laboratory workers were demoralized and unhappy and several were untrained. Only 12% of smears sent from the units were judged to be of good quality in Addis Ababa, only 1% were considered to be properly stained and only 59% showed a good correlation in the reading of the BI.

The author describes the situation as deplorable. He makes recommendations for improvement in training, supervision and support, [but the situation, as described in this article, is so bad that it may be more realistic to first establish one central reference laboratory of high quality, for the accurate examination of smears from selected patients in whom the examination is considered to be essential].—A. C. McDougall (Trop. Dis. Bull.)

Grossman, D., Rapini, R. P., Osborne, B. and Duvic, M. Emergence of leprosy in a patient with mycosis fungoides. J. Am. Acad. Dermatol. **30** (1994) 313–315.

A patient with mycosis fungoides that had progressed to tumor stage responded to chemotherapy and electron beam treatment, but 6 years later a peripheral neuropathy, extensive plaques, erythroderma, and enlarged pinnae containing acid-fast organisms developed while he was being treated with photopheresis. The skin lesions cleared with administration of rifampin and dapsone, but a reversal reaction biopsy specimen showed features of both mycosis fungoides and leprosy. This case raises the question of whether there may be an association between mycosis fungoides and leprosy.—Authors' Abstract

Kimura, T. and Goto, M. Existence of senile plaques in the brain of elderly leprosy patients. (Letter) Lancet **342** (1994) 1364.

In a previous publication (*Lancet,* 1992, 340, 978) Namba, *et al.* had postulated that their failure to detect senile plaques in the hippocampal, parahippocampal, and occipitotemporal regions of the brain in elderly non-demented leprosy patients was due to their *Mycobacterium leprae* infection or to use of antiinflammatory drugs. The present correspondents present contrasting findings. They found senile plaques in parahippocampal regions of 13 (48%) of 27 non-demented leprosy patients aged 70–97 years who had died in their leprosarium in Japan. In comparison, plaques were found in 4 (33%) of 12 non-demented patients without leprosy who had died in acute medical wards of local general hospitals. The correspondents concede that different detection methods were used in the two studies but conclude that the hypothesis that the use of antiinflammatory drugs and/or *M. leprae* infection suppresses senile plaque formation "is dubious."—C. A. Brown (Trop. Dis. Bull.)

Kirsztajn, G. M., Nishida, S. K., Silva, M. S., Lombardi, C., Ajzen, H. and Pereira, A. B. Specific and nonspecific aspects of humoral immune response in leprosy. Braz. J. Med. Biol. Res. **27** (1994) 43–54.

We have studied some generic and specific aspects of the humoral immune response in 96 patients with leprosy (29 paucibacillary and 67 multibacillary individuals). We determined serum immunoglobulins (IgM, IgG and IgA), CH50, C1q, C3 and C4, circulating immune complexes (CIC), C-reactive protein (CRP), rheumatoid factor (RF) and antinuclear antibodies. No specific pattern of general humoral immune changes could be observed. The specific immune response was studied by the detection of specific IgM anti-*Mycobacterium leprae* antibodies. An immunoradiometric assay (IRMA) and an ELISA were compared for clinical effectiveness. IRMA showed greater sensitivity for the serodiagnosis of leprosy as compared to ELISA (88.1% vs 58.2% for multibacillary patients and 20.7% vs 10.3% for paucibacillary leprosy patients). Specificity was 96% for IRMA and 97% for ELISA. Our results indicate

that nonspecific changes in the humoral immune response are of little value in assessing leprosy patients and that immune assays for the detection of specific anti-*M. leprae* antibodies may be of value in the diagnosis, study and follow-up of these patients.—Authors' Abstract

Lucas, S. Human immunodeficiency virus and leprosy. (Editorial) Lepr. Rev. **64** (1993) 97–103.

The editorialist discusses potential interactions between infections with HIV and *Mycobacterium leprae*: namely, the 2 infections could be reciprocally promoting; there could be clinical, therapeutic and epidemiological associations between leprosy and HIV infection; leprosy may influence the course of HIV disease; false-positive HIV serology in leprosy patients (eliminated with recent tests); enhanced neuropathy in co-infected individuals; non-leprosy lesions resembling leprosy. The conclusion reached is that there is little evidence for the expected interactions. Clinical leprosy does not appear to be more common in HIV-positive than in HIV-negative people in areas where both infections are endemic. Similarly, there is no evidence that the paucibacillary/multibacillary distribution of patients is altered by HIV infection. Reports that neuritis is more severe and that reversal reactions after therapy are more common in co-infected patients are inconclusive.—C. A. Brown (Trop. Dis. Bull.)

Mennen, U., Howells, C. and Wiese, A. J. Serum zinc, sodium, calcium, magnesium and potassium levels and standard diet in leprosy patients. Indian J. Lepr. **54** (1993) 415–421.

Serum zinc levels were estimated in different types of leprosy by means of the atomic absorption spectrophotometry method in 64 leprosy patients, composed of tuberculoid tuberculoid (TT) (5), borderline tuberculoid (BT) (6), borderline borderline (BB) (10), borderline lepromatous (BL) (13), lepromatous (LL) (14) and burnt-out leprosy (BO) (16). These findings were evaluated in comparison to 86 normal control subjects who were served the same standard diet. Serum zinc levels were significantly low in the total leprosy group. The findings of this study are of clinical importance since zinc deficiency can be one of the factors involved in suppression of cell-mediated immunity (CMI) in lepromatous leprosy. This again has a bearing on the management of wounds and wound healing. This study also reveals that altered levels of the serum elements (e.g., calcium and sodium) have a direct association with the disease and not with food deprivation.—Authors' Abstract

Sharma, V. K., Kaur, S., Radotra, B. D. and Kaur, I. Tongue involvement in lepromatous leprosy. Int. J. Dermatol. **32** (1993) 27–29.

Involvement of the oral cavity in lepromatous leprosy is well-documented. The tongue may demonstrate multiple nodules, thickening, and scarring. Ten consecutive untreated patients [in India] with lepromatous leprosy with a bacterial index of 4+ or more were clinically and histopathologically studied for evidence of tongue involvement. Three patients showed clinical tongue involvement, as a nodulo-plaque lesion in 1 patient and fissured tongue (lingua plicata) in 2 patients; the tongue was clinically normal or showed nonspecific changes in the remaining 7 patients. Histologic evidence of tongue involvement by lepromatous process was seen in 6 patients, including 3 without clinical involvement. [The authors] conclude from this study that the tongue is as prone to involvement by lepromatous process as buccal and palatal mucosa.—S. B. Lucas (Trop. Dis. Bull.)

Immuno-Pathology

Anderton, S. M., Vanderzee, R., Noordzij, A. and van Eden, W. Differential mycobacterial 65-kDa heat shock protein T cell epitope recognition after adjuvant arthritis-inducing or protective immunization protocols. J. Immunol. **152** (1994) 3656–3664.

Immunization of Lewis rats with heat-killed *Mycobacterium tuberculosis* (Mt) in

mineral oil induces adjuvant arthritis (AA), associated with T-cell responses to residues 180–188 of the mycobacterial 65-kDa heat-shock protein (hsp65). Preimmunization with hsp65 protects rats against AA and other forms of arthritis. Several explanations for these protective effects have been proposed, including enhanced responsiveness to protective epitopes in hsp65, down-regulation of T-cell responses to the 180–188 epitope, and activation of self-hsp60-reactive T cells. To assess the potential of these hypotheses, we analyzed hsp65 T-cell epitopes recognized after immunization of Lewis rats with Mt or hsp65. Here we identify nine RT1.B-1-restricted T-cell epitopes in hsp65. Mt immunization induced T-cell responses in which the 180–188 epitope was dominant; whereas hsp65 immunization resulted in a co-dominance of this and two further epitopes, 216–225 and 226–235. Two minor epitopes were recognized after hsp65 but not Mt immunization. These results indicate that hsp65 preimmunization does not down-regulate responses to the AA-associated epitope, but does enhance responses to several hsp65 epitopes that are minor or absent after the AA-inducing immunization protocol. Crossreactive T-cell recognition of hsp65 and rat hsp60 was limited to a single epitope (256–265), recognized after hsp65 immunization, but poorly recognized after Mt immunization. This study provides the necessary basis for elucidating the T-cell events involved in the protective effects of hsp65 preimmunization.—Authors' Abstract

Constant, P., Davodeau, F., Peyrat, M. A., Poquet, Y., Puzo, G., Bonneville, M. and Fournie, J. J. Stimulation of human gamma/delta T cells by nonpeptidic mycobacterial ligands. Science **264** (1994) 267–270.

Most human peripheral blood gamma/delta T lymphocytes respond to hitherto unidentified mycobacterial antigens. Four ligands from *Mycobacterium tuberculosis* strain H37Rv that stimulated proliferation of a major human gamma/delta T-cell subset were isolated and partially characterized. One of these ligands, TUBag4, is a 5′ triphosphorylated thymidine-containing compound, to which the three other stimulatory molecules are structurally related. These findings support the hypothesis that some gamma/delta T cells recognize nonpeptidic ligands.—Authors' Abstract

Damle, A. and Mahadevan, P. R. Nature of peritoneal macrophages from DCC immunized mice. Indian J. Lepr. **65** (1994) 405–414.

Peritoneal macrophages from mice immunized with the delipidified cell component (DCC) of *Mycobacterium leprae* showed changes in various parameters such as increased protein synthesis, levels of hydrolytic enzyme and augmented phagocytic ability indicating activation of the cells. Furthermore, the surface structure of the cells was quite different from that of the macrophages of normal mice. These observations indicate that the peritoneal macrophages have been activated to phagocytose and kill *M. leprae* better in the immunized mice. The ability to kill the pathogen by these cells was reported by us earlier.—Authors' Abstract

Drowart, A., Chanteau, S., Huygen, K., Yernault, J. C. and Van Vooren, J. P. The effects of chemotherapy on antibody levels directed against PGL-1 and 85A and 85B protein antigens in lepromatous leprosy patients. (Abstract) Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 54–55.

IgG antibodies against antigen 85A and 85B from *Mycobacterium bovis* BCG, IgM antibodies against PGL-I and circulating PGL-I antigen were measured in the serum of 11 patients with lepromatous leprosy receiving multidrug therapy. Before treatment, 6 patients were reactive to antigen 85A, 10 patients to antigen 85B, and 11 patients to PGL-I and to circulating PGL-I. After 2 years of multidrug therapy, PGL-I antigen negativated in all of the patients, except for two who were not compliant to treatment. IgG antibodies directed against the 85A and 85B antigens and IgM antibodies against the PGL-I antigen also decreased significantly during treatment but more slowly. Determination of circulating PGL-I antigen remains the most appropriate tool for monitoring lepromatous leprosy under polychemotherapy.

Falcone, V., Bassey, E. B., Toniolo, A., Conaldi, P. G. and Collins, F. M. Differential release of tumor necrosis factor-alpha from murine peritoneal macrophages stimulated with virulent and avirulent species of mycobacteria. FEMS Immunol. Med. Microbiol. **3** (1994) 225–232.

The ability of *Mycobacterium tuberculosis* H37Rv and H37Ra, *M. bovis* BCG and *M. smegmatis* to induce the secretion of tumor necrosis factor-alpha (TNF-α) by cultured murine peritoneal macrophages is inversely related to their virulence. The avirulent species of mycobacteria which were unable to persist in macrophages were capable of inducing significant levels of TNF-α compared to that formed in cultures infected with the virulent *M. tuberculosis* H37Rv. This difference was also associated with an inherent toxicity by live H37Rv for macrophage cultures. Heat-killed H37Rv was nontoxic and induced significant levels of TNF-α; in contrast, live and heat-killed suspensions of avirulent mycobacteria had an equivalent ability to trigger TNF-α secretion. The TNF-α response was dose-dependent, related directly to the percentage of infected cells, and peaked 6–12 hr post-infection. An early and vigorous TNF-α response appears to be a marker of macrophage resistance, while the downregulation of this response seems associated with macrophage toxicity and unrestricted mycobacterial growth.—Authors' Abstract

Gilburd, B., Ziporen, L., Zharhary, D., Blank, M., Zurgil, N., Scheinberg, M. A., Guedes, L. H., Gershwin, M. E. and Shoenfeld, Y. Antimitochondrial (pryuvate dehydrogenase) antibodies in leprosy. J. Clin. Immunol. **14** (1994) 14–19.

Sera from 69 patients with leprosy but without liver involvement were assayed for the presence of mitochondrial pyruvate dehydrogenase (PDH)-specific autoantibodies by enzyme-linked immunoabsorbent assay (ELISA), immunoblotting using PDH as an antigen and by enzymatic inhibition test. Twenty-seven of the leprosy serum samples (39.1%) were found to react with PDH by ELISA. However, unlike sera from primary biliary cirrhosis (PBC) patients, none of these were able to inhibit the PDH enzymatic activity. By immunoblotting, it was found that only 2 of the 27 positive sera recognized the 74-kDa protein of the PDH complex, which is recognized by sera of most PBC patients. The antimitochondrial antibodies in leprosy most probably recognize different epitopes than those in PBC. These findings may indicate that anti-PDH autoantibodies in patients with leprosy may arise by polyclonal B-cell stimulation and may represent natural anti-PDH autoantibodies.—Authors' Abstract

Goto, M., Minauchi, Y., Nobuhara, Y. and Sato, E. Immunohistochemical demonstration of *Mycobacterium leprae* in the nervous system of long-term cured leprosy patients using a *M. leprae* specific anti-PGL antibody. Jpn. J. Trop. Med. Hyg. **21** (1993) 117–121.

The authors report on an immunocytochemical study of neural tissue of leprosy patients, using anti-BCG and anti-phenolic glycolipid-I (PGL-I) antibodies. The presence of persistent *Mycobacterium leprae* antigen in peripheral nerves after effective chemotherapy is well known. This study purports to demonstrate the presence of antigen also in the spinal cord and cerebrum of treated patients, both lepromatous and tuberculoid.—S. B. Lucas (Trop. Dis. Bull.)

Huygen, K., Launois, P., De Bruyn, J., Drowart, A., Van Vooren, J. P., N'Diaye, M., Sarthou, J. L., Grimaud, J. and Millan, J. T and B cell response to purified filtrate antigen 85 from *M. bovis* BCG in leprosy patients and their contacts. (Abstract) Ann. Soc. Belg. Med. Trop. **73** Supp. 1 (1993) 76–77.

The precise immune reactions involved in the actual protection against mycobacteria remain unclear, but living mycobacteria have generally been reported to be more effective in the generation of specific acquired resistance than killed mycobacterial preparations. It has therefore been argued that secreted, extracellular antigens, present in large amounts in mycobacterial culture filtrates, and produced only by actively metabolizing bacteria, could be essential for the induction of protective immunity. One of the major secreted antigens from *Mycobacterium bovis* BCG is the 30–32 kDa protein, also called antigen 85. Ag85 is a protein

family with three members, i.e., 85A, 85B and 85C. Little is known about its function except that it binds to fibronectin and is not a stress protein. The genes encoding for these three components in *M. leprae* have been cloned and demonstrate about 85% homology with the genes in BCG or *M. tuberculosis*. Ag85 has proven to be a dominant B-cell antigen in leprosy. High antibody levels against antigen 85 can be detected in leprosy patients and the 85B component is particularly useful for the serodiagnosis of the multibacillary, lepromatous form of leprosy. T cells from leprosy patients also react to Ag85 and show a marked parallelism of responsiveness toward whole *M. leprae* and the purified Ag85 from BCG. Indeed positive lymphoproliferation and IFN-gamma secretion in response to Ag85 could be demonstrated in 10/10 tuberculoid leprosy patients reactive to *M. leprae*; whereas T cells from 25/29 patients with lepromatous leprosy were completely unreactive to Ag85. Furthermore, Mitsuda-negative healthy controls (N = 11) did not react to Ag85, whereas all Mitsuda-positive controls (N = 14) did react to Ag85. Recently we have analyzed T-cell reactivity against Ag85 in 45 leprosy contacts. All 14 Mitsuda-positive contacts reacted to *M. leprae* and to Ag85. Three Mitsuda-negative contacts reacted weakly to *M. leprae* and to Ag85. The other 28 Mitsuda-negative contacts did not react to *M. leprae* but 9 of them reacted to Ag85. Thirty-four contacts could be retested 16 months later. None of them developed leprosy. Eleven initial Mitsuda-positive contacts remained lepromin-positive at follow-up and reactive to *M. leprae* and Ag85. Fourteen initially Mitsuda-negative contacts converted and all reacted *in vitro* to *M. leprae* and Ag85. Finally 9 contacts remained Mitsuda-negative and 7 of them were unreactive to Ag85. *In vitro* reactivity against Ag85 at baseline in Mitsuda-negative contacts was associated with subsequent conversion to lepromin reactivity in 7/9 subjects. These results indicate that reactive T cells against Ag85 develop very early during *M. leprae* infection (even before actual Mitsuda skin test conversion) and that Ag85 is a potentially protective T-cell immunogen. Furthermore, these data underline the immunodominant character of Ag85 in leprosy and are suggestive of important common T-cell epitopes shared between *M. leprae* and *M. bovis* BCG.

Kashala, O., Marlink, R., Ilunga, M., Diese, M., Gormus, B., Xu, K. Y., Mukeba, P., Kasongo, K. and Essex, M. Infection with human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic viruses among leprosy patients and contacts—correlation between HIV-1 cross-reactivity and antibodies to lipoarabinomannan. J. Infect. Dis. **169** (1994) 296–304.

To determine the association between leprosy and human retroviral infections, 57 leprosy patients, 39 leprosy contacts, and 500 pregnant women were investigated serologically for antibodies to human immunodeficiency virus type 1 (HIV) and human T-cell lymphotropic virus (HTLV) types I and II. Antibodies to *Mycobacterium leprae* phenolic glycolipid-I (PGL-I), and lipoarabinomannan (LAM) were also analyzed. A low prevalence of HIV-1 infection was observed among leprosy patients (3.5%), leprosy contacts (0), and pregnant women (3.6%). Antibodies to HTLV-I but not -II were found more often in leprosy patients (8.7%) and contacts (12.8%) than in pregnant women (0). Sera from leprosy patients and leprosy contacts were often false-positive for HIV-I by ELISA and were indeterminate by Western blot. LAM IgM and PGL-I IgM antibodies in sera from leprosy patients yielded significant crossreactivities with HIV-1 pol and gag proteins. These data suggest that mycobacterial cell wall antigens may share common epitopes with HIV. Caution should be exercised when interpreting HIV-1 ELISA and Western blot data from regions where leprosy or other mycobacterial diseases are endemic.—Authors' Abstract

Kumar, B., Jaswal, S., Vaishnavi, C., Thakur, M., Kaur, S. and Ganguly, N. K. Release of acid hydrolases in spectrum of human leprosy. J. Hyg. Epidemiol. Microbiol. Immunol. **36** (1992) 405–411.

Release of acid hydrolases by blood monocytes (BM) of leprosy patients both before and after 6 months of chemotherapy was measured fluorimetrically. Monocyte cultures were set up for spontaneous as well

as zymosan-dependent enzyme release measured after 2 hr and 24 hr of culture. In the untreated multibacillary group (BL/LL) a significantly higher ($p < 0.001$) release of both β-glucuronidase (BG) and *N*-acetyl glucosaminidase (NAG) was observed compared to the paucibacillary group (BT/TT) and healthy controls. On comparing the BT/TT group with controls a significant decrease ($p < 0.001$) in zymosan-dependent NAG release was observed in the former group at 2 hr culture. After 6 months of antileprosy therapy, a significant decrease ($p < 0.05$) in BG release was observed from BM of multibacillary patients; whereas NAG activity increased significantly ($p < 0.05$) in the paucibacillary group compared to the controls. The results of the present study suggest that nonoxidative metabolic status of BM varies within the leprosy spectrum.—Authors' Abstract

Mauff, G., de Messias, I. J. T., Santamaria, J., Reis, A. and Brenden, M. Erythema nodosum leprosum is highly associated with deficiency of the complement isoprotein C4B (C4B*Q0). (Abstract) Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 60.

Genetic susceptibility to manifestation, clinical course and immunopathology of leprosy has been postulated for many years. Associations were predominantly observed with major histocompatibility complex (MHC) class II (HLA-DR, DQ) antigens, in some studies with class III complement allotypes of C2, factor B (BF), and C4A and B. In the present investigation 109 leprosy patients from southern Brazil with lepromatous leprosy (LL, N = 73), and with tuberculoid, borderline and indeterminate leprosy (TIBL, N = 36) were studied for MHC class III alleles and compared with 172 healthy control individuals. The patients were classified according to Ridley and Jopling, and also with regard to erythema nodosum leprosum (ENL). C2, BF, and C4A and B allotypes were determined by standard technologies including Western blots for C2 and C4 variant alleles with monoclonal and polyclonal antibodies. Non-expressed ("silent") C4 alleles in hemizygously deficient individuals were estimated semiquantitatively on the basis of C4A to B isotype ratio and by the MASC ("minimal chi-Square") method. Among the LL patients 46 presented with ENL. In all LL patients taken together, the C4 deficiency allele (C4B*Q0) was significantly more often seen than in the controls (26/73 against 20/172, $p < 0.00003$; level of significance if corrected for the number of observed alleles: $p = 0.0045$). This difference was even more pronounced when only LL patients with ENL were compared with the controls (22/46, $p < 0.0000004$), and still highly significant between ENL-positive and -negative LL patients (22/46 against 4/27, $p < 0.006$). All patients who were homozygously C4B deficient had ENL and the majority of these possessed the BF*F1 allele. Our findings are further support for the role of the C4B isoprotein in immune complex diseases involving bacterial or fungal antigens, and especially for the MHC class III dependent regulation of immunity in leprosy.

Narayanan, R. B., Natarajan, M., Bagga, A. K. and Katoch, K. Reduction in CDI positive epidermal Langerhan's cell numbers in leprosy lesions following epicutaneous application of 2:4 dinitrochlorobenzene. Indian J. Lepr. **65** (1993) 423–427.

A study was made on Langerhans cells (LC) in the dermal lesions of leprosy after epicutaneous application of 2:4 dinitrochlorobenzene (DNCB) to the lesion. LC were quantitated with OKT6 monoclonal antibody and indirect immunofluorescence. A depletion or reduction in the numbers of CD1 + epidermal LC was observed at both 4 and 24 hours after the application of DNCB in the lesions of both tuberculoid and lepromatous leprosy, compared to untreated lesions.—Authors' Abstract

Scollard, D. M. Time and change: new dimensions in the immunopathologic spectrum of leprosy. Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 5–11.

Accumulating evidence of diversity within leprosy types suggests that the traditional application of the concept of an immunopathologic spectrum has been too narrow; new dimensions are necessary to accommodate these observations. The spectrum is a continuous one, not a series of steps,

and heterogeneity within each type may be based on factors both inherited ("causal") and acquired ("consequences"). Current clinical and epidemiological data indicate that time is an important variable in several respects. Long increments of time must be considered; major shifts in immune responsiveness from one end of the spectrum to the other may occur in individual patients over many years. The age of onset and duration of illness may generate different risks of immuno-inflammatory reactions in leprosy, also after many years. New methods must therefore be devised to evaluate the immunologic status of patients over several years. The powerful tools of molecular biology and immunology are likely to be more fruitful if applied with new questions in mind to help us understand the diversity within the spectrum and the differing potential for changes in immunologic response over a long period of time.—Authors' Summary

Shaw, M. A., Atkinson, S., Dockrell, H., Hussain, R., Linslainson, Z., Shaw, J., Ramos, F., Silveira, F., Mehdi, S. Q., Kaukab, F., Khaliq, S., Chaing, J. and Blackwell, J. An RFLP map for 2Q33-Q37 from multicase mycobacterial and leishmanial disease families—no evidence for an *Lsh/Ity/Bcg* gene homologue influencing susceptibility to leprosy. Ann. Hum. Genet. **57** (1993) 251–271.

The mycobacterial diseases leprosy and tuberculosis (TB) and the leishmaniases are characterized by a wide spectrum of disease phenotypes, and by the fact that the majority of individuals exposed to the causative organisms *Mycobacterium leprae, M. tuberculosis* and *Leishmania* sp. become infected but do not present with clinical disease. In order to determine whether a human homolog to the murine macrophage-resistance gene *Lsh/Ity/Bcg* influences susceptibility to human disease, multicase families for all three diseases have been collected, and linkage analysis performed using a panel of markers in the region of human chromosome 2q33-q37 known to be conserved with the *Lsh/Ity/Bcg*-containing region of murine chromosome 1. Because of the paucity of available polymorphic markers/linkage information for 2q33-q37 data from 35 multicase leprosy, TB and visceral leishmaniasis families (310 individuals) were first pooled to produce a detailed RFLP map of the region. Peak LOD scores well in excess of 3 were observed for linkage between adjacent pairs of a more proximal (2q33-q35) set of markers CRYGP1, MAP2, FN1, TNP1, VIL1 and DES, and between adjacent pairs of a more distal (2q35-q37) set COL6A3, D2S55 and D2S3. These peak LOD scores and the corresponding values for B were used in the MAP92 program to generate a multiple two-point map with gene order/map intervals (cM) of: CRYGP1-4.65-MAP2-3.45-FN1-5.95-TNP1-3.41- VIL1-3.01DES-20 (14-COL6A-10.91-D2S55-3.67-D2S3. Although local support for the placement of loci in this order was weak (LOD < 2, except for DES-COL6A3 where LOD = 6.02), the map is consistent with the gene order for those loci (Cryg, Fn-1, Tp-1, Vil, Des, Col6a3) previously mapped in the mouse. Data from 17 multicase leprosy families (149 individuals) were further analyzed for linkage between a putative disease susceptibility locus (DSL) controlling susceptibility to leprosy *per se* and each of the marker loci. Assuming 100% penetrance for the susceptibility allele, no positive LOD score was obtained for linkage between the DSL and any of the marker genes. Instead, the data provide convincing evidence (LOD scores < -2) that a DSL does not fall within 10–20 cM of CRYGP1, MAP2, TNP1, VIL1, DES or D2S55, or within 5–10 cM of FN1, COL6A3 or D2S3. This effectively excludes a putative DSL controlling susceptibility to leprosy *per se* from the entire region 2q33-q37. Even with reduced penetrance (80% and 60%) for the susceptibility allele, the data argue against a putative DSL within the region TNP1-DES where the murine *Lsh/Ity/Bcg* gene is located. Analysis of the data for these loci using affected pedigree-member linkage analysis also failed to provide evidence for cosegregation of these markers with susceptibility to disease *per se*. Nor could any evidence be found for a gene in this region controlling susceptibility to the tuberculoid form of disease, or to T-cell responder phenotypes for proliferative responses to the mycobacterial antigens purified protein derivative (PPD) or *M. leprae*-soluble antigen (MLSA). The RFLP map generated is now the most detailed genetic map of the region 2q33-q37, mostly com-

prising genes encoding molecules of known function. This now provides an anchor map and a set of typed families around which new highly polymorphic microsatellite markers can be ordered.—Authors' Abstract

Song, X., *et al.* [Determination of the antibody in the sera of leprosy patients with NT-P-BSA ELISA.] China Lepr. J. **9** (1993) 207–210. (in Chinese)

NT-P-BSA ELISA has been used to determine the specific antibody in the sera of leprosy patients and healthy persons. The results showed that its specificity is 95.6% and the sensitivity 92.7% in MB and 50% in PB. The antibody level was highest in newly detected cases, declining in MB as time went on and treatment was given to normal levels after over 10 years of disease duration. And the level became normal shortly after the beginning of treatment in PB. The authors suggest that the percentile method in 95% is more suitable than $\bar{X}$ + 2 S.D. method for determination of the threshold value in the test.—Authors' English Abstract

Sun, X., *et al.* [Determination of anti-PGL1 antibody in the blood of leprosy patients who completed MDT and their contacts.] China Lepr. J. **9** (1993) 213–215. (in Chinese)

NT-O-BSA ELISA has been used to determine anti-PGL-I IgM antibody in the sera of 1715 contacts of leprosy and 6 new cases of leprosy before and after wide application of MDT. The positivity rates of the antibody among the contacts was 33.4% before MDT, 32.8% 2–4 months after MDT, and 21.8% 48 months after completion of MDT. The OD values decayed by 72.7% among 6 newly detected cases of leprosy the 5th year after the introduction of MDT. Among 243 contacts with the positivity, 7 new cases of leprosy have been found after 2–5 years of continuous follow up, including 1 BT, 4 BL, and 2 LL.—Authors' English Abstract

Wang, C. R., Liu, M. F., Jeng, G. W., *et al.* Autoantibodies and related immunity of leprosy patients from leprosarium in Taiwan. Chin. J. Microbiol. Immunol. **25** (1992) 181–188.

Autoantibodies and related immunological examinations were measured in 60 leprosy patients from a leprosarium in Taiwan; 31 lepromatous, 24 tuberculoid, and 5 borderline type patients were identified. The measured autoantibodies included antinuclear antibodies, anti-nDNA, anti-cardiolipin and rheumatoid factor. Serum protein electrophoresis and immunofixation were performed to detect the monoclonal and polyclonal status of immunoglobulins. Circulating immune complex and complements were also quantitated. Delayed-type skin tests were performed during patients' visits. A higher frequency of autoantibodies, especially the antinuclear antibodies and anticardiolipin antibodies, were detected in lepromatous type patients. Higher levels of circulating immune complex and frequency of polyclonal and monoclonal gammopathy were noted in lepromatous type patients. Anergy skin tests were only noted in lepromatous type patients. It was concluded that the more impaired cell-mediated immunity in leprosy patients, lepromatous type in particular, the greater the production of autoantibodies.—Authors' Abstract

Wang, T., Izumi, S., Butt, K. I., Kawatsu, K. and Maeda, Y. Demonstration of PGL-I and LAM-B antigens in paraffin sections of leprosy skin lesions. Jpn. J. Lepr. **61** (1992) 165–174.

An investigation on the demonstration of PGL-I and LAM-B antigens in 34 paraffin-embedded skin biopsies taken from leprosy patients who covered the whole spectrum of the disease and in 4 control specimens was carried out. Neither the PGL-I antigen nor the LAM-B antigen was demonstrated in the normal skin specimens that were used as negative controls; and only the LAM-B antigen appeared in the tuberculosis specimens in which the PGL-I antigen was negative. The PGL-I antigen was demonstrated on 33 leprosy samples except 1 TT sample and the LAM-B antigen, on all samples by immunochemical staining technique. The antigens were identified as intracytoplasmic bacillary staining, in solitary, granular as well as debris patterns; and as soluble antigenic staining, in vacuolar or amorphous pattern. In LL and BL cases, the antigens were detected predominantly from macro-

phages and peripheral nerves in all five staining patterns; in BB cases, from macrophages mostly in the granular as well as debris patterns, from the nerves in the vacuolar pattern; while in TT and the majority of BT cases, they were mainly from nerve remnants inside the granuloma in the vacuolar or amorphous staining pattern. In addition, it is interesting to note that the immunochemical staining was able to differentiate the foamy change from the hydropic degeneration. [The authors] also found that the antigens distributed in arrector pili muscles and the walls of muscular vessels were obviously related to the unmyelinated nerve fibers innervating the smooth muscle cells. The results indicate that the specificity and sensitivity of the immunohistochemical staining technique used in this study are suitable for both the application of the diagnostic pathology and the research on pathogenesis of leprosy.—Authors' Abstract

Zhang, Y., Broser, M. and Rom, W. N. Activation of the interleukin 6 gene by *Mycobacterium tuberculosis* of lipopolysaccharide is mediated by nuclear factors NF-IL6 and NF-κB. Proc. Natl. Acad. Sci. U.S.A. **91** (1994) 2225–2229.

The host response to *Mycobacterium tuberculosis* includes granuloma formation at sites of infection and systemic symptoms. Cytokines have been identified by immunohistochemistry in granulomas in animal models of bacillus Calmette-Guérin (BCG) infection and are released by mononuclear phagocytes upon stimulation by mycobacterial proteins. In this regard, the cytokine interleukin 6 (IL-6) may play a role in the clinical manifestations and pathological events of tuberculosis infection. We have demonstrated that lipoarabinomannan (LAM) from the mycobacterial cell wall, which was virtually devoid of lipopolysaccharide (LPS), stimulated mononuclear phagocytes to release IL-6 in a dose-response manner. LAM and LPS were potent inducers of IL-6 gene expression in peripheral blood monocytes. Both LAM- and LPS-inducible IL-6 promoter activity were localized to a DNA fragment, positions −158 to −49, by deletion analysis and chloramphenicol acetyltransferase assay. Two nuclear factor NF-IL6 (positions −153 to −145 and −83 to −75) and one nuclear factor NF-κB (positions −72 to −63) motifs are present within this fragment. Site-directed mutagenesis of one or more of these motifs within the IL-6 promoter demonstrated that each has positive regulatory activity and that they could act in a function- and orientation-independent manner. Deletion of all three elements abolished inducibility of IL-6 promoter activity by both LAM and LPS. We conclude that the NF-IL6 and NF-κB sites mediate IL-6 induction in response to both LPS and LAM, acting as bacterial or mycobacterial response elements.—Authors' Abstract

Microbiology

Bramhne, H. G., Porichha, D., Samal, R. C. and Reddy, B. N. Non-acid-fast fluorescent *M. leprae* (?) in skin smears from leprosy patients. Indian J. Lepr. **65** (1994) 439–442.

Smears from 74 known smear-negative cases of leprosy were examined after staining with auramine "O"; 40.54% cases were positive for fluorescent bacilli; 60.52% of cases on treatment and 19.44% cases after RFT had fluorescent bacilli in the skin smears. Results suggest the possibility of a non-acid-fast fluorescent positive variant of *M. leprae*.—Authors' Abstract

Cambau, E., Sougakoff, W. and Jarlier, V. Amplification and nucleotide sequence of the quinolone resistance-determining region in the *gyrA* gene of mycobacteria. FEMS Microbiol. Lett. **116** (1994) 49–54.

Chromosomal DNA of different species of mycobacteria, *Mycobacterium tuberculosis, M. leprae, M. avium* and *M. smegmatis,* has been submitted to polymerase chain reaction using two oligonucleotide primers highly homologous to DNA sequences flanking the quinolone resistance-determining region in the *gyrA* gene of *Esch-*

erichia coli and *Staphylococcus aureus*. For each of these mycobacterial species, a 150-bp DNA fragment hybridizing with an intragenic probe of the *gyrA* gene of *E. coli* K12 was obtained. The nucleotide sequences of the 108-bp fragments amplified from *M. tuberculosis* and *M. avium* were determined. The two sequences were 87% homologous. Except for one residue, their deduced amino acid sequences were identical and shared 67% homology with the quinolone resistance-determining region of the gyrase A subunits of *E. coli* and *S. aureus*. Sequencing of the 108-bp, fragment amplified from an *in vitro* mutant of *M. avium*, highly resistant to fluoroquinolones, showed a point mutation leading to the substitution of Ala for Val at a position corresponding to residues involved in quinolone resistance in *E. coli* and *S. aureus*, i.e., Ser 83 for *E. coli* and Ser 84 for *S. aureus*. —Authors' Abstract

Cirillo, J. D., Weisbrod, T. R., Pascopella, L., Bloom, B. R. and Jacobs, W. R. Isolation and characterization of the aspartokinase and aspartate semialdehyde dehydrogenase operon from mycobacteria. Mol. Microbiol. **11** (1994) 629–639.

Diaminopimelic acid (DAP) is a major component of the peptidoglycan layer of the mycobacterial cell wall. The mycobacterial cell wall has been implicated as a potential virulence factor and is highly immunogenic. The pathway for biosynthesis of DAP may serve as a target in the design of antimycobacterial agents and construction of *in vivo* selection systems. Despite its significance, this biosynthetic pathway is poorly understood in mycobacteria. In order to develop a better understanding of mycobacterial DAP biosynthesis, the aspartate semialdehyde dehydrogenase (asd) genes of *Mycobacterium smegmatis*, bacille Calmette-Guerin (BCG), *M. avium*, *M. leprae*, and *M. tuberculosis* were isolated. The *M. smegmatis* asd gene was isolated by complementation in *Escherichia coli*. This gene was then used to isolate the asd genes from other mycobacteria. The asd-complementing fragments from BCG and *M. smegmatis* were sequenced. An open reading frame upstream of the mycobacterial asd gene was identified as the mycobacterial aspartokinase gene (ask). Primer extension analysis revealed that the only transcriptional start in this region is found 5′ of the ask gene. This observation indicates that the mycobacterial ask and asd genes are in an operon. —Authors' Abstract

Cook, S. M., Bartos, R. E., Pierson, C. L. and Frank, T. S. Detection and characterization of atypical mycobacteria by the polymerase chain reaction. Diagn. Mol. Pathol. **3** (1994) 53–58.

The purpose of this study was to develop a simple protocol of nested reamplification polymerase chain reaction (PCR) to detect and characterize diverse mycobacterial species. DNA extracted from 126 pure mycobacterial cultures isolated from clinical specimens was amplified by nested PCR with use of a novel set of oligonucleotide primers specific for the 65-kDa antigen gene of mycobacteria. The PCR products were each digested with three restriction enzymes and electrophoresed on an agarose gel. The observed DNA fragment sizes of the different species with each enzyme were compiled into a simple algorithm. This method can rapidly detect and characterize a wide variety of mycobacterial species, including the most common pathogens *Mycobacterium tuberculosis*, *M. avium-intracellulare*, and *M. kansasii*, without hybridization to labeled probes. The application of this method to surgical pathology was demonstrated by amplification and identification of atypical mycobacteria, including *M. kansasii* and *M. leprae*, in formalin-fixed paraffin-embedded tissue. This protocol broadens the diagnostic potential of PCR for rapidly diagnosing mycobacterial infection in clinical samples, particularly in paraffin-embedded tissue sections. —Authors' Abstract

Davis, E. O., Thangaraj, H. S., Brooks, P. C. and Colston, M. J. Evidence of selection for protein introns in the recAs of pathogenic mycobacteria. EMBO Journal **13** (1994) 699–703.

Protein introns are recently discovered genetic elements whose intervening sequences are removed from a precursor protein by an unusual protein splicing reaction. This involves the excision of a central spac-

er molecule, the protein intron, and the religation of the amino- and carboxy-terminal fragments of the precursor. The recA gene of *Mycobacterium tuberculosis* contains one such element, and we now show that the other major mycobacterial pathogen, *M. leprae*, also possesses a protein intron in its recA, although other mycobacterial recA genes do not. However, these two protein introns are different in size, sequence and location of insertion of their coding sequences into the recAs of *M. tuberculosis* and *M. leprae*, indicating that acquisition of the protein introns has occurred independently in the two species, and thus suggesting that there has been selection for splicing in the maturation of recA in the pathogenic mycobacteria. The *M. leprae* protein intron provides an example of conditional protein splicing, splicing occurring in *M. leprae* itself but not when expressed in *Escherichia coli*, unlike most previously described protein introns. These observations suggest that protein introns may perform a function for their host, rather than being just selfish elements.—Authors' Abstract

De Beenhouwer, H., de Rijk, P., Douglas, J. and Portaels, F. Diagnosis of mycobacterial diseases, including leprosy, by PCR with nested primers on 16S rRNA sequence. (Abstract) Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 71.

Diagnosis of mycobacterial diseases in the laboratory is a lengthy and cumbersome process when using traditional methods. Molecular biological approaches have been described which are much more rapid but difficult to apply in nonresearch circumstances. We developed a very rapid and efficient nonenzymatical extraction method allowing processing of all kinds of clinical samples (sputa, biopsies, feces, . . .) together with a polymerase chain reaction (PCR) with primers designed on 16S rRNA sequences which could specifically detect organisms belonging to the genus *Mycobacterium*. Nested primers were designed to enhance sensitivity. This set-up allowed the detection of < 500 bacteria/ml in diverse clinical specimens. Moreover the nesting procedure allowed rapid identification of different mycobacteria (*M. tuberculosis, M. leprae*, . . .). For the discrimination between mycobacteria belonging to the *M. avium-intracellulare* complex (MAC) a rapid and easy nonradioactive labeled probe system was developed. Results: 210 clinical samples (sputum, homogenized biopsies, buffy coats, feces, etc.) were examined in parallel with PCR and culture. Overall sensitivity and specificity was comparable between the two systems with a correct identification of *M. tuberculosis* (nesting), *M. avium* and *M. intracellulare* (probe). Skin biopsies from leprosy patients and mouse foot pad homogenates were examined with PCR and histopathology. Overall PCR results correlated with the histopathology indicating that the level of detection with PCR was < 1000 *M. leprae*/ml. Biopsies kept in formaldehyde however always gave negative results.

Ji, Y., Colston, M. J. and Cox, R. A. Nucleotide sequence of the leader region of transcripts of the *rrn* operon of mycobacteria. (Abstract) Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 67–68.

There is a broad correlation between the rate of growth of a bacterium and the number of its ribosomes. In turn, the number of ribosomes is determined by the production of rRNA. The rate at which mature rRNAs are produced will depend on factors which include the number of ribosomal RNA (*rrn*) operons present, the strength of their promoters and the efficiency with which the operons are transcribed. The latter factor is important to slow-growing mycobacteria which have only a single *rrn* operon. A fully efficient transcription process is one where each initiation event leads to a complete transcript from which a copy of mature 16S rRNA, 23S rRNA and 5S rRNA is produced. Premature termination, which would lower the efficiency of transcription, is reduced if antitermination signals are incorporated in the transcript. Three such signals or elements (namely Box B, Box A and Box C, in that order) are found in the leader region (sequences up stream from the 5′-end of 16S rRNA) of transcripts of *rrn* operons of both *Escherichia coli* and *Bacillus subtilis*.

Previously, we reported sequence data for the leader region of two mycobacteria (*M. tuberculosis* and *M. leprae*). We now present

data for several others (*M. paratuberculosis, M. avium, M. intracellulare, M. marinum, M. habana, M. lufu* and for both operons of *M. smegmatis*). The principal result is that elements homologous to Box B, Box A and Box C found in *E. coli* and *B. subtilis* were identified in the mycobacteria studied. The sequence of each of these elements was highly conserved (in fact they were identical) in all the slow-growers studied. One *rrn* operon of *M. smegmatis* was found to have all the three antitermination signals found on slow-growers. In the second operon Box A and Box C (but not Box B) elements were slow-grower like. The region from the start of Box B to the 5'-end of 16S rRNA ranged from 186 nucleotides (*M. paratuberculosis*) to 207 nucleotides (*M. leprae, M. habana*). Comparison revealed regions of highly conserved (identical sequences) and others of nonidentical but homologous sequences. The leader region of the transcript of the *rrn* operon is known to form a long bihelical stem structure through interaction with approx. 130 nucleotides downstream from the 3'-end of 16S rRNA. A pattern of secondary structure common to all the mycobacterial species studied (including *M. leprae* and *M. smegmatis*) was discerned. Apart from theoretical considerations the leader region is of interest because it is a potential target for species specific probes. In contrast, few homologies were detected between the mycobacterial sequences and the leader region of the *rrn* D operon of *Streptomyces ambofaciens.*

Katoch, V. M., Katoch, K., Kanaujia, G. V., Shivannavar, C. T., Sharma, R. K., Natrajan, M., Sharma, V. D., Bhatia, A. S. and Patil, M. A. Application of gene probes & gene amplification techniques to early diagnosis and relapses/reactions in leprosy. Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 58–59.

Large scale use of multidrug therapy (MDT) has been helping to reduce prevalence of established cases of leprosy. As a result, the diagnosis of early and suspected cases is assuming greater importance. Further, the persistent clinical activity, relapses and reactions after MDT are posing therapeutic problems. In recent years, several *Mycobacterium leprae*-specific probes and gene amplification techniques targeting 16S RNA have been standardized. These new systems along with gene amplification (targeting 18 kDa and 36 kDa protein genes) described by other investigators are being evaluated in clinical specimens from untreated and treated established cases of different types of leprosy: early suspected cases, paucibacillary cases with persistent residual activity, late reactions and relapses. Initial results suggest that these techniques may have a role in confirming the diagnosis of early disease and possible relapse requiring additional chemotherapy. The suboptimal sensitivity of all available gene amplification techniques in paucibacillary cases and persistence of weak signals in multibacillary cases even after prolonged chemotherapy are two of the important limitations for which further improvements are still necessary.—Authors' Abstract

Miller, L. P., Crawford, J. T. and Shinnick, T. M. The *rpoB* gene of *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **38** (1994) 805–811.

A portion of the *Mycobacterium tuberculosis* gene encoding the β subunit of RNA polymerase (*rpoB*) was amplified by PCR using degenerate oligonucleotides and used as a hybridization probe to isolate plasmid clones carrying the entire *rpoB* gene of *M. tuberculosis* H37Rv, a virulent, rifampin-susceptible strain. Sequence analysis of a 5084-bp *Sac*I genomic DNA fragment revealed a 3534-bp open reading frame encoding an 1178-amino-acid protein with 57% identity with the *Escherichia coli* β subunit. This *Sac*I fragment also carried a portion of the *rpoC* gene located 43 bp downstream from the 3' end of the *rpoB* open reading frame; this organization is similar to that of the *rpoBC* operon of *E. coli.* The *M. tuberculosis rpoB* gene was cloned into the shuttle plasmid pMV261 and electroporated into the LR223 strain of *M. smegmatis,* which is highly resistant to rifampin (MIC > 200 μg/ml). The resulting transformants were relatively rifampin susceptible (MIC = 50 μg/ml). Using PCR mutagenesis techniques, we introduced a specific *rpoB* point mutation (associated with clinical strains of rifampin-resistant *M. tu-*

berculosis) into the cloned *M. tuberculosis rpoB* gene and expressed this altered gene in the LR222 strain of *M. smegmatis*, which is susceptible to rifampin (MIC = 25 μg/ml). The resulting transformants were rifampin resistant (MIC = 200 μg/ml). The mutagenesis and expression strategy of the cloned *M. tuberculosis rpoB* gene that we have employed in this study will allow us to determine the *rpoB* mutations that are responsible for rifampin resistance in *M. tuberculosis*.—Authors' Abstract

Minnikin, D. E., Besra, G. S., Bolton, R. C., Datta, A. K., Mallet, A. I., Sharif, A., Stanford, J. L., Ridell, M. and Magnusson, M. Identification of the leprosy bacillus and related mycobacteria by analysis of mycocerosate profiles. Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 25–34.

Members of the phthiocerol dimycocerosate family of waxes were extracted from *Mycobacterium bovis* BCG, *M. tuberculosis*, *M. kansasii*, *M. marinum*, *M. ulcerans* and a skin biopsy from a leprosy patient. The waxes were degraded by alkaline hydrolysis and the mycocerosic acids converted to pentafluorobenzyl ester. Profiles of the esters, recorded using electron-capture gas-chromatography, gave characteristic profiles for the mycocerosates from *M. leprae*, but those from *M. bovis*, *M. tuberculosis* and *M. kansasii* were superficially similar. The mycocerosate profiles from *M. marinum* and *M. ulcerans* were similar, but distinct from the others. Selected ion monitoring negative ion-chemical ionization gas chromatography-mass spectrometry of the pentafluorobenzyl esters allowed the analysis of mycocerosate isomers not revealed on gas chromatography alone. *M. bovis* and *M. tuberculosis* had similar profiles of C_{29}, C_{30} and C_{32} mycocerosates; an additional C_{33} component was also present in *M. kansasii*. The mycocerosates from *M. marinum* and *M. ulcerans* were C_{27}, C_{29} and C_{30} and those from *M. leprae* were distinct in having C_{29}, C_{30}, C_{32}, C_{33} and C_{34} components. These methods have excellent potential for use in the detection of mycobacterial disease by direct analysis of infected tissue without prior cultivation of the causative agent.—Authors' Summary

Minnikin, D. E., Bolton, R. C., Hartmann, S., Besra, G. S., Jenkins, P. A., Mallet, A. I., Wilkins, E., Lawson, A. M. and Ridell, M. An integrated procedure for the direct detection of characteristic lipids in tuberculosis patients. Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 13–24.

An integrated method is described for the sensitive detection of tuberculostearic, mycocerosic and mycolic acids in infected materials from tuberculosis patients. Tuberculostearic acid is analyzed by two-dimensional gas chromatography of pentafluororobenzyl esters, the key component being switched from a short nonpolar column to a high-resolution polar column with final electron capture detection. Mycocerosic acids are identified by simple one-dimensional electron-capture gas chromatography of pentafluorobenzyl esters, with the use of negative-ion chemical ionization gas chromatography in indecisive cases. Conversion of mycolic acids to anthrylmethyl esters produces compounds which are suitable for sensitive detection by fluorescence high-performance liquid chromatography. Application of all three of these methods to sputum samples from tuberculosis patients gave profiles characteristic of *Mycobacterium tuberculosis*.—Authors' Summary

Munk, M. E., De Bruyn, J., Gras, H. and Kaufmann, S. H. E. The *Mycobacterium bovis* 32-kilodalton protein antigen induces human cytotoxic T-cell responses. Infect. Immun. **62** (1994) 726–728.

The 30-kDa protein (P32) is a mycobacterial secreted antigen which is homologous in *Mycobacterium bovis* and *M. tuberculosis*. *In vitro*, P32 induced T-cell proliferation. *M. tuberculosis*- or P32-stimulated T-cell lines lysed macrophages pulsed with P32 or *M. tuberculosis*, respectively. We conclude that P32 stimulates cytotoxic T cells specifically.—Authors' Abstract

Plum, G. and Clark-Curtiss, J. E. Induction of *Mycobacterium avium* gene expression following phagocytosis by human macrophages. Infect. Immun. **62** (1994) 476–483.

Little is known about the bacterial factors that enable pathogenic mycobacteria to sur-

vive and multiply within the macrophages of the infected host. By preparing cDNA from *Mycobacterium avium* bacilli grown in human-derived macrophages and in broth culture and using subtractive hybridization to remove commonly expressed genes, a procedure was developed to identify genes of *M. avium* that are specifically expressed when the bacilli are growing within macrophages. Total RNA was isolated from *M. avium* recovered 5 days after infection of human macrophages and from bacilli grown *in vitro* in broth. Mycobacterial mRNAs were converted to cDNA by reverse transcription. Biotin-modified cDNAs prepared from *M. avium* grown in broth culture were used to subtract the housekeeping genes from the cDNAs of the macrophage-derived *M. avium*. After each round of subtraction, a sample of the unsubtracted cDNA was amplified, labeled, and hybridized to cosmid clones of *M. avium* DNA. After three rounds of subtraction, the amplified DNA hybridized to approximately 1% of the cosmid clones under stringent conditions. Although the majority of the genes that are induced in phagocytized *M. avium* cells are expressed in the broth-grown bacilli, one DNA fragment that was identified coded for an mRNA that is highly specific for *M. avium* in phagosomes. This procedure will be especially useful for identifying genes that are expressed in response to growth in specific environments from organisms with genetic systems that are not well characterized.—Authors' Abstract

Ridell, M. Enzyme electrophoresis in taxonomy of mycobacteria. Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 35–39.

Taxonomical analyses of mycobacteria were performed by using multilocus enzyme electrophoresis (MEE). The studies showed that the mycobacteria tested advantageously could be differentiated on species level. The MEE method could also be used for subspecies differentiation, but the method seemed less well suited than some other methods for this purpose. The results are discussed in relation to MEE analyses of other microorganisms.—Authors' Summary

Takiff, H. E., Salazar, L., Guerrero, C., Philipp, W., Huang, W. M., Kreiswirth, B., Cole, S. T., Jacobs, W. R., Jr., and Telenti, A. Cloning and nucleotide sequence of *Mycobacterium tuberculosis gyrA* and *gyrB* genes and detection of quinolone resistance mutations. Antimicrob. Agents Chemother. **38** (1994) 773–780.

The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* has resulted in increased interest in the fluoroquinolones (FQs) as antituberculosis agents. To investigate the frequency and mechanisms of FQ resistance in *M. tuberculosis,* we cloned and sequenced the wild-type *gyrA* and *gyrB* genes, which encode the A and B subunits of the DNA gyrase, respectively; DNA gyrase is the main target of the FQs. On the basis of the sequence information, we performed DNA amplification for sequencing and single-strand conformation polymorphism analysis to examine the presumed quinolone resistance regions of *gyrA* and *gyrB* from reference strains (N = 4) and clinical isolates (N = 55). Mutations in codons of *gyrA* analogous to those described in other FQ-resistant bacteria were identified in all isolates (N = 14) for which the ciprofloxacin MIC was > 2 μg/ml. In addition, we selected ciprofloxacin-resistant mutants of *M. bovis* BCG and *M. tuberculosis* Erdman and H37Ra. Spontaneously resistant mutants developed at a frequency of 1 in 10^7 to 10^8 at ciprofloxacin concentrations of 2 μg/ml, but no primary resistant colonies were selected at higher ciprofloxacin concentrations. Replating of those first-step mutants selected for mutants with high levels of resistance which harbored *gyrA* mutations similar to those found among clinical FQ-resistant isolates. The *gyrA* and *gyrB* sequence information will facilitate analysis of the mechanisms of resistance to drugs which target the gyrase and the implementation of rapid strategies for the estimation of FQ susceptibility in clinical *M. tuberculosis* isolates.—Authors' Abstract

Experimental Infections

Wang, H., *et al.* [A study of multiple route inoculation of *M. leprae* to nude mouse.] China Lepr. J. **9** (1993) 210–212. (in Chinese)

Immunodeficient nude mice have been inoculated with nude mouse-derived *M. leprae* through multiple routes, i.e., intravenously, subcutaneously at the foot pad, and into the ear. The results showed that the inoculated animals were capable of producing a large number of *M. leprae*, amounting to 10^{11-12} per gram of tissue and distinct histopathological changes of the lepromatous form. The disease could disseminate to sites with lower body temperature. The organism demonstrated a partiality for the striated muscles and peripheral nerves. The authors regard the nude mouse model as a very useful tool for leprosy research, especially in countries without armadillos. As compared with inoculation through a single route reported previously, the inoculation through multiple routes seemed to be more practical.—Authors' English Abstract

Epidemiology and Prevention

Klatser, P. R., de Wit, M. Y. L., Madjid, B. and van Beers, S. Detection of *Mycobacterium leprae* in nasal swab specimens. (Abstract) Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 77–78.

We have developed a polymerase chain reaction (PCR) for the specific detection of *Mycobacterium leprae* DNA. This PCR has shown to be applicable for the rapid detection of small number of *M. leprae* in clinical samples, in which existing methods fail to do so. The common problems with PCR of false-positive and false-negative reactions have been solved by introducing a UTP/UNG anti-contamination kit and a modified template, respectively. In order to determine the role of *M. leprae* nasal carriage in the maintenance of infection reservoirs and transmission of leprosy, we applied this technology for the detection of bacilli in nasal swab specimens. When applied to nasal swab specimens, amplifications were found in 55% of the untreated multibacillary leprosy patients, in 19% of the occupational contacts, in 12% of the endemic controls and in none of the nonendemic controls. This study suggested that not only leprosy patients but also healthy persons may carry *M. leprae*. To further investigate *M. leprae* carriage among healthy people, we performed total population surveys in South Sulawesi, Indonesia. The PCR for the specific detection of *M. leprae* DNA was applied on nasal swabs collected through these total population surveys from individuals (N = 1228) living in two isolated villages in Indonesia with expected high and low prevalence of leprosy, respectively. However, upon actual screening of the villages it was found that both had a similar prevalence (10.7/1000 and 9.0/1000). No significant difference in PCR-positivity was found between the two villages. There were 7.8% PCR-positives among the total population tested (N = 1228). No clear age or sex relationship was found among PCR-positives. A small minority of households tested (3.1%) was associated with 27% of all PCR-positive individuals. These results show that carriage of *M. leprae* is common among healthy individuals and that it seems to be clustered around certain households.

Pattyn, S. R., Ursi, D., Ieven, M., Grillone, G. and Raes, V. Detection of *Mycobacterium leprae* by the polymerase chain reaction in nasal swabs of contacts of leprosy patients. (Abstract) Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 61.

In view of the large number of bacilli present in nasal secretions of lepromatous patients, transmission of leprosy through inhalation with deposition of bacilli on the nasal mucosa seems to be a most probable route of infection. Up to now studies of the nasal mucosa of contacts of leprosy patients

were exclusively based on Ziehl-Neelsen-stained nasal swabs, with all the inevitable shortcomings of the lack of sensitivity and specificity. We undertook a study on the detection of a *M. leprae*-specific DNA in nose swabs of contacts of paucibacillary and multibacillary patients in Anjouan, Comores. All swabs were tested for the presence of a rRNA coding DNA gene in the presence of an internal control for the detection of nonspecific inhibitors. All positive samples were confirmed in a second PCR amplifying another region of the *M. leprae* genome. Overall 30% of the samples contained inhibitors for the PCR. All samples from PB patients were negative, 1/55 (1.9%) samples from their contacts was positive. Three samples from two MB patients were positive, 13/164 (7.9%) samples from their contacts were positive. If these figures can be confirmed, it would seem that most leprosy infections are contracted outside the home. This study, which had to be stopped for reasons beyond our will, merits to be continued and expanded.

Seidenbaum, M., Slater, P. E., Ever-Hadani, P., Costin, C. and Leviatan, A. [Epidemiology of Hansen's disease in Israel.] Harefuah **125** (1993) 65–69.

There are currently 200 patients with Hansen's disease in Israel who are being followed by the Hansen's Disease Government Hospital and the Ministry of Health (prevalence 4.4/100,000). Most of them immigrated from countries where the disease is endemic. Dermatological findings dominated the initial clinical picture, although 5% of patients are asymptomatic contacts of known cases. Age at onset of disease was less than 20 years in ¼ of the cases. The incidence in Israel is falling: 0.4/100,000 in 1985–1989 compared to 3.6/100,000 in 1950–1954. Neurologic and dermatologic findings in an immigrant of any age originating from countries where Hansen's disease is endemic should prompt appropriate diagnostic evaluation even years after immigration to Israel. Contacts of known cases of Hansen's disease should be aggressively screened, even if asymptomatic.—Authors' Abstract

Zarate, N. O. V. [Leprosy in Ecuador.] Hansen. Int. **17** (1992) 33–41. (in Spanish)

As recommended by WHO/PAHO and sponsored by the German Aid for Leprosy Patients (AYU), MDT/OMS was introduced in Ecuador in the second half of 1983 for treatment of all known cases of leprosy in the country. Since then, the prevalence shows a steady decrease, from 2399 (0.27/1000) in 1983 to 839 (0.08/1000) in 1990. The incidence has been decreasing since 1983, from 1.7/1000 to 1.01/1000 in 1990. The coverage of MDT is 100% since 1987. Patients are mainly lepromatous form due to its greater period of incubation and the need of a long treatment. The incidence of patients below 15 years old is minimal which leads to the conclusion that there is a low risk of infection in the community. The proportion of multibacillary cases among new cases is 48.15% in 6 years of control activities in Ecuador, less than the average of paucibacillary cases, which means that the endemy is still active in the country.—Authors' English Abstract

Rehabilitation

Trindade, M. A. B. and Nemes, M. I. B. [Physical disabilities in HD patients at the time of diagnosis: epidemiologic characteristics of the cases registered from 1983 to 1988 in the state of Sao Paulo (Brazil).] Hansen. Int. **17** (1992) 8–14. (in Portuguese)

The evaluation of physical disabilities caused by hanseniasis at the moment of the diagnosis was carried out through a sample study of the cases recorded in the state of São Paulo, Brazil, from 1984 to 1988. The records of the physical disabilities were studied by two different methods: the disabilities at their highest grades and the absolute disabilities frequency. The study suggested the necessity of improving the diagnosis and the information register of the control program and a proper system of attention to the physical disabilities.—Authors' English Abstract

Wang, Z., *et al.* [Effect of the temporal muscle transposition on lagophthalmos in leprosy.] China Lepr. J. **9** (1993) 205–207. (in Chinese)

Thirty cases of lagophthalmos in 21 leprosy patients have been corrected with Gillies temporal muscle transposition and the method modified by Johnson. Among them 18 cases are male and 3 female with ages from 33 to 54; including tuberculoid type disease in 12, borderline in 7, and lepromatous in 2. The follow up for 10 months–15 years after the transpositions, averaging 7.2 years, showed that the eyes were closed, the palpebral tissues were < 2 mm in 21 eyes, 3 mm or more in 4, and unchanged in 2; they had been as wide as 3–9 mm before the operations. The failures were due to adhesion of the transplants. The authors judged that both the operation methods, the original and the modified, are the same in efficiency but the modified one only needs a smaller incision so as to have a favorable reception from the patients.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Agarwal, A., Kandpal, H., Gupta, H. P., Singh, N. B. and Gupta, C. M. Tuftsin-bearing liposomes as rifampin vehicles in treatment of tuberculosis in mice. Antimicrob. Agents Chemother. **38** (1994) 588–593.

The antitubercular activity of rifampin was considerably increased when it was encapsulated in egg phosphatidylcholine liposomes. A further increase in the activity was observed when the macrophage activator tetrapeptide tuftsin was grafted on the surface of the drug-loaded liposomes. Intermittent treatments (twice weekly) with these preparations were significantly more effective than continuous treatments. Rifampin delivered twice weekly for 2 weeks in tuftsin-bearing liposomes was at least 2000 times more effective than the free drug in lowering the load of lung bacilli in infected animals. However, pretreatment with drug-free tuftsin-bearing liposomes did not render the pretreated animals resistant to the *Mycobacterium tuberculosis* infections, neither did it appreciably increase the chemotherapeutic efficacy of the liposomized rifampin. These results clearly demonstrate that liposome targeting to macrophages could considerably increase the antitubercular activity of liposomized drugs such as rifampin. Also, it shows that immunoprophylactic treatment with macrophage activators such as tuftsin does not afford any advantage in treatment of tuberculosis infections, presumably because of inactivation of the primed macrophages by the mycobacterial sulfatides.—Authors' Abstract

Barihuta, T., Rigouts, L., Barette, M., Collart, J. P., De Bruyn, J., Kadende, P., Kamamfu, G., Douglas, J. T. and Portaels, F. Rapid, early and specific diagnosis of tuberculosis and other mycobacterial diseases in Burundi. Ann. Soc. Belg. Med. Trop. **73** Suppl. 1 (1993) 41–51.

The potential usefulness of ELISA-based serological tests to assist in rapid, early and specific diagnosis of tuberculosis was investigated. The materials were selected based on published data and on our preliminary findings. Initially screening tests were performed using crude antigens such as purified protein derivate (PPD) and a BCG-filtrate. Unfortunately, the results with these antigens were not promising. The specificity of both antigens using sera from 94 healthy controls was 64%. As a consequence of these findings, the crude antigens were excluded from further tests, and the study was continued with purified antigens. The work focused on two purified proteins: antigen 60 (A60), a lipopolysaccharide-protein complex, and P32, a stress protein produced in zinc-deprived cultures, identified as antigen 85A in the BCG reference system, both isolated from *Mycobacterium bovis* BCG. The commercial A60-based ELISA and our own P32-based ELISA were used to test a total of 300 sera from HIV-positive, -negative and unscreened individuals, mainly originating from Burundi. These sera were collected from clinical established cases of pulmonary tuberculosis (TB), extrapulmonary TB, and patients with nontuberculous tropical diseases such as salmonellosis, try-

panosomiasis, malaria, etc. and healthy individuals. The A60-based ELISA had a sensitivity of 76.8% for the proven cases of active pulmonary TB and 61.9% for the extrapulmonary TB cases. No difference was shown between HIV-positive and HIV-negative patients. Specificity reached 95.2% for healthy individuals, but dropped to 68.1% when persons with active nontuberculous tropical diseases were included. Eight-six percent of the pulmonary cases and 87.7% of the extrapulmonary cases were detected by the ELISA-P32. These findings suggest that this test might be useful as a confirmatory test for the diagnosis of extrapulmonary TB. Again no difference was noticed between HIV-negative and -positive patients. The main contraindication for the use of the ELISA-P32 for the diagnosis of TB is its low specificity: 70.2% with sera from healthy controls and 22.2% for hospitalized patients and persons with nontuberculous tropical diseases. In a small recent prospective study 4 out of 10 HIV+ persons with no evidence for TB yielded a positive result for the ELISA-P32. Two of them developed pulmonary TB within 6 months, whereas 2 P32-positives and 6 P32-negatives remained up to now without any manifestations of TB. The difference was not significant, but the number of cases was limited. To avoid false-positive reactions, two specific lipid antigens isolated from *M. tuberculosis* were selected: sulfolipid IV (SLIV), a diacyl trehalose sulfate, and phenolic glycolipid Tb1 (PGLTb1), a glycolipid. Most of the testing was done with SLIV, where a specificity of 81.2% was reached among control sera. When only "healthy" persons (HIV negatives and positives) were included, the specificity increased to 87%. The test detected 75.0% of the pulmonary TB cases and 52.0% of the tuberculous lymphadenitis cases. Our results are promising and further studies on the usefulness of the ELISA-P32, ELISA-SLIV and other antigens, in confirming active TB or predicting reactivation in HIV+ persons, should be pursued on a larger number of subjects. In view of the low specificity of the ELISA-P32 among patients with other diseases, research on the possible occurrence of crossreactions with shared epitopes among other etiological agents is being studied in collaboration with the Erasmus Hospital in Brussels (Drowart and coworkers). Although a good diagnostic ELISA test (with both high sensitivity and specificity) for the detection of active TB still needs to be developed, our findings and those of others would suggest that it is possible to improve or modify existing tests by identifying specific epitopes and careful application of these tests to high-risk groups.—Authors' Abstract

Bloch, A. B., Cauthen, G. M., Onorato, I. M., Dansbury, K. G., Kelly, G. D., Driver, C. R. and Snider, D. E. Nationwide survey of drug-resistant tuberculosis in the United States. JAMA **271** (1994) 665–671.

Objective.—To determine antituberculosis drug-resistance patterns, geographic distribution, demographic characteristics, and risk factors of reported tuberculosis (TB) patients in the Untied States. Design.—Survey of reported TB cases in the United States. For culture-positive cases reported to the Centers for Disease Control and Prevention, we asked health departments to provide drug susceptibility test results from initial *Mycobacterium tuberculosis* isolates. Study Population.—Culture-positive TB cases in the United States reported during the first quarter of 1991. Main Outcome Measures.—Individual TB case reports submitted to the Centers for Disease Control and Prevention and drug susceptibility test results. Result.—Resistance to one or more antituberculosis drugs was found in 14.2% of cases. Resistance to isoniazid and/or rifampin was found in 9.5% of cases whose isolates were tested against one or both drugs; such cases were found in 107 counties in 33 states. Resistance to both isoniazid and rifampin (multidrug-resistant [MDR] TB) was found in 3.5% of cases whose isolates were tested against both drugs; such cases were found in 35 counties in 13 states. New York City accounted for 61.4% of the nation's MDR TB cases. The 3-month population-based incidence rate of MDR TB in New York City was 52.4 times (95% confidence interval [CI], 35.5 to 78.3) that of the rest of the nation (9.559 vs 0.182 cases per million population). Compared with the rate in non-Hispanic whites in the rest of the nation (0.032 cases per million), the relative risk of MDR TB in New York City non-Hispanic whites was 39.0 (95% CI, 8.1 to 164.5), 299.3 (95% CI, 112.5 to 927.1)

in Hispanics, 420.9 (95% CI, 121.0 to 1515.8) in Asian/Pacific islanders, and 701.0 (95% CI, 296.4 to 2018.1) in non-Hispanic blacks. Conclusions.—With nearly 10% of TB patients resistant to isoniazid and/or rifampin, greater use of four-drug regimens and directly observed therapy is indicated. Aggressive intervention to prevent the further spread of MDR TB is needed to find every TB patient and to provide optimal patient management to ensure completion of chemotherapy.—Authors' Abstract

Bothamley, G. H. and Rudd, R. M. Clinical evaluation of a serological assay using a monoclonal antibody (TB72) to the 38 kDa antigen of *Mycobacterium tuberculosis*. Eur. Respir. J. **7** (1994) 240–246.

We examined an enzyme-linked immunosorbent assay (ELISA) modification of a radioimmunoassay, using the TB72 monoclonal antibody, as a serological test for tuberculosis in a clinical setting. Sera were obtained from 238 patients with suspected pulmonary tuberculosis, 30 patients treated for tuberculosis, 28 contacts, and 480 random samples from inpatients. Antibody levels were measured as the dilution of serum causing 50% inhibition of binding of the TB72 monoclonal antibody, which binds to an epitope of the 38-kDa antigen specific to the *Mycobacterium tuberculosis* complex, a positive titer being > 3. Positive antibody titers were present in 21 out of 25 (84%) patients with smear-positive and 22 out of 27 (82%) patients with smear-negative, culture-positive tuberculosis, and 37 out of 41 (90%) patients successfully treated for tuberculosis but without bacteriological confirmation of disease. Three out of 82 (4%) patients with a firm alternative diagnosis to tuberculosis gave a positive result. Serological tests were negative within 2.5 years of successful treatment. Patients without a definite diagnosis 1 year after tuberculosis had been suspected, and those who had received inadequate treatment for tuberculosis, were frequently positive (21 out of 31 and 21 out of 32, respectively). Positive tests concurred with tuberculin reactivity in 8 out of 11 contacts given chemoprophylaxis. Screening of 480 random serum samples gave 22 positive titers, 16 of which were not associated with tuberculosis; none of these 16 had an antibody titer > 10. We conclude that the TB72 test provides additional information in the diagnosis and treatment of tuberculosis. Antibody titers > 10 suggest active tuberculosis; titers of 3–10 merit observation.—Authors' Abstract

Braibant, M., De Wit, L., Peirs, P., Kalai, M., Ooms, J., Drowart, A., Huygen, K. and Content, J. Structure of the *Mycobacterium tuberculosis* antigen 88, a protein related to the *Escherichia coli* PstA periplasmic phosphate permease subunit. Infect. Immun. **62** (1994) 849–854.

We report the cloning and sequencing of the gene coding for antigen 88 from *Mycobacterium tuberculosis* by using monoclonal antibodies to screen an expression library in lambda gt11. The gene encodes a 403-amino-acid-residue protein, with a calculated molecular mass of 43,790 Da which contains seven putative transmembrane orhelical domains and presents a significant homology to the PstA protein of *Escherichia coli*. In its N-terminal region, it contains a 61-amino-acid region highly homologous to the fifth transmembrane helix off *E. coli* PstC. PstA and PstC are the two hydrophobic subunits of an *E. coli* periplasmic phosphate permease. Since the phosphate-binding subunit of this putative permease in *M. tuberculosis* has previously been characterized, i.e., the 38-kDa mycobacterial protein (also called protein antigen b, Ag5, and Ag78) homologous to PstS off *E. coli*, it seems likely that functional permeases analogous to the periplasmic permeases of gram-negative bacteria also exist in mycobacteria.—Authors' Abstract

Cage, G. D. Direct identification of *Mycobacterium* species in BACTEC 7H12B medium by high-performance liquid chromatography. J. Clin. Microbiol. **32** (1994) 521–524.

Primary cultures of mycobacteria grown in BACTEC 7H12B medium with and without the addition of oleic acid-albumin-dextrose-catalase (OADC) enrichment were analyzed for their mycolic acid patterns by high-performance liquid chromatography. Of the 126 isolates grown in medium to which OADC was added, 117 (93%) were successfully identified to the species level.

The time to identification of *Mycobacterium tuberculosis* (N = 65) averaged 19 days, and the average time was 21 days for nontuberculosis mycobacteria (N = 52) from initial specimen processing. None of the 10 isolates cultured without OADC were identified. The mycolic acid patterns were considered reliable for identification if the height of the tallest peak in the chromatogram was at least 50% of the internal standard peak height.—Authors' Abstract

Dellisola, B., Poyart, C., Goulet, O., Mougenot, J. F., Sadounjourno, E., Brousse, N., Schmitz, J., Ricour, C. and Berche, P. Detection of *Mycobacterium paratuberculosis* by polymerase chain reaction in children with Crohn's disease. J. Infect. Dis. **169** (1994) 449–451.

Mycobacterium paratuberculosis, the causative agent of Johne's disease (a chronic enteritis in ruminants), has been suspected to be involved in Crohn's disease. In this study, polymerase chain reaction was used to detect the presence of IS900 DNA sequences specific to *M. paratuberculosis* genomes in biopsies and surgical resections from 53 children with various gastrointestinal diseases and disorders. IS900 sequences were found in 13 of 18 samples from patients with Crohn's disease (72%; $p < 0.01$ vs samples from patients without Crohn's disease), in 1 of 5 with ulcerative colitis, in 2 of 6 with severe unclassified colitis, and in 7 of 24 with other gastrointestinal illnesses. These results appear to support the hypothesis that *M. paratuberculosis* is involved in the pathogenesis of Crohn's disease.—Authors' Abstract

Denis, M. and Ghadirian, E. Interleukin-1 is involved in mouse resistance to *Mycobacterium avium*. Infect. Immun. **62** (1994) 457–461.

In this study, we examined the contribution of the monokine interleukin-1 (IL-1) in mouse resistance to the intracellular pathogen *Mycobacterium avium*. The effect of neutralizing endogenous IL-1 in mouse macrophage resistance to *M. avium* infection was investigated. Infection of mouse peritoneal macrophages with *M. avium* B101 was shown to result in significant IL-1 beta release by cells at 4 and 7 days postinfection. Addition of IL-I receptor antagonist (IL-1ra) at doses of 5 μg daily, which neutralized endogenous IL-1, failed to significantly modify the intracellular growth of *M. avium*. Mice were injected with *M. avium* B101 by the intravenous route, and the growth of the mycobacteria was monitored in the organs of intact mice and in those of mice that received repeated high doses of IL-1ra. The infection with *M. avium* elicited the production of large amounts of IL-1 in the lungs, livers, and spleens. Repeated injections of IL-1ra into *M. avium*-infected mice resulted in moderately enhanced growth of the bacilli in the livers and spleens but in much enhanced growth in the lungs. The enhanced growth of *M. avium* in the lungs correlated with a diminished inflammatory influx of cells (particularly neutrophils) in the bronchoalveolar space. These data argue for a role for IL-1 in host resistance to *M. avium* infections.—Authors' Abstract

Fattorini, L., Xiao, Y., Li, B., Santoro, C., Ippoliti, F. and Orefici, G. Induction of IL-1 beta, IL-6, TNF-alpha, GM-CSF and G-CSF in human variants of *Mycobacterium avium*. J. Med. Microbiol. **40** (1994) 129–133.

Both smooth transparent (SmT) and smooth domed-opaque (SmD) colonial variants were obtained from a strain of *Mycobacterium avium* isolated from a patient with AIDS. The two variants showed similar biochemical characteristics but SmT bacteria proliferated better than SmD bacteria inside human macrophages and were much less capable than the SmD variant of inducing the release of IL-1 beta, IL-6, TNF-alpha, GM-CSF and G-CSF, after incubation for either 3 or 6 days. Since cytokines are important extracellular signals for immune cells, the lack of induction observed in SmT-infected macrophages may be one of the pathogenic mechanisms of *M. avium*.—Authors' Abstract

Fifis, T., Corner, L. A., Rothel, J. S. and Wood, P. R. Cellular and humoral immune responses of cattle to purified *Mycobacterium bovis* antigens. Scand. J. Immunol. **39** (1994) 267–274.

Cellular responses to several purified antigens of *Mycobacterium bovis* were exam-

ined in experimentally infected cattle over a period of 36 months, using *in vitro* cellular proliferation and interferon-gamma assays. These antigens (12, 19, 22a, b, 24, 25, 30, 32, 39, 65 and 70 kDA) included the majority of *M. bovis* protein antigens described to date and are highly homologous to those purified from *M. tuberculosis*. Cellular responses were examined at 3-month time intervals during the 36-month course of infection. All purified antigens induced cellular immune responses in the infected animals. The onset and magnitude of response to individual antigens varied among the animals. At any specific time during the period of infection one or more antigens appeared to be immunodominant, but the immunodominance profile changed as the infection progressed. Humoral immune response were low or absent in the first half of the infection period, but increased substantially for some of the antigens during the second half. Variation was observed among the different animals as to which antigens they recognized.—Authors' Abstract

Goodger, J., Russell, W. P., Nolan, A. and Newell, D. G. Production and characterization of a monoclonal badger anti-immunoglobulin G and its use in defining the specificity of *Mycobacterium bovis* infection in badgers by Western blot. Vet. Immunol. Immunopathol. **40** (1994) 243–252.

A mouse monoclonal anti-badger IgG antibody was produced to investigate the specificity of the antibody response of badgers infected with *Mycobacterium bovis*. The monoclonal antibody generated was directed against badger IgG heavy chain and appeared to be species restricted, reacting only with badger and dog IgGs but not cat, rabbit, mouse, guinea pig, bovine or ferret IgGs. This monoclonal antibody detection system functioned well in both ELISA and Western blot analyses, and was successfully used to investigate the humoral response of the badger to *M. bovis* infection. Sera from infected badgers detected a 25-kDa antigen which was not detected by sera from *M. bovis* culture-negative animals. This antigen was conserved in all field strains of *M. bovis* tested, and seroconversion to it was detected during experimental infection. The immunodominance of this antigen in the badger during infection with *M. bovis* suggests that this 25-kDa polypeptide is a suitable candidate on which to base an antibody detection test for *M. bovis* infection.—Authors' Abstract

Hoffner, S. E., Heurlin, N., Petrini, B., Svenson, S. B. and Kallenius, G. *Mycobacterium avium* complex develop resistance to synergistically active drug combinations during infection. Eur. Respir. J. 7 (1994) 247–250.

Isolates of *Mycobacterium avium* complex from five patients on long-term (3–5 years) antimycobacterial drug treatment were collected during the early and late phase of disease, and studied *in vitro* for their susceptibility to antimycobacterial drugs and drug combinations. All isolates were resistant or moderately resistant to ethambutol, rifampin and streptomycin when given singly; however, all strains isolated early in the disease were susceptible to the combination of ethambutol with either rifampin or streptomycin. All late isolates had developed resistance to one or both of these combinations. Three of the patients died within a year after the last isolation of *M. avium* complex, and the two remaining patients still have severe chronic disease. It is concluded that the susceptibility of *M. avium* strains to combinations of drugs should be monitored during the course of treatment in order to guide the selection of effective drug combinations throughout the infection.—Authors' Abstract

Honore, N. and Cole, S. T. Streptomycin resistance in mycobacteria. Antimicrob. Agents Chemother. **38** (1994) 238–242.

Streptomycin, the first antibiotic used in tuberculosis control programs, perturbs protein synthesis at the ribosome level. It is shown here that streptomycin resistance in some clinical isolates of *Mycobacterium tuberculosis* is associated either with missense mutations in the *rpsL* gene, which encodes ribosomal protein S12, or with base substitutions at position 904 in the 16S rRNA. The primary structure of the S12 protein is well conserved among the mycobacteria, even those, such as *M. avium,*

M. gordonae, and *M. szulgai,* that are naturally resistant to streptomycin. This suggests that permeability barriers may be responsible for the resistance to the antibiotic.—Authors' Abstract

Huygen, K., Lozes, E., Gilles, B., Drowart, A., Palfliet, K., Jurion, F., Roland, I., Art, M., Dufaux, M., Nyabenda, J., De Bruyn, J., Van Vooren, J. P. and Deleys, R. Mapping of Th1 helper T-cell epitopes on major secreted mycobacterial antigen 85A in mice infected with live *Mycobacterium bovis* BCG. Infect. Immun. **62** (1994) 363–370.

TH1 cytokine secretion was examined in response to synthetic peptides of the 85A component of the major secreted, fibronectin-binding antigen 85 complex from *Mycobacterium tuberculosis* in seven different mouse strains infected with live *M. bovis* BCG. Twenty-eight overlapping 20-mer peptides covering the complete mature 295-amino-acid (AA) protein were synthesized. Significant interleukin-2 (IL-2) and gamma interferon (IFN-γ) secretion could be measured following *in vitro* stimulation of spleen cells with these peptides. H-2(d) haplotype mice reacted preferentially against the amino-terminal half of the protein, i.e., against peptide 5 (AA 41 to 60) and especially against peptide 11 (AA 101 to 120), which contained an I-Ed binding motif. H-2(b) haplotype mice, on the other hand, reacted against peptides from both amino- and carboxyl-terminal halves of the protein, peptide 25 (AA 241 to 260) being the most potent stimulator of IL-2 and IFN-γ production. (BALB/c × C57BL/6)F, animals with the H-2(d/b) haplotype weakly recognized peptides specific for both parental lines. Finally, CBA/J (H-2(k)) and major histocompatibility complex class II mutant B6.C.bm12 mice, carrying a mutant I-A beta (bm12) allele on an H-2(b) background, reacted only very weakly to the 85A peptides. Reactive T cells isolated from lungs of BCG-infected H-2(b) haplotype mice recognized the same epitopes as spleen cells, especially peptide 25. These data confirm previous findings regarding the powerful IL-2 and IFN-γ-inducing properties of antigen 85 during infection with live *M. bovis* BCG.—Authors' Abstract

Kanyok, T. P., Reddy, M. V., Chinnaswamy, J., Danziger, L. H. and Gangadharam, P. R. J. *In vivo* activity of paromomycin against susceptible and multidrug-resistant *Mycobacterium tuberculosis* and *M. avium* complex strains. Antimicrob. Agents Chemother. **38** (1994) 170–173.

Encouraged by *in vitro* results, we have assessed the *in vivo* activity of paromomycin (PRM) against *Mycobacterium tuberculosis,* multidrug-resistant (MDR) *M. tuberculosis* (resistant to isoniazid, rifampin, and streptomycin), and *M. avium* complex in C57BL/6 mice and their beige counterparts. In all these experiments, PRM was effective in preventing mortality from a mycobacterial infection and was significantly more active than the drug-free control ($p < 0.0005$) in reducing the CFU relative to the mean log CFU in the lungs, livers, and spleens of infected animals. In the drug-susceptible *M. tuberculosis* experiment, PRM given at 100 and 200 mg/kg of body weight was significantly less active than isoniazid at 25 mg/kg ($p < 0.0005$) in reducing the mean log CFU in the lungs, livers, and spleens of infected mice. In the MDR *M. tuberculosis* experiment, PRM given at 200 mg/kg was effective, relative to the drug-free control, in reducing the mean log CFU of an isolate of *M. tuberculosis* resistant to isoniazid, rifampin, and streptomycin. In the *M. avium* complex experiment, PRM given at 200 mg/kg was as effective as amikacin at 50 mg/kg in reducing the mean log CFU in the lungs, livers, and spleens of infected mice. On the basis of our experiments, we believe that PRM has promising activity *in vivo* in the treatment of infections caused by *M. tuberculosis,* MDR *M. tuberculosis,* and *M. avium* complex.—Authors' Abstract

Kapur, V., Li, L.-L., Iordanescu, S., Hamrick, M. R., Wanger, A., Kreisworth, B. N. and Musser, J. M. Characterization by automated DNA sequencing of mutations in the gene (*rpoB*) encoding the RNA polymerase β subunit in rifampin-resistant *Mycobacterium tuberculosis* strains from New York City and Texas. J. Clin. Microbiol. **32** (1994) 1095–1098.

Automated DNA sequencing was used to characterize mutations associated with ri-

fampin resistance in a 69-bp region of the gene, *rpoB*, encoding the β subunit of RNA polymerase in *Mycobacterium tuberculosis*. The data confirmed that greater than 90% of rifampin-resistant strains have sequence alterations in this region and showed that most are missense mutations. The analysis also identified several mutant *rpoB* alleles not previously associated with resistant organisms and one short region of *rpoB* that had an unusually high frequency of insertions and deletions. Although many strains with an identical IS*6110* restriction fragment length polymorphism pattern have the same variant *rpoB* allele, some do not, a result that suggests the occurrence of evolutionary divergence at the clone level.—Authors' Abstract

Kellner, H., Wen, J., Wang, J., Raybourne, R. B., Williams, K. M. and Yu, D. T. Y. Serum antibodies from patients with ankylosing spondylitis and Reiter's syndrome are reactive with HLA-B27 cells transfected with the *Mycobacterium tuberculosis* hsp60 gene. Infect. Immun. **62** (1994) 484–491.

HLA-B27-related arthritis is probably mediated by an immune response against HLA-B27 complexed with peptides derived from proteins of arthritis-causing bacteria. Immunogenic proteins, with a high degree of homology among bacteria, such as in the hsp60 family, are likely candidates. To create such complexes experimentally, we transfected an HLA-B27 cell line with the *Mycobacterium tuberculosis* hsp60 gene. Because of previous observations that HLA-B27-peptide complexes can be distinguished by antibodies, we tested the transfected cell line with a panel of sera from 24 HW-B27(+) arthritis patients. Significant antibodies were detected in at least eight of the sera. Several cell lines and peptides were used as negative controls to ensure that the antibody reactivity was specific to HLA-BZ7-peptide complexes. A panel of nine peptides derived from the sequence of the *Mycobacterium* hsp60 were synthesized and tested. At least three were identified as being responsible for the serological activities.—Authors' Abstract

Lemassu, A. and Daffe, M. Structural features of the exocellular polysaccharides of *Mycobacterium tuberculosis*. Biochem. J. **297** Part 2 (1994) 351–357.

The cell envelope which surrounds pathogenic mycobacteria is postulated to be a defense barrier against phagocytic cells and its outermost constituents have a tendency to accumulate in the culture medium. The present work demonstrates that the exocellular material of *Mycobacterium tuberculosis* contains large amounts of polysaccharides with only traces, if any at all, of lipids. Three types of polysaccharides were purified by anion-exchange and gel-filtration chromatography; all were found to be neutral compounds devoid of acyl substituents. They consisted of D-glucan, D-arabino-D-mannan and D-mannan, which were eluted from gel-filtration columns in positions corresponding to molecular masses of 123, 13 and 4 kDa, respectively. Their predominant structural features were determined by the characterization of the per-O-methyl derivatives of enzymic, acetolysis and Smith-degradation products and by H-1- and C-13-n.m.r. spectroscopy of the purified polysaccharides, using mono- and two-dimensional homonuclear chemical-shift correlated spectroscopy and two-dimensional heteronuclear (H-1/C-13) spectroscopy. The glucan which represented up to 90% of the polysaccharides was composed of repeating units of five or six → 4-alpha-D-Glcp-1 → residues and a → 4-alpha-D-Glcp substituted at position 6 with an alpha-D-Glcp, indicating a glycogen-like highly branched structure not related to the so-called polysaccharide-II previously identified in tuberculin. The arabinomannan consisted of a mannan segment composed of a → 6-alpha-D-Man-1 → core substituted at some positions 2 with an alpha-D-Manp. The arabinin termini of the arabinomannan were found to be extensively capped with mannosyl residues. The possibility that these polysaccharides contribute to the persistence of the tubercle bacillus in the macrophage by molecular mimicry is discussed.—Authors' Abstract

Mastroianni, C. M., Paoletti, F., Valenti, C., Massetti, A. P., Vullo, V. and Delia, S. Tumour necrosis factor-alpha production and immune cell activation in tuberculous meningitis. Mediat. Inflamm. **3** (1994) 57–60.

The local production of tumor necrosis factor-alpha (TNF-α) was evaluated in the cerebrospinal fluid (CSF) from ten patients with tuberculous meningitis (TBM). The degree of intrathecal immune activation was also studied by assessing the CSF levels of beta2-microglobulin (beta2-M) and adenosine deaminase activity (ADA). Results indicate that elevated CSF concentrations of TNF-α, beta2-M and ADA were found in all TBM patients. Moreover, TNF-α is produced and selectively concentrated for a long period of time, while beta2-M and ADA values progressively decline during the course of TBM. Our findings suggest that in TBM patients, after an early activation of immune cells, there is an enhanced and continuous production of TNF-α at the site of infection.—Authors' Abstract

Nolan, C. M., Sandblom, R. E. Thummel, K. E., Slattery, J. T. and Nelson, S. D. Hepatotoxicity associated with acetaminophen usage in patients receiving multiple drug therapy for tuberculosis. Chest **105** (1994) 408–411.

We report three patients who experienced hepatotoxic reactions in association with acetaminophen ingestion while undergoing treatment for active tuberculosis with isoniazid, rifampin, and other agents; all were young adult women. One patient intentionally took a large amount of acetaminophen and had typical signs and symptoms of acetaminophen overdosage; another took acetaminophen in combination form for a minor upper respiratory illness. She experienced no symptoms. The remaining patient took acetaminophen to ameliorate the symptoms of fever and malaise that were subsequently attributed to tuberculosis. She had the rapid onset of signs and symptoms of isoniazid hepatotoxicity. The patterns of liver function abnormalities were similar: each patient experienced pronounced serum elevations of hepatocellular enzymes with at most only modest rises in those of bilirubin. All antituberculous drugs were withheld until symptoms resolved and laboratory values became normal; then treatment for tuberculosis was resumed without isoniazid and was successfully completed in all three patients. These cases plus similar reports in the literature suggest that isoniazid or rifampin, or both, may potentiate the hepatotoxicity of acetaminophen, perhaps by induction of cytochrome P450 isozymes that oxidize acetaminophen to its toxic metabolites.—Authors' Abstract

Onyeji, C. O., Nightingale, C. H., Nicolau, D. P. and Quintiliani, R. Efficacies of liposome-encapsulated clarithromycin and ofloxacin against *Mycobacterium avium-M. intracellulare* complex in human macrophages. Antimicrob. Agents Chemother. **38** (1994) 523–527.

The therapeutic effects of liposome-encapsulated ofloxacin and clarithromycin against *Mycobacterium avium-M. intracellulare* (MAI) were evaluated in a model of intramacrophage infection. Liposome encapsulation was found to markedly enhance the uptake of each of the drugs by human macrophages. The human blood-derived macrophages were infected at day 7 of culture with MAI. Treatment was initiated 24 hr after the infection, and the number of intracellular bacteria was determined at days 2, 3, and 4. Liposome entrapment of either ofloxacin or clarithromycin significantly ($p < 0.005$) enhanced the activities of the drugs when compared with the antimycobacterial effects of equivalent concentrations of the free (unentrapped) drugs. The drugs were used at concentrations close to their clinically achievable peak levels. The efficacy of clarithromycin, either in the free or liposome-entrapped form, was markedly higher than that of ofloxacin. Liposome-encapsulated ofloxacin or clarithromycin plus ethambutol was, in each case, more effective in organism eradication ($p < 0.005$) than each agent used singly. These results suggest that liposome-encapsulated clarithromycin may be more effective than the free form of the drug against MAI infections *in vivo,* and the use of a combination therapy with ethambutol could further enhance the efficacy.—Authors' Abstract

Peltola, H., Mohamed, O. N., Kataja, M., Salminen, S., Tuittula, T., Peltola, T. L. and Brander, E. Risk of infection with *Mycobacterium tuberculosis* among children and mothers in Somalia. Clin. Infect. Dis. **18** (1994) 106–111.

The prevalence of infection with *Mycobacterium tuberculosis* in prewar Somalia

was surveyed by testing the tuberculin sensitivity of 2792 infants and children and 446 mothers in two towns: Burao in the dry north and Kismayo in the humid south. Sensitivity increased with age, but considerable differences prevailed between the towns. In Burao a roughly linear increase in sensitivity was found, with no sensitivity in infancy, sensitivity in 19% of children at 7 years, and sensitivity in 54% of children at 15 years; in Kismayo the corresponding figures were 9%, 28%, and 47%, respectively. Together, the correlation of prior BCG vaccination with a positive tuberculin test in Burao and the lack of these findings in Kismayo suggested that vaccination had partly failed in Kismayo, where living conditions also favored the transmission of *M. tuberculosis.* The annual risk of *M. tuberculosis* infection was similar to 1% higher in the south than in the north and was much higher during the first 3 years of life than later. This study—the first defining the risk of *M. tuberculosis* infection among children of various ages in Somalia—indicates that this risk is greatest in the southern parts of the country and among infants and young children.—Authors' Abstract

Penneys, N. S., Leonardi, C. L., Cook, S., Blauvelt, A., Rosenberg, S., Eells, L. D., Konwiser, M. and Aaronson, C. M. Identification of *Mycobacterium tuberculosis* DNA in five different types of cutaneous lesions by the polymerase chain reaction. Arch. Dermatol. **129** (1993) 1594–1598.

A spectrum of skin lesions are believed to be secondary to the presence of *Mycobacterium tuberculosis.* Demonstration of *M. tuberculosis* directly or in culture in some of these eruptions can be difficult. We used the polymerase chain reaction (PCR) and a primer/probe set specifically for *M. tuberculosis* complex DNA to evaluate five types of skin lesions clinically considered to represent infection by, or reaction to, *M. tuberculosis. M. tuberculosis* DNA was demonstrated in paraffin-embedded sections of these five cases, representing a variety of clinical and histologic patterns. In two cases, *M. tuberculosis* could not be demonstrated by routine cultural methods. DNA diagnostic methods such as the PCR can be used to rapidly identify cutaneous lesions produced by *M. tuberculosis.*—Authors' Abstract

Peters, J. and Spicher, G. Model tests for the efficiency of disinfectants on surfaces. 3. Communication—Dependence on test results upon the kind of the active substances and the test germs (*Staphylococcus aureus, Mycobacterium terrae*)—review. Zentralbl. Hyg. Umweltmed. **195** (1994) 97–110. (in German)

A new test method for surface disinfectants was applied to investigate the efficacy of the most important active components of disinfectants to *Staphylococcus aureus* and *Mycobacterium terrae.* The test germs were embedded in coagulated blood. Frosted glass served as the test surface. The disinfection was performed by applying a fixed amount of the disinfectant and mixing it with the contamination by rubbing. The number of surviving germs was determined quantitatively. Generally, the two test germs showed a distinctly different behavior toward the active substances applied. Mycobacteria proved to be clearly more resistant than staphylococci, except with formaldehyde and the cresol-soap solution. To formaldehyde, the mycobacteria were only a little more resistant, while to cresol-soap solution they were even a little more sensitive than were staphylococci. Compounds containing active chlorine showed a sufficient effect on mycobacteria only if the consumption of the active component by blood was nearly excluded. The quaternary ammonium compound and glyoxal, even the high concentrations, showed a totally insufficient efficacy to mycobacteria. The results shall provide the basis for a new guideline to be established by the Federal Health Office concerning the efficacy testing of surface disinfectants effective against mycobacteria, especially tuberculosis bacteria.—Authors' English Abstract

Pfyffer, G. E., Kissling, P., Wirth, R. and Weber, R. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J. Clin. Microbiol. **32** (1994) 918–923.

A total of 938 respiratory specimens (633

sputa, 249 bronchial and tracheal aspirates, and 56 bronchoalveolar lavages) from 589 patients were tested for direct detection of *Mycobacterium tuberculosis* complex by the Gen-Probe amplified *M. tuberculosis* direct test (MTD), and the results were compared with those of the conventional methods of fluorescence microscopy and cultivation (solid and radiometric media). One series of specimens (N = 515) was decontaminated with *N*-acetyl-L-cysteine (NALC)-NaOH; the other one (N = 423) was decontaminated with sodium dodecyl (lauryl) sulfate (SDS)-NaOH. Of the specimens decontaminated with NALC, 39 were MTD and culture positive, 455 were MTD and culture negative, 18 were MTD positive and culture negative, and 3 were MTD negative and culture positive, indicating a sensitivity of 92.9% and a specificity of 96.2% for the MTD. Of the specimens decontaminated with SDS, 35 were MTD and culture positive, 372 were MTD and culture negative, 15 were MTD positive and culture negative, and 1 was MTD negative and culture positive, indicating a sensitivity of 97.2% and a specificity of 96.1% for the MTD. After resolution of discrepant results by review of the patients' clinical data, the sensitivity of the MTD was 93.9%, the specificity was 97.6%, the positive predictive value was 80.7%, and the negative predictive value was 99.3% for the NALC series; the corresponding values were 97.4%, 96.9%, 76.0%, and 99.7%, respectively, for the SDS series. In conclusion, the MTD is a highly sensitive and specific technique for detecting *M. tuberculosis* complex within hours in both smear-positive and smear-negative respiratory specimens.—Authors' Abstract

Rodriques, L., Diwan, V. K. and Wheeler, J. G. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis; a meta-analysis. Int. J. Epidemiol. **22** (1993) 1154–1158.

The protective effect of BCG against tuberculosis (TB) estimated in randomized controlled trials and observational studies ranges from negative to close to 100%. One of the many explanations offered for this is that different immunological mechanisms may be associated with the protective effect against different forms and sites of disease. In this investigation, we then recalculated vaccine protective effect separately for pulmonary disease and for meningeal/miliary disease in randomized controlled trials and case-control studies, tested for heterogeneity in site-specific estimates of protective effect, and calculated a summary measure when appropriate. We found the protective effect against pulmonary disease to be heterogenous to a statistically significant degree, and thus we did not calculate a summary measure of protection. The protective effect against meningeal and miliary TB was higher than against pulmonary disease and, except for a single study with two cases only, appeared to be homogeneous. Summary BCG protective effect against miliary or meningeal TB in randomized controlled trials was 86% (95% confidence interval [CI] 65, 95) and in case-control studies 75% (95% CI: 61, 84). The fact that the protective effect appeared to be homogeneous against meningitis and miliary TB but not against pulmonary disease may result from the fact that patients with meningitis are, on average, younger and thus less likely to have been exposed to atypical bacteria; to a waning of the protective effect of BCG; or from the diversity of mechanisms of pathogenesis of pulmonary disease, which can originate from reinfection, reactivation or primary progression.—Authors' Abstract

Shiratsuchi, H., Johnson, J. L., Toossi, Z. and Ellner, J. J. Modulation of the effector function of human monocytes for *Mycobacterium avium* by human immunodeficiency virus-1 envelope glycoprotein gp120. J. Clin. Invest. **93** (1994) 885–891.

Disseminated *Mycobacterium avium* infection in AIDS is associated with high tissue burdens (10^9–10^{10} mycobacteria/g tissue) of organism. The basis for the extraordinary susceptibility of AIDS to *M. avium* infection is unclear. HIV or its constituents may alter mononuclear phagocyte functions resulting in enhanced intracellular *M. avium* growth. The effects of an envelope glycoprotein (gp120) a transmembrane protein (p121), and core proteins of HIV-1 on *M. avium* infection of human monocytes were examined. Preculturing monocytes with gp120 inhibited *M. avium* phagocytosis and consistently enhanced intracellu-

lar growth of six *M. avium* strains. Pretreatment with p121, gag5, or p24 did not inhibit phagocytosis nor enhance intracellular growth of *M. avium*. Incubation of gp120 with soluble CD4 before addition to monocyte cultures or pretreatment of monocytes with OKT4A abrogated gp120 effects on *M. avium* phagocytosis and intracellular growth. gp120 also augmented cytokine production by infected monocytes. These results suggest that gp120, but not p121 or core proteins, modulate monocyte phagocytosis and enhance intracellular growth of *M. avium* at least in part through monocyte CD4 receptors. Direct effects of HIV-1 products may, therefore, contribute to the diathesis of AIDS to develop disseminated *M. avium* infection and to the extensive replication of the organisms within tissue macrophages.—Authors' Abstract

Srivastava, L., Prasanna, S. and Srivastava, V. K. Diagnosis of tuberculous meningitis by ELISA test. Indian J. Med. Res. **99** (1994) 8–12.

Detection of IgG antibodies to *Mycobacterium tuberculosis* H37Ra antigen in cerebrospinal fluid (CSF) by ELISA test appears to be highly sensitive. In 90% (18/20) of proven cases of tuberculous meningitis (TBM) antibodies were present in CSF; in 75% (15/20) antibodies were also present in the sera. In the patients clinically suspected to have TBM, antibodies in CSF and sera were present in 87.5% (42/48) and 70.8 (34/48), respectively; whereas in the control group antibodies were present in only one serum sample and in none of the CSF samples. The results indicate that ELISA test using sonicated *M. tuberculosis* H37Ra as antigen is a sensitive and specific test for diagnosis of TBM.—Authors' Abstract

Stanford, J. L., Onyebujoh, P. C., Rook, G. A. W., Grange, J. M. and Pozniak, A. Old plague, new plague, and a treatment for both? (Letter) AIDS **7** (1993) 1275–1276.

In a placebo-controlled study of immunotherapy as an adjunct to chemotherapy for pulmonary tuberculosis in Nigeria, 8 HIV-seropositive patients were immunized with *Mycobacterium vaccae* and 9 HIV-seropositive patients received placebo. After 10–12 months all 8 recipients of immunotherapy were still alive compared with only 3 placebo recipients. The 2 immunotherapy recipients who were retested at follow-up were found to have become seronegative for HIV-1; all placebo recipients remained seropositive. The correspondents hypothesize that *M. vaccae*, which restores protective immunity against *M. tuberculosis*, simultaneously corrects the response to HIV in dually infected patients.—H. Richardson (Trop. Dis. Bull.)

Surcel, H. M., Troye Blomberg, M., Paulie, S., Andersson, G., Moreno, C., Pasvol, G. and Ivanyi, J. Th1/Th2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigen. Immunology **81** (1994) 171–176.

Proliferation and cytokine production profiles by blood mononuclear cells in response to *in vitro* stimulation with mycobacterial antigens were compared in patients with active tuberculosis and in sensitized healthy controls. Interleukin-4 (IL-4) and interferon-gamma (INF-γ) were detected at single-cell level using the ELISPOT assay. Patients showed significantly ($p < 0.01$) increased numbers of IL-4-secreting cells and decreased thymidine incorporation, but no significant difference in IFN-γ-producing cells in response to the 38,000 MW or 19,000 MW antigens and their immunodominant peptide epitopes. Pronounced individual variations were found in both patient and control groups when comparing the responsiveness to the mycobacterial extract, two protein antigens and five synthetic peptides. None of the antigens or peptides tested showed preferential stimulation of either IL-4- or IFN-γ-secreting T cells, and proliferation was not correlated with either IL-4 or IFN-γ production. In particular, cytokine responsiveness was of similar frequency in subjects who did or did not show positive proliferation, indicating that the latter test was not fully representative of the active T-cell repertoire. It is concluded that the demonstrated Th2 type of profile in response to two prominent mycobacterial antigens may play a role in the mechanisms of defective host

resistance in tuberculosis.—Authors' Abstract

Telenti, M., Dequiros, J. F. B., Alvarez, M., Rionda, M. J. S. and Mendoza, M. C. The diagnostic usefulness of a DNA probe for *Mycobacterium tuberculosis* complex (Gen-Probe®) in BACTEC cultures versus other diagnostic methods. Infection **22** (1994) 18–23.

The combination of the radiometric BACTEC system and the Gen-Probe (R) for *Mycobacterium tuberculosis* complex (MTB) in detection and identification of mycobacteria was evaluated. Lowenstein-Jensen and Coletsos media for isolation, and BACTEC-NAP(R) test and cellular morphology and grouping for identification of MTB and mycobacteria other than tuberculosis (MOTT) were also evaluated. The study included all specimens submitted to our laboratory for mycobacteria detection over a 6-month period. The mean recovery times of MTB were 13.7, 23.3, and 21.2 days for BACTEC, Lowenstein-Jensen and Coletsos, respectively. BACTEC system recovered 87.8% of MTB strains and 98% of MOTT, and the conventional media 82.9% of MTB and 19.2% of MOTT. Ziehl-Neelsen smears and BACTEC-NBPO were effective in differentiating MTB and MOTT strains (3.3% and 9.6%, respectively, of the cultures were uninterpretable). Gen-Probe(R) (cutoff point for MTB = 5% of hybridization) was applied to 100 positive vials of BACTEC. All cultures of MTB with a growth index (GI) > 400 displayed hybridization > 9% (average 28.9%) but for a GI < 400, 17% of cultures showed < 5% of hybridization (average 18.6%). All cultures for MOTT had < 5% hybridization. With the combination BACTEC/Gen-Probe the average time of the final report could be reduced to 15.5 days.—Authors' Abstract

Vandenbroek, J., Borgdorff, M. W., Pakker, N. G., Chum, H. J., Klokke, A. H., Senkoro, K. P. and Newell, J. N. HIV infection as a risk factor for the development of tuberculosis—a case-control study in Tanzania. Int. J. Epidemiol. **22** (1993) 1159–1165.

A population-based, case-control study was carried out in Mwanza Region, Tanzania, to determine the relative and population-attributable risk of the human immunodeficiency virus type 1 (HIV-1) infection for developing active tuberculosis. Cases were 441 consecutively diagnosed patients with tuberculosis (all types), aged 15–54 years. Controls were a representative population sample of 4161 people, drawn in a stratified cluster sample from urban areas, roadside settlements, and rural villages. HIV-1 infection was determined by ELISA and if the ELISA result was indeterminate by Western blot. The HIV-1 prevalence in cases was 23.0% in rural, 32.1% in roadside, and 54.1% in urban areas, while in controls these prevalences were 3.4%, 7.2% and 12.1%, respectively. The relative risk (RR) of HIV-1 infection for the development of active tuberculosis was estimated to be 8.3 (95% confidence interval [CI] 6.4–11.0). This risk varied little by sex or residence, but appeared to be more pronounced in the age group 25–34 years. The case-detection rate of tuberculosis in those aged 15–54 years was 125/100,000 people per year. The population-attributable risk was 36/100,000 people per year, implying that 29% of tuberculosis cases at present may be attributable to HIV-1 infection. It is concluded that HIV-1 infection is a major contributing factor to the increased case-detection rate of tuberculosis observed over the past 10 years in Mwanza Region; if the prevalence of HIV-1 continues to increase, the incidence of tuberculosis will continue to rise as well. Maintaining a high cure rate of tuberculosis patients will be imperative to prevent an increased risk of tuberculosis infection to HIV-1 infected and uninfected people.—Authors' Abstract

Voeller, D., Kovacs, J., Andrawis, V., Chu, E., Masur, H. and Allegra, C. Interaction of *Pneumocystis carinii* dihydropteroate synthase with sulfonamides and diaminodiphenyl sulfone (dapsone). J. Infect. Dis. **169** (1994) 456–459.

Dihydropteroate synthase is the target enzyme for the sulfonamide compounds which are the mainstay of therapy for *Pneumocystis carinii* pneumonia, a common infection in patients with impaired immunity. The stability of this enzyme, its kinetic con-

stants with respect to substrates, and the 50% inhibitory concentration (IC50) of several sulfonamides and the sulfone dapsone have been characterized using both cell-free and intact organism assay systems. Stability of the enzyme is dependent on storage temperature, reducing reagents, and to a lesser extent, protease inhibitors. The sulfonamides sulfadiazine and sulfamethoxazole were found to be highly potent inhibitors of *P. carinii* dihydropteroate synthase with IC50s of 0.42 and 0.71 μM, respectively. Dapsone had equivalent potency when compared with the most potent sulfonamides tested in both assay systems. Data suggest that sulfamethoxazole, sulfadiazine, and dapsone may represent equivalent choices as *P. carinii* dihydropteroate synthase inhibitors, assuming an equivalent *in vivo* drug exposure can be achieved.—Authors' Abstract

Vogetseder, W., Fille, M., Patscheider, S., Dierich, M. P. and Allerberger, F. Molecular epidemiology of tuberculosis in Austria. Clin. Invest. **72** (1994) 107–110.

The incidence of pulmonary tuberculosis among newspaper vendors, tram operators, and other exposed groups leads to repeated discussions about the importance of single cases for the spread of tuberculous infection. We subjected 36 strains of *Mycobacterium tuberculosis,* isolated in 1992 from 31 patients, to restriction fragment length polymorphism analysis using PvuII and an insertion element 986 probe. Only two isolates obtained from a married couple showed the same DNA fingerprinting pattern, all of the other strains had unique and clearly distinguishable banding patterns. Our investigation revealed no dominating strains, except in the case of one married couple where the chain of infection was obvious (the wife being diagnosed during the course of testing of her alcoholic and tuberculous husband's contacts). The main emphasis in the fight against tuberculosis still rests on securing the availability of diagnostic and therapeutic means for all patients with tuberculosis. The importance of single infected source cases for the spread of tuberculosis should not be overestimated.—Authors' Abstract

Watanabe, M., Yamada, Y., Iguchi, K. and Minnikin, D. E. Structural elucidation of new phenolic glycolipids from *Mycobacterium tuberculosis.* Biochim. Biophys. Acta **1210** (1994) 174–180.

From one clinical isolate of *Mycobacterium tuberculosis,* two new phenolic glycolipids (PGLs) were obtained as its major PGLs. These were dimycocerosyl esters of 2,4-di-*O*-methyl-fucopyranosyl-(alpha 1 → 3)-rhamnopyranosyl-(alpha 1 → 3)-2-*O*-methyl-rhamnopyranosyl-(alpha 1 →)-phenolphthiocerol A and-phenolphthriotriol A, which were produced by this strain at a ratio of about 5:1. Another clinical isolate of this species was found to produce PGL-tb1, and its analog 2,3,4-tri-*O*-methyl-fucopyranosyl-(alpha 1 → 3)-rhamnopyranosyl-(alpha 1 → 3)-2-*O*-methyl-rhamnopyranosyl-(alpha 1 →)-phenolphthriotriol A at a ratio of about 1:3. The fact that different strains of *M. tuberculosis* produce chemically different PGLs as their major PGLs may be related to the diversity of virulence of the clinical isolates of *M. tuberculosis.*—Authors' Abstract

Yakrus, M. A. and Straus, W. L. DNA polymorphisms detected in *Mycobacterium haemophilus* by pulsed-field gel electrophoresis. J. Clin. Microbiol. **32** (1994) 1083–1084.

Nineteen isolates of *Mycobacterium haemophilum* were analyzed by pulsed-field gel electrophoresis of large restriction fragments generated by digestion of chromosomal DNA with *Xba*I. Six patterns were observed. Twelve of 6 *M. haemophilum* isolates (75%) collected in the New York Metropolitan Area from 1990 to 1991 shared the same pattern, including all six isolates submitted from one hospital. Two different patterns were seen among the other four isolates. Individual isolates from Albany, N.Y., Florida, and Texas had unique patterns. Pulsed-field gel electrophoresis is the first method reported with the capability to type strains of *M. haemophilum* and will hopefully provide insight into the source and transmission of this emerging pathogen.—Authors' Abstract