CURRENT LITERATURE

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

Chemotherapy

Chong, P. Y. and Ti, T. K. Severe abdominal pain in low dosage clofazimine. Pathology **25** (1993) 24–26.

We describe a 41-year-old leprosy patient treated for 10 years with clofazimine who underwent laparotomy for severe abdominal pain. At surgery, the only significant findings were that the celiac lymph nodes were enlarged and stained purplish-black as were the omentum and the intraperitoneal fat. No other cause of abdominal pain was identified. On histological examination, reddish-purple crystals were identified at frozen section but not in the paraffin sections.—Authors' Abstract

Edstein, M. D. and Rieckmann, K. H. Lack of effect of proguanil on the pharmacokinetics of dapsone in healthy volunteers. Chemotherapy **39** (1993) 235–241.

The multiple-dose kinetics of dapsone (DDS) and its principal metabolite monoacetyldapsone (MADDS) were determined in six healthy volunteers after daily administration of low-dose dapsone (10 mg). Comparison with a previous study involving the same volunteers on a daily regimen of proguanil (200 mg) plus dapsone (10 mg) revealed no statistically significant differences in the maximum plasma concentrations, area under the plasma drug concentration curves, and elimination half-lives of both DDS and MADDS in the presence of proguanil. Although these findings suggest that proguanil does not alter the pharmacokinetics of DDS and MADDS, the possibility that proguanil affects the disposition of hydroxylated metabolites of dapsone, which appear to mediate dapsone toxicity, cannot be excluded.—Authors' Abstract

González, A. B., Maestre, J. L., Hernández, O., Columbié, Y., Atrio, N., Martin, M., Fernández, A. M. and Rodríguez, J. Survey for secondary dapsone and rifampicin resistance in Cuba. Lepr. Rev. **64** (1993) 128–135.

A total of 1211 Cuban multibacillary leprosy patients treated for at least 5 years were clinically and bacteriologically examined. They were being treated according to a two-phase monotherapy regimen with RMP first and DADDS afterward. On skin-smear examination 50 patients were found positive, of which 9 showed a BI of 3+ or higher at any site. With regard to the clinical status, the only cases found with clinical signs of relapse were 5 out of 7 long-standing patients with BI of 4+ and 5+. A sixth patient of this high BI group who showed good clinical condition, except for a heavy infiltration in both earlobes, was receiving a second RMP course when examined and biopsied for this research. These 9 patients were biopsied and susceptibility tests to RMP and DDS performed. The results showed that in 1 case the *Mycobacterium leprae* were resistant to both drugs; the organisms from 2 other patients were susceptible to RMP but low-grade resistant to DDS. Those from another patient were susceptible to RMP and fully resistant to DDS. In 3 other cases the bacilli did not multiply in any of the mice but 1 of these strains was from the patient taking a second RMP course, and, therefore, this strain might also be susceptible to RMP and resistant to DDS. In the last 2 cases multiplication was only observed in 2 of the controls and in 1 of the 0.0001% DDS-treated mice; therefore, these experiments were not conclusive, and the AFB recovered were inoculated into fresh mice to repeat the tests but these failed to multiply.—Authors' Summary

Ji, B. H., Jamet, P., Perani, E. G., Bobin, P. and Grosset, J.-H. Powerful bactericidal activities of clarithromycin and

minocycline against *Mycobacterium leprae* in lepromatous leprosy. J. Infect. Dis. **168** (1993) 188–190.

Thirty-six patients with newly diagnosed lepromatous leprosy were allocated randomly to three groups and treated for 56 days with minocycline (100 mg daily), clarithromycin (500 mg daily), or clarithromycin (500 mg) plus minocycline (100 mg daily). All groups had rapid and remarkable clinical improvement and significant decline of the bacterial and morphologic indices in skin smears during treatment. More than 99% and > 99.9% of the viable *Mycobacterium leprae* had been killed by 28 and 56 days of treatment, respectively, as measured by inoculation of organisms recovered from skin samples, taken before and during treatment, into the foot pads of immunocompetent and nude mice. Clinical improvement and bactericidal activity did not differ significantly among the three groups. Adverse reactions were rare and mild, and no laboratory abnormality was detected during the trial. Both clarithromycin and minocycline displayed powerful bactericidal activities against *M. leprae* in leprosy patients, and may be considered important components of new multidrug regimens for the treatment of multibacillary leprosy.—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 remaining 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 (N) 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.—Authors' Summary

Poitineau, Y., Barthelemy, J., Rouby, D., Fauron, P., Barrau, P. and Beligon, C. Fatal poisoning by rifampicin—a case report. Therapie **48** (1993) 271–273.

Successful suicide attempts by rifampin are not commonly reported in literature. Fatal cases and mechanisms of death are most of the time unexplained. We report a suicidal case in a 33-year-old man with fatal course occurring 27 hr after acute overdosage with 15 g rifampin. Criteria of prognostic value are discussed: clinical signs, in particular the red man syndrome, and biological data. None of them allows us to prevent fatal issue, but since cardiac arrest of unknown origin may rapidly occur, admission in intensive care must be carried out promptly with a total dose absorbed of 12 g and/or evident clinical signs.—Authors' Abstract

Singh, R. P., Tiwari, V. D. and Chattopadhyay, S. P. Comparative study of short term results in two multidrug regimens in multibacillary leprosy. Indian J. Lepr. **65** (1993) 173–180.

Thirty lepromatous and borderline lepromatous leprosy patients were treated with multidrug therapy in an open trial. Fifteen of them received the standard WHO multidrug regimen, i.e., rifampin 600 mg and clofazimine 300 mg monthly, supervised, and dapsone 100 mg daily and clofazimine 100 mg on alternate days as self administered; the other 15 received a modified multidrug therapy regimen comprising of rifampin 600 mg, clofazimine 100 mg and dapsone 100 mg daily for 21 days as suggested by the Indian Association of Leprologists, followed by the standard WHO regimen. The observation period was 6 months.

Clinical, bacteriological, histological and immunological parameters were studied. The fall in morphological index was much faster in patients receiving the modified multidrug therapy regimen compared to those receiving the standard WHO regimen. Otherwise, there was no difference between the two groups of patients. Five patients developed type 1 (upgrading) reaction, with one developing ulnar nerve paralysis. No untoward effects of drugs were noted in the study subjects except for darkening of skin color of all the patients.—Authors' Abstract

Thomas, A., Joseph, P. and Prabhakar, R. "Flu" syndrome associated with other systemic manifestations with once a month rifampicin in the treatment of multibacillary leprosy. Indian J. Lepr. **65** (1993) 219–224.

Rifampin, a potent bactericidal drug, has become an essential component of multidrug treatment regimens for tuberculosis and leprosy. One of the adverse reactions described following twice weekly or once weekly administration of rifampin is the "flu" syndrome. This syndrome is immunological in nature and is often associated with the presence of circulating rifampin-dependent antibodies. Initially it was thought to be uncommon with longer intervals between doses of the drug, i.e., once a month administration, as advocated in the chemotherapy of leprosy. However, there have been a few reports about the occurrence of "flu" syndrome with a regimen containing rifampin once a month. We report here three cases of leprosy developing "flu" syndrome, associated with other systemic manifestations, during the monthly phase of their treatment.—Authors' Abstract

Venkatesan, K., Chauhan, S. L., Girdhar, A. and Girdhar, B. K. Bioavailability of dapsone on oral administration of Dapsomine® —a comparative evaluation. Indian J. Lepr. **65** (1993) 157–161.

This study describes a comparative evaluation of dapsone kinetics in humans on administration of Dapsomine®, a capsule containing dapsone 100 mg dispersed in an oily base suspension of clofazimine 50 mg. Seven untreated lepromatous leprosy patients were given one capsule of Dapsomine® a day for 7 days and the pharmacokinetics parameters in this group were compared with those from another group of seven patients who received dapsone 100 mg and clofazimine 50 mg separately. There were no statistically significant differences in parameters such as peak dapsone plasma concentration (C_{max}), basal plasma level (C_{24h}), time to peak level (t_{max}), absorption half-life ($t_{1/2}\alpha$), elimination half-life ($t_{1/2}\beta$) and areas under plasma concentration-time curves ($AUC_{0\text{-}8h}$ and $AUC_{0\text{-}24h}$) between the two groups.—Authors' Abstract

Clinical Sciences

Avelleira, J. C. R., Vianna, F. R., Coutinho, R. B., Marques Boechat, A. and Gomes de Andrade, V. R. [Distribution of single cutaneous lesions in paucibacillary leprosy.] Acta Leprol. **8** (1993) 127–131. (in French)

In this paper the authors study the sites of single lesions in 317 paucibacillary patients registered at the outpatient units of the CMS Jorge Saldanha and the Curupaiti State Hospital in Rio de Janeiro, Brazil. The preferential sites of lesions in the population studied, their relation with age and sex and factors likely to influence their distribution are discussed. The findings are compared with other similar studies performed in Asia and Africa.—Authors' English Abstract

Girdhar, B. K., Girdhar, A., Chauhan, S. L., Malaviya, G. N., Husain, S. and Mukherjee, A. Borderline tuberculoid relapse in lepromatous leprosy. Lepr. Rev. **64** (1993) 157–163.

We report details of two patients who had been treated for a long time by monotherapy and who had remained smear negative for

over 10 years found to have relapsed with borderline-tuberculoid (BT) leprosy.—Authors' Summary

Husain, A., Husain, S., Malaviya, G. N. and Bahadur, R. R. Myiasis in leprosy. Acta Leprol. **8** (1993) 137–141.

During the 1989–1991 period, leprosy patients with various ulcers attending the surgical outpatient department at Central JALMA Institute for Leprosy, Agra, India, were seen. Of these, 64 cases were found to be infested with maggots. Live maggots were collected in all cases from different sites, i.e., nasal cavity, hand, great toe and second toe. It was possible to rear the maggots into flies in 53 out of 64 cases. In 11 cases the maggots did not survive and died in the early part of their life cycle. Four different types of flies were identified: *Sarcophaga haemorrhoidalis, Chrysomya bezziana, Callitroga americana* and *Musca domestica.*—Authors' Summary

Jayakumar, J., Aschhoff, M. and Gomathi, A. Reaction in borderline tuberculoid leprosy presenting with multiple subcutaneous nodules. Indian J. Lepr. **65** (1993) 239–242.

Reaction in borderline leprosy usually manifests with exacerbation of existing lesions, or with the appearance of new skin lesions, or both. The skin patches become more erythematous with distinct edges. There is edema of the lesions and the extremities; even macules become plaques. The new lesions in the skin usually resemble the pre-existing lesions. In this paper we record the sudden appearance of numerous fairly large subcutaneous nodules as a manifestation of (upgrading, type 1) reversal reaction in a patient with borderline tuberculoid leprosy.—Authors' Abstract

Jayakumar, J., Aschhoff, M. and Renuka, G. Mycetomas in leprosy. Indian J. Lepr. **65** (1993) 229–233.

Neuropathic plantar ulcer is one of the most common complications of leprosy and is seen in 10% to 15% of leprosy patients. Even with such high prevalence of plantar ulcers, mycetomas of the feet in leprosy patients seem to be very rare. Ordinarily mycetomas are produced following the introduction of these exogenous pathogenic organisms into the tissues as a result of trauma. Yet there seems to be hardly any association between anesthetic feet in leprosy so prone to trauma and mycetomas. Leprosy is a chronic granulomatous disease and is known to be associated with other granulomatous infections like tuberculosis and syphilis. Recently, there has been a report of a case of chromoblastomycosis in a patient with borderline tuberculoid leprosy. We report here two instances of leprosy patients having mycetoma of the foot.—Authors' Abstract

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

A 37-year-old black South African man was admitted to Kalafong Hospital (South Africa) in November 1989 with an 18-month history of pain in both hands followed by weakness and loss of sensation. Both his father and sister had leprosy but the patient himself denied ever having skin lesions. Clinical, neurophysiological and histopathological examination led to a diagnosis of primary neuritic leprosy, with all 4 limbs involved and 14 nerves affected. Selective involvement of the facial nerve branches, with normal blink reflexes, was observed. A biopsy of the sural nerve suggested borderline leprosy. The authors comment that cases of primary neuritic leprosy (without skin lesions) are not infrequent on the Indian subcontinent. However, they are unaware of previous formal reports of such cases in black Africans; they cite a personal communication from R. E. Pfalzgraff stating that neuritic leprosy does occur in Africa but in < 1% of leprosy cases (compared with 18% of all leprosy cases in Chingleput District in India).—C. A. Brown (Trop. Dis. Bull.)

Pavithran, K. Shoreline nails following type II lepra reaction. Indian J. Lepr. **65** (1993) 225–227.

The growing portion of the nail is the nail matrix, and any disturbance in the growth of the cells of the nail matrix results in morphological changes in the nail plate. In lep-

rosy the growth of the nails may be affected causing them to become curved, brittle and thin. They may also appear dry, lusterless, shrunken, narrowed and longitudinally ridged. Singh, *et al.* reported Terry's nails in a patient with BT leprosy and Patki and Mehta noted Beau's illness on the nails of a patient with BL leprosy who had associated dapsone-induced erythroderma. We report a patient with lepromatous leprosy who developed "Shoreline nails" following type 2 lepra reaction.—Author's Abstract

Rao, S. P. and Bharambe, M. S. Electro-neuro-physiological studies in early tuberculoid leprosy. Indian J. Lepr. **65** (1993) 181–187.

Electrophysiological studies were carried out in early tuberculoid type of leprosy in order to study their utility in detecting nerve damage before the onset of obvious functional deficit. Fifty-three cases showing one mixed nerve thickening in one limb were selected. Nerve conduction studies (both motor and sensory) were done using the single-blind technique. There was no statistically significant difference between the findings obtained from clinically thickened and nonthickened nerves. There was also no direct relationship between clinical sensory deficit and electrophysiological abnormality. Clinical motor power loss was well correlated with electrophysiological abnormalities.—Authors' Abstract

Reddy, B. S. N., Sheriff, M. O., Garg, B. R. and Ratnakar, C. Solitary neurofibroma mimicking nerve abscess of leprosy. Indian J. Lepr. **65** (1993) 235–238.

Pathological thickening of a peripheral nerve in an area endemic for leprosy should make one suspect this disease as the primary cause, since the other etiological factors are rare. Nevertheless, it is necessary that one should also keep in mind these causes in the differential diagnosis. We report here an interesting instance of solitary neurofibroma arising from the left lateral popliteal (common peroneal) nerve clinically simulating pure neuritic leprosy with nerve abscess in a 15-year-old girl.—Authors' Abtract

Immuno-Pathology

Chen, Z.-L., Tang, Q.-G., Wang, Z.-M. and Chen, J. Pilot study to determine acceptability and ability of heat-killed *Mycobacterium leprae* plus BCG (HKML + BCG) vaccine to induce skin test conversion. Lepr. Rev. **64** (1993) 117–127.

Although local reactions, including erythema, induration and ulcers, appeared in every patient after the injection of the combined heat-killed *Mycobacterium leprae* (HKML) + BCG vaccine, they were accepted by the patients. There was no tendency for the local reaction to become aggravated after repeated vaccination. However, systemic reactions, mainly iridocyclitis and complaint of numbness of the fingers and toes, became quite common after the fifth vaccination and, therefore, significantly reduced the acceptability of vaccine by injection. It seems that repeated vaccination might activate the iridocyclitis, but the relationship between the complaint of numbness and vaccination has not been well established. Neither typical erythema nodosum leprosum (ENL) nor reversal reaction had been observed throughout the trial. A significant proportion of patients converted to soluble *M. leprae* skin test antigen (SMLA) positivity after repeated vaccination. However, it seems the positive status was not stable, since many of them reverted to negative after the following vaccination. After the seventh vaccination, the positive conversion rate to SMLA-I was 45% and to SMLA-II was 35%. After the eighth vaccination, 66.7% of patients converted to Mitsuda reaction positive, which has been confirmed by histopathological examination. Nevertheless, further follow up is required in order to determine whether or not such conversion will be of a long duration. The reactions to SMLA-I and SMLA-II were associated but only correlated at a moderate level. Overall, the positive conversion rate to SMLA-I was significantly higher than that

to SMLA-II after repeated vaccination. Neither the early reaction nor the late (Mitsuda) reaction of the lepromin test were correlated to either SMLA reaction. The repeated vaccination of HKML + BCG vaccine did not affect the weakly positive anti-PGL-1 *M. leprae* antibody level seen in the skin-smear-negative lepromatous patients participating in this study.—Authors' Summary

Doherty, T. M., Backstrom, B. T., Love, S. G., Harding, D. R. K. and Watson, J. D. Identification of T-cell stimulatory epitopes from the 18-kDa protein of *Mycobacterium leprae.* Int. Immunol. **5** (1993) 673–680.

We have used different mouse strains to examine *in vivo* and *in vitro* responses to the 18 kDa protein of *Mycobacterium leprae,* which appears to be strongly immunogenic in both mice and humans. B- and T-cell stimulatory epitopes recognized by different strains of mice have been mapped using overlapping peptides that span the entire 18-kDa protein. Previous work established that immunization of mice with the 18-kDa protein results in specific antibody production to common B-cell epitopes, and immunization of mice with peptides containing these B-cell epitopes resulted in the induction of specific IgG to only a limited subset of epitopes in each strain. Now we report that T cells purified from mice immunized with peptides that stimulate antibody production, proliferate *in vitro* when rechallenged. The proliferating T cells produce levels of IL-2 and IFN-gamma that indicate antigen-specific T-helper type 1 cells are present in significant numbers. Thus, a comparison of *in vivo* and *in vitro* data suggests that T cells bearing the phenotype associated with potentially protective cell-mediated responses can be primed *in vivo* by epitopes on small peptides. Since T cells from both strains of mice are capable of responding to the immunogenic synthetic peptides *in vitro,* but give different responses to the same peptides *in vivo,* factors other than epitope structure appear to influence T-cell subset activation. This may have important implications for diseases such as leprosy where a polarized T-cell response appears to develop and for the development of synthetic subunit vaccines.—Authors' Abstract

Esaguy, N., Freire, O., Van Embden, J. D. A. and Aguas, A. P. Lactoferrin triggers *in vitro* proliferation of T cells of Lewis rats submitted to mycobacteria-induced adjuvant arthritis. Scand. J. Immunol. **38** (1993) 147–152.

We have recently reported antigenic (B-cell) crossreactivity between the mycobacterial 65-kDa heat-shock protein (hsp65) and human lactoferrin (LF), and we suggested that this crossreactivity might have a role in mycobacteria-associated autoimmune disease. Here, we have searched for anti-LF T-cell reactivity in Lewis rats submitted to a mycobacteria-triggered autoaggressive disorder (adjuvant arthritis, AA), an autoimmune disorder characterized by high anti-hsp65 reactivity. We have quantified the *in vitro* proliferative response to LF of lymph node and spleen cells of Lewis rats killed 9, 14 and 21 days after the immunization with the AA-triggering, mycobacteria-containing adjuvant (complete Freund's adjuvant, CFA). We found that LF induced significant proliferation of lymph node T cells of rats undergoing AA. This T-cell proliferation was not as marked as the one provoked by hsp65; it was, nevertheless, significantly higher ($p < 0.05$) than that produced by a nonarthritogenic antigen (i.e., albumin). T cells from naive or mineral oil (incomplete Freund's adjuvant, IFA) injected rats did not respond to LF or hsp65. These data indicate that LF may work as an accessory stimulatory factor of the T-cell autoreactivity associated with mycobacteria-induced arthritis.—Authors' Abstract

Kaur, S., Sharma, V. K., Basak, P., Kaur, I. and Radotra, B. D. Concurrent skin and nerve histology in leprosy and its role in the classification of leprosy. Lepr. Rev. **64** (1993) 110–116.

Concurrent skin and nerve histology was evaluated in 60 leprosy patients (25 BT, 28 BL and 7 LL). The twin aims were to study the comparative histology and the usefulness of nerve histology in the classification of the disease. In BT patients, clinical and histological classification was in agreement in 11 (44%) skin and 17 (68%) nerve biopsies. Concurrent skin and nerve histology was in consonance in 14 (56%) BT patients, while in 6 (24%) patients only nerve his-

tology was helpful in the classification of the disease, the skin histology being nonspecific. Nerve histology was classified as BL in 3 (12%) BT patients, the skin histology was nonspecific. In the BL group, the histology of 23 (82.4%) nerve biopsies correlated with the clinical classification, in contrast to skin histology which correlated with clinical assessment in 19 (68%) patients only. In the LL patients, the histology of nerve correlated with the clinical classification in 5 patients (71.4%), compared to histology of the skin in 4 (57%) patients only. The GF was higher in the nerves than in the skin throughout the leprosy spectrum (BT, BL, LL); the difference was, however, marginal in BL leprosy. The average bacterial index (BI) was higher in nerves (4+) compared to that of skin histology and slit-skin smears (3+) in BL leprosy. There was, however, no difference in the BI of the slit-skin smears, skin and nerve biopsies in lepromatous leprosy. It is inferred that the neural histology is often more useful than skin histology in the classification of leprosy patients ($p < 0.01$) and it correlates better with clinical classification, particularly in the borderline tuberculoid disease. The neural histology gave a better idea about the bacterial load in the BT, BL patients. It is proposed that bacteriologically negative patients clinically and histologically classified as BT, but with nerve histology more consistent with BL, should be considered multibacillary for purposes of therapy.—Authors' Summary

Launois, P., Niang, M. B. N., Sarthou, J. L., Rivier, F., Drowart, A., Van Vooren, J. P., Millan, J. and Huygen, K. T-cell stimulation with purified mycobacterial antigens in patients and healthy subjects infected with *Mycobacterium leprae*-secreted antigen-85 is another immunodominant antigen. Scand. J. Immunol. **38** (1993) 167–176.

Peripheral blood leukocytes from 9 paucibacillary and 12 multibacillary leprosy patients, from 18 healthy controls and from 34 healthy leprosy contacts were stimulated with three mycobacterial heat-shock proteins with respective molecular weights of 70, 65 and 18 kDa and with the secreted 30-32-kDa protein, also called antigen 85. Antigen 85 was found to be the most powerful T-cell antigen (as measured by lymphoproliferation and IFN-gamma secretion), eliciting a positive response in all (100%) paucibacillary patients and in all lepromin-positive controls and contacts. The three heat-shock proteins (hsp) were less active T-cell stimuli. Reactivity to the 70-kDa hsp was found in only 44% of the paucibacillary patients, in 80% of the lepromin-positive controls and in 60% of the lepromin-positive leprosy contacts. The 65-kDa hsp stimulated T cells in 89% of the paucibacillary patients and in 80% of the lepromin-positive controls and contacts. Responsiveness to the 18-kDa hsp, finally, was clearly more frequent in tuberculoid leprosy patients (78%) than in lepromin-positive controls (40%) or lepromin-positive leprosy contacts (4%). T-cell reactivity of 8 lepromin-negative controls, of 9 lepromin-negative contacts, and of 12 multibacillary leprosy patients was low to all the antigens tested. Although proliferative and IFN-gamma responses were generally closely related, some subjects demonstrated a dissociation of these two immune parameters. Our data confirm previous findings on the powerful T-cell stimulatory properties of antigen 85 during *M. leprae* infection, and suggest that this antigen is indeed a potentially protective T-cell immunogen.—Authors' Abstract

Launois, P., Van den Bussche, P., Niang, N. M., Drowart, A., Van Vooren, J.-P., Sarthou, J.-L., Millan, J. and Huygen, K. IL-6 production in response to purified mycobacterial heat-shock proteins and to antigen 85 in leprosy. Cell. Immunol. **148** (1993) 283–290.

IL-6 production was examined in PBMC cultures from healthy leprosy contacts and from leprosy patients stimulated with the purified mycobacterial 18-, 65-, and 70-kDa heat-shock proteins (hsp) and the secreted fibronectin-binding antigen 85 (Ag85). In lepromin-negative contacts, the 70-kDa hsp was the only antigen capable of eliciting significant IL-6 production. In lepromin-positive contacts, Ag85, the 65- and the 70-kDa hsp induced substantial IL-6 titers. IL-6 levels induced with the 70-kDa antigen were about fourfold higher than with the 65-kDa hsp or with Ag85. The 18-kDa antigen did not induce any IL-6 in these healthy contacts. PBMC from tuberculoid leprosy pa-

tients produced even more elevated levels of IL-6, and PBMC from lepromatous leprosy patients produced extremely high levels of IL-6. All antigens were capable of inducing IL-6 in leprosy patients. Highest levels were found in cultures stimulated with the 65-kDa hsp, and lowest levels were in cultures stimulated with the 18-kDa hsp.—Authors' Abstract

Méndez-Samperio, P. Antigen presentation of mycobacterial peptides to human T cell clones can be immunomodulated by adding an MHC-specific inhibitor. Cell. Immunol. **148** (1993) 1–9.

The immunomodulation of T-cell recognition by mycobacterial antigens was investigated using T-cell clones activated with peptide-pulsed EBV-B cells. An HLA-DR1-restricted T-cell clone from a patient with tuberculosis responded to peptide 65–85 from the 65-kDa protein of *Mycobacterium tuberculosis* in a dose-dependent manner, while no significant response was induced by antigen-nonpulsed EBV-B cells or EBV-B cells pulsed with an unrelated antigen (streptokinase/streptodornase). The observed binding to HLA-DR1 could be inhibited when the EBV-B cells were cultured in the presence of an excess of an HLA-DR1-restricted T-cell epitope (residues 1–20) from the 19-kDa protein of *M. tuberculosis*. This inhibition was dose-dependent. In other experiments, proliferation of a DR1-restricted T-cell clone from a healthy individual which responded to peptide 1–20 was inhibited by an excess of peptide 65–85, confirming that these peptides are able to compete for the same DR1-binding site. Nevertheless, the T-cell clone from the healthy individual showed a relatively lower percentage of inhibition compared with the T-cell clone from a patient with tuberculosis. Furthermore, the intensity of this inhibition was reversed as the concentration of stimulatory peptide was increased. The experiments described in this paper demonstrate the immunomodulation of mycobacterial antigen presentation by peptide competition at the level of MHC-binding sites. These data may be important for an understanding of the interactions involved in the mycobacterial cell-mediated immune recognition.—Author's Abstract

Negesse, Y., Beimnet, K., Miko, T., Wondimus, A. and Berhan, T. Y. In leprosy the presence of mycobacteria in the nerve is an essential factor in the cycle and spectrum of *Mycobacterium leprae* infection. Lepr. Rev. **64** (1993) 104–109.

A total of 220 untreated leprosy patients who underwent parallel skin and nerve biopsies are included in this study, which is intended to evaluate the extent of previously reported differences in bacillary load between skin and nerve lesions in leprosy and to describe the response of peripheral blood lymphocytes to *Mycobacterium leprae* antigens in such patients. In 161 patients out of the 220, the skin and nerve biopsies were diagnostic for leprosy. When patients were grouped according to their skin and nerve lesions, the three groups observed were (1) paucibacillary skin and nerve lesions; (2) multibacillary skin and nerve lesions; and (3) paucibacillary skin and multibacillary nerve lesions. There was no observation of a group of patients with multibacillary skin and paucibacillary nerve lesions. In all patients with multibacillary nerve lesions, regardless of the type of skin lesions, a low response of peripheral blood lymphocytes to *M. leprae* was consistently noted. These results suggest that the bacillary load in the nerve is certainly one of the factors determining the immunological spectrum observed in leprosy.—Authors' Summary

Prakash, K., Aggarwal, R. and Sehgal, V. N. Significance of antibodies to phenolic glycolipid-I in leprosy diagnosis. J. Dermatol. **19** (1992) 953–958.

A gelatin particle agglutination assay for the detection of anti-PGL-I antibodies in 40 clinically diagnosed and variously classified groups of leprosy cases revealed elevated PGL-I antibody titers in 85% of cases. In contrast, the slit-skin smear examination was positive in only 30% of cases. It was further observed that out of 28 cases with bacterial index (BI) of 0, 22 cases (78.5%) had significant levels of PGL-I antibodies. There was no case in which the slit-skin smear was positive and the PGL-I antibody titer was not significant. The elevated titers of PGL-I antibody better correlated (84%) with histopathological findings than did BI.

Thus, it was concluded that estimation of PGL-I antibody titer is a better supplement to clinical diagnosis than BI. Significant levels of PGL-I antibody were seen in 85% of cases who had no earlier chemotherapy or were treated for less than 2 months. Similar findings were observed in 12 patients who were on MDT for more than 5 months but for less than 2 years. In order to determine the significance of anti-PGL-I antibodies in monitoring the response of patients to chemotherapy, a longer follow up with a greater number of cases should be contemplated.—Authors' Abstract

Shende, R. K., Bardapurker, J., Patil, V. and Gaikwad, A. Adenosine deaminase activity in leprosy. Indian J. Lepr. **65** (1993) 201–205.

Serum adenosine deaminase (ADA) was studied in 60 patients of different types of leprosy and 50 healthy control subjects. ADA levels in patients with tuberculoid (50.50 ± 5.22 U/L), borderline (41.14 ± 3.89 U/L) and lepromatous leprosy (30.10 ± 0.03 U/L) were higher than that in controls (17.84 ± 2.78 U/L), thus correlating with the immunological status of patients. Patients with lepra reaction showed decreased ADA levels and higher grade of lepromin test positivity was associated with increased ADA activity.—Authors' Abstract

Sieling, P. A., Abrams, J. S., Yamamura, M., Salgame, P., Bloom, B. R., Rea, T. H. and Modlin, R. L. Immunosuppressive roles for IL-10 and IL-4 in human infection—*in vitro* modulation of T-cell responses in leprosy. J. Immunol. **150** (1993) 5501–5510.

IL-10 and IL-4 have been shown to exert an inhibitory effect on cell-mediated immune responses. Our previous studies of leprosy demonstrated that IL-10 and IL-4 mRNA were preferentially expressed in lesions from lepromatous patients, those immunologically unresponsive individuals who manifest widespread infection. To define more precisely the regulatory roles of these two cytokines in the immune response to infection, we studied *in vitro* responses to *Mycobacterium leprae. M. leprae* triggered IL-10 release from PBMC of patients and healthy donors; the predominant source of the IL-10 was found to be monocytes/macrophages. Stimulation of PBMC in the presence of neutralizing anti-IL-10 mAb indicated that endogenous IL-10 production inhibits PBMC proliferation and release of TNF-alpha, GM-CSF, and IFN-gamma. Paradoxically, studies using neutralizing anti-IL-4 mAb indicated that endogenous IL-4 production enhances PBMC proliferative responses most strikingly in lepromatous patients. We found that rIL-4 expanded CD8+ T cells from lepromatous patients *in vitro*. CD8+ T cells from lepromatous patients have been shown to suppress CD4+ T-cell responses, in part by the release of IL-4. Our study indicated that endogenous IL-4 production inhibited IL-10 secretion and, concomitantly, increased TNF-alpha and GM-CSF release. The present data suggest that, on balance, IL-4 and IL-10 contribute to immunosuppression in human infectious disease.—Authors' Abstract

Microbiology

Bhatia, V. N. and Thawani, G. Observations on attempted leprosy cultures in two media. Indian J. Lepr. **65** (1993) 163–171.

Suspensions of skin-tissue material collected from lepromatous leprosy patients and material from mouse foot pad harvests were inoculated into two media, i.e., a biphasic medium and a minimal basal medium. The cultures were incubated at 37°C and 15°C. Small oval (or round) cells appeared in these cultures around the tenth day along with a few cystic structures; and they increased in number later, reaching the maximum around 6–7 weeks. The above cells appeared acid-fast in some cultures and some of them appeared to split into pairs

of acid-fast bacilli. The cells were most often seen in the biphasic medium at 37°C. The identity of these structures is not known at this stage.—Authors' Abstract

Godard, C. M. Attempts to cultivate *Mycobacterium leprae* in fat tissue. Acta Leprol. **8** (1993) 133–135.

The behavior of *Mycobacterium leprae* in fat tissue was studied. Preadipocyte cells were infected with *M. leprae* and injected intradermally (i.d.) into nude mice. Adipose nodules obtained by *in vivo* differentiation of infected cells were maintained *in vivo* for 3 months and subsequently incubated *in vitro* for 3 months. Counts of bacilli showed no increase over this 6-month period. It is concluded that undifferentiated preadipocyte and mature fat cells are not permissive for *M. leprae.* The morphological changes observed following passage of *M. leprae* into adipose nodules might be related to the process of adipose cell differentiation.—Author's Summary

Sareen, M., Kaur, H. and Khuller, G. K. Regulation of phospholipid synthesis in *Mycobacterium smegmatis* by cyclic adenosine monophosphate. J. Biosci. **18** (1993) 207–212.

Forskolin, an adenylate cyclase activator, and a cyclic AMP analog, dibutyryl cyclic AMP, have been used to examine the relationship between intracellular levels of cyclic AMP and lipid synthesis in *Mycobacterium smegmatis.* Total phospholipid content was found to be increased in forskolin-grown cells as a result of increased cyclic AMP levels caused by activation of adenylate cyclase. Increased phospholipid content was supported by increased [C-14] acetate incorporation as well as increased activity of glycerol-3-phosphate acyltransferase. Pretreatment of cells with dibutyryl cyclic AMP had similar effects on lipid synthesis. Taking all these observations together, it is suggested that lipid synthesis is being controlled by cyclic AMP in mycobacteria.—Authors' Abstract

Epidemiology and Prevention

Arain, G. M., Alam, S. E., and Chiang, T. Leprosy in Karachi. J. Pakistan Med. Assoc. **42** (1992) 160–161.

In Karachi, most of the 18,300 registered cases of leprosy are in former Indian refugees who came to Pakistan at the time of partition from India. Between 1981–1985, a total of 8779 new cases, of whom about 60% were Indians, were registered in the city. The remainder were mostly from Baluchistan, the North-west Frontier Province (NWFP), Punjab, Sindh, and Afghanistan. Tuberculoid leprosy prevailed except in the Afghani, 60% of whom had lepromatous leprosy (LL). LL was also relatively common (40% of cases) in individuals from the NWFP. As a rule, leprosy presented in the second decade in Indians and Baluchis and in the third decade in Afghanis. Control measures should be concentrated in those districts of Karachi where leprosy is unduly prevalent, e.g., in the Afghan district.—E. M. Scrimgeour (Trop. Dis. Bull.)

Chantraprachoom, C. and Kittampol, K. Leprosy control program in Thailand. Jpn. J. Lepr. **60** (1991) 85–96.

This paper describes the status of leprosy in Thailand under the following main headings: historical background, National Leprosy Control Program, training, research, multiple drug therapy (MDT), and epidemiological evaluation. The National Leprosy Control Program started in 1953; in 1971 leprosy control activities were integrated into the general health services, with the exception of six hyperendemic provinces which remained specialized (vertical). Detailed information is given on totals of registered patients, newly detected patients, those released from control between 1975 and 1989, and on the prevalence rate per 1000 of the population between 1959 and 1989. The prevalence fell from nearly 5 to 0.9 per 1000 during a long period of "village survey and domiciliary treatment with dapsone monotherapy" in 1959–1984. From

1984, when MDT based on WHO recommendations was introduced, prevalence has fallen to 0.31 per 1000. Newly detected cases numbered 2000 in 1975, rose to about 6000 in 1982, and have steadily declined since that year to 1594 in 1989. Around 92% of all patients in Thailand have been treated with MDT.—A. C. McDougall (Trop. Dis. Bull.)

Global estimates of the number of people needing medical treatment and care as a result of leprosy. Lepr. Rev. **63** (1992) 375–376.

This Special Article, endorsed by the ILEP Medical Commission in June 1992, comments that the WHO's revised estimates of the size of the leprosy problem globally need careful interpretation. It is considered that a distinction should be made between the estimates of those leprosy patients who require chemotherapy and those who have disabilities as a result of the disease and also need treatment and care. For better comparisons with earlier estimates of the leprosy problem the estimated number of 2–3 million people with disabilities (probably an underestimate) should be added to the revised figure of 5.5 million leprosy patients requiring chemotherapy. Multidrug therapy has been partly responsible for the decline in numbers of active leprosy cases. However, some 40% of leprosy patients are probably not being reached by multidrug therapy. Not until the number of new cases arising each year (currently estimated by WHO as 600,000–800,000) shows a steady decline can it be said that leprosy has been brought under control.—C. A. Brown (Trop. Dis. Bull.)

Kamaludin, F. Leprosy control programme in Malaysia. Jpn. J. Lepr. **59** (1990) 169–182.

The author gives an overview of the status of leprosy in Malaysia under the main headings: historical background, National Leprosy Control Program, training, multiple drug therapy (MDT), prevalence survey, epidemiological evaluation, clinical and biomedical research. The National Leprosy Control Program started in 1969 and MDT, based on WHO recommendations, was introduced in 1985. The author describes significant reductions since that date in the number of dapsone-resistant cases, reaction cases, the prevalence rate, the number of patients admitted to the National Leprosy Control Center at Sungai Buluh and the defaulter rate. The case detection rate has declined from 2.50% in 1981 to 1.27% in 1988 and the number of institutionalized patients has been reduced by 55% in a period of 19 years (1970–1989).—A. C. McDougall (Trop. Dis. Bull.)

Other Mycobacterial Diseases and Related Entities

Abbot, N. C., Beck, J. S., Harrison, D. K. and Wilson, S. B. Dynamic thermographic imaging for estimation of regional perfusion in the tuberculin reaction in healthy adults. J. Immunol. Methods **162** (1993) 97–107.

A sensitive method for measurement of the volume of blood flow through the skin, based on the kinetics of reheating after localized cooling, is described in this paper. This method has been used to study the tuberculin reaction as a model of cutaneous delayed-type hypersensitivity (DHS) in man. Over the positive reaction there is accelerated reheating similar in kinetics and extent to that seen after maximal hyperemia induced by intradermal injection of histamine or prostaglandin E2. The earlier phase of reheating (10–100 sec) is more dependent on blood flow; whereas the later phase (100–300 sec) is apparently more dependent on nonperfusion heat exchange mechanisms, including conduction. The reheat kinetic method is largely dependent on blood flow in the deep dermal vessels (diameter > 50 μm); whereas the alternative approach of measurement of the velocity of flow of

erythrocytes in the microcirculation by laser Doppler (LD) flowmetry gives results biased toward the most superficial dermal circulation. Previous studies with LD flowmetry have shown that the blood velocity is greatest at the center of weak and strong reactions, while in the most intense reactions it is raised at the center but maximal at the periphery (central relative slowing, CRS) raising the possibility of central ischemia. The reheat kinetics approach has now indicated that the deep dermal circulation is not impaired in CRS reactions. It is concluded that there must be partial obstruction of the parts of the microcirculation communicating between the deep and superficial dermal plexuses, presumably from the accumulation of exudate edema in the most intense tuberculin reactions.—Authors' Abstract

Alugupalli, S., Olsson, B. and Larsson, L. Detection of 2-eicosanol by gas chromatography-mass spectrometry in sputa from patients with pulmonary mycobacterial infections. J. Clin. Microbiol. **31** (1993) 1575–1578.

A total of 96 sputum specimens from patients with suspected or known mycobacterial and nonmycobacterial pulmonary infections were analyzed by gas chromatography-mass spectrometry for the presence of 2-eicosanol. This secondary alcohol was detected in all of the 25 sputum specimens culture positive for *Mycobacterium tuberculosis,* in 7 of the 9 sputum specimens culture positive for *M. avium* complex, and in all 3 of the studied sputum specimens associated with *M. malmoense.* The alcohol was not detected in any of the 45 culture-negative sputum specimens or in 14 sputum specimens culture positive for *Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae,* and *Streptococcus pneumoniae.* The ratio of tuberculostearic acid to 2-eicosanol was much lower in sputum samples culture positive for mycobacteria than in the corresponding *in vitro*-grown cultures. The present findings indicate that 2-eicosanol may be useful as a chemical marker for rapid diagnosis of pulmonary infections caused by the *M. avium* complex, *M. malmoense,* and *M. tuberculosis.*—Authors' Abstract

Arbeit, R. D., Slutsky, A., Barber, T. W., Maslow, J. N., Niemczyk, S., Falkinham, J. O., O'Connor, G. T. and Von Reyn, C. F. Genetic diversity among strains of *Mycobacterium avium* causing monoclonal and polyclonal bacteremia in patients with AIDS. J. Infect. Dis. **167** (1993) 1384–1390.

To define the genetic diversity among *Mycobacterium avium* isolates from human immunodeficiency virus-infected patients, specimens were cultured prospectively, and isolates obtained from 14 patients (4 with positive blood, stool, and sputum; 6 with positive blood and stool; 3 with positive blood only; and 1 with positive stool only) were studied. Both serotyping and ribotyping had limited ability to discriminate among isolates from different patients; whereas the distinctive restriction fragment profiles resolved by pulsed-field gel electrophoresis indicated that each patient was infected by a unique strain. Of the 13 bacteremic patients, 2 were bacteremic concurrently with two distinct strains. The fact that *M. avium* isolates from AIDS patients exhibit considerable genetic diversity supports the hypothesis that the infection is acquired from various environmental sources. Further, individual patients are not infrequently bacteremic with more than one strain simultaneously, which may need to be considered in protocols for the diagnosis and management of *M. avium* disease.—Authors' Abstract

Barradell, L. B., Plosker, G. L. and McTavish, D. Clarithromycin—a review of its pharmacological properties and therapeutic use in *Mycobacterium avium intracellulare* complex infection in patients with acquired immune deficiency syndrome. Drugs **46** (1993) 289–312.

Results from noncomparative and placebo-controlled studies demonstrate the efficacy of clarithromycin in the treatment of disseminated *Mycobacterium avium-intracellulare* complex (MAC) infection in patients with acquired immune deficiency syndrome (AIDS). Whether given alone or in combination with other antimycobacterial treatments, doses of 500 to 2000 mg (typically 1000 mg) administered twice daily are effective in controlling bacteremia in these

patients. Clarithromycin has also been shown to improve clinical symptoms of infection and may improve the quality of life in AIDS patients with MAC infection. Clarithromycin is generally well tolerated when used in the doses typically required for the treatment of MAC infection (1000 or 2000 mg/day). Gastrointestinal disturbances are the most commonly occurring adverse events, and occur most frequently at dosages of 4000 mg/day. Thus, clarithromycin, as monotherapy or in combination with other antimycobacterial agents, is well tolerated and effectively eradicates MAC from the blood in the short term in patients with AIDS; however, short term monotherapy may lead to bacterial resistance, underscoring the importance of long-term treatment with a combination of antimycobacterial agents. While the optimal combination regimen to prevent the development of resistance to antimycobacterial agents by MAC remains to be determined, clarithromycin will almost certainly be a valuable agent in any such combination.—Authors' Abstract

Bermudez, L. E. and Champsi, J. Infection with *Mycobacterium avium* induces production of interleukin-10 (IL-10), and administration of anti-IL-10 antibody is associated with enhanced resistance to infection in mice. Infect. Immun. **61** (1993) 3093–3097.

Organisms of the *Mycobacterium avium* complex are associated with disseminated infection in patients with AIDS. The mechanisms that account for the survival of the intracellular bacteria are unknown. We document here that infection of C57BL/6 black mice with *M. avium* 101 triggered interleukin-10 (IL-10) production. The synthesis of IL-10 peaked after 2 weeks of infection and remained elevated throughout the period of infection. Treatment of *M. avium*-infected peritoneal macrophages with recombinant IL-10 suppressed the stimulatory effect of tumor necrosis factor-alpha and granulocyte-macrophage colony-stimulating factor. To confirm the possible role of IL-10 in the infection *in vivo,* mice were infected with *M. avium* 101 and simultaneously received treatment with neutralizing anti-11, 10 antibody. After 4 weeks the animals were harvested and the numbers of viable bacteria were quantitated in the liver, spleen, and blood. The liver and spleen of animals receiving anti-IL-10 antibody had 2 to 3 log units fewer bacteria than did those of control animals. These results suggest a role for IL-10 in the pathogenesis of *M. avium* infection.—Authors' Abstract

Birnbaum, G., Kotilinek, L. and Albrecht, L. Spinal fluid lymphocytes from a subgroup of multiple sclerosis patients respond to mycobacterial antigens. Ann. Neurol. **34** (1993) 18–24.

Immune responses to heat shock or stress proteins are observed in several chronic autoimmune diseases. Such proteins are major antigens of many bacteria, especially mycobacteria. To determine whether immune responses to stress proteins occur in chronic inflammatory diseases of the central nervous system such as multiple sclerosis (MS), we measured proliferative responses of lymphocytes from spinal fluids and bloods of patients with MS and other neurological diseases to a sonicate of *M. tuberculosis,* an acetone extract of *M. tuberculosis,* a recombinant 65-kDa heat shock protein of *M. leprae,* and tetanus toxoid as a control recall antigen. Significantly increased spinal fluid lymphocyte responses to mycobacterial sonicate, relative to responses from paired peripheral blood lymphocytes, were present in 14 of 20 specimens from patients with MS ($p < 0.025$) and 2 of 9 specimens from patients with other neurological diseases. Spinal fluid lymphocytes also responded to tetanus toxoid, but differences between blood and spinal fluid were not statistically significant. Lymphocytes from one patient with MS responded only to *M. leprae.* There were no proliferative responses to the *M. tuberculosis* acetone extract. When patients with MS were classified according to duration of disease (< 2- or > 2-yr duration) 9 of 10 patients with recent onset had cerebrospinal fluid cells that responded to *M. tuberculosis* compared with 5 of 10 with longer duration symptoms ($p < 0.012$). Our data suggest a selective recruitment and/or expansion of mycobacterial reactive cells to the central nervous system of a subpopulation of patients with MS. Immune responses to antigens present in mycobacteria, possibly stress proteins, may be

important in perpetuating and amplifying chronic inflammatory diseases of the central nervous system such as MS.—Authors' Abstract

Drowart, A., Cambiaso, C. L., Huygen, K., Serruys, E., Yernault, J. C. and Van Vooren, J. P. Detection of mycobacterial antigens present in short-term culture media using particle counting immunoassay. Am. Rev. Respir. Dis. **147** (1993) 1401–1406.

Particle counting immunoassay (PACIA) was compared with the BACTEC® system for detecting mycobacterial growth after short-term culture, and was used to identify *Mycobacterium tuberculosis.* The latex particles were coated with polyclonal anti-BCG or with specific 2A1-2 monoclonal antibodies. Bottles containing nonradioactive Middlebrook 7H9 liquid medium and BACTEC® 12B vials were inoculated with equal amounts of mycobacteria from four reference strains (*M. tuberculosis, M. kansasii, M. avium,* and *M. xenopi*). Using anti-BCG, PACIA detected mycobacterial antigens 3 to 6 days before the BACTEC system. *M. tuberculosis* was differentiated from the other mycobacteria using 2A1-2. Seventeen clinical samples were also studied. In the same 10, the two techniques detected mycobacteria, PACIA with anti-BCG after 9 days and BACTEC® 1 to 5 days later. For 9 of the 10 samples, PACIA with 2A1-2 detected *M. tuberculosis* after 20 days, a result confirmed with the AccuProbe® system. *M. xenopi* was biochemically identified in Specimen 10. Nonmycobacterial diseases were diagnosed in the 7 remaining unreactive specimens. We conclude that PACIA detects mycobacterial growth earlier than BACTEC® and that *M. tuberculosis* can be distinguished from other mycobacteria in PACIA performed with specific monoclonal antibodies.—Authors' Abstract

Forbes, B. A. and Hicks, K. E. S. Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chain reaction. J. Clin. Microbiol. **31** (1993) 1688–1694.

The emergence of epidemic multiple-drug-resistant (MDR) strains of *Mycobacterium tuberculosis* in conjunction with an increase in the number of reported cases of tuberculosis (TB) represents a major public health problem. In light of a recent outbreak of MDR *M. tuberculosis* at our center, we began the development of a polymerase chain reaction (PCR) assay for the rapid diagnosis of pulmonary TB using two sets of primers, one based on the IS*6110* repeated sequence of *M. tuberculosis* and the other based on the protein antigen b (PAB). Reaction conditions were first optimized as to the appropriate extraction protocol and the concentrations of primer pairs, nucleotides, and $MgCl_2$. Following a preliminary evaluation of the assay with clinical specimens, extraction and amplification procedures were further modified. PAB and IS*6110* primers detected between 2 and 23 and 0.023 and 0.23 CFU of *M. tuberculosis,* respectively, in pooled, *M. tuberculosis*-negative sputa by our optimized PCR assay. After routine processing for mycobacteria, 734 specimens were subsequently amplified. DNA for amplification was obtained by boiling and beating the sediments with Tween 20. For each reaction, DNA (10 μl) was added to an amplification mixture containing 12 pmol of IS*6110* primers, 20 pmol of PAB primers, 2 mM $MgCl_2$, 200 μM nucleotides, and 2.5 U of Taq polymerase and the mixture was then amplified for 40 cycles. The sensitivity and specificity of our PCR assay were 87.2% and 97.7%, respectively. We were unable to interpret the results for seven specimens (1%). In our experience, PCR proved to be a useful, rapid diagnostic test for TB in a clinical setting and a valuable epidemiological tool for determining exposure groups in the hospital setting. Our findings also underscore the need for the systematic optimization of PCR assay conditions.—Authors' Abstract

Godeau, B., Oksenhendler, E. and Bierling, P. Dapsone for autoimmune thrombocytopenic purpura. Am. J. Hematol. **44** (1993) 70–72.

Twenty-one human immunodeficiency virus (HIV)-free and six HIV-infected adults with autoimmune thrombocytopenic purpura (AITP) were treated with dapsone (100 mg/day). A response was observed in 13 patients (median platelet count before 25 ×

10^9/L, range 3–49; after 109 × 10^9/L, range 69–241). Thrombocytopenia recurred in four of the responders in whom dapsone was discontinued. No response was observed in 12 patients. Dapsone had to be withdrawn after 2 weeks of treatment in the remaining two patients and after 6 to 8 weeks in three other patients due to intolerance. No serious hematological complications were observed. These results confirm that dapsone is a safe, inexpensive, and effective treatment of AITP.—Authors' Abstract

Godfrey-Faussett, P. and Stoker, N. G. Aspects of tuberculosis in Africa. 3. Genetic "fingerprinting" for clues to the pathogenesis of tuberculosis. Trans. R. Soc. Trop. Med. Hyg. **86** (1992) 472–475.

In a study of 117 isolates of *Mycobacterium tuberculosis* collected in Malawi and Kenya most paired isolates from individuals were identical. Two exceptions were found in HIV-infected patients: discordant isolates were obtained from a single episode of disease. Thus, multiple strains may exist within a single patient with active tuberculosis.—D. W. FitzSimons (Trop. Dis. Bull.)

Harris, D. P., Vordermeier, H. M., Friscia, G., Roman, E., Surcel, H. M., Pasvol, G., Moreno, C. and Ivanyi, J. Genetically permissive recognition of adjacent epitopes from the 19-kDa antigen of *Mycobacterium tuberculosis* by human and murine T cells. J. Immunol. **150** (1993) 5041–5050.

The specificity of the T-cell-immune repertoire at the level of individual antigenic determinants could play a fundamental role in the immunopathogenesis of tuberculous infections. Therefore, we analyzed the immunogenicity, genetic restriction, and epitope core structure of two adjacent, yet distinct, immunodominant T-cell determinants from the 19-kDa antigen of *Mycobacterium tuberculosis*. After immunization with two peptides comprising residues 45 to 64 and 61 to 80, vigorous *in vitro* proliferative responses to the homologous peptide were elicited in five different strains of C57BL/10 mice (H-2b,k,d,s,f), indicating that both epitopes were recognized in a genetically permissive manner. When immunized with intact 19-kDa protein, lymph node cells from the same mouse strains responded to both peptides, with the exception of H-2b mice which did not respond to p45–64. Delayed-type hypersensitivity responses in C57BL/10 (H-2b) mice were elicited by p61–80 only; whereas in H-2d mice both peptides were delayed-type hypersensitivity negative, despite eliciting pronounced proliferative responses. Analysis of the proliferative responses of human PBMC in purified protein derivative-positive healthy subjects and tuberculosis patients revealed significant differences in the antigenicity to the two peptides. All purified protein derivative-positive healthy and diseased individuals manifested strong responses to p45–64, indicating HLA permissive recognition. In contrast, pronounced responses to p61–80 were detected only in patients with lymphatic tuberculosis. Epitope core structures, composed of 6 or 7 residues within each peptide, have been mapped with peptides of overlapping sequence. Significantly, for both epitopes, the core sequences recognized by both human and murine T cells were almost identical. We conclude that despite many similarities between murine and human T-cell epitope recognition, distinct differences in the responsiveness of the infected host could play a role in pathogenesis. Furthermore, the genetically permissive nature of the identified epitopes is a potentially important attribute for the development of peptide-based diagnostic reagents and vaccines.—Authors' Abstract

Heym, B., Zhang, Y., Poulet, S., Young, D. and Cole, S. T. Characterization of the katG gene encoding a catalase-peroxidase required for the isoniazid susceptibility of *Mycobacterium tuberculosis*. J. Bacteriol. **175** (1993) 4255–4259.

The isoniazid susceptibility of *Mycobacterium tuberculosis* is mediated by the product of the *katG* gene which encodes the heme-containing enzyme catalase-peroxidase. In this study, the chromosomal location of *katG* has been established and its nucleotide sequence has been determined so that the primary structure of catalase-peroxidase could be predicted. The *M. tuberculosis* enzyme is an 80,000-dalton protein containing several motifs characteristic of

peroxidases, and shows strong similarity to other bacterial catalase-peroxidases. Expression of the *katG* gene in *M. tuberculosis, M. smegmatis,* and *Escherichia coli* was demonstrated by Western blotting (immunoblotting). Homologous genes were detected in other mycobacteria, even those which are naturally insensitive to isoniazid.—Authors' Abstract

Huygen, K., Drowart, A., Harboe, M., Tenberg, R., Cogniaux, J. and Van Vooren, J. P. Influence of genes from the major histocompatibility complex on the antibody repertoire against culture filtrate antigens in mice infected with live *Mycobacterium bovis* BCG. Infect. Immun. **61** (1993) 2687–2693.

C57BL/10 and C57BL/6 mice (H-2b); BIO congenic mice with f, k, p, q, r, and s H-2 haplotypes; B10 mice with recombinant g2, o2, a, h2, h4, i5, and bq1 H-2 haplotypes; and B6 mice with major histocompatibility complex (MHC) mutant bm1 and bm13 (class I) and bm12 (class II) haplotypes were infected intravenously with 4×10^6 CFU of live *Mycobacterium bovis* BCG and examined by Western immunoblot analysis for serum antibodies against BCG culture filtrate antigens, following a boost injection with live BCG or with BCG culture filtrate. Parental B10 and B6 mice reacted very intensely with three culture filtrate protein bands with estimated molecular masses of 37, 38, and 40 kDa. Response against the 40-kDa protein was stronger following a boost injection with live BCG than following a boost with culture filtrate. Sera from mice with f, p, i5, bm1, and bm13 haplotypes reacted strongly, with both the 37-38- and 40-kDa antigens, and sera from mice with q and bq1 haplotypes showed a somewhat weaker reaction. Sera from mice with r, s, and bm12 haplotypes reacted against the 37-38-kDa antigen but not against the 40-kDa antigen, and sera from mice with the h2 haplotype reacted only with the 40-kDa antigen but not with the 37-38-kDa antigen. Sera from mice with the k, g2, o2, a, and h4 haplotypes showed, at most, a very weak reaction with the 37-38- and 40-kDa antigens. These results demonstrate that MHC genes profoundly affect the antibody repertoire used against culture filtrate antigens in mice infected with live *M. bovis* BCG. In particular, as shown in mice with the recombinant H-2 haplotype and in class II mutant bm12 mice, the I-A heterodimer controls the recognition of the immunodominant 40-kDa antigen. By using crossed immunoelectrophoresis, this 40-kDa antigen was identified as antigen 88 according to the reference system of Closs, *et al.* for BCG antigens.—Authors' Abstract

Iralu, J. V., Sritharan, V. K., Pieciak, W. S., Wirth, D. F., Maguire, J. H. and Barker, R. H. Diagnosis of *Mycobacterium avium* bacteremia by polymerase chain reaction. J. Clin. Microbiol. **31** (1993) 1811–1814.

We describe a rapid polymerase chain reaction (PCR)-based test for diagnosing *Mycobacterium avium* directly from blood specimens. Blood was collected in anticoagulant (EDTA) from patients who also had blood cultures performed by the lysis-centrifugation method. Blood samples were centrifuged on a Ficoll-Hypaque gradient to purify peripheral blood mononuclear cells. The purified cells were washed and incubated in the presence of Chelex-100 (a divalent cation-binding resin), boiled to release mycobacterial DNA, and then amplified with *M. avium*-specific PCR primers. Amplification was detected by hybridization with radiolabelled probe, and the results were compared with the culture results. The PCR assay gave positive results for 12 of 15 specimens that were taken from patients with positive cultures for *M. avium* complex (sensitivity, 80%). The three PCR-negative specimens in this group showed evidence of PCR inhibition. The PCR assay gave positive results for 32 of 228 specimens taken from patients with negative cultures (specificity, 86%). Of these 32 PCR-positive culture-negative specimens, 27 were also positive when amplified with primers specific for the genus *Mycobacterium,* suggesting that PCR may be more sensitive than culture.—Authors' Abstract

Klopman, G., Wang, S., Jacobs, M. R., Bajaksouzian, S., Edmonds, K. and Ellner, J. J. Anti-*Mycobacterium avium* activity of quinolones: *in vitro* activities. Antimi-

crob. Agents Chemother. **39** (1993) 1799–1806.

The minimal inhibitory concentrations (MICs) of 88 quinolones against 14 selected reference and clinical strains of *Mycobacterium avium-M. intracellulare* complex were determined. Agents tested included ciprofloxacin, sparfloxacin (PD 131501), and 86 other experimental quinolones. Test strains were selected to represent various susceptibilities to ciprofloxacin and other drug resistance profiles. MICs were determined by the microdilution method in 7HSF broth, with incubation for 14 days at 35°C. The results showed 25 of the quinolones to be active against the strains, with MICs for 90% of the strains (MIC_{90}s) of 2 to 32 μg/ml. Ten of these compounds had activities equivalent to or greater than that of ciprofloxacin. The most active compound was PD 125354, with an MIC_{50} of 0.5 μg/ml and an MIC_{90} of 2 μg/ml; comparable values for ciprofloxacin were 4 and 8 μg/ml, respectively. The next most active compounds, with MIC_{90}s of 4 μg/ml, were sparfloxacin (PD 131501), PD 123982, PD 135144, and PD 119421. MIC_{90}s of PD 131575, PD 126889, PD 122642, PD 139586, and PD 143289 were 8 μg/ml. Further evaluation of the most active agents is warranted, as is assessment of structure-activity relationships of active and inactive agents to elucidate the active portions of the compounds and to lead to the development of compounds with enhanced activity.—Authors' Abstract

Kocagoz, T., Yilmaz, E., Ozkara, S., Kocagoz, S., Hayran, M., Sachedeva, M. and Chambers, H. F. Detection of *Mycobacterium tuberculosis* in sputum samples by polymerase chain reaction using a simplified procedure. J. Clin. Microbiol. **31** (1993) 1435–1438.

A repetitive sequence of *Mycobacterium tuberculosis* DNA was amplified by polymerase chain reaction (PCR), from sputum samples, for the diagnosis of pulmonary tuberculosis. The method of heating the sample in a boiling water bath to break down the bacterial cell wall and to release the DNA was compared with that of enzymatic lysis of bacteria and then phenol-chloroform extraction of DNA. Heating the sample was the better method with a sensitivity of approximately 10 microorganisms. A total of 78 sputum specimens prepared by heating were examined by PCR, and the results were compared with the results of acid-fast-stained smears, cultures, and clinical data. *M. tuberculosis* was detected by PCR in all smear- and culture-positive and smear-negative, culture-positive cases. Additionally, PCR was capable of detecting 4 of 9 cases which were smear and culture negative but clinically suspected of tuberculosis. DNA amplification by PCR is a sensitive and specific method for the diagnosis of tuberculosis, and with this simplified DNA isolation procedure it can be used in routine clinical practice.—Authors' Abstract

Lazard, T., Perronne, C., Grosset, J.-H., Vilde, J. L. and Pocidalo, J. J. Clarithromycin, minocycline, and rifabutin treatments before and after infection of C57BL/6 mice with *Mycobacterium avium*. Antimicrob. Agents Chemother. **37** (1993) 1690–1692.

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

Limb, D. I., Wheat, P. F., Spencer, R. C., Harris, G. S., Rayner, A. B. and Watt, B. Comparison of techniques for antimicrobial susceptibility testing of mycobacteria. J. Clin. Pathol. **46** (1993) 403–407.

Aims—To evaluate adenosine triphosphate (ATP) bioluminescence as a rapid technique for antimicrobial susceptibility testing of *Mycobacterium* spp. by comparing it with conventional and radiometric methods, and to assess its potential for use in clinical microbiology laboratories.

Methods—115 clinical isolates from a wide range of mycobacterial species and four control organisms of known susceptibility were tested against six antimicrobial agents. Minimum inhibitory concentrations (MICs) were determined after 4–6 weeks' incubation on Middlebrook 7H10 agar. Susceptibility was also determined radiometrically using a BACTEC® 460, and by bioluminescent assay of ATP using a 1250 luminometer (LKB-Wallac).

Results—Susceptibility results after 7 days showed excellent correlation with conventionally determined MICs; 714 susceptibility tests were performed by both techniques, with seven major discrepancies between the two systems. For pyrazinamide, agreement was 100%, but five strains of *M. tuberculosis,* including one control, and 11 mycobacteria other than *M. tuberculosis* (MOTT) failed to grow on Middlebrook agar at pH 5.5; 606 tests were performed by radiometry, with four major discrepancies between this technique and ATP bioluminescence. No particular species of *Mycobacterium* gave aberrant results. Contamination was a problem; 12 of the 119 strains tested were contaminated at day 1 and had to be repeated before results were obtained. Contamination of individual tests increased significantly after 7 days of incubation.

Conclusions—ATP bioluminescence can be used to monitor mycobacterial growth in fluid culture media; the technique has considerable potential for rapid susceptibility testing. Advantages include lower initial cost of analytical equipment, lower reagent cost per test, and the use of nonradioactive substrates.—Authors' Abstract

Mackall, J. C., Bai, G. H., Rouse, D. A., Armoa, G. R. G., Chuidian, F., Nair, J. and Morris, S. L. A comparison of the T-cell delayed-type hypersensitivity epitopes of the 19-kD antigens from *Mycobacterium tuberculosis* and *Mycobacterium intracellulare* using overlapping synthetic peptides. Clin. Exp. Immunol. **93** (1993) 172–177.

Mycobacterial disease remains a serious international public health concern. Improved methods to rapidly and specifically detect mycobacterial infections would greatly enhance clinical management of these diseases. To define species-specific T-cell epitopes that may be useful for the immunodiagnosis of mycobacterial infections, polymerized synthetic peptides from the 19-kD *Mycobacterium tuberculosis* and *M. intracellulare* protein homologs were tested in guinea pig DTH assays. Five *M. tuberculosis* and eight *M. intracellulare* peptides evoked skin test responses. Although all of the active *M. tuberculosis* and seven of the *M. intracellulare* peptides elicited nonspecific DTH reactions, the peptide IN13 induced a *M. intracellulare*-specific skin test reaction, and thus represents a specific *M. intracellulare* T-cell DTH epitope. This result suggests that the development of monospecific peptide-based immunodiagnostic reagents may be feasible for future clinical use.—Authors' Abstract

Marin, L. M. L., Laneelle, M. A., Prome, D. and Daffe, M. Structures of the glycopeptidolipid antigens of two animal pathogens—*Mycobacterium senegalense* and *Mycobacterium porcinum.* Eur. J. Biochem. **215** (1993) 859–866.

The structures of the major glycolipid antigens of two animal pathogens *Mycobacterium senegalense* and *M. porcinum* were elucidated by a combination of fast-atom bombardment mass spectrometry, nuclear magnetic resonance spectroscopy, chemical analyses and radiolabeling experiments. Five glycoconjugates belonging to the class of C-mycoside glycopeptidolipids were characterized in each species. They shared with those recently described in *M. peregrinum* the same unusual distribution of the disaccharides on the alaninol end of the molecules. Both species showed the presence of the novel sulfated glycopeptidolipid. In addition, some acetylated forms of the glycolipids were also present in the species examined. Identical seroreactivities were observed between the glycolipid antigens extracted from *M. senegalense, M. porcinum* and *M. peregrinum* and an antiserum raised against the whole lipid antigens of *M. peregrinum.* These data reinforce the close taxonomic relationships between the three mycobacterial species and demonstrate the antigenicity of the new variants of mycobacterial glycopeptidolipids.—Authors' Abstract

Matoba, A. Y., Lee, B. L., Robinson, N. M., Penland, R. and Osato, M. S. Combination drug testing of *Mycobacterium chelonae.* Invest. Ophthalmol. Vis. Sci. **34** (1993) 2786–2789.

Medical therapy of *Mycobacterium chelonae* keratitis is difficult because there are so few effective antimicrobial agents and single-agent therapy frequently fails clinically. To identify more effective medical treatment regimens, the *in vitro* antimicrobial efficacy of amikacin, the most frequently used single agent, was investigated in combination with four antibiotics previously reported to have activity against *M. chelonae*: erythromycin, imipenem, ciprofloxacin, and vancomycin. The drug combinations were tested by the checkerboard method against seven corneal isolates of *M. chelonae.* The combination of amikacin with erythromycin or vancomycin consistently led to synergistic or additive effect; however the minimum inhibitory concentrations for vancomycin were very high. The combination of amikacin with imipenem or ciprofloxacin led to results ranging from antagonism to additive effects. Of the antibiotics tested, erythromycin showed the most activity against *M. chelonae* in combination with amikacin. *In vitro* combination drug testing of *M. chelonae* by the checkerboard method should be further evaluated for clinical relevance in microbial keratitis.—Authors' Abstract

McDonough, K. A., Kress, Y. and Bloom, B. R. Pathogenesis of tuberculosis—interaction of *Mycobacterium tuberculosis* with macrophages. Infect. Immun. **61** (1993) 2763–2773.

Central to understanding the pathogenesis of tuberculosis is the interaction between the pathogen and mononuclear phagocytes. A key question about that interaction is whether *Mycobacterium tuberculosis* exerts an effect on phagolysosome fusion. We have reexamined the dynamics of phagolysosome fusion and its effect on intracellular bacterial replication in *M. tuberculosis*-infected macrophages by performing an extensive study at the electron microscopic level. Thoria-labeled murine and human macrophages were infected with a virulent (H37Rv) or avirulent (H37Ra) strain of *M. tuberculosis* or with *M. bovis* BCG vaccine for times ranging from 2 hr to 7 days. In all cases, by 2-hr postinfection, approximately 85% of the bacteria clearly resided in fused vacuoles. However, at 4 days postinfection, fusion levels for viable H37Rv and H37Ra were reduced by half; whereas the fusion profiles of BCG and of heat-killed H37Rv and H37Ra were unchanged. A comparison of the numbers of bacteria per fused and nonfused vacuoles suggests both a net transfer of bacteria out of fused vacuoles and preferential bacterial multiplication in nonfused vacuoles. H37Rv and H37Ra appeared to bud from the phagolysosomes into tightly apposed membrane vesicles that did not fuse with secondary lysosomes. In some cases, no such membrane was seen and the bacteria appeared to be free in the cytoplasm. Only viable H37Rv showed a significant increase in bacterial counts during the course of infection. Thus, both of the attenuated strains we examined differed from the virulent strain H37Rv in their abilities to replicate successfully within macrophages, but each diverged from H37Rv at a different point in the process. Viable tubercle bacilli H37Rv and H37Ra had the capacity to escape from fused vesicles as the infection progressed; BCG did not. After extrusion from the phagolysosome, H37Rv, but not H37Ra, was able to multiply. These results suggest a novel mechanism by which virulent *M. tuberculosis* eludes the microbicidal mechanisms of macrophages by escaping from fused phagolysosomes into nonfused vesicles or the cytoplasm.—Authors' Abstract

Nikaido, H., Kim, S. H. and Rosenberg, E. Y. Physical organization of lipids in the cell wall of *Mycobacterium chelonae.* Mol. Microbiol. **8** (1993) 1025–1030.

Mycobacterial cell wall functions as an effective permeability barrier, making these bacteria resistant to most antibacterial agents. It has been assumed that this low permeability was due to the presence of a large amount of unusual lipids in the cell wall, but it was not known how these lipids are able to produce such an exceptional barrier. We report here the first experimental evidence on the physical arrangement of these lipids based on X-ray diffraction stud-

ies of purified *Mycobacterium chelonae* cell wall, a result suggesting that the hydrocarbon chains of the cell-wall lipids are arranged predominantly in a direction perpendicular to the cell-wall surface, probably producing an asymmetric bilayer structure.—Authors' Abstract

Nolte, F. S., Metchock, B., McGowan, J. E., Jr., Edwards, A., Okwumabua, O., Thurmond, C., Mitchell, P. S., Plikaytis, B. and Shinnick, T. Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. J. Clin. Microbiol. **31** (1993) 1777–1782.

A polymerase chain reaction (PCR) assay for the rapid diagnosis of pulmonary tuberculosis was developed by using oligonucleotide primers to amplify a fragment of IS*6110*, an insertion sequence repeated multiple times in the chromosome of *Mycobacterium tuberculosis*. Sediment obtained from sputa processed by the *N*-acetyl-L-cysteine-NaOH method was suspended in a simple lysis buffer and was heated at 100°C for 30 min prior to amplification. A dUTP-uracil *N*-glycosylase PCR protocol was used to prevent false-positive test results because of the carryover of products from previous amplification reactions. The 317-bp amplicon was detected by direct gel analysis and Southern blotting and then hybridization with a biotin-labeled internal probe. Hybrid molecules were detected by using a commercially available avidin-alkaline phosphatase-chemiluminescent substrate system (Tropix, Inc., Bedford, Mass.). The analytical sensitivity of the assay was 10 fg of purified mycobacterial DNA. The limits of detection by culture (Middlebrook 7H11 agar and Lowenstein-Jensen medium) and by PCR were equivalent in terminal dilution experiments for organism suspensions and positive sputa. An internal control was used to detect the presence of amplification inhibitors in each negative reaction mixture. DNA was purified from inhibitory specimens by phenol-chloroform extraction and ethanol precipitation. PCR results were compared with results of microscopy and conventional culture for the detection of *M. tuberculosis* in 313 sputum specimens. There were 124 specimens that were positive for *M. tuberculosis* by conventional methods and 113 (91%) that were positive by PCR. PCR detected 105 of 110 (95%) of the smear-positive and 8 of 14 (57%) of the smear-negative specimens. There were no false-positive results by PCR (specificity, 100%). This PCR assay incorporates several innovations that make application of this new technology feasible in clinical microbiology laboratories.—Authors' Abstract

Orme, I. M., Andersen, P. and Boom, W. H. T-cell response to *Mycobacterium tuberculosis*. J. Infect. Dis. **167** (1993) 1481–1497.

The T-cell-mediated, acquired immune response to infection with *Mycobacterium tuberculosis*, both in humans and in experimental models in the mouse, is a complex event believed to involve a variety of T-cell subsets that manifest themselves in numerous functions, including protection, delayed-type hypersensitivity, cytolysis, and the establishment of a state of memory immunity. These functions in turn involve the secretion of an array of cytokines, several of which direct cells of the monocyte/macrophage axis to contain and destroy the invading bacilli. This article reviews the development of these ideas, both from clinical experience and from basic research in animal models. In addition, the newly emerging hypothesis that the secreted or export proteins of *M. tuberculosis* are the key protective antigens leading to the initial expression of acquired specific resistance to this organism is examined.—Authors' Abstract

Orme, I. M., Roberts, A. D., Griffin, J. P. and Abrams, J. S. Cytokine secretion by CD4 T-lymphocytes acquired in response to *Mycobacterium tuberculosis* infection. J. Immunol. **151** (1993) 518–525.

The results of this study in which CD4 T-cells were harvested from mice at various times during the course of a virulent *Mycobacterium tuberculosis* infection and examined for their secretion of cytokines during culture *in vitro* with bone-marrow-derived macrophages presenting mycobacterial antigen, provide evidence for the existence of two separate waves of cytokine-producing CD4 cells. The first, which peaks at the time at which protective immunity was maximally

expressed, was characterized as a IFN-gamma-secreting cell population that preferentially recognized macrophages presenting mycobacterial culture filtrate proteins, or that were infected with the live organism. A second population, which emerged 20 to 40 days later at a time when the infection had been contained, secreted IL-4 in response to the filtrate proteins, but also reacted particularly strongly to the 65-kDa (hsp60) heat-shock protein molecule of *M. tuberculosis*. These data indicate that acquired immunity to tuberculosis infection involves the production of both Th1- and Th2-like cell populations that differ in terms of their kinetics of emergence and loss, and in terms of their antigen specificity.—Authors' Abstract

Pervin, K., Childerstone, A., Shinnick, T., Mizushima, Y., Vanderzee, R., Hasan, A., Vaughan, R. and Lehner, T. T-cell epitope expression of mycobacterial and homologous human 65-kilodalton heat shock protein peptides in short term cell lines from patients with Behcet's disease. J. Immunol. **151** (1993) 2273–2282.

T-cell epitopes of the 65-kDa heat-shock protein (HSP) were mapped in patients with Behcet's disease (BD) by stimulating T cells with the overlapping synthetic peptides derived from the sequences of the *Mycobacterium tuberculosis* 65-kDa HSP. Significant lymphoproliferative responses were stimulated with four HSP peptides in BD, as compared with the related disease (recurrent oral ulcers), unrelated disease, and healthy controls ($p < 0.05$ to 0.005). In order to assess the relative frequency of sensitized lymphocytes by these peptides, 7353 short-term cell lines (STCL) were generated from the lymphocytes of patients and controls. Peptides 111–125, 154–172, and 311–325 ($p < 0.001$) and peptide 219–233 ($p < 0.02$) yielded significantly greater frequency of STCL in BD than in healthy and disease controls. All but peptide 154–172 stimulated only the CD4+ subset of T cells, although there was no evidence that reactivity to the selected peptides is restricted by DR2 to DR7 antigen. HLA-B51 is significantly associated with BD, but there was no evidence that B51 was a restricting element, when B51+ patients were compared with B51− patients with BD, and with B51+ healthy control subjects. A comparative investigation was then carried out between the corresponding mycobacterial and human HSP peptides. Similar or higher lymphoproliferative responses were stimulated by the human peptides compared with the mycobacterial peptides. These results suggest that the four peptide determinants within the 65-kDa HSP might be involved in the pathogenesis of BD. Whereas the high microbial load and associated stress proteins found in oral ulceration of BD may initiate an immune response to these conserved epitopes, expression of autoreactive T-cell clones might be stimulated by immunodominant T-cell epitopes of endogenous HSP which may induce immunopathologic changes.—Authors' Abstract

Pourshafie, M., Ayub, Q. and Barrow, W. W. Comparative effects of *Mycobacterium avium* glycopeptidolipid and lipopeptide fragment on the function and ultrastructure of mononuclear cells. Clin. Exp. Immunol. **93** (1993) 72–79.

Among the various lipids associated with the cell envelope of the *Mycobacterium avium* complex, the species-specific glycopeptidolipids (GPL) are responsible for distinguishing one serovar from another. In a continuing effort to study the immunomodulatory capabilities of these mycobacterial lipids, we have examined and compared the effects of the GPL and its lipopeptide fragment (beta-lipid) on mononuclear cell function. It was observed that the lymphoproliferative response of murine splenic mononuclear cells to mitogen stimulation was reduced by both the GPL and its lipopeptide fragment. Although the responsiveness appeared to be down-regulated to a greater degree by the beta-lipid, treatment with either GPL or beta-lipid resulted in the release of soluble factors from peritoneal macrophages that caused suppression of the lymphoproliferative responsiveness of splenic mononuclear cells. Flow cytometric analysis of peritoneal macrophages revealed that treatment with the beta-lipid fragment caused a marked decrease in expression of the C3bi complement receptor, Mac-1, on macrophages; whereas treatment with GPL resulted in a marked increase in the ex-

pression of Mac-2 receptor on macrophages. Treatment of peritoneal macrophages with either GPL or beta-lipid resulted in the release of tumor necrosis factor (TNF), as determined by an L929 biological cytotoxicity assay. Perturbation of macrophage membrane ultrastructure by both GPL and beta-lipid was confirmed by electron microscopy, and may be a possible explanation for the resulting alterations in mononuclear cell function observed in this study.—Authors' Abstract

Radhakrishnan, V. V., Mathai, A. and Sundaram, P. Diagnostic significance of circulating immune complexes in patients with pulmonary tuberculosis. J. Med. Microbiol. **36** (1992) 128–131.

A polyethylene glycol (PEG) precipitation method was used to examine sera of patients [in India] with active pulmonary tuberculosis (PT), leprosy and nontuberculous pulmonary diseases, and of healthy control subjects for immune complexes (ICs). *Mycobacterium tuberculosis* antigen 5 was detected in the ICs in 80% of patients with PT by the indirect (sandwich) enzyme-linked immunosorbent assay (ELISA). Detection of mycobacterial antigen in ICs has diagnostic potential as an adjunct in the laboratory diagnosis of PT, particularly when repeated bacteriological investigations for *M. tuberculosis* in clinical specimens are negative. Levels of ICs tend to decrease with the duration of antituberculosis chemotherapy and their detection can also be used to assess the clinical response to therapy in patients with PT.—Authors' Summary

Raviglione, M. C., Sudre, P., Rieder, H. L., Spinaci, S. and Kochi, A. Secular trends of tuberculosis in Western Europe. Bull. WHO **71** (1993) 297–306.

Deaths due to tuberculosis have decreased uniformly in all countries in Western Europe, and most have occurred among those aged ≥ 65 years. In recent years, tuberculosis case notifications have continued to decline in Belgium, Finland, France, Germany, and Spain, and have levelled off in Sweden and the United Kingdom; increases have, however, been recorded in Austria, Denmark, Ireland, Italy, The Netherlands, Norway, and Switzerland. In Denmark, The Netherlands, Norway, Sweden, and Switzerland an increasing number of cases of tuberculosis among foreign-born residents has resulted in a change from the expected downward trend. Human immunodeficiency virus (HIV) infection appears to contribute only marginally to the overall tuberculosis morbidity; however, it appears to be important in Paris and its surrounding areas, and tuberculosis is very common among HIV-infected persons in Italy and Spain. Despite these recent changes in the incidence of tuberculosis, there is currently no evidence of its increased transmission among the youngest age groups of the indigenous populations. Properly designed disease surveillance systems are critical for monitoring the tuberculosis trends so that each country can identify its own high-risk groups and target interventions to prevent, diagnose, and treat the disease. Tuberculosis remains a global disease and because of increasing human migrations, its elimination in Western Europe cannot be envisaged without concomitant improvements in its control in high-incidence, resource-poor countries.—Authors' Abstract

Ratnaker, P. and Murthy, P. S. Trifluoperazine inhibits the incorporation of labeled precursors into lipids, proteins and DNA of *Mycobacterium tuberculosis* H37Rv. FEMS Microbiol. Lett. **110** (1993) 291–294.

We have recently demonstrated that the calmodulin antagonist trifluoperazine has antitubercular activity *in vitro* against *Mycobacterium tuberculosis* H37R(v) susceptible and resistant to isoniazid. It is now shown that trifluoperazine at a concentration of 50 μg/ml when added to the cells along with the labeled precursors inhibited the incorporation of [C-14]acetate into lipids (63%) and uptake of [C-14]glycine (74%) and [H-3]thymidine (52%) by whole cells of *M. tuberculosis* H37R by 6 hr of exposure. After 48 hr, the inhibition was 87%, 97% and 74%, respectively. However, when the drug was added to cells taking up and metabolizing the labeled precursors at a later point (3 hr for [C-14]acetate and [H-3]thymidine and 12 hr for [C-14]glycine), it inhibited completely the uptake of all the precursors, at least up to 24 hr. The onset

of inhibitory action was very rapid, i.e., 3 hr. It is suggested that trifluoperazine has multiple sites of action and acts probably by affecting the synthesis of lipids, proteins and DNA.—Authors' Abstract

Riviere, M., Auge, S., Vercauteren, J., Wisingerova, E. and Puzo, G. Structure of a novel glycopeptidolipid antigen containing a *O*-methylated serine isolated from *Mycobacterium xenopi* complete H-1-NMR and C-13-NMR assignment. Eur. J. Biochem. **214** (1993) 395–403.

GPL X-1, a novel glycopeptidolipid (GPL) isolated from *Mycobacterium xenopi* (CIPT 140 35004), has recently been found to typify a new class of mycobacterial glycopeptidolipids devoid of C-mycoside core structure, the so-called serine-containing glycopeptidolipid [Riviere, M. and Puzo, G. (1991) J. Biol. Chem. 266, 9057–9063]. Here we report the purification and characterization of a novel serine-containing GPL termed GPL X-IIb, isolated from the *M. xenopi* strain NCTC 10042. On thin-layer chromatography, this GPL was found to be present in some other *M. xenopi* strains isolated from patients with pulmonary infections. The sugar and amino-acid compositions of this GPL were elucidated from the native form using a combination of two-dimensional homonuclear and heteronuclear scalar coupling NMR. The peptide and sugar sequences, as well as the methoxyl group locations on the C-3 of the 6-deoxy-alpha-L-talopyranoside (6dTalp) and on a Ser, were unambiguously determined by heteronuclear multiple-bond correlation experiments. GPL X-IIb was found to be composed of a lipotetrapeptide of the following structure C-12-Ser-OMe-Ser-Phe-aThr-OMe (aThr = allothreonine). The sugar part is made up of 3OMe-alpha-L-6dTalp and the following disaccharide: alpha-L-Rhap-(1→3)-2-*O*-Lau-alpha-L-Rhap (Rhap = rhanmopyranose). Unlike GPL X-I, the sugar attachment sites on the tetrapeptide were successfully determined from heteronuclear three-bond coupling correlation observed in the heteronuclear multiple-bond correlation spectrum between the anomeric carbon resonances and the beta protons of aThr-OMe and Ser. It was established that the 3OMe-6dTalp glycosylates the Ser while the disaccharide is linked to the aThr-OMe. Thus, both GPL X-1 and GPL X-IIb share a common lipotetrapeptide core [with the exception of Ser(OMe)] but drastically differ in their oligosaccharide appendage. Thus, by analogy with the *M. avium* complex, the present report suggests that *M. xenopi* species can be divided in various serovars characterized by the unique structure of their C-mycoside GPL oligosaccharide appendage, enhancing the interest for this new type of serine-containing glycopeptidolipid.—Authors' Abstract

Thibert, L. and Lapierre, S. Routine application of high-performance liquid chromatography for identification of mycobacteria. J. Clin. Microbiol. **31** (1993) 1759–1763.

Mycolic acid analysis by high-performance liquid chromatography (HPLC) was introduced in our laboratory as the routine technique for identifying all clinical isolates of mycobacteria referred to us. HPLC identified 96.1% of the 1103 strains analyzed; whereas the biochemical procedures and/or the commercial DNA probes identified 98.3% of strains, for an overall agreement of 94.4%. Compared with the probes, there was 100% specificity and 98.9% sensitivity for *Mycobacterium tuberculosis* identification. HPLC allowed early detection and identification of the rare mycobacterial species *M. haemophilum, M. malmoense, M. shimoidei,* and *M. fallax* as well as uncharacteristic strains of *M. simiae.* After 18 months of routine use, HPLC proved to be reliable, easy to perform, rapid, and less costly than other identification methods.—Authors' Abstract

Venisse, A., Berjeaud, J.-M., Chaurand, P., Gilleron, M. and Puzo, G. Structural features of lipoarabinomannan from *Mycobacterium bovis* BCG; determination of molecular mass by laser desorption mass spectrometry. J. Biol. Chem. **268** (1993) 12401–12411.

It was recently shown that mycobacterial lipoarabinomannan (LAM) can be classified into two types (Chatterjee, D., Lowell, K., Rivoire, B., McNeil, M. R., and Brennan, P. J. (1992) J. Biol. Chem. 267, 6234–6239) according to the presence or absence of

mannosyl residues (Man*p*) located at the nonreducing end of the oligoarabinosyl side chains. These two types of LAM were found in a pathogenic *Mycobacterium tuberculosis* strain and in an avirulent *M. tuberculosis* strain, respectively, suggesting that LAM with Man*p* characterizes virulent and "disease–inducing strains." We now report the structure of the LAM from *M. bovis* bacille Calmette-Guérin (BCG) strain Pasteur, largely used throughout the world as vaccine against tuberculosis. Using an up-to-date analytical approach, we found that the LAM of *M. bovis* BCG belongs to the class of LAMs capped with Man*p*. By means of two-dimensional homonuclear and heteronuclear scalar coupling NMR analysis and methylation data, the sugar spin system assignments were partially established, revealing that the LAM contained two types of terminal Man*p* and 2-*O*-linked Man*p*. From the following four-step process: (i) partial hydrolysis of deacylated LAM (dLAM), (ii) oligosaccharide derivatization with aminobenzoic ethyl ester, (iii) HPLC purification, (iv) FAB/MS-MS analysis; it was shown that the dimannosyl unit α-D-Man*p*-(1→2)-α-D-Man*p* is the major residue capping the termini of the arabinan of the LAM. In this report, LAM molecular mass determination was established using matrix-assisted UV-laser desorption/ionization mass spectrometry which reveals that the LAM molecular mass is around 17.4 kDa. The similarity of the LAM structures between *M. bovis* BCG and *M. tuberculosis* H37Rv is discussed in regard to their function in the immunopathology of mycobacterial infection.—Authors' Abstract

Verbon, A., Weverling, G. J., Kuijper, S., Speelman, P., Jansen, H. M. and Kolk, A. H. J. Evaluation of different test for the serodiagnosis of tuberculosis and the use of likelihood ratios in serology. Am. Rev. Respir. Dis. **148** (1993) 378–384.

A serologic test for the diagnosis of tuberculosis was evaluated in 91 newly diagnosed tuberculosis (TB) patients of whom 15 were HIV positive, in 17 TB patients during treatment, and in 220 control subjects (including individuals from endemic areas and patients with sarcoidosis or Crohn's disease). Purified proteins of *Mycobacterium tuberculosis* with molecular weights of 10,000, 16,000, 24,000, 30,000, 38,000, and 70,000 were tested by ELISA. In addition, monoclonal antibody TB72 was tested by competition ELISA. The cutoff values were set at the mean plus three standard deviations of the values obtained in 100 healthy Dutch army recruits. Only the ELISA with the 10,000, 16,000, and 24,000 antigens and the TB72 assay discriminated between patients with TB who were not HIV positive and control subjects. Specificity varied from 95% to 98% and sensitivity from 29% to 51% with the different antigens. Combination of the test results of the ELISA with the 16,000 antigen and the TB72 assay had a sensitivity of 65% (95% confidence interval, 53% to 75%) and a specificity of 96% (95% confidence interval, 92% to 98%). The assay was useful for the diagnosis of both pulmonary and extrapulmonary TB. Optimal use of the serologic assay could be obtained when likelihood ratios for each test value are calculated instead of using the test dichotomized (positive or negative). High post-test probabilities indicate the presence of TB; low post-test probabilities do not exclude the disease and should lead to additional investigations.—Authors' Abstract

Wheeler, P. R., Besra, G. S., Minnikin, D. E. and Ratledge, C. Inhibition of mycolic acid biosynthesis in a cell-wall preparation from *Mycobacterium smegmatis* by methyl 4-(2-octadecylcyclopropen-1-Y1) butanoate, a structural analog of a key precursor. Lett. Appl. Microbiol. **17** (1993) 33–36.

[C-14]acetate was incorporated into mycolic acids by a cell-free, cell-wall fraction from *Mycobacterium smegmatis*. This activity was inhibited by methyl 4-(2-octadecylcyclopropen-1-yl) butanoate which was designed as a structural analog of cis-tetracos-5-enoate, a precursor of mycolic acid biosynthesis. Other fatty acids and their methyl esters failed to inhibit mycolic acid biosynthesis at the concentration 1–2 mg/ml-1, at which methyl 4-(2-octadecylcyclopropen-1-yl) butanoate was effective. Thus, a novel agent was shown to act against an enzyme activity or target involved specifically in biosynthesis of a characteristic, mycobacterial, cell-wall component.—Authors' Abstract

Witzig, R. S. and Franzblau, S. G. Susceptibility of *Mycobacterium kansasii* to ofloxacin, sparfloxacin, clarithromycin, azithromycin, and fusidic acid. Antimicrob. Agents Chemother. **37** (1993) 1997–1999.

The MICs of ofloxacin, sparfloxacin, clarithromycin, azithromycin, and fusidic acid for clinical isolates of *Mycobacterium kansasii* were determined by the radiometric (BACTEC®) method. All drugs except azithromycin elicited MICs for 90% of the strains tested that were lower than previously reported achievable maximum concentrations in serum. Ofloxacin, sparfloxacin, and clarithromycin had the largest maximum concentration in serum/MIC for 90% of strains ratio of the drugs tested.—Authors' Abstract

Zhang, Y. and Rom, W. N. Regulation of interleukin-1-beta (IL-1-beta) gene by mycobacterial components and lipopolysaccharide is mediated by two nuclear factor-IL6 motifs. Mol. Cell. Biol. **13** (1993) 3831–3837.

The cytokines interleukin-1beta (IL-1beta) and tumor necrosis factor alpha (TNF-alpha) are released by mononuclear phagocytes *in vitro* after stimulation with mycobacteria and are considered to mediate pathophysiologic events, including granuloma formation and systemic symptoms. We demonstrated that the *Mycobacterium tuberculosis* cell-wall component lipoarabinomannan (LAM) is a very potent inducer of the IL-1beta gene expression in human monocytes, and investigated the mechanism of this effect. We localized the LAM-, lipopolysaccharide (LPS)-, and TNF-alpha-inducible promoter activity to a −131/+15 (positions −131 to +15) DNA fragment of the IL-1beta gene by deletion analysis and chloramphenicol acetyltransferase assay. Within this DNA fragment, there were two novel 9-bp motifs (−90/−82 and −40/−32) with high homology to the nuclear factor-IL6 (NF-IL6) binding site. Site-directed mutagenesis demonstrated that the two NF-IL-6 motifs could be independently activated by LAM, LPS, or TNF-alpha, and that they acted in an orientation-independent manner. DNA mobility shift assay revealed specific binding of nuclear protein(s) from LAM-, LPS-, or TNF-alpha-stimulated THP-1 cells to the NF-IL6 motifs. We conclude that the two NF-IL6 sites mediate induction of IL-1beta in response to the stimuli LAM, LPS, and TNF-alpha.—Authors' Abstract