CURRENT LITERATURE

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

General and Historical

Green, A. T. and Jochem, K. Sustaining leprosy services in the changing context of health sector reform. Lepr. Rev. **69** (1998) 134–144.

National leprosy control programs currently face a number of changes to the environment within which they operate. This paper examines the issues arising from these. It focuses, in particular, on those arising from changes in the structure of the health sector as a result of policies of health sector reform which are being considered or adopted in many developing countries. These include decentralization, financing strategies, greater role for the private and nongovernmental organization (NGO) sectors and the integration of vertical programs. The paper is structured around a number of key steps in the development of a strategy for sustainability of appropriate leprosy services. These are the assessment of the epidemiological, social and health services context, development of the strategy, mapping the roles of potential actors, development of regulatory and incentive mechanism, action planning and managing change and, finally, re-evaluation of the program objectives and service delivery organization. The paper stresses the importance of process in developing ownership of a strategy. It concludes with a set of key questions which it suggests need to be addressed by leprosy program managers in the development of a pro-active response to the changes.—Authors' Abstract

Japanese Leprosy Association. [71st General Meeting of the Japanese Leprosy Association.] Jpn. J. Lepr. **67** (1998) 1–71. (in Japanese)

This issue is devoted to papers from the Japanese Leprosy Association 71st General Meeting. The symposium discussed treatment for leprosy patients in Japan, topics including current problems and chemotherapy, trends in treatment, and implementation of WHO multiple drug therapy (MDT). Experiences of implementing WHO/MDT in Bangladesh were presented. A special lecture considered the future of medical information systems.—Trop Dis. Bull. **95** (1998) 1080

Schafer, J. Leprosy and disability control in the Guéra Prefecture of Chad, Africa: do women have access to leprosy control services? Lepr. Rev. **69** (1998) 267–278.

In a retrospective study, data from the Guéra Leprosy and Disability Control Project in Chad, covering the years from 1992 to 1996, were analyzed in order to determine whether there was any indication that the quality of care provided to female leprosy sufferers is inferior to the care provided for male patients. Data from a total of 741 patients registered for MDT, of whom 351 were newly diagnosed cases, are presented and discussed. The data indicate that women have access to diagnosis and treatment and health education. They do not present for treatment later than men, disability rates are lower and they have slightly higher treatment completion rates. Both women and men benefit from footwear and loan programs. More women than men are involved in patient self-help groups. The study shows that in this part of central Chad, there is no evidence of disadvantage for women with leprosy in either diagnosis, treatment or follow up, but more qualitative data is needed to confirm these findings.—Author's Summary

Chemotherapy

Deidda, D., Lampis, G., Fioravanti, R., Biava, M., Porretta, G. C., Zanetti, S. and Pompei, R. Bactericidal activities of the pyrrole derivative BM212 against multidrug-resistant and intramacrophagic *Mycobacterium tuberculosis* strains. Antimicrob. Agents Chemother. **42** (1998) 3035–3037.

The pyrrole derivative BM212 [1,5-diaryl-2-methyl-3-(4-methylpiperazin-1-yl)-methyl-pyrrole] was shown to possess strong inhibitory activity against both *Mycobacterium tuberculosis* and some nontuberculosis mycobacteria. BM212 was inhibitory to drug-resistant mycobacteria and also exerted bactericidal activity against intracellular bacilli residing in the U937 human histiocytic lymphoma cell line.—Authors' Abstract

Dong, Y., Xu, C., Zhao, X., Domagala, J. and Drlica, K. Fluoroquinolone action against mycobacteria: effects of C-8 substituents on growth, survival, and resistance. Antimicrob. Agents Chemother. **42** (1998) 2978–2984.

Fluoroquinolones trap gyrase on DNA as bacteriostatic complexes from which lethal DNA breaks are released. Subsituents at the C-8 position increase activities of *N*-1-cyclopropyl fluoroquinolones against several bacterial species. In the present study, a C-8-methoxyl group improved bacteriostatic action against *gyrA* (gyrase-resistant) strains of *Mycobacterium tuberculosis* and *M. bovis* BCG. It also enhanced lethal action against gyrase mutants of *M. bovis* BCG. When cultures of *M. smegmatis, M. bovis* BCG, and *M. tuberculosis* were challenged with a C-8-methoxyl fluoroquinolone, no resistant mutant was recovered under conditions in which more than 1000 mutants were obtained with a C-8-H control A C-8-bromo substituent also increased bacteriostatic and lethal activities against a *gyrA* mutant of *M. bovis* BCG. When lethal activity was normalized to bacteriostatic activity, the C-8-methoxyl compound was more bactericidal than its C-8-H control, while the C-8-bromo fluoroquinolone was not. The C-8-methoxyl compound was also found to be more effective than the C-8-bromo fluoroquinolone at reducing selection of resistant mutants when each was compared to a C-8-H control over a broad concentration range. These data indicate that a C-8-methoxyl substituent, which facilitates attack of first-step gyrase mutants, may help make fluoroquinolones effective antituberculosis agents.—Authors' Abstract

Horgen, L., Jerome, A. and Rastogi, N. Pulsed-exposure and postantibiotic leukocyte enhancement effects of amikacin, clarithromycin, clofazimine and rifampin against intracellular *Mycobacterium avium.* Antimicrob. Agents Chemother. **42** (1998) 3006–3008.

We investigated the postantibiotic effects (PAEs) of four agents against *Mycobacterium avium* in a human macrophage model under two different experimental conditions. For postantibiotic leukocyte enhancement (PALE), bacteria were exposed to antibiotics prior to their phagocytosis, whereas for pulsed exposure (PE), antibiotics were added after phagocytosis. In both cases, the drugs were used at their peak concentrations in serum (C_{max}) for 2 hr. The results showed two different patterns: one for the drug for which results under PE and PALE test conditions did not significantly differ (amikacin) and one for drugs for which PAE values were significantly higher under PE test conditions (clarithromycin, clofazimine, and rifampin). These data suggest that even a brief exposure of *M. avium* to peak concentrations of certain drugs in serum may result in prolonged and persistent suppression of bacterial growth inside human macrophages.—Authors' Abstract

Oleksijew, A., Meulbroek, J., Ewing, P., Jarvis, K., Mitten, M., Paige, L., Tovcimak, A., Nukkula, M., Chu, D. and

Alder, J. D. *In vivo* efficacy of ABT-225 against drug-sensitive and -resistant *Mycobacterium tuberculosis* strains. Antimicrob. Agents Chemother. **42** (1998) 2674–2677.

Current therapy for pulmonary tuberculosis involves 6 months of treatment with isoniazid, pyrazinamide, rifampin, and ethambutol or streptomycin for reliable treatment efficacy. The long treatment period increases the probability of noncompliance, leading to the generation of multidrug-resistant isolates of *Mycobacterium tuberculosis.* A treatment option that significantly shorted the course of therapy, or a new class of antibacteria effective against drug-resistant *M. tuberculosis* would be of value. ABT-255 is a novel 2-pyridone antibacterial agent which demonstrates *in vitro* potency and *in vivo* efficacy against drug-susceptible and drug-resistant *M. tuberculosis* strains. By the Alamar blue reduction technique, the MIC of ABT-255 against susceptible strains of *M. tuberculosis* ranged from 0.016 to 0.031 μg/ml. The MIC of ABT-255 against rifampin- or ethambutol-resistant *M. tuberculosis* isolates was 0.031 μg/ml. In a murine model of pulmonary tuberculosis, 4 weeks of oral ABT-255 therapy produced a 2- to 5-$\log_{10}$ reduction in viable drug-susceptible *M. tuberculosis* counts from lung tissue. Against drug-resistant strains of *M. tuberculosis,* ABT-255 produced a 2- to 3-$\log_{10}$ reduction in viable bacterial counts from lung tissue. ABT-255 is a promising new antibacterial agent with activity against *M. tuberculosis.* —Authors' Abstract

Paramasivan, C. N., Kubendiran, G. and Herbert, D. Action of metronidazole in combination with isoniazid and rifampicin on persisting organisms in experimental murine tuberculosis. Indian J. Med. Res. **108** (1998) 115–119.

To study the activity of metronidazole on persisting tubercle bacilli, BALB/c mice were infected with *Mycobacterium tuberculosis* and, after 14 days, treated with isoniazid (H) or rifampin (R) or isoniazid + rifampin (HR) for 2 months. An untreated group and a group treated with metronidazole (M) alone served as controls. At the end of 2 months, M was added to the H, R, and HR regimen in half the mice, and the treatment was continued for 1 more month in all mice. At the end of treatment, no viable organisms were detected in the lung or spleen of mice treated with HR or HRM regimens. In contrast, compared to the mice treated with R alone, the $\log_{10}$ colony forming units (cfu) of mice treated with RM were lower by 1.84 and 0.52 in the lung and spleen, respectively. Similarly, compared to the H group, the $\log_{10}$ cfu were lower by 0.67 in the spleen of mice treated with HM, and no additional effect due to M was seen in the lung. Three months after stopping treatment, viable organisms were isolated from both the organs of all the groups. However, the $\log_{10}$ cfu in the lung and spleen for the groups with metronidazole were below the $\log_{10}$ cfu for the respective single or two drug groups, except for the $\log_{10}$ cfu in the lung for the RM group. These findings suggest that metronidazole, given with bactericidal drugs such as rifampin and isoniazid may be of value in eliminating persisting tubercle bacilli, but further studies are warranted.—Authors' Abstract

Terencio de las Aguas, J., Chofre, C. and Contreras, F. [Ineffective therapy in a case of dimorphous leprosy.] Rev. Leprol. Fontilles **21** (1998) 593–601. (in Spanish)

A paucibacillary leprosy patient (dimorphous type) with persistence and appearance of new skin lesions after 10 years of treatment with six different drugs is presented. An immunotherapeutical treatment is recommended.—Authors' English Summary

Clinical Sciences

Batistella, G. G. G., Maakaroun, M. and de Castro, A. V. Extracapsular cataract extraction and intraocular lens implantation in leprosy patients: visual outcome and complications. Indian J. Lepr. **70** (1998) 5–10.

The outcome of extracapsular cataract extraction and intraocular lens implantation, conducted on 70 eyes of 53 leprosy patients (24 male, 29 female: age range 43–84 years) in Belo Horizonte, Brazil, during 1993–1996, was analyzed. Pre-operative ocular manifestations included iris atrophy (22 eyes), miosis (8 eyes) and corneal damage (5 eyes); 48 eyes did not show any major abnormality. When examined at ≥10 weeks after surgery, visual acuity improved in 92.9% of the eyes; in 65.7% the acuity had improved by ≥4 lines on the Snellen chart; 39 eyes (55.7%) had at least one postoperative complication; the complications could not be associated only to leprosy infiltration, were not too serious and could be controlled.—Authors' Abstract

Bhargava, P., Kuldeep, C. M. and Mathur, N. K. Erythema nodosum leprosum in subgroups of lepromatous leprosy. Lepr. Rev. **68** (1997) 373–375.

A total of 659 lepromatous leprosy patients seen in a 19-year period in India were studied for the development of erythema nodosum leprosum (ENL). ENL was observed in 26.2% of patients with subpolar lepromatous leprosy (LL_S) and 11.2% of immunologically stable and anergic polar lepromatous leprosy (LL_P) patients. All LL_P patients who developed ENL were on antileprosy drugs and 25% of LL_S patients developed ENL before treatment. It is concluded that these results can be explained by the decreased cell-mediated immunity in LL_P patients compared to LL_S patients.—Authors' Abstract

Boratto, L. M., Orefice, F., Werner, L. P. and Antunes, C. M. F. Dacryocystographic examination does not identify early seventh nerve failure in leprosy patients. Indian J. Lepr. **70** (1998) 287–289.

Dacryocystographic examination was performed in two groups of patients: patients having leprosy and those not having leprosy, in order to look for early failure of the facial nerve. The results of this study show that this kind of examination does not improve diagnosis of leprosy.—Authors' Abstract

Brandsma, J. W., van Brakel, W. H., Anderson, A. M., Kortendijk, A. J., Gurung, K. S. and Sunwar, S. K. Intertester reliability of manual muscle strength testing in leprosy patients. Lepr. Rev. **69** (1998) 257–266.

This study reports the results of a study on the intertester reliability of manual muscle strength testing in leprosy patients with confirmed motor function loss of at least one nerve. Three testers graded the muscle strength of 72 patients in random order. both hands and feet were graded. Strength was graded on a modified Medical Research Council scale (9 points, 5, 4+, 4, 3+, 3, 2+, 2, 1, 0). The following movements were tested for strength: littler finger and index finger abduction, intrinsic position of all four fingers, thumb abduction and opposition, foot dorsiflexion and eversion and extension of the big toe. The weighted kappa statistic was used to calculate the chance-corrected percentage of agreement between observers. Overall agreement for each of the 11 tests appeared to be good or very good (0.61–1.00). However, when data for hands or feet with normal strength or complete paralysis were excluded from the analysis, the reliability of the remaining mid-range scale was not acceptable (kappa 0.55–0.88; direct agreement range 11%–41%). While the reliability of this scale could possibly be improved by special training, we feel that, for the evaluation of nerve function for leprosy patients with (suspected) nerve function loss, the extended 9-point VMT scale should only be used when direct intra- or intertester agreement is more than 80%.—Authors' Summary

Campos, W. B., Orefice, F., Sucena, M. A. and Rodrigues, C. A. F. Conjunctival biopsy in patients with leprosy. Indian J. Lepr. **70** (1998) 291–294.

The authors examined the eyes of 120 leprosy patients comprising of 30 cases each of tuberculoid, indeterminate, borderline and lepromatous leprosy. The investigation included biopsy of the bulbar conjunctiva on the upper temporal quadrant of the right eye. The study patients included those who were untreated, those that were being treated and those who were in observation after the end of treatment. The aim of the study was to identify the presence of *Mycobacterium leprae* in the conjunctiva. Four such cases were found: one borderline patient with no treatment and three lepromatous patients who were being treated with MDT.—Authors' Abstract

Croft, R. A., Richardus, J. H. and Smith, W. C. S. Field treatment of acute nerve function impairment in leprosy using a standardized corticosteroid regimen—first year's experience with 100 patients. Lepr. Rev. **68** (1997) 316–325.

A fixed regimen of prednisolone for the treatment of acute nerve function impairment (NFI) in leprosy patients was developed and introduced at the field level in one area (Thakurgaon) of the Danish-Bangladesh Leprosy Mission's field in Bangladesh [date not given]. The assessment, management and follow up of patients was undertaken by leprosy control supervisors and physiotechnicians; 100 patients were treated and followed up 6–8 months after completion of a 4-month course of prednisolone (40 mg/day tapering to 5 mg/day over 16 weeks for adults >35 kg). At a level of change of two points (where a change of at least two points in the motor/sensory score was taken to indicate a change of status, i.e., full or partial recovery, or deterioration); 42/65 (64.6%) patients with sensory loss experienced some sensory recovery at completion of prednisolone treatment, and 40/65 (61.5%) at 6–8 months' follow up, 41/85 (48.3%) of patients with motor loss experienced improvement, and 42/85 (49.4%) at follow up. Analysis of the mean scores at start of prednisolone treatment, completion and at follow up using Student's *t* test showed highly significant ($p < 0.001$) differences between scores before and after treatment. The benefit is maintained as seen after a period of 6–8 months follow up. It was concluded that treatment of acute nerve function impairment at the field level by paramedical workers, using a standardized regimen of prednisolone is feasible, practical and effective.—Authors' Abstract

Knuuttila, J. P., van Brakel, W. H. and Anderson, A. M. Ocular impairments in an impairment survey of leprosy-affected persons in Nepal. Indian J. Lepr. **70** (1998) 93–96.

Of 318 study subjects with leprosy in the western region of Nepal [date of survey not given], 101 had been admitted to a hospital or were attending the outpatient clinic and 217 were examined in the field. The patients studied included 232 on multidrug therapy and 71 attending for care-after-cure. Ocular impairments were found in 79 patients (25%). The most common ocular impairment was poor vision and was more common in patients aged >45 years. Lagophthalmos was present in 30 of 318 (9%) and corneal anesthesia in 23 of 318 patients (7%); 25 patients (8%) had at least one blind eye.—Authors' Abstract

Nwosu, C. M. and Nwosu, S. N. N. Sociocultural factors in leprosy: implications for control programmes in the post-leprosaria years in Nigeria. W. Afr. J. Med. **16** (1997) 126–132.

A questionnaire was used to investigate the impact of sociocultural factors on leprosy control and to predict the chances of compliance and regular attendance at clinics in the post-leprosaria abolition years. The questionnaire was administered to 53 male and female leprosy patients aged between 17 and 78 years who were randomly selected from four clinics in two local government areas of eastern Nigeria. Approximately 60% of the patients indicated that traditional concepts were the likely factors explaining the etiology of leprosy; 4 pa-

tients had definite knowledge of the microbial etiology of leprosy. Patients who held a traditional etiological concept of leprosy were introduced to the control program more frequently by their relatives than by other mean of referral ($p < 0.025$) and they were also more likely to live within the vicinity of the leprosy clinics ($p < 0.01$); however, a traditional etiological concept was not significantly associated with the clinic attendance rate of leprosy patients. The distance of a patient's home from the clinic attended, some formal education, and death of a spouse were not significantly associated with clinic attendance rate. Clinic attendance was more irregular (defined as three or more monthly sessions missed in the preceding year) in males ($p < 0.025$), patients aged below 55 years ($p < 0.025$), and those patients whose first degree relatives showed a negative family attitude ($p < 0.05$). It is concluded that it appears necessary to categorize the groups at risk of irregular clinic attendance at the first time of contact, and target patient-holding methods and health education toward patients, their relatives and the community.—Authors' Abstract

Thompson, K. and Daniel, E. Management of ocular problems in leprosy. Indian J. Lepr. **70** (1998) 295–315.

This is an excellent review of ocular leprosy and its management. It bears careful study in the original.—RCH

Turkof, E., Tambwekar, S., Kamal, S., El Dahrawi, M., Mansukhani, K., Soliman, H., Ciovica, R. and Mayr, N. Leprosy affects facial nerves at the main trunk: neurolysis can possibly avoid transfer procedures. Plas. Reconstr. Surg. **102** (1998) 1565–1573.

The predilective sites of lesions in leprous peripheral nerves are well established, and their surgical decompression is common practice when sensorimotor disorders persist after medication. By contrast, the precise localization of leprous facial neuropathy still remains unclear, and musculofascial transfers have been the only type of surgical treatment. The goal of this study was to clarify where leprosy affects facial nerves and to determine whether neurolysis might suffice to restore facial function. In five Indian and two Egyptian patients suffering from leprous facial neuritis, the nerves were stimulated transcranially at the brainstem to evoke efferent motor nerve action potentials, which were recorded from the exposed nerves. lesions were detected at the main trunk proximally from the first bifurcation in all cases. Epineuriotomy revealed fibrosis of the interfascicular epineurium in all instances, as an indication for interfascicular neurolysis. One patient was able to close his eye and showed a better smile soon after surgery. After 16 and 21 months, respectively, 1 patient had improved distinctly, 2 patients slightly, 2 patients showing no progress, and 2 patients were lost to follow up. It is concluded that: (1) leprous facial neuropathy is located at the main trunk close to the first bifurcation and not exclusively at the peripheral zygomatic branches, (2) microsurgical neurolysis can be considered in leprous facial neuropathy before transfer procedures as long as voluntary or spontaneous activity is present in the affected muscles, and (3) intra-operative transcranial electrical stimulations is an effective means of localizing the site and proximal extent of leprous facial neuropathy.—Authors' Abstract

Immuno-Pathology

Bansal, A. S., Bruce, J., Wilson, P. B. and Anyiwo, C. E. Serum sCD23 in patients with lepromatous and tuberculoid leprosy. Scand. J. Infect. Dis. **30** (1998) 133–135.

Tuberculoid and lepromatous leprosy (TL and LL) manifest exaggerated Th1 and Th2 type immunity, respectively. Serum soluble CD23, which is regulated by the stimulatory action of IL-4 and inhibitory

action of IFN-gamma, was significantly elevated in LL relative to TL and healthy controls. These results confirm the state of cellular and humoral immunity in TL and LL.—Authors' Abstract

Bonorino, C., Nardi, N. B., Zhang, X. H. and Wysocki, L. J. Characteristics of the strong antibody response to mycobacterial hsp70: a primary T-cell-dependent IgG response with no evidence of natural priming or gamma delta T cell involvement. J. Immunol. **161** (1998) 5210–5216.

Despite its high degree of evolutionary conservation, hsp70 is a surprisingly robust antigen (Ab), to such a degree that it is under consideration as a potential substrate in vaccine development. The cellular basis of the strong humoral response, however, is unknown, although it is often hypothesized to derive from restimulation of memory T cells that have been primed by hsp of intestinal flora. In this study, we tested this hypothesis and performed additional studies on the immune response to hsp70 of *Mycobacterium tuberculosis.* Superficially, the primary Ab response to this protein resembles a T cell-dependent secondary one, constituted almost exclusively by IgG. However, there is no evidence of natural priming, as revealed both by *in vitro* stimulation experiments and by immunity in germ-free mice. Although hsp70 stimulates gamma delta and alpha beta T cells from unprimed mice to proliferate *in vitro,* gamma delta cells are not required for the strong humoral response, which is indistinguishable in normal and gamma delta T cell-deficient mice. Thus, the unusual immunogenicity of this protein in eliciting a humoral response appears to be due to a strong alpha beta T cell response with no evidence of natural priming or a gamma delta T cell involvement.—Authors' Abstract

Buhrer Sekula, S., Cunha, M. D. S., Ferreira, W. A. and Klatser, P. R. The use of whole blood in a dipstick assay for detection of antibodies to *Mycobacterium leprae*: a field evaluation. FEMS Immunol. Med. Microbiol. **21** (1998) 197–201.

We describe a further simplification of a dipstick assay for the detection of antibodies to phenolic glycolipid-I of *Mycobacterium leprae* by using whole blood, and evaluated the assay performance in the leprosy-endemic area of Amazonas in Brazil. The agreement with the "gold" standard ELISA was 94.9% (kappa value = 0.87). This simple assay may be useful to identify those at risk of developing leprosy, for example, among contacts of leprosy patients at lower levels in the health services.—Authors' Abstract

Castells Rodellas, A., Valero Geli, G. and Terencio de las Aguas, J. [The immunology of leprosy, 1991.] Rev. Leprol. Fontilles **21** (1998) 507–580. (in Spanish)

The macrophage immune response, humoral and cellular immunity against *Mycobacterium leprae* and its different antigens are studied. Total and partial defects of cellular immunity exist with disturbed secretion patterns of cytokines while humoral immunity is unaltered. The secretion of various cytokines under the effect of *M. leprae* can alter the immune function, creating important deficiencies. The immunodeficiency is not satisfactorily explained (384 references).—Authors' English Abstract

Hussain, R., Dockrell, H. M., Shahid, F., Zafar, S. and Chiang, T. J. Leprosy patients with lepromatous disease recognize crossreactive T cell epitopes in the *Mycobacterium leprae* 10-kD antigen. Clin. Exp. Immunol. **114** (1998) 204–209.

T-cell responses play a critical role in determining protective responses to leprosy. Patients with self-limiting tuberculoid leprosy show high T-cell reactivity, while patients with the disseminated lepromatous form of the disease show absent to low levels of T-cell reactivity. Since the T-cell reactivity of lepromatous patients to purified protein derivative (PPD), a highly crossreactive antigen, is similar to that of tuberculoid patients, we queried if lepromatous patients could recognize crossreactive epitopes in *Mycobacterium leprae* antigens as well. T-cell responses were analyzed to a recom-

binant antigen 10-kD (a heat shock cognate protein) which is available from both *M. tuberculosis* (MT) and *M. leprae* (ML) and displays 90% identity in its amino acid sequence. Lymphoproliferative responses were assessed to ML and MT 10 kD in newly diagnosed leprosy patients (lepromatous, N = 23; tuberculoid, N = 65). Lepromatous patients showed similar, but low, lymphoproliferative responses to ML 10 and MT 10 kD, while tuberculoid patients showed much higher responses to ML kD. This suggests that the tuberculoid patients may be recognizing both species-specific and crossreactive epitopes in ML 10 kD, while lepromatous patients may be recognizing only crossreactive epitopes. This was further supported by linear regression analysis. Lepromatous patients showed a high concordance in T-cell responses between ML and MT 10 kD ($r = 0.658$; $p < 0.0006$) not observed in tuberculoid patients ($r = 0.203$; $p > 0.01$). Identification of crossreactive T-cell epitopes in *M. leprae* which could induce protective responses should prove valuable in designing second generation peptide-based vaccines.—Authors' Abstract

Khanolkar Young, S., Snowdon, D. and Lockwood, D. N. J. Immunocytochemical localization of inducible nitric oxide synthase and transforming growth factor-beta (TGF-β) in leprosy lesions. Clin. Exp. Immunol. **113** (1998) 438–442.

Inducible nitric oxide synthase (INOS) and TGF-beta were localized by immunocytochemistry in skin lesions from patients across the leprosy spectrum and from patients undergoing reversal reaction. INOS expression was highest at the tuberculoid pole of the spectrum and increased during reversal reaction. TGF-beta was observed throughout the leprosy spectrum but was highest at the lepromatous pole. Levels of TGF-beta decreased during reversal reaction. Reduced levels of TGF-beta may contribute to unregulated inflammatory responses during reactional episodes.—Authors' Abstract

Meyer, C. G., May, J. and Stark, K. Human leukocyte antigens in tuberculosis and leprosy. Trends Microbiol. **6** (1998) 148–154.

A review is given of studies concerning the associations between human leukocyte antigens (HLAs) and mycobacterial disease, particularly *Mycobacterium leprae* and *M. tuberculosis* infection; the associations are summarized in a table and the limitations of the studies discussed. A model for immunological mechanisms in human mycobacterial infection is presented and the role of HLA-restricted mycobacterial epitopes, immunosuppression, cytotoxic T cells and T-cell dichotomy in mycobacterial infection is discussed.—Authors' Abstract

Mustafa, A. S., Lundin, K. E. A. and Oftung, F. Isolation of recombinant phage clones expressing mycobacterial T cell antigens by screening a recombinant DNA library with human CD4+ Th1 clones. FEMS Immunol. Med. Microbiol. **22** (1998) 205–216.

A lambda g11 recombinant DNA library of *Mycobacterium leprae* was screened to isolate recombinant phage clones expressing mycobacterial antigens important for T-cell reactivity. The library was plated on a lawn of *Escherichia coli* Y1090 and recombinant antigens were expressed from isolated phage clones in 96-well plates. Pools of recombinant antigens from 12 wells were tested in T-cell proliferation assays with MHC class II restricted human CD4+ Th1 clones secreting interferon-gamma and cytotoxic for antigen-pulsed antigen-presenting cells. By screening 1750 pools of recombinant antigens with a mixture of eight Th1 clones, we identified two recombinant phage clones that expressed recombinant mycobacterial antigens stimulatory for T cells. MHC restriction analysis and reactivity to a battery of mycobacterial antigens suggested that the two responding Th1 clones recognized mycobacterial antigens/epitopes with different MHC class II (HLA-DR) restriction requirements. Our results suggest that the methodology described in this paper is suited to isolate recombinant phage clones expressing mycobacterial recombinant antigens stimulatory for T cells of protective

phenotype. Such antigens may be useful in designing new vaccines and diagnostic reagents against mycobacterial diseases.—Authors' Abstract

Ragno, S., Estrada Garcia, I., Butler, R. and Colston, M. J. Regulation of macrophage gene expression by *Mycobacterium tuberculosis*: down-regulation of mitochondrial cytochrome c oxidase. Infect. Immun. **66** (1998) 3952–3958.

We have investigated changes in gene expression in mouse peritoneal macrophages following infection with virulent *Mycobacterium tuberculosis*. Using differential-display reverse transcription-PCR (RT-PCR), we have identified a gene that was markedly downregulated within 6 hr of infection and remained so for the duration of the experiment (5 days). On sequencing, this gene was found to encode the murine cytochrome c oxidase subunit VIIc (COX VIIc). Downregulation of COX VIIc during *M. tuberculosis* infection was confirmed by three independent techniques: limiting-dilution RT-PCR, RNase protection assay, and Northern analysis. Limiting-dilution RT-PCR and Northern analysis were also used to analyze the specificity of this regulation; heat-killed *M. tuberculosis, M. bovis* BCG, and latex beads had no effect on expression of COX VIIc. Downregulation of this enzyme was also confirmed by using adherent cells isolated from spleens of *M. tuberculosis*-infected mice. These *ex vivo* macrophages showed apoptotic features, suggesting a possible involvement of cytochrome c oxidase in the programmed cell death of the host cells.—Authors' Abstract

Ruth, J. H., Lukacs, N. W., Warmington, K. S., Polak, T. J., Burdick, M., Kunkel, S. L., Strieter, R. M. and Chensue, S. W. Expression and participation of eotaxin during mycobacterial (type 1) and schistosomal (type 2) antigen-elicited granuloma formation. J. Immunol. **161** (1998) 4276–4282.

Eotaxin participation was analyzed during types 1 and 2 lung granuloma formation induced by embolizing Sepharose beads coupled to purified protein derivative (PPD) of *Mycobacterium bovis* or soluble Ags derived from *Schistosoma mansoni* eggs. Eotaxin was monitored by protein ELISA and semiquantitative reverse-transcriptase PCR mRNA analysis. Both types 1 and 2 granulomas released eotaxin, but levels were six-fold greater (on day 4) in the type 2 than for the type 1 or foreign body granulomas. Transcripts for eotaxin, IL-4, and CCR3 (eotaxin receptor) were also enhanced during type 2 granuloma formation. Anti-IL-4 treatment impaired eotaxin mRNA in lungs with type 2 granulomas, indicating that IL-4 promoted local eotaxin expression. *In vivo,* anti-eotaxin treatment caused modest reductions in the size of both types 1 and 2 lesions, with negligible effect on eosinophil recruitment. Surprisingly, anti-eotaxin treatment abrogated IFN-gamma-producing cells in regional lymph nodes during the type 1 PPD response. Lymph nodes draining both types 1 and 2 lesions showed enhanced CCR3 mRNA, but this followed the time of maximum eotaxin protein and mRNA expression. Correlative, *in vitro* studies revealed that graded doses of eotaxin increased IFN-gamma production from PPD-sensitive regional lymph node cultures, while monocyte-chemotactic regional lymph node cultures, while monocyte-chemotactic protein-1, an important macrophage chemoattractant, had the opposite effect. These findings indicate that eotaxin expression is not limited to type 2 hypersensitivity granulomas, but also promotes IFN-gamma production during mycobacterial responses.—Authors' Abstract

Sasiain, M. D., delaBarrera, S., Fink, S., Finiasz, M., Aleman, M., Farina, M. H. and Pizzariello, G. Interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) are necessary in the early stages of *Mycobacterium leprae* heat shock protein (hsp) 65 kD. Clin. Exp. Immunol. **114** (1998) 196–203.

Cytotoxic T cells (CTL) may play an important role in host defense against mycobacterial infections. CD4 CTL are preferentially induced by mycobacteria, but both CD4 and CD8 CTL may be necessary components of a protective immune response.

The 65-kD mycobacterium heat shock protein (hsp65) is a poor inducer of CTL in multibacillary leprosy (MB) patients. In this study we evaluate the possible role of cytokines in modulating the cytotoxic activity of CTL from leprosy patients and normal individuals (N) against autologous macrophages presenting *Mycobacterium leprae* hsp65. Our results show that hsp65-specific CTL were generated from both CD4 and CD8 lymphocytes. In N, individual cytokines as well as the combination of them were able to modify the hsp65-induced cytotoxic activity. The effect of cytokines on leprosy patients' lymphocytes was different in MB and paucibacillary (PB) patients. Thus, IL-6, IL-2, IFN-gamma or TNF-alpha did not modify the generation of hsp65-CTL from either MB [with or without an erythema nodosum episode (ENL)] or PB. In all of the patients the simultaneous addition of two cytokines was required in order to increase CTL generation. In MB, IL-6 plus IFN-gamma or IL-2 increased both CD4 and CD8 CTL, while TNF-alpha plus IFN-gamma upregulated only CD4 CTL. In PB, CD8 CTL were prominent with IL-6 plus IFN-gamma, while the increase was significant in CD4 CTL with IL-6 plus IL-2. Downregulation of CTL was observed by addition of IL-4, IL-10, anti-IFN-gamma or anti-TNF-alpha in N controls. Our data demonstrate that IFN-gamma and TNF-alpha must be present for at least the first 60 hr of the induction stage in order to generate full hsp65 CTL. Hence, IFN-gamma and TNF-alpha would be key factors in the generation of hsp65 CTL.—Authors' Abstract

Schlienger, K., Uyemura, K., Jullien, D., Sieling, P. A., Rea, T. H., Linsley, P. S. and Modlin, R. L. B7-1 but not CD28 is crucial for the maintenance of the CD4+ T cell responses in human leprosy. J. Immunol. **161** (1998) 2407–2413.

We used human leprosy as a model to compare patterns of costimulatory molecule expression in respect to the clinical/immunologic spectrum of disease. We found that B7-1, B7-2, and CD28 transcripts dominated in tuberculoid leprosy patients, who have potent T-cell responses to *Mycobacterium leprae.* In contrast, CTLA-4 was more strongly expressed in lesions from lepromatous patients, who manifest specific T-cell anergy to the leprosy bacterium. T-cell clones from tuberculoid lesions were CD4+ CD28+ or CD4+ CD28–, and T-cell clones from lepromatous lesions were predominantly CD8+ CD28–. The *M. leprae*-specific recall response of CD4+ T-cell clones from tuberculoid lesions was blocked by anti-B7-1 monoclonal antibody (mAb), but not by anti-B7-2 mAb or CTLA-Ig. However, anti-CD28 and anti-CTLA-4 mAbs did not block activation of clones from tuberculoid lesions, suggesting that B7-1 may utilize another costimulatory pathway. Peripheral blood T-cell responses in the lepromatous form were strongly regulated by CD28 during T-cell activation in contrast to the tuberculoid form. Thus, B7-1 costimulation could play a role in maintaining a strong immune response to the pathogen.—Authors' Abstract

Sharma, N., Sharma, V. K., Gupta, A., Kaur, I., Kaur, S. and Ganguly, N. K. Alterations in early biochemical events following T cell activation in leprosy patients. Clin. Immunol. Immunopathol. **88** (1998) 142–149.

The early events of activation and cytokine profiles (IL-2, -4, and -6) were studied in lymphocytes of paucibacillary (TT/BT) and multibacillary (BL/LL) leprosy patients after stimulation with PMA/A23187 and *Mycobacterium leprae* antigen (PGL-I). Lymphocytes from BT/TT patients showed proliferation in response to both PMA/A23187 and PGL-I compared to BL/LL. The levels of early activation signaling molecules such as IP3, calcium, and protein kinase C (PKC) in the particulate fraction were found to be elevated in BT/TT and BL/LL patients and showed a further significant increase after stimulation with PMA/A23187 in BT/TT patients. PGL-I marginally increased the IP3 levels in BT/TT patients; whereas in BL/LL patients, it had no effect. The levels of IL-2 were enhanced in lymphocytes of BT/TT leprosy patients and were further augmented by PPD and PGL-I, while the levels

of IL-4 and IL-6 were increased in LL/BL lymphocytes and further augmented by PGL-I. Thus PGL-I seems to be a major culprit in inducing the Th2-type cytokine response observed in lepromatous leprosy patients.—Authors' Abstract

Singh, N., Birdi, T. J., Chandrashekar, S. and Antia, N. H. *In vitro* studies on extracellular matrix production by *M. leprae*-infected murine neurofibroblasts. Lepr. Rev. **69** (1998) 246–256.

Fibroblasts and a host of macrophage secretory products have been implicated in a number of diseases where excess extracellular matrix (ECM) deposition is the main pathological feature. Fibrosis characterized by excessive deposition of collagen also contributes to the irreversible nerve damage observed in leprosy. Since *Mycobacterium leprae* are seen within neurofibroblasts (Nf) in the advanced stages of the disease and macrophages form a common infiltrating cellular constituent of leprous nerves at all stages, secretion of ECM proteins by Nf was studied, *in vitro* following infection with *M. leprae* and in the presence of macrophage secretory products. These studies were compared in cells derived from two strains of mice, Swiss white (SW) and C57BL/6, as they differ in their response to *M. leprae* infection and parallel those observed in lepromatous and tuberculoid patients, respectively. On infection with *M. leprae,* Nfs showed a decrease in secretion of collagen type IV in SW and type I in C57BL/6 strain. Macrophages caused a further decrease in the secretion of collagen types affected by *M. leprae* infection *per se,* while the other collagen types, viz. I and III in SW strain and III and IV in C57BL/s strain, were unaffected. This study indicates that neural collagenization in nerves in advanced leprosy may be of Nf origin. However, unlike other diseases with excess collagen deposition, ECM proteins produced by Nfs in response to nerve damage may not be of prime importance in the progression of leprous neuropathy and occur as a general response to loss of cellular content in leprous nerves.—Authors' Summary

Stenger, S., Niazi, K. R. and Modlin, R. L. Down-regulation of CD1 on antigen-presenting cells by infection with *Mycobacterium tuberculosis.* J. Immunol. **161** (1998) 3582–3588.

Intracellular pathogens have developed efficient evasion strategies to survive the defenses of the host immune system. In this study, we describe a new escape mechanism utilized by *Mycobacterium tuberculosis* that involves the down-regulation of the antigen (Ag)-presenting molecule CD1 from the cell surface of CD1+ APCs. The loss of CD1 from the cell surface is associated with a complete inhibition of the ability of the infected cells to present Ag to CD1-restricted T cells. The downregulation of Ag-presenting molecules on CD1+ APC by infection with *M. tuberculosis* is unique for CD1, since the expression of the classical Ag-presenting molecules MHC class I and MHC class II is not influenced. Our data show that efficient downregulation of CD1 requires infection of the cells with live mycobacteria, since heat killing of the bacteria completely abrogates the effect. The observed downregulation is not due to the secretion of cytokines or other host- or pathogen-derived factors. Investigation of upstream events responsible for the down-regulation of CD1 revealed that infection with live *M. tuberculosis* decreased the steady state CD1-mRNA levels. This study introduces a novel evasion mechanism of *M. tuberculosis* that could contribute to persistence of intracellular infection by avoiding immune recognition.—Authors' Abstract

Sterne, J. A. C., Fine, P. E. M., Ponnighaus, J. M., Rees, R. J. W. and Chavula, D. Delayed-type hypersensitivity to *Mycobacterium leprae* soluble antigens as a test for infection with the leprosy bacillus. Int. J. Epidemiol. **27** (1998) 713–721.

Background: *Mycobacterium leprae* soluble antigen (MLSA) reagents have been developed with the aim of finding a reagent, comparable to tuberculin, which could identify individuals infected with the leprosy bacillus. They have yet to be evaluated fully in human populations.

Methods: More than 15,000 individuals living in a leprosy-endemic area of northern Malawi were skin tested with one of five batches of MLSA prepared using two different protocols. The main difference in preparation was the introduction of a high G centrifugation step in the preparation of the last three ("second-generation") batches.

Results: The prevalence of skin-test positivity [delayed-type hypersensitivity (DTH)] and association with the presence of a BCG scar were greater for first- (batches A5, A22) than second- (batches AB53, CD5, CD19) generation reagents. The association of positivity with *M. leprae* infection was investigated by comparing results among known (household) contacts of leprosy cases, and among newly diagnosed leprosy patients with those in the general population. While positivity to "first-generation" antigens appeared to be associated with *M. leprae* infection, positivity to later antigens was unrelated either to exposure to leprosy cases or presence of leprosy disease. There were geographical differences in the prevalence of DTH to the various batches, probably reflecting exposure to various mycobacteria in the environment.

Conclusions: Our results suggest that the "second-generation" batches have lost antigens that can detect *M. leprae* infections, but that they retain one or more antigens which are shared between *M. leprae* and environmental mycobacteria. Natural exposure to these both sensitizes individuals and provides natural protection against *M. leprae* infection or disease. Identification of antigens present in these groups of skin test reagents may assist in production of improved skin test reagents.—Authors' Abstract

Sterne, J. A. C., Rodrigues, L. C. and Guedes, I. N. Does the efficacy of BCG decline with time since vaccination. Int. J. Tuberc. Lung Dis. **2** (1998) 200–207.

A quantitative review was conducted of all 10 randomized trials of bacille Calmette-Guérin (BCG) against tuberculosis in purified protein derivative-negative individuals who presented data for discrete periods. For each trial, log rate ratios were derived for the annual change in the efficacy of BCG. Efficacy in the first 2 years and the first 10 years was compared to that in the rest of the trial. There was considerable heterogeneity between trials in the annual change in the efficacy of BCG. In 7, efficacy decreased over time, while in 3 it increased. Average annual change in efficacy was not related to overall efficacy. Efficacy also varied between trials in the first 2 years after vaccination, at >2 years after vaccination and in the first 10 years after vaccination. The variation in efficacy between trials >10 years after vaccination was not statistically significant ($p = 0.26$). The average efficacy >10 years after vaccination was 14% (95% confidence interval –9% to 32%). It is concluded that there is no good evidence that BCG provides protection >10 years after vaccination.—Authors' Abstract

Sugawara, I., Yamada, H., Kazumi, Y., Doi, N., Otomo, K., Aoki, T., Mizuno, S., Udagawa, T., Tagawa, Y. and Iwakura, Y. Induction of granulomas in interferon-gamma gene-disrupted mice by avirulent but not by virulent strains of *Mycobacterium tuberculosis.* J. Med. Microbiol. **47** (1998) 871–877.

To gain a better understanding of the pathological role of interferon-gamma (IFN-γ) in specific granuloma formation, IFN-γ gene-deficient mice (BALB/c and C57BL/6) were produced. The IFN-γ gene in embryonic stem (ES) cells was disrupted by inserting the beta-galactosidase gene (lacZ) and the neomycin resistance gene (neo) at the translation initiation site in exon 1 by homologous recombination. Six-week-old IFN-γ-deficient and wild-type mice were inoculated with 10^3–10^7 bacilli of various strains of *Mycobacterium tuberculosis* (Kurono, H37Rv, H37Ra and BCG Pasteur) through their tail veins. The mice were examined 7 weeks later for granuloma formation. The avirulent BCG Pasteur and H37Ra strains (10^3–10^4 bacilli/ml) induced granulomas in the spleen, liver and lungs of IFN-γ-deficient mice. The granulomas consisted of epithelioid macrophages and Langhans' multinucleate giant cells, but lacked caseous necrosis. The virulent Kurono and H37Rv strains induced disseminated abscesses but not granulomas in var-

ious organs of IFN-γ-deficient mice and Mac-3-positive macrophages were not detected in the abscess lesions. These results suggest that IFN-γ may be primarily responsible for macrophage activation and that other factor(s) may be involved in the granuloma formation mechanism.—Authors' Abstract

Thompson Snipes, L., Skamene, E. and Radzioch, D. Acquired resistance but not innate resistance to *Mycobacterium bovis* bacillus Calmette-Guerin is compromised by interleukin-12 ablation. Infect. Immun. **66** (1998) 5268–5278.

Interleukin-12 (IL-12) is one of the first cytokines produced by macrophages, key mediators of innate resistance, during the host's immune response to infections. Therefore, in this study we propose that IL-12 has an important role in the early phase of the immune response to *Mycobacterium bovis* BCG. IL-12 has been shown to enhance the maturation of protective Th1 cells and gamma interferon (IFN-γ) production during mycobacterial infection. Therefore, it may play a crucial role during the immune phase of infection as well. To examine the role of IL-12 in both the innate and the immune phase of infection, we compared BCG-resistant mice, B10.A (Bcg^r), to the susceptible congenic strain B10.A (Bcg^s) following administration of a blocking monoclonal antibody to IL-12 (10F6). Anti-IL-12-treated susceptible animals exhibited a two- to threefold increase in spleen CFU by day 21. In contrast, anti-IL-12 treatment had little or no effect on the response of the genetically resistant animals to infection. The B10.A (Bcg^r) but not the B10.A (Bcg^s) mice had an increase in IFN-γ mRNA relative to baseline levels as early as day 1 of infection irrespective of anti-IL-12 treatment. By day 14, B10.A (Bcg^r) mice showed a decrease in IFN-γ mRNA while the B10.A (Bcg^s) mice showed a significant increase in IFN-γ mRNA levels. Thus, during BCG infection, the B10.A (Bcg^r) mice mount an early IFN-γ response against BCG; whereas the B10.A (Bcg^s) mice have a delayed IFN-γ response correlating with their genetic permissiveness expressed as an increased mycobacterial load by day 21. Overall, our data demonstrate that the inherent resistance of B10.A (Bcg^r) mice to mycobacteria does not depend on optimal levels of IL-12 to maintain effective control of the bacteria; whereas IL-12 is important for the susceptible animal's response to BCG during the peak of infection.—Authors' Abstract

Vallishayee, R. S., Anantharaman, D. S. and Gupte, M. D. Tuberculin sensitivity and skin lesions in children after vaccination with two batches of BCG vaccine. Indian J. Lepr. **70** (1998) 277–286.

BCG is one of the vaccines used, as a control arm in an on-going, large-scale comparative leprosy vaccine trial in South India. The objective of the present study was to examine, in the local population, the sensitizing ability as measured by skin test reactions to tuberculin, and reactogenecity, in terms of skin lesions at the site of vaccination, for the two batches of BCG vaccine used in the above trial. The study was undertaken in 816 tuberculin-negative, previously not vaccinated school children, aged 5 to 14 years. Each child received one of the two batches of BCG vaccine or normal saline (control) by random allocation. At 12 weeks from vaccination, the character and the size of the local response at the vaccination site were recorded. At the same time, the children were re-tested with tuberculin, and post-vaccination reactions to the test were measured after 72 hr. At 3 years after vaccination all available children were re-examined for the presence and size of the BCG scar at the site of vaccination. It was found that healing of vaccination lesions was uneventful with both batches of BCG. The mean size of the lesion was similar for the two batches, the overall mean being 6.3 mm. The mean size of post-vaccination tuberculin sensitivity increased with age, and it was 14.5 mm and 15.6 mm. The sensitizing effect attributable to the vaccine was 11 mm and 12 mm for the two batches of BCG, respectively. This study showed that the two batches of BCG, in a dose of 0.1 mg, used in the on-going leprosy vaccine trial were acceptable in terms of vaccination lesion and were highly satisfactory in

terms of development of hypersensitivity.—Authors' Abstract

Wilkinson, K. A., Vordermeier, H., Wilkinson, R. J., Ivanyi, J. and Hudecz, F. Synthesis and *in vitro* T-cell immunogenicity of conjugates with dual specificity: attachment of epitope peptides of 16 and 38 kDa proteins from *Mycobacterium tuberculosis* to branched polypeptide. Bioconjug. Chem. **9** (1998) 539–547.

T-cell epitope-containing peptides covalently attached to macromolecular carriers can be considered as synthetic immunogens for the development of skin-test diagnostics and of vaccines. As a carrier, an amphoteric branched chain polypeptide, poly[Lys-(Glu(i)-DL-Ala(m))] (EAK) with poly(L-lysine) backbone has been used. This polypeptide with free alpha-amino and gamma-carboxyl groups at the end of the side chains was conjugated with peptides representing two immunodominant regions of the 16- and 38-kDa proteins of *Mycobacterium tuberculosis,* respectively. Peptide C(91)SEFAYGSFVRTVSLPVGADE(110) was elongated by Cys at the N-terminal and attached to the carrier containing protected SH groups to form disulfide bridges. Peptide (65)FNLWGPAFHERYPNVTITA(83) was conjugated to the 3-(2-pyridyldithio) propionic acid N-hydroxysuccinimide ester (SPDP) modified and acetylated EAK by introducing amide bond between the free alpha-amino group of peptide and the free gamma-COOH group of Glu at the terminal position of the branches. This strategy led to chemically well-defined synthetic immunogens that contain two different epitopes in multiple copies covalently linked to a synthetic branched polypeptide carrier. *In vitro* T-cell immunogenicity of a prototype conjugate was studied using T-cell hybridomas, lymph node cells from 38-kDa protein immunized mice, and human peripheral blood mononuclear cell (PBMC) cultures from sensitized individuals. These data document that the specific T-cell stimulatory effect of each mycobacterial epitope was maintained in this conjugate. Taken together, these findings suggest that it is feasible to use a biodegradable polymeric polypeptide for producing macromolecular bioconjugates for the stimulation of T-cell responses.—Authors' Abstract

Microbiology

Alland, D., Kramnik, I., Weisbrod, T. R., Otsubo, L., Cerny, R., Miller, L. P., Jacobs, W. R. and Bloom, B. R. Identification of differentially expressed mRNA in prokaryotic organisms by customized amplification libraries (DECAL): the effect of isoniazid on gene expression in *Mycobacterium tuberulosis.* Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 13227–13232.

Understanding the effects of the external environment on bacterial gene expression can provide valuable insights into an array of cellular mechanisms including pathogenesis, drug resistance, and, in the case of *Mycobacterium tuberculosis,* latency. Because of the absence of poly (A+) mRNA in prokaryotic organisms, studies of differential gene expression currently must be performed either with large amounts of total RNA or rely on amplification techniques that can alter the proportional representation of individual mRNA sequences.

We have developed an approach to study differences in bacterial mRNA expression that enables amplification by the PCR of a complex mixture of cDNA sequences in a reproducible manner that obviates the confounding effects of selected highly expressed sequences, e.g., ribosomal RNA. Differential expression using customized amplification libraries (DECAL) uses a library of amplifiable genomic sequences to convert total cellular RNA into an amplified probe for gene expression screens. DECAL can detect 4-fold differences in the mRNA levels of rare sequences and can be per-

formed on as little as 10 ng of total RNA. DECAL was used to investigate the *in vitro* effect of the antibiotic isoniazid on *M. tuberculosis,* and three previously uncharacterized isoniazid-induced genes, iniA, iniB, and iniC, were identified. The iniB gene has homology to cell wall proteins, and iniA contains a phosphopantetheine attachment site motif suggestive of an acyl carrier protein. The iniA gene is also induced by the antibiotic ethambutol, an agent that inhibits cell wall biosynthesis by a mechanism that is distinct from isoniazid. The DECAL method offers a powerful new tool for the study of differential gene expression.—Authors' Abstract

Asano, R. L. and Davies, J. Molecular characterization of the thioredoxin system of *Mycobacterium smegmatis.* Res. Microbiol. **149** (1998) 567–576.

Thiol-disulfide exchanges are involved in many important biological processes; they are normally regulated by the glutaredoxin and thioredoxin systems. The thioredoxin system (TX) is composed of thioredoxin (TrxA) and thioredoxin reductase (TrxB) and requires NADPH as a cofactor. The thioredoxin genes trxA and trxB of *Mycobacterium smegmatis* mc(2)6 were cloned and sequenced. The complete nucleotide sequences revealed that the TX genes of *M. smegmatis* were clustered, similar to the organization of trxA and trxB of *S. clavuligerus, M. tuberculosis* and *M. leprae.* Alignment with the *M. tuberculosis* and *M. leprae* protein sequences showed that the deduced amino acid sequences for *M. smegmatis* trxA and trxB have a very high degree of similarity. Sequence alignments and phylogenetic analysis of known TrxAs and TrxBs clearly identify the two gene products of *M. smegmatis* as members of the TX family grouped with other mycobacteria.—Authors' Abstract

Chamberlain, D., Keeley, A., Aslam, M., Arenas Licea, J., Brown, T., Tsaneva, I. R. and Perkins, S. J. A synthetic Holliday junction is sandwiched between two tetrameric *Mycobacterium leprae* RuvA structures in solution: new insights from neutron scattering contrast variation and modelling. J. Mol. Biol. **284** (1998) 385–400.

The interaction between homologous DNA molecules in recombination and DNA repair leads to the formation of crossover intermediates known as Holliday junctions. Their enzymatic processing by the RuvABC system in bacteria involves the formation of a complex between RuvA and the Holliday junction. To study the solution structure of this complex, contrast variation by neutron scattering was applied to *Mycobacterium leprae* RuvA (MleRuvA), a synthetic analog of a Holliday junction with 16 base-pairs in each arm, and their stable complex.

Unbound MleRuvA was octameric in solution, and formed an octameric complex with the DNA junction. The radii of gyration at infinite contrast were determined to be 3.65 nm, 2.74 nm and 4.15 nm for MleRuvA, DNA junction and their complex, respectively, showing that the complex was structurally more extended than MleRuvA. No difference was observed in the presence or absence of Mg2+. The large difference in R-G values for the free and complexed protein in 65% (H_2O) -H-2, where the DNA component is "invisible," showed that a substantial structural change had occurred in complexed MleRuvA. The slopes of the Stuhrmann plots for MleRuvA and the complex were 19 and 15 or less ($\times 10^{-5}$), respectively, indicating that DNA passed through the center of the complex. Automated constrained molecular modelling based on the *Escherichia coli* RuvA crystal structure demonstrated that the scattering curve of octameric MleRuvA in 65% and 100% (H_2O) -H-2 is explained by a face-to-face association of two MleRuvA tetramers stabilized by salt-bridges. The corresponding modelling of the complex in 65% (H_2O) -H-2 showed that the two tetramers are separated by a void space of about 1–2 nm, which can accommodate the width of B-form DNA. Minor conformational changes between unbound and complexed MleRuvA may occur. These observations show that RuvA plays a more complex role in homologous recombination than previously thought.—Authors' Abstract

Cole, S. T. Comparative mycobacterial genomics. Curr. Opin. Microbiol. **1** (1998) 567–571.

Genomics is providing us with a mass of information about the biochemistry, physiology and pathogenesis of *Mycobacterium tuberculosis* and *M. leprae*. Comparison of the two genome sequences is mutually enriching and indicates that the *M. leprae* genome appears to have undergone shrinkage and large-scale gene inactivation, which may account for the exceptionally slow growth of this organism.— Author's Abstract

Eckstein, T. M., Silbaq, F. S., Chatterjee, D., Kelly, N. J., Brennan, P. J. and Belisle, J. T. Identification and recombinant expression of *Mycobacterium avium* rhamnosyltransferase gene (rtfA) involved in glycopeptidolipid biosynthesis. J. Bacteriol. **180** (1998) 5567–5573.

The *Mycobacterium avium* complex is a source of disseminated infections in patients with advanced AIDS. This group of mycobacteria is distinguished by the presence of highly antigenic, surface-exposed glycopeptidolipids, and these glycolipids possess variant oligosaccharide structures that are the chemical basis of the 28 distinct serovars of the *M. avium* complex. We previously described the ser2 gene cluster, encoding the synthesis of the haptenic oligosaccharide (2,3-dimethylfucose-rhamnose-6-deoxytalose-) of the serovar 2-specific glycopeptidolipid, and revealed a locus (ser2A) encoding a putative rhamnosyltransferase. Sequencing of the ser2A locus demonstrated the presence of three open reading frames, two of which yielded significant homology to several glycosyltransferases, and the deduced amino acid sequences of these two putative glycosyltransferases had 63% identity. These two genes were expressed in *M. smegmatis*, and the resulting recombinant glycopeptidolipids were characterized by thin-layer chromatography and gas chromatography-mass spectrometry. These analyses demonstrated that only one of these genes, termed rtfA, encoded the rhamnosyltransferase responsible for the transfer of rhamnose to 6-deoxytalose. The identification of rtfA will permit further evaluation of glycopeptidolipid biosynthesis and the construction of isogenic mutants of multiple *M. avium* complex serovars. Moreover, such mutants will help define the role of glycopeptidolipids in the intracellular survival of these bacteria.—Authors' Abstract

Gonzalez y Merchand, J. A., Colston, M. J. and Cox, R. A. Roles of multiple promoters in transcription of ribosomal DNA: effects of growth conditions on precursor rRNA synthesis in mycobacteria. J. Bacteriol. **180** (1998) 5756–5761.

The roles of multiple promoters in the synthesis of rRNA under different conditions of growth were investigated using two mycobacterial species as model organisms. When *Mycobacterium smegmatis* was grown under optimal conditions, its two rRNA operons contributed equally, with two promoters, one from each operon, being responsible for most transcripts. In stationary-phase growth or balanced growth under carbon starvation conditions, one operon ($rrnA^{1}$) dominated and its three promoters contributed more equally to the generation of transcripts. *M. tuberculosis* has a single operon with two promoters, one of which generated 80% of transcripts, at all stages of growth. We infer that each promoter functions independently according to its intrinsic strength when cells are growing slowly so that one operon with three promoters is roughly equivalent to three operons with one promoter; at high growth rates, occlusion effects reduce the efficiency of multiple promoters to that of a single promoter.—Authors' Abstract

Lamb, D. C., Kelley, D. E., Manning, N. J. and Kelly, S. L. A sterol biosynthetic pathway in *Mycobacterium*. FEBS Lett. **437** (1998) 142–144.

The genome sequence of *Mycobacterium tuberculosis* (and also *M. leprae*) revealed a significant number of homologies to *Saccharomyces cerevisiae* sterol biosynthetic

enzymes. We addressed the hypothesis of a potential sterol biosynthetic pathway existing in *Mycobacterium* using cultures of *M. smegmatis.* Non-saponifiable lipid extracts subjected to analysis by gas chromatography-mass spectrometry (GC-MS) showed cholesterol was present. Sterol synthesis by *M. smegmatis* was confirmed using C-14-radiolabelled mevalonic acid and incorporation into C4-desmethyl sterol co-migrating with authentic cholesterol on TLC. The sterol biosynthetic pathway has provided a rich source of targets for commercially important bioactive molecules and such agents represent new opportunities for *Mycobacteria* in chemotherapy.—Authors' Abstract

Robb, C. W., Ni, H. L., Wang, H. M., Barrett, A. D. T. and Niesel, D. W. Expression of an 18 kDa::PhoA fusion protein in *Mycobacterium* spp. J. Microbiol. Meth. **33** (1998) 245–254.

Recent advances with mycobacterial vectors hold promise for the development of recombinant mycobacterial vaccines. Production of heterologous proteins by mycobacteria can elicit strong cellular and humoral immune responses. Importantly, expression of proteins at the surface of *Mycobacterium* spp. Results in significant humoral responses as compared to those against cytoplasmic proteins. We have developed pCR7, a plasmid vector that expresses the *M. leprae* 18-kDa antigen fused in-frame to *E. coli* alkaline phosphatase (PhoA). The fusion sequence is flanked by insertion sequence (IS900) elements, allowing stable integration into the mycobacterial chromosome. A 59-kDa protein, the predicted size of the fusion product, was detectable by immunoblotting with monoclonal antibody to PhoA and to the 18-kDa antigen. *M. smegmatis* and *M. vaccae* transformed with pCR-7 exhibited alkaline phosphatase (PhoA) activity, indicating transport of the heterologous protein across the mycobacterial membrane. PCR7 transformants: (a) had a single copy of the gene construct, (b) varied in the level of PhoA activity and (c) were cultivated stably in the absence of antibiotic pressure. Furthermore, production of the 18 kDa::PhoA fusion protein in pCR7 transformants was significantly enhanced during intracellular incubation in J774 macrophage monolayers. Thus, pCR7 may offer several advantages as a recombinant vaccine vector. Target antigens can be expressed in-frame with the 18 kDa::PhoA construct. Such recombinant *Mycobacterium* spp. would express the target antigen at the mycobacterial surface, co-express the immunostimulatory *M. leprae* 18-kDa sequences, and allow enhanced production of target antigens *in vivo.* Importantly, production of heterologous proteins could be verified by screening for PhoA activity, providing a potential alternative to antibiotic selection.—Authors' Abstract

Sethna, K. B., Mistry, N. F., Dholakia, Y., Antia, N. H. and Harboe, M. Longitudinal trends in serum levels of mycobacterial secretory (30 kD) and cytoplasmic (54 kD) antigens during chemotherapy of pulmonary tuberculosis patients. Scand. J. Infect. Dis. **30** (1998) 363–369.

Antigen 85 (mol. wt. 30,000; 30 kD), secreted by actively growing mycobacteria under axenic conditions, and mol. wt. 65,000 (65 kD), a cytoplasmic antigen released during mycobacterial lysis, were used to monitor the efficacy of chemotherapy in previously untreated pulmonary tuberculosis (UPTB) patients using enzyme-linked immunosorbent assay. Sera from 125 UPTB patients were examined for each of the two antigens individually and for the ratio of secretory (30 kD) to cytoplasmic (65 kD) antigen (SCR) before commencement of treatment, after intensive phase (IP), completion of optimum period of treatment (COPT) and 6 months' post-COPT; 116 controls (normals and contacts) were also checks for these antigens. The detection of 30 kD and 65 kD antigens in UPTB patients had a sensitivity ranging from 50%–57% (mean 30 kD value: 0.64 ± 1.24 ngs/ml) to 20%–22% (mean 65 kD value: 0.51 ± 1.87 ngs/ml), respectively, whereas in controls it ranged from 2%–8% (0.05 ± 0.28 ngs/ml) to 14%–47% (0.09 ± 0.22 ngs/ml), respectively. Although the decline in 30 kD positivity was more evident at COPT, computation of the SCR denoted efficacy of

chemotherapy more readily at LP. Similarly, SCR resolved the ambiguity between individual antigen levels and the clinical status of a patient. Since significant numbers of patients demonstrated 30 kD at IP, it may be computed that the lifespan of circulating 30 kD in serum could be at least 2 months after the start of treatment, declining gradually thereafter. Although seromonitoring for secretory antigen generally reflects the efficacy of chemotherapy, the interpretation of findings clearly requires further elucidation.—Authors' Abstract

Silbaq, F. S., Cho, S.-N., Cole, S. T. and Brennan, P. J. Characterization of a 34-kilodalton protein of *Mycobacterium leprae* that is isologous to the immunodominant 34-kilodalton antigen of *Mycobacterium paratuberculosis.* Infect. Immun. **66** (1998) 5576–5579.

During DNA sequence analysis of cosmid L373 from the *Mycobacterium leprae* genome, an open reading frame of 1.4 kb encoding a protein with some homology to the immunodominant 34-kDa protein of *M. paratuberculosis,* but lacking significant serological activity, was detected. The DNA sequence predicted a signal peptide with a modified lipoprotein consensus sequence, but the protein proved to be devoid of lipid attachment.—Authors' Abstract

Triccas, J. A., Parish, T., Britton, W. J. and Gicquel, B. An inducible expression system permitting the efficient purification of a recombinant antigen from *Mycobacterium smegmatis.* FEMS Microbiol Lett. **167** (1998) 151–156.

A novel expression vector utilizing the highly inducible acetamidase promoter of *Mycobacterium smegmatis* was constructed. High-level induction of a model antigen, the *M. leprae* 35-kDa protein, was demonstrated in recombinant *M. smegmatis* grown in the presence of the acetamidase inducer acetamide. The recombinant protein could be simply and efficiently purified from the bacterial sonicate by virtue of a C-terminal 6-histidine tag, demonstrating that this purification strategy can be used for the mycobacteria. The histidine tag had no apparent effect on the protein conformation or immunogenicity, suggesting that the vector described may prove useful for the purification of native-like recombinant mycobacterial proteins from fast-growing mycobacterial hosts.—Authors' Abstract

Wang, P. F., Marcinkeviciene, J., Williams, C. H. and Blanchard, J. S. Thioredoxin reductase thioredoxin fusion enzyme from *Mycobacterium leprae*: comparison with the separately expressed thioredoxin reductase. Biochemistry **37** (1998) 16378–16389.

Thioredoxin reductase (TrxR) catalyzes the reduction of thioredoxin (Trx) by NADPH. A unique gene organization of TrxR and Trx has been found in *Mycobacterium leprae,* where TrxR and Trx are encoded by a single gene and, therefore, are expressed as a fusion protein (M1TrxR-Trx). This fusion enzyme is able to catalyze the reduction of thioredoxin or 5,5′-dithiobis (2-nitrobenzoic acid) or 1,4-naphthoquinone by NADPH, although the activity is much lower than that of *Escherichia coli* TrxR. It has been proposed that a large conformational change is required in catalysis of *E. coli* TrxR. Because the reductase portion of the enzyme from *M. leprae* shows significant primary structure similarity with *E. coli* TrxR, it is possible that M1TrxR-Trx may require a similar conformational change and that the change in conformation may be affected by the tethered Trx. The reductase has been expressed without Trx attached (M1TrxR). As reported here, comparison of the steady-state and pre-steady-state kinetics of M1TrxR-Trx with those of M1TrxR suggests that the low reductase activity of the fusion enzyme is an inherent property of the reductase, and that any steric limitation caused by the attached thioredoxin in the fusion protein makes only a minor contribution to the low activity. Titration of M1TrxR-Trx and M1TrxR with 3-aminopyridine adenine dinucleotide phosphate (AADP+), an NADP (H) analog, results in only slight quenching of FAD fluorescence, suggesting an enzyme conformation in which the binding site of AADP+ is not

close to the FAD, as in one of the conformations of *E. coli* TrxR.—Authors' Abstract

Webb, J. R., Vedvick, T. S., Alderson, M. R., Guderian, J. A., Jen, S. S., Ovendale, P. J., Johnson, S. M., Reed, S. G. and Skeiky, Y. A. W. Molecular cloning, expression, and immunogenicity of MTB12, a novel low-molecular-weight antigen secreted by *Mycobacterium tuberculosis.* Infect. Immun. **66** (1998) 4208–4214.

Proteins secreted into the culture medium by *Mycobacterium tuberculosis* are thought to play an important role in the development of protective immune responses. In this report, we describe the molecular cloning of a novel, low-molecular-weight antigen (MTB12) secreted by *M. tuberculosis.* Sequence analysis of the MTB12 gene indicates that the protein is initially synthesized as a 16.6-kDA precursor protein containing a 48-amino-acid hydrophobic leader sequence. The mature, fully processed form of MTB12 protein found in culture filtrates has a molecular mass of 12.5 kDa. MTB12 protein constitutes a major component of time *M. tuberculosis* culture supernatant and appears to be at least as abundant as several other well-characterized culture filtrate proteins, including members of the 85B complex. MTB12 is encoded by a single-copy gene which is present in both virulent and avirulent strains of the *M. tuberculosis* complex, the BCG strain of *M. bovis,* and *M. leprae.* Recombinant MTB12 containing an N-terminal six-histidine tag was expressed in *Escherichia coli* and purified by affinity chromatography. Recombinant MTB12 protein elicited *in vitro* proliferative responses from the peripheral blood mononuclear cells of a number of purified protein derivative-positive (PPD+) human donors but not from PPD-donors.—Authors' Abstract

Wu, X. M., Marino Albernas, J. R., Auzanneau, F. I., Verez Bencomo, V. and Pinto, B. M. Synthesis and NMR analysis of C-13-labeled oligosaccharides corresponding to the major glycolipid from *Mycobacterium leprae.* Carbohyd. Res. **306** (1998) 493–503.

An improved synthesis of propyl 4-O-(3,6-di-(3-methyl-beta-D-glucopyranosyl)-2, 3-di-O-methyl-alpha-L-rhamnopyranoside, a disaccharide corresponding to the phenolic glycolipid of *Mycobacterium leprae* using a trichloroacetimidate as a glycosyl donor is described. The synthetic strategy is also applied to the preparation of three corresponding disaccharide analogs containing C-13-labeled methyl groups. The preparation of the trisaccharide, propyl 2-O-[4-O-(3,6-di-O-methyl-beta-D-glucopyranosyl)-2,3-di-O-methyl-alpha-L-rhamnopyranosyl]-3-O-methyl-alpha-L-rhamnopyranoside is also reported. The di- and tri-saccharides were characterized by H-1 and C-13-NMR spectroscopy.—Authors' Abstract

Yuan, Y., Crane, D. D., Simpson, R. M., Zhu, Y. Q., Hickey, M. J., Sherman, D. R. and Barry, C. E. The 16-kDa alpha-crystallin (Acr) protein of *Mycobacterium tuberculosis* is required for growth in macrophages. Proc. Am. Acad. Sci. U.S.A. **95** (1998) 9578–9583.

Although the 16-kDa alpha-crystallin homolog of *Mycobacterium tuberculosis* (MTB) is the dominant protein produced by stationary phase cultures *in vitro,* it is undetectable in logarithmically growing cultures. By growing bacilli at defined oxygen concentrations, acr transcription was shown to be strongly induced by mildly hypoxic conditions. acr expression also was found to be induced during the course of *in vitro* infection of macrophages. The acr gene was replaced with a hygromycin-resistance cassette by allelic exchange in MTB H37Rv. The resulting Delta acr::hpt strain was shown to be equivalent to wild-type H37Rv *in vitro* growth rate and infectivity but was significantly impaired for growth in both mouse bone marrow-derived macrophages and THP-1 cells. In addition to its proposed role in the maintenance of long-term viability during latent, asymptomatic infections, these results establish a role for the acr protein in replication during initial MTB infection.—Authors' Abstract

Experimental Infections

Gormus, B. J., Xu, K., Baskin, G. B., Martin, L. N., Bohm, R. P., Jr., Blanchard, J. L., Mack, P. A., Ratterree, M. S., Meyers, W. M. and Walsh, G. P. Experimental leprosy in rhesus monkeys: transmission, susceptibility, clinical and immunological findings. Lepr. Rev. **69** (1998) 235–245.

A total of 46 rhesus monkeys (RM) was inoculated with *Mycobacterium leprae* (ML) and followed clinically and immunologically for extended periods. Twenty-one (45.7%) of the RM developed leprosy spanning the known leprosy spectrum, with 6 of 21 (28.6%) having disease in the borderline lepromatous to lepromatous area of the spectrum. RM with paucibacillary forms of leprosy produced predominantly IgG antiphenolic glycolipid (PGL-I) antibodies and a positive lepromin skin test and/or *in vitro* blastogenesis responses; IgM anti-PGL-I predominated in animals with BB-LL leprosy and correlated with negative immune responses to lepromin. IgG anti-PGL-I antibodies persisted in a number of RM for several years without histopathological evidence of leprosy, suggesting possible persisting subclinical infection. The data show that RM are a valuable model for the study of leprosy. Eleven of the 46 RM were inoculated with ML from sources infected with simian immunodeficiency virus (SIV), the monkey counterpart to the human immunodeficiency virus (HIV). The possible effect of SIV on the clinical outcome of ML infection could not be determined due to insufficient numbers of animals to yield statistically significant results.—Authors' Summary

Kobayashi, K., Kai, M., Gidoh, M., Nakata, N., Endoh, M., Singh, R. P., Kasama, T. and Saito, H. The possible role of interleukin (IL)-12 and interferon-gamma-inducing factor/IL-18 in protection against experimenal *Mycobacterium leprae* infection in mice. Clin. Immunol. Immunopathol. **88** (1998) 226–231.

Cell-mediated-immunity participates in host defense against mycobacterial infection. Both interleukin 12 (IL-12) and interferon-gamma-inducing factor (IGIF/IL-18), produced mainly by macrophages, play a critical role in expression of cell-mediated immunity. To investigate the role of IL-12 and IGIF/IL-18 *in vivo,* we examined cytokine profile, bacterial growth, and the potential benefit of cytokine therapy in susceptible and resistant mice infected with *Mycobacterium leprae.* The early expression of IL-12 p40 and IGIF/IL-18 at the site of inoculation was found in resistant mice 3–72 hr after the infection, but not in susceptible mice. Both strains of mice did not show expression of IFN-gamma and IL-4. IL-12 administration resulted in a significant reduction of bacterial counts in mice with established *M. leprae* infection. The results imply that susceptible mice exhibit decreased expression of type 1 helper T (Th1) response without reciprocal increased Th2 response, and show responsiveness to exogenous IL-12. IL-12 therapy may be a possible rationale for treatment of *M. leprae* infection.—Authors' Abstract

Singh, N., Birdi, T. J. and Antia, N. H. Differential *in vitro* modulation of Schwann cell proliferation by *Mycobacterium leprae* and macrophages in the murine strains, Swiss white and C57BL/6. J. Periph. Nerv. Sys. **3** (1998) 207–216.

The special susceptibility of Schwann cells (SCs) to parasitization by *M. leprae* and of macrophages to *M. leprae*-induced defects implicates them in leprous nerve pathogenesis. SC proliferation is an important prerequisite for peripheral nerve regeneration and is regulated by a number of secretory factors. Several of these factors are secreted by SCs themselves as well as by the macrophages which are recruited at the site of lesion to assist in regeneration. SC proliferation, as indicated by 3-H-thymidine incorporation, was therefore studied in response to *M. leprae* infection and in the

presence of macrophages in order to determine the role of SC in leprous neuropathy. Cells derived from two strains of mice, Swiss white (SW) and C57BL/6 were used, as macrophages from these strains have been shown to differ in their response to *M. leprae;* such differences are similar to those observed in macrophages from lepromatous and tuberculoid leprosy patients, respectively. Infection with *M. leprae* for a duration of 9 days resulted in reduced proliferation of SCs from SW strain, while SCs from C57BL/6 remained unaffected. However, in the presence of macrophages, SCs from both strains not only showed enhanced proliferation, but SW SCs also overcame the *M. leprae*-induced suppression of their proliferation. Altered SC proliferation, therefore, can be implicated as a factor in leprous nerve pathogenesis. The strain variation observed in the response of SCs indicate different nerve damage mechanisms in lepromatous and tuberculoid patients.—Author's Abstract

Epidemiology and Prevention

El-Orfi, A. H. A., Singh, M. and Giasuddin, A. S. M. Conjugal leprosy among Libyan patients. Dermatology **196** (1998) 271–272.

Data from 269 couples on the Leprosy Register at Jamahiriya Hospital, Benghazi, Libya, were analyzed to determine the prevalence of leprosy among consanguineous couples. Of the 25 couples who gave a history of consanguinity, both partners were affected in eight cases (32%) and one partner only was affected in each of the remaining 17 couples. Of 244 patients without any history of consanguinity, both partners were affected by leprosy in seven cases (2.87%) and one partner only was involved in each of the remaining 237 cases. There was a significant difference among the groups studied ($p < 0.01$). The possibility of a genetic predisposition to the disease is suggested.—Authors' Abstract

Feliciano, K. V. de O., Kovacs, M. H., Sevilla, E. and Alzate, A. [Perceptions regarding leprosy and resulting handicaps prior to diagnosis in Recife, Brazil.] Pan Am. J. Publ. Health **3** (1998) 293–302. (in Spanish)

A case-control study was conducted in Recife, Brazil, between November 1993 and July 1994, to determine how leprosy patients' perceptions and notions influence disease management and use of health services. The sample was composed of 183 residents of Recife between the ages of 20 and 70 years who sought diagnostic services in the dermatology clinics of two referral centers; 64 patients having handicaps or their precursor lesions were classified as cases, the remaining 119 were used as controls. All were diagnosed during the study period. For the analysis, adjustments were made for sex, age, schooling, and a previous history of Hansen's disease among patients. The study revealed the simultaneous presence of two types of "invisibility" of the disease in an area where endemicity is increasing: firstly, for patients in both groups, the low frequency of spontaneous explanatory models related to the illness, even in the presence of disease, and secondly, for health professionals, the limitations of detection methods. It is concluded that since such deficiencies affect decisions bearing on individual and collective disease management, they are a risk factor in and of themselves and stand in the way of eliminating leprosy as a public health problem.—Authors' English Abstract

Leprosy elimination campaigns (LECs); progress during 1997–1998. Wkly. Epidemiol. Rec. **73** (1998) 177–182.

This report on progress made during 1997–1998, includes information on 11 of the 16 countries which account for 92% of the global registered leprosy cases and are

particularly targeted for LECs. During 1997–May 1998, the total number of cases detected in the 11 countries (Philippines, Nigeria, Guinea, Madagascar, Myanmar, Niger, Bangladesh, Brazil, Sudan, Cambodia and India) was approximately 407,000 covering a population of >850 million. The proportion of cases classified as multibacillary (MB) ranged from 24% in Brazil to 74% in Sudan, and the proportion of newly detected cases presenting with grade 2 disabilities varied from 2% in Nigeria to 44% in Sudan. In most areas, more cases were detected during the campaign period than the number detected during the previous year. The strategy for LECs, country experiences with LECs during 1997–1998 in Myanmar, Madagascar and India, and the impact of LECs are discussed.—Authors' Abstract

Trends in leprosy detection. Wkly. Epidemiol. Rec. **73** (1998) 169–175.

During 1997, a total of 113 countries/areas notified the number of leprosy cases detected. A total of 642,167 cases were notified, of which 266,840 (41.5%) were multibacillary cases. This indicated an increase in leprosy detection of 16%, although the overall trend over the last 13 years does not show major variations. Definitions of leprosy cases and case detection rate are provided and global leprosy trends during 1985–1997 in 32 endemic countries (representing 93% of the current global leprosy burden and 85% of that of 1985) are analyzed. The leprosy prevalence rate was reduced by 85% and the detection rate by only 4% at the global level. Data showing prevalence and detection trends during 1985–1997 are also presented for selected countries which recently reached a prevalence below 1 per 10,000 population at the national level, for high-endemic countries which started implementing multidrug therapy (MDT) nationwide after 1991, and for the top three leprosy-endemic countries (Brazil, India and Indonesia).—Trop. Dis. Bull. **95** (1998) 1195

WHO Expert committee on Leprosy, 1998. WHO Tech. Rep. Ser. 874.

The WHO Expert Committee on Leprosy was commissioned to review the global leprosy situation in 1997, to assess the adequacy of current technology for elimination of the disease as a public health problem and to identify the remaining obstacles. The results of several recent studies were reviewed to determine whether diagnostic tools could be simplified and treatment regimens shortened. This report presents the conclusions reached and is divided into the following sections: global leprosy situation in 1997, epidemiology, chemotherapy, management of reactions and neuritis, disabilities and rehabilitation, the global strategy for the elimination of leprosy by the year 2000, monitoring the elimination of leprosy, integration of antileprosy activities within general health services, community action and participation, and research priorities.—Trop. Dis. Bull. **95** (1998) 1194

World Health Organization. Progress towards leprosy elimination. Wkly. Epidemiol. Rec. **73** (1998) 153–160.

This report updates figures published in June 1997 of progress made toward the global elimination of leprosy. At the end of 1997, leprosy was still considered a public health problem in 32 countries (population >1 million and prevalence rate >1 case/10,000 population) situated mainly in the intertropical belt of the world; 16 countries from the Asian, African and American continents account for 92% of the global leprosy burden with a reported prevalence rate of 3.9/10,000. At the start of 1998, 828,803 leprosy cases were registered for treatment globally; only a small decrease (6.7%) in the number of registered cases was noted between 1997 and 1998. During 1997, around 642,000 cases of leprosy were detected, notified by 104 countries, however the endemic countries Brazil, Congo, Côte d'Ivoire and Gabon had not sent information at the time of reporting. The global detection was estimated to be 685,000 (a detection rate of 11.5 per 100,000 population). Around 652,500 cases (95%) were detected in the 16 major endemic countries, with 76% of the newly detected cases in India alone; 10.3% of newly detected cases, for

which detailed information was provided, were children aged <15 years, and 42% were multibacillary (MB) patients. Almost all leprosy patients registered for treatment are now being treated with multidrug therapy (MDT), and during 1997 the total number of patients treated with MDT was over 1.5 million (old and new patients). Data are presented showing the distribution and treatment of leprosy cases in each WHO Region, the detailed situation in the top 16 endemic countries and the leprosy situation as reported by countries in 1998.—Trop. Dis. Bull. **95** (1998) 1194

Rehabilitation

Grauwin, M. Y., Ndiaye, A., Sylla, P. M., Gaye, A. B., Mane, I., Cartel, J. L. and Lepers, J. P. [Can plantar ulcers be treated in the field? The Senegalese experience.] Cah. Etud. Res. Francoph. **8** (1998) 199–204. (in French)

The introduction of a program for the treatment of plantar ulcers (PU) in field conditions in Senegal was studied. The program was complementary to the Health Education and Protective Footwear to Prevent Disability (POD) initiatives within the Senegalese antileprosy program. The wound care given in health centers was coded and simplified, and access to hospitals was made easier for those patients requiring surgery. As a result, >30% of patients with PU were treated each year, with a mean of 62% cured, and an increasing number of leprosy patients were admitted to regional hospitals for surgery. Never before have patients with signs of leprosy had access to general hospitals. It is suggested that there is a need for regular supervision of individuals treating wounds.—Authors' English Abstract

Seboka, G., Saunderson, P. and Currie, H. Footwear for farmers affected by leprosy. Lepr. Rev. **69** (1998) 182–183.

This study in central Ethiopia compared two commercially available types of footwear for use by farmers: canvas shoes and PVC boots; 110 male farmers aged 20–70 years were randomly assigned to the canvas shoe group or the PVC boot group and were followed for 1 year from June 1996. All were former leprosy patients who either had one or more plantar ulcers at intake, or had the scar of a healed ulcer; all had loss of sensation as tested by a 10 g monofilament. Many had clawed toes and bone loss. It is concluded that PVC boots are more suitable for the agricultural working environment in Ethiopia than canvas shoes.—Trop. Dis. Bull. **95** (1998) 1204

Other Mycobacterial Diseases and Related Entities

Aberg, J. A., Yajko, D. N. and Jacobson, M. A. Eradication of AIDS-related disseminated *Mycobacterium avium* complex infection after 12 months of antimycobacterial therapy combined with highly active antiretroviral therapy. J. Infect. Dis. **178** (1998) 1446–1449.

To determine if microbiologic cure of AIDS-related disseminated *Mycobacterium avium* complex (MAC) is possible in patients receiving highly active antiretroviral therapy (HAART), 4 patients with a history of disseminated MAC received ≥12 months of macrolide-based antimycobacterial therapy. All were asymptotic and had absolute CD4 cell count >100/μL (range, 137–301) and <10,000 copies/mL of human immunodeficiency virus RNA (range, <500–1250). A bone marrow aspirate and peripheral

blood were obtained for mycobacterial culture. Follow-up blood cultures were obtained routinely at 4 weeks and every 8 weeks thereafter. All 4 patients had negative bone marrow and blood cultures and then discontinued antimycobacterial therapy. All patients' subsequent cultures remain sterile and all are clinically asymptomatic (range, 8–13 months follow up). It appears that disseminated MAC infection can be cured by prolonged antimycobacterial therapy in some persons who experience sustained CD4 lymphocyte increases while receiving HAART.—Authors' Abstract

Ainsa, J. A., Blokpoel, M. C. J., Otal, I., Young, D. B., DeSmet, K. A. L. and Martin, C. Molecular cloning and characterization of tap, a putative multidrug efflux pump present in *Mycobacterium fortuitum* and *Mycobacterium tuberculosis*. J. Bacteriol. **180** (1998) 5836–5843.

A recombinant plasmid isolated from a *Mycobacterium fortuitum* genomic library by selection for gentamicin and 2-N′-ethylnetilmicin resistance conferred low-level aminoglycoside and tetracycline resistance when introduced into *M. smegmatis*. Further characterization of this plasmid allowed the identification of the *M. fortuitum* tap gene. A homologous gene in the *M. tuberculosis* H37Rv genome has been identified. The *M. tuberculosis* tap gene (Rv1258 in the annotated sequence of the *M. tuberculosis* genome) was cloned and conferred low-level resistance to tetracycline when introduced into *M. smegmatis*. The sequences of the putative Tap proteins showed 20% to 30% amino acid identity to membrane efflux pumps of the major facilitator superfamily (MFS), mainly tetracycline and macrolide efflux pumps, and to other proteins of unknown function but with similar antibiotic resistance patterns. Approximately 12 transmembrane regions and different sequence motifs characteristic of the MFS proteins also were detected. In the presence of the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP), the levels of resistance to antibiotics conferred by plasmids containing the tap genes were decreased. When tetracycline accumulation experiments were carried out with the *M. fortuitum* tap gene, the level of tetracycline accumulation was lower than that in control cells but was independent of the presence of CCCP. We conclude that the Tap proteins of the opportunistic organism *M. fortuitum* and the important pathogen *M. tuberculosis* are probably proton-dependent efflux pumps, although we cannot exclude the possibility that they act as regulatory proteins.—Authors' Abstract

Altare, F., Jouanguy, E., Lamhamedi, S., Doffinger, R., Fischer, A. and Casanova, J. L. Medelian susceptibility to mycobacterial infection in man. Curr. Opin. Immunol. **10** (1998) 413–417.

Selective susceptibility to poorly pathogenic mycobacteria, such as bacille Calmette-Guerin vaccine and environmental nontuberculous mycobacteria, has long been suspected to be a mendelian disorder but its molecular basis has remained elusive. Recently, recessive mutations in the interferon-gamma-receptor ligand-binding chain, interferon-gamma-receptor signalling chain, IL-12 p40 subunit and IL-12-receptor beta 1 chain genes have been identified in a number of patients with disseminated mycobacterial infection. Although genetically distinct, these conditions are immunologically related and highlight the essential role of interferon-gamma-mediated immunity in the control of mycobacteria in man.—Authors' Abstract

Bala, S., Hastings, K. L., Kazempour, K., Inglis, S. and Dempsey, W. L. Inhibition of tumor necrosis factor alpha alters resistance to *Mycobacterium avium* complex infection in mice. Antimicrob. Agents Chemother. **42** (1998) 2336–2341.

Increased production of tumor necrosis factor alpha (TNF-α) appears to play an important role in the progression of human immunodeficiency virus disease. One treatment strategy being explored is the use of TNF-α inhibitors. TNF-α also appears to be important in conferring resistance to infections, and the inhibition of this cytokine may exacerbate the emergence of oppor-

tunistic pathogens, such as *Mycobacterium avium* complex (MAC). The present study examines the possibility that inhibition of TNF-α will increase the progression of disease in mice infected with MAC. C57BL/6 beige (*bg/bg*) mice have been shown to be highly susceptible to infection with MAC and are routinely used for testing of antimycobacterial drugs. However, *bg/bg* mice are known to exhibit impaired phagocyte and natural killer cell function. Since these cell types are important sources of TNF-α, the susceptibility of the *bg/bg* strain to infection with MAC was compared with those of the heterozygous (*bg/+*) and wild-type (+/+) strains of C57BL/6 mice. The susceptibilities of the *bg/bg* and *bg/+* strains of mice infected with MAC were found to be comparable. The +/+ strain was the least susceptible. Mycobacterial burden and serum TNF-α levels increased over time in all the strains of mice tested. The *bg/+* strain of C57BL/6 mice was then chosen to measure the activity of TNF-α antagonists. Treatment with dexamethasone decreased serum TNF-α levels and increased mycobacterial burden. Treatment with anti-TNF-α antibody or pentoxifylline did not significantly alter serum TNF-α levels but increased mycobacterial burden. Treatment with thalidomide neither consistently altered mycobacterial burden in the spleens or livers of infected mice nor affected serum TNF-α levels.—Authors' Abstract

Balcewicz Sablinska, M. K., Keane, J., Kornfeld, H. and Remold, H. G. Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-alpha. J. Immunol. **161** (1998) 2636–2641.

Infection by *Mycobacterium tuberculosis* (MTB) induces human alveolar macrophage (AM Φ) apoptosis by a TNF-alpha-dependent mechanism. The apoptotic response is postulated to be a defense mechanism, limiting the growth of this intracellular pathogen. Consistent with that model, recent studies showed that the virulent MTB strain H37Rv induces substantially less AM Φ, apoptosis than the attenuated strain H37Ra. We now report that AM infection with either H37Rv or H37Ra induces comparable levels of levels of TNF-alpha measured by ELISA but that TNF-alpha bioactivity is reduced in supernatants of H37Rv-infected AM Φ. Differential release of soluble TNFR2 (sTNFR2), with formation of inactive TNF-alpha-TNFR2 complexes accounted for the difference in TNF-alpha bioactivity in these cultures. Release of sTNFR2 by H37Rv-infected AM Φ was IL-10 dependent since it was inhibited by neutralizing anti-IL-10 antibody. Thus, the effect of TNF-alpha produced by AM Φ following infection can be modulated by virulent MTB, using IL-10 as an upstream mediator.—Authors' Abstract

Banerjee, A., Sugantino, M., Sacchettini, J. C. and Jacobs, W. R., Jr. The *mabA* gene from the *inhA* operon of *Mycobacterium tuberculosis* encodes a 3-ketoacyl reductase that fails to confer isoniazid resistance. Microbiology **144** (1998) 2697–2707.

A target of the antituberculosis drugs isoniazid (INH) and ethionamide (ETH) has been shown to be an enoyl reductase, encoded by the *inhA* gene. The *mabA* (mycolic acid biosynthesis A) gene is located immediately upstream of *inhA* in *Mycobacterium tuberculosis, M. bovis* and *M. smegmatis*. The MabA protein from *M. tuberculosis* was expressed in *Escherichia coli* and shown to have 3-ketoacyl reductase activity, consistent with a role in mycolic acid biosynthesis. In *M. smegmatis, inhA* and *mabA* are independently transcribed, but in *M. tuberculosis* and *M. bovis* BCG, *mabA* and *inhA* constitute a single operon. Several INH-ETH-resistant *M. tuberculosis* clinical isolates contain point mutations in the ribosome-binding site of *MabA* in the *mabA-inhA* operon. However, genetic dissection of this operon reveals that the INH-ETH-resistance phenotype is encoded only by *inhA,* and not by *mabA*.—Authors' Abstract

Bellamy, R., Ruwende, C., Corrah, T., McAdam, K. P. W. J., Whittle, H. C. and Hill, A. V. S. Variations in the *NRAMP1* gene and susceptibility to tuberculosis in West Africans. N. Engl. J. Med. **338** (1998) 640–644.

Sequence-specific oligonucleotide hybridization and microsatellite analysis were used to type polymorphisms in the natural-resistance-associated macrophage protein 1 gene (*NRAMP1*) in 410 adults (mean age 34.7 years; 67.4% male) with, smear-positive pulmonary tuberculosis and 417 ethnically matched, healthy controls in the Gambia, West Africa [date not given]. The ethnic groups Mandinka, Wolof, Jola, Fula, Manjago, Serrahule, Aku and Serere were represented. Patients with human immunodeficiency virus infection were excluded. The four *NRAMP1* polymorphisms, 5′ $(CA)_n$, INT4, D543N and 3′UTR were each significantly associated with tuberculosis ($p = 0.03$, $p = 0.009$, $p = 0.008$ and $p < 0.001$, respectively). Subjects who were heterozygous for two *NRAMP1* polymorphisms in intron 4 and the 3′ of the gene were particularly over-represented among those with tuberculosis, compared with those with the most common *NRAMP1* genotype (odds ratio 4.07; 95% confidence interval, 1.86–9.12; chi-square = 14.58; $p < 0.001$). It is concluded that genetic variation in *NRAMP1* affects susceptibility to tuberculosis in West Africans.—Authors' Abstract

Bermudez, L. E., Petroksky, M., Wu, M. and Young, L. S. Clarithromycin significantly improves interleukin-12-mediated anti-*Mycobacterium avium* activity and abolishes toxicity in mice. J. Infect. Dis. **178** (1998) 896–899.

Treatment of experimental murine *Mycobacterium avium* (MAC) infection with interleukin-12 (IL-12) significantly decreased MAC organisms in tissue but resulted in toxicity. Because IL-12-related toxicity was seen only in infected mice, IL-12 was combined with clarithromycin in an attempt to decrease bacterial burden. Clarithromycin (200 mg/kg/day) was administered alone to *M. avium*-infected mice for 1 week, and from week 2, IL-12 (20 μg/kg twice per week) was added to the regimen for 4 weeks. Treatment with IL-12 resulted in 60% mortality, compared with 40% mortality in untreated control mice and 20% when IL-12 was given with clarithromycin ($p < 0.05$). Clarithromycin plus IL-12 resulted in increased activity compared with either clarithromycin or IL-12 alone in reducing the number of bacteria in spleen and blood. Although potentially toxic, IL-12 is an effective immunotherapy for MAC infection, and combination with clarithromycin reduces IL-12 toxicity.—Authors' Abstract

Berthet, F.-X., Rasmussen, P. B., Rosenkrands, I., Andersen, P. and Gicquel, B. A *Mycobacterium tuberculosis* operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10). Microbiology **144** (1998) 3195–3202.

The early secreted antigenic target 6 kDa protein (ESAT-6) is a potent T-cell protein antigen synthesized by *Mycobacterium tuberculosis.* Its corresponding gene (*esat-6*) is located in RD1, a 10-kb DNA region deleted in the attenuated tuberculosis vaccine strain *M. bovis* BCG. The promoter region of *M. tuberculosis esat-6* was cloned and characterized. A new gene, designated *lhp* and cotranscribed with *esat-6,* was identified. Moreover, computer searches in the *M. tuberculosis* genome identified 13 genes related to the *lhp/esat-6* operon, defining a novel gene family. The transcription initiation sites of the *lhp/esat-6* operon were mapped using *M. tuberculosis* RNA. The corresponding promoter signals were not recognized in *M. smegmatis,* in which transcription of *lhp/esat-6* is initiated at different locations. The *M. tuberculosis lhp* gene product was identified as CFP-10, a low-molecular-mass protein found in the short-term culture filtrate. These results show that the genes encoding CFP-10 and ESAT-6 are transcribed together in *M. tuberculosis* and that both code for small exported proteins.—Authors' Abstract

Ching, L. M., Browne, W. L., Tchernegovski, R., Gregory, T., Baguley, B. C. and Palmer, B. D. Interaction of thalidomide, phthalimide analogues of thalidomide and pentoxifylline with the antitumour agent 5,6-dimethylxanthenone-4-acetic acid: concomitant reduction of serum tumour necrosis factor-alpha and enhancement of anti-tumour activity. Br. J. Cancer **78** (1998) 336–343.

DMXAA (5,6-dimethylxanthenone-4-acetic acid), a novel antitumor agent currently undergoing clinical evaluation, appears to mediate its antitumor effects through immune modulation and the production of the cytokine tumour necrosis factor-alpha (TNF-α). Our previous studies have shown that thalidomide, a potent inhibitor of TNF biosynthesis that has numerous biological effects, including inhibition of tumor angiogenesis, unexpectedly augments the antitumor response in mice to DMXAA. We show here that thalidomide (100 mg kg^{-1}) has no effect when administered with inactive doses of DMXAA, and that it must be given simultaneously with an active dose of DMXAA to have its maximum potentiating effect on the growth of the murine colon 38 adenocarcinoma. To address the issue of whether inhibition of serum TNF production is important for potentiation of anti-tumor activity, we have tested three potent analogs of thalidomide. All three analogs, when co-administered with DMXAA to mice at doses lower than those used with thalidomide, inhibited TNF production and were effective in potentiating the antitumor activity of DMXAA against transplanted colon 38 tumors. One of the analogs, N-phenethyltetrafluorophthalimide, was 1000-fold more potent than thalidomide and at a dose of 0.1 mg kg^{-1} in combination with DMXAA (30 mg kg^{-1}) cured 100% of mice, compared with 67% for the group treated with DMXAA alone. We also tested pentoxifylline and found it to suppress TNF production in response to DMXAA and to potentiate the antitumor effect of DMXAA. The results are compatible with the hypothesis that pharmacological reduction of serum TNF levels might benefit the antitumor effects of DMXAA and suggest new strategies for therapy using this agent.—Authors' Abstract

Coler, R. N., Skeiky, Y. A. W., Vedvick, T., Bement, T., Ovendale, P., Campos Neto, A., Alderson, M. R. and Reed, S. G. Molecular cloning and immunologic reactivity of a novel low molecular mass antigen of *Mycobacterium tuberculosis.* J. Immunol. **161** (1998) 2356–2364.

Polypeptide antigens (Ags) present in the culture filtrate of *Mycobacterium tuberculosis* (Mtb) were purified and evaluated for their ability to stimulate PBMC from purified protein derivative (PPD)-positive healthy donors. One such Ag, which elicited strong proliferation and IFN-gamma production, was further characterized. The N-terminal amino acid sequence of this polypeptide was determined and used to design oligonucleotides for screening a recombinant *M. tuberculosis* genomic DNA library. The gene (Mtb 8.4) corresponding to the identified polypeptide was cloned, sequenced, and expressed in *Escherichia coli.* The predicted molecular weight of the recombinant protein without its signal peptide was 8.4 kDa. By Southern analysis, the DNA encoding this mycobacterial protein was found in the *M. tuberculosis* substrains H37Rv, H37Ra, Erdman, and "C" strain, as well as in certain other mycobacterial species, including *M. avium* and *M. bovis* BCG (bacillus Calmette-Guerin, Pasteur). The Mtb 8.4 gene appears to be absent from the environmental mycobacterial species examined thus far, including *M. smegmatis, M. gordonae, M. chelonae, M. fortuitum,* and *M. scrofulaceum.* Recombinant Mtb 8.4 Ag induced significant proliferation as well as production of IFN-gamma, IL-10, and TNF-alpha, but not IL-5, from human PBMC isolated from PPD-positive healthy donors. Mtb 8.4 did not stimulate peripheral blood mononuclear cells from PPD-negative donors. Furthermore, immunogenicity studies in mice indicate that Mtb 8.4 elicits a Th1 cytokine profile, which is considered important for protective immunity to tuberculosis. Collectively, these results demonstrate that Mtb 8.4 is an immunodominant T cell Ag of *M. tuberculosis.*—Authors' Abstract

Croft, R. A. and Croft, R. P. Expenditure and loss of income incurred by tuberculosis patients before reaching effective treatment in Bangladesh. Int. J. Tuberc. Lung Dis. **2** (1998) 252–254.

A total of 21 tuberculosis (TB) patients registered serially in March 1996 at the TB clinic run by the Danish Bangladesh Lep-

rosy Mission (which provides free services and medicines) in northwestern Bangladesh, were interviewed after completing 1 month of treatment to assess the loss of income resulting from the illness and the actual expenditure incurred by medicines and doctors' fees before attending the clinic for treatment. Patients were treated in their homes and were not hospitalized. Mean duration of illness was 16 months (range 2–60 months); 12 of the 21 patients were unable to work and mean loss of work time was 14 months (range 5 days to 60 months). The mean financial loss to the patient was US$245, almost 4 months of the average annual income for a Bangladeshi family. It is suggested that the economic impact of TB on the individual, the family and the community could be included as a measure of success of TB control programs.—Authors' Abstract

Geluk, A., Taneja, V., van Meijgaarden, K. E., Zanelli, E., Abou Zeid, C., Thole, J. E. R., de Vries, R. R. P., David, C. S. and Ottenhoff, T. H. M. Identification of HLA class II restricted determinants of *Mycobacterium tuberculosis*-derived proteins by using HLA-transgenic, class II deficient mice. Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 10797–10802.

T helper 1 cells play a major role in protective immunity against mycobacterial pathogens. Since the antigen (Ag) specificity of CD4+ human T cells is strongly controlled by HLA class II polymorphism, the immunogenic potential of candidate Ags needs to be defined in the context of HLA polymorphism. We have taken advantage of class II-deficient (Ab^0) mice, transgenic for either HLA-DRA/B1*0301 (DR3) or HLA-DQB1*0302/DQA*0301 (DQ8) alleles. In these animals, all CD4+ T cells are restricted by the HLA molecule. We reported previously that human DR3-restricted T cells frequently recognize heat shock protein (hsp) 65 of *Mycobacterium tuberculosis,* and only a single hsp65 epitope, p1-20. DR3.Ab^0 mice, immunized with bacillus Calmette-Guerin or hsp65, developed T-cell responses to *M. tuberculosis,* and recognized the same hsp65 epitope, p1–20. Hsp65-immunized DQ8.Ab^0 mice mounted a strong response to bacillus Calmette-Guerin but not to p1–20. Instead, we identified three new DQ8-restricted T-cell epitopes in the regions 171–200, 311–340, and 411–440. DR3.Ab^0 mice immunized with a second major *M. tuberculosis* protein, Ag85 (composed of 85A, 85B, and 85C), also developed T-cell responses against only one determinant, 85B p51–70, that was identified in this study. Importantly, subsequent analysis of human T-cell responses revealed that HLA-DR3+, Ag85-reactive individuals recognize exactly the same peptide epitope as DR3.Ab^0 mice. Strikingly, both DR3-restricted T-cell epitopes represent the best DR3-binding sequences in hsp65 and 85B, revealing a strong association between peptide-immunodominance and HLA binding affinity. Immunization of DR3.Ab^0 with the immunodominant peptides p1–20 and p51–70 induced T-cell reactivity to *M. tuberculosis.* Thus, for two different Ags, T cells from DR3.Ab^0 mice and HLA-DR3+ humans recognize the same immunodominant determinants. Our data support the use of HLA-transgenic mice in identifying human T-cell determinants for the design of new vaccines.—Authors' Abstract

Giuliani, A., Porcelli, S. A., Tentori, L., Graziani, G., Testorelli, C., Prete, S. P., Bussini, S., Cappelletti, D., Brenner, M. B., Bonmassar, E. and Aquino, A. Effect of rifampin on CD1b expression and double-negative T cell responses against mycobacteria-derived glycolipid antigen. Life Sci. **63** (1998) 985–994.

Nonclassical antigen-presentation by CD1 molecules expressed on cytokine-activated monocytes (CAM) and cell-mediated responses supported by double-negative (DN) and by CD8 + responder alpha beta T cells are involved in host resistance against mycobacterial infections. The CD1b protein is responsible for presentation of non-peptide, lipid antigens to T cells. In this context, a pivotal role is played by induction of CD1b protein on the membrane of human monocytes activated by GM-CSF

alone, and more efficiently by GM-CSF combined with IL-4. Rifampin (RFP), a drug which is extensively utilized for chemoprophylaxis or treatment of *Mycobacterium tuberculosis,* is known to reduce a number of B or T cell-dependent responses. Therefore we undertook immunopharmacological studies on RFP to determine the effects of this agent on human macrophage function relative to antigen presentation by CD1b molecules and on DN T-cell cytolytic function. The result showed that: a) graded concentration of RFP (2 or 10 μg/ml) induced a significant increase of CD1 expression in CAM as evaluated by FAGS analysis; b) RFP increased significantly the specific mAb binding to CD1 on CAM surface; c) treatment of effector cells with RFP did not reduce DN T cell-mediated cytolysis against lymphoblastoid cells transfected with CD1b cDNA (C1R.b6 cells) pulsed with *M. tuberculosis.* These results suggest that RFP could be of potential value in improving mycobacterial antigen presentation without impairing responder T-cell function.—Authors' Abstract

Glynn, J. R., Warndorff, D. K., Fine, P. E. M., Munthali, M. M., Sichone, W. and Ponnighaus, J. M. Measurement and determinants of tuberculosis outcome in Karonga District, Malawi. Bull. WHO **76** (1998) 295–305.

Evaluation of disease outcome is central to the assessment of tuberculosis (TB) control programs. In the study reported in this article we examined the factors influencing the measurement of outcome, survival rates during and after treatment, smear conversion rates, and relapse rates for patients diagnosed with TB in a rural area of Malawi between 1986 and mid-1994.

Patients with less certain diagnoses of TB were more likely to die than those with confirmed TB, both among those who were seropositive and those who were seronegative to human immunodeficiency virus (HIV). The mortality rate among smear-positive patients with a separate culture-positive specimen was half that of patients with no such diagnostic confirmation. Patients not registered by the Ministry of Health had much higher mortality and default rates than did registered patients. Among smear-positive patients, HIV serostatus was the most important influence on mortality both during and after treatment [crude hazard ratios (95% confidence intervals) = 5.6 (3.0–10) and 7.7 (3.4–17) respectively], but HIV serostatus did not influence smear conversion rates. The initial degree of smear positivity influenced smear conversion rates, but not mortality rates. No significant predictors of relapse were identified.

Unless considerable care is taken to include all TB patients, and to exclude nontuberculous patients, recorded TB outcome statistics are difficult to interpret and may be misleading. In populations with high rates of HIV infection, TB target cure rates of 85% are unrealistic. When new interventions are assessed it cannot be assumed that factors which influence the smear conversion rate will also influence the mortality rate.—Authors' Abstract

Gomes, M. S. and Appelberg, R. Evidence for a link between iron metabolism and Nramp1 gene function in innate resistance against *Mycobacterium avium.* Immunology **95** (1998) 165–168.

In the mouse, the progression of the *Mycobacterium avium* infection is highly dependent on the Nramp1 gene. Strains of mice that express the Nramp1 (D169) allele are highly susceptible to *M. avium* infections, while Nramp1 (G169) strains of mice can control them. Recently, the Nramp1 gene has been cloned and characterized as coding a transmembrane protein, probably involved in divalent cation transport. One possible function of this protein could be the transport of iron out of the parasite-harboring phagosome. Previous work in our lab has shown both *in vitro* (in macrophage cultures) and *in vivo,* that the growth rate of *M. avium* is highly dependent on the amount of iron available in the system. To try to correlate this with the Nramp1 gene function, BALB/c (susceptible) and CD2 (resistant) congenic mice were treated for 20 days with different doses of iron-dextran, so as to induce different degrees of iron overload, and in-

fected with *M. avium* 2447. Iron administration increased *M. avium* growth in infected organs in a dose-dependent manner at the same time as it decreased the difference in mycobacterial growth between the two mouse strains. These results indicate that an excess of iron hampers Nramp1-encoded function, strongly suggesting a direct involvement of the Nramp1-encoded protein in the transport of this cation.—Authors' Abstract

Hall Stoodley, L. and Lappin Scott, H. Biofilm formation by the rapidly growing mycobacterial species *Mycobacterium fortuitum.* FEMS Microbiol. Lett. **168** (1998) 77–84.

Rapidly growing mycobacteria (RGM) are found in soil and diverse aquatic environments. Two species, *Mycobacterium fortuitum* and *M. chelonae,* are associated with disease and are difficult to eradicate. Biofilm formation may be a contributing factor to their mode of transmission and their resistance to antimicrobial agents. We investigated the ability of the RGM species *M. fortuitum* to colonize surfaces using a modified Robbins device. *M. fortuitum* formed dense biofilms within 48 hr. The high numbers of sessile organisms recovered and the swiftness of colonization suggest that *M. fortuitium* readily forms biofilms. These results suggest a novel mechanism for mycobacteria in evading antimicrobial treatment and also indicate that biofilms should be considered possible sites for mycobacterial contamination.—Authors' Abstract

Hetland, G., Wiker, H. G., Hogasen, K., Hamasur, B., Svenson, S. B. and Harboe, M. Involvement of antilipoarabinomannan antibodies in classical complement activation in tuberculosis. Clin. Diagn. Lab. Immunol. **5** (1998) 211–218.

Alternative and classical complement activation induced by whole bacilli of *Mycobacterium bovis* BCG and *M. tuberculosis* products was examined. After exposure to BCG, there were higher levels of the terminal complement complex in sera from Indian tuberculosis patients than in sera from healthy controls. The addition of BCG with or without EGTA to these sera indicated that approximately 70%–85% of the total levels of the terminal complement complex was formed by classic activation. Sera from Indian tuberculosis patients contained more antibody to lipoarabinomannan (LAM) than sera from healthy Indians. Levels of anti-LAM immunoglobulin G2 (IgG2), but not anti-LAM IgM, correlated positively with classical activation induced by BCG in the sera. By flow cytometry, deposition of C3 and terminal complement complex on bacilli incubated with normal human serum was demonstrated. The anticomplement staining was significantly reduced in the presence of EGTA and EDTA. Flow cytometry also revealed the binding of complement to BCG incubated with rabbit anti-LAM and then with factor B-depleted serum. It is concluded that classical activation plays a major role in complement activation induced by mycobacteria and that anti-LAM IgG on the bacilli can mediate this response. It is suggested that classical complement activation may be important for the extent of phagocytosis of *M. tuberculosis* by mononuclear phagocytes, which may influence the course after infection.—Authors' Abstract

Hetzel, C., Janssen, R., Ely, S. J., Kristensen, N. M., Bunting, K., Cooper, J. B., Lamb, J. R., Young, D. B. and Thole, J. E. R. An epitope delivery system for use with recombinant mycobacteria. Infect. Immun. **66** (1998) 3643–3648.

We have developed a novel epitope delivery system based on the insertion of peptides within a permissive loop of a bacterial superoxide dismutase molecule. This system allowed high-level expression of heterologous peptides in two mycobacterial vaccine strains, *Mycobacterium bovis* bacille Calmette-Guerin (BCG) and *M. vaccae.* The broader application of the system was analyzed by preparation of constructs containing peptide epitopes from a range of infectious agents and allergens.

We report detailed characterization of the immunogenicity of one such construct, in which an epitope from the Der pi house dust mite allergen was expressed in *M. vaccae.* The construct was able to stimulate T-cell hybridomas specific for Der pi, and it induced peptide-specific gamma interferon responses when used to immunize naive mice. This novel expression system demonstrates new possibilities for the use of mycobacteria as vaccine delivery vehicles.—Authors' Abstract

Hirsch, D., Stahl, A. and Lodish, H. F. A family of fatty acid transporters conserved from mycobacterium to man. Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 8625–8629.

Long chain fatty acids (LCFAs) are an important source of energy for most organisms. They also function as blood hormones, regulating key metabolic functions such as hepatic glucose production. Although LCFAs can diffuse through the hydrophobic core of the plasma membrane into cells, this nonspecific transport cannot account for the high affinity and specific transport of LCFAs exhibited by cells such as cardiac muscle, hepatocytes, and adipocytes. Transport of LCFAs across the plasma membrane is facilitated by fatty acid transport protein (FATP), a plasma membrane protein that increases LCFA uptake when expressed in cultured mammalian cells [Schaffer, J. E. and Lodish, H. F. (1994) Cell 79, 427–436]. Here, we report the identification of four novel murine FATPs, one of which is expressed exclusively in liver and another only in liver and kidney. Both genes increase fatty acid uptake when expressed in mammalian cells. All five murine FATPs have homologs in humans in addition to a sixth FATP gene. FATPs are found in such diverse organisms as *Fugu rubripes, Caenorhabditis elegans, Drosophila melanogaster, Saccharomyces cerevisiae,* and *Mycobacterium tuberculosis.* The function of the FATP gene family is conserved throughout evolution as the *C. elegans* and mycobacterial FATPs facilitate LCFA uptake when overexpressed in COS cells or *Escherichia coli,* respectively. The identification of this evolutionarily conserved fatty acid transporter family will allow us to gain a better understanding of the mechanisms whereby LCFAs traverse the lipid bilayer as well as yield insight into the control of energy homeostasis and its dysregulation in diseases such as diabetes and obesity.—Authors' Abstract

Hiserodt, R. D., Franzblau, S. G. and Rosen, R. T. Isolation of 6-, 8-, and 10-gingerol from ginger rhizome by HPLC and preliminary evaluation of inhibition of *Mycobacterium avium* and *Mycobacterium tuberculosis.* J. Agri. Food Chem. **46** (1998) 2504–2508.

Diseases caused by *Mycobacterium avium* and *M. tuberculosis* have reached pandemic proportions with some strains being resistant to existing chemotherapies. Complex therapies requiring four to six drugs are sometimes required to prevent the emergence of resistant strains. There is a need for the discovery of new drugs or compounds that are potential drug templates that can be used to treat diseases caused by these bacteria. The research reported in this paper describes the isolation of 6-, 8-, and 10-gingerol from fresh ginger rhizome and the identification of 10-gingerol as the most active inhibitor of *M. avium* and *M. tuberculosis in vitro.* The gingerols were isolated by fractionation of a crude methylene chloride extract of fresh ginger rhizome by normal phase HPLC. Identification was based on mass spectral data. The identification of 10-gingerol was confirmed by synthesis.—Authors' Abstract

Horgen, L., Sola, C., Devallois, A., Goh, K. S. and Rastogi, N. Follow up of *Mycobacterium tuberculosis* transmission in the French West Indies by IS6110-DNA fingerprinting and DR-based spoligotyping. FEMS Immunol. Med. Microbiol. **21** (1998) 203–212.

A total of 115 *Mycobacterium tuberculosis* isolates from 80 patients were typed using IS6110-DNA fingerprinting and DR-based spoligotyping to describe the active transmission of tuberculosis in a Caribbean

setting over a 2-year period. A total of 61 different pattern types were observed by IS6110-RFLP and 10 clusters containing between two and 15 patients could be defined. By spoligotyping, 45 different pattern types were observed with 12 clusters containing two to 11 patients. Thirty-two patients could be included in eight spoligotype-defined clusters and in nine RFLP-defined clusters when strictly concordant matching results were put together. In conclusion, about 40% of the patient isolates were clustered by DNA fingerprinting, suggesting recent transmission of tuberculosis in our region. This study confirmed the increased accuracy and discriminatory power of the association of IS6110-RFLP and spoligotyping for studies on the molecular epidemiology of *M. tuberculosis,* and suggests that despite good implementation of tuberculosis control programs in Guadeloupe, active transmission of tuberculosis may be far more important than suspected.—Authors' Abstract

Hutter, B. and Dick, T. Increased alanine dehydrogenase activity during dormancy in *Mycobacterium smegmatis.* FEMS Microbiol. Lett. **167** (1998) 7–11.

The aerobic fast-growing *Mycobacterium smegmatis* has, like its slow-growing pathogenic counterpart *M. tuberculosis,* the capability to adapt to anaerobiosis by shifting down to a drug-resistant dormant state. Here, we report the identification of the first enzyme, L-alanine dehydrogenase, whose specific activity is increased during dormancy development in *M. smegmatis.* This mycobacterial enzyme activity was previously identified as the 40-kDa antigen in *M. tuberculosis* and shows a preference for the reductive amination of pyruvate to alanine at physiological pH. The determination of the temporal profile of alanine dehydrogenase activity during dormancy development showed that the activity stayed at a low baseline level during the initial aerobic exponential growth phase (0.7 mU mg^{-1} min^{-1}. After termination of aerobic growth, alanine dehydrogenase activity increased rapidly 5-fold. As oxygen becomes more and more limiting, the enzyme activity declined until it reached a level about two thirds that of the peak value. The strong induction immediately after deflection from aerobic growth suggests that alanine might be required for the adaptation from aerobic growth to anaerobic dormancy. As alanine synthesis is coupled to NADH oxidation, we propose that the induction of alanine dehydrogenase activity might also support the maintenance of the NAD pool when oxygen as a terminal electron acceptor becomes limiting.—Authors' Abstract

Kumar, R. H., Kumar, M. V. and Thappa, D. M. Dapsone syndrome—a five year retrospective analysis. Indian J. Lepr. **70** (1998) 271–276.

Seventeen cases of dapsone syndrome were seen in 5 years from 1992 onward. Their mean age was 27.8 years (range 11 to 60 years). Male-to-female ratio was 1.1:1. Of these cases, 7 had confirmed leprosy, 9 were cases of suspected leprosy and 1 case had lichen planus. On average, they developed the symptoms 27 days after the intake of dapsone. The cutaneous lesions were in the form of erythematous papules and plaques (13 cases), eczematous lesions (4 cases) and associated bullous lesions (2 cases). The other manifestations were: fever (16 cases), pruritus (15 cases), lymphadenopathy (14 cases), hepatomegaly (10 cases), icterus and oral erosions (5 cases each), photosensitivity (4 cases) and splenomegaly (2 cases). Previous drug allergy was present in four cases. Elevated ESR and liver enzyme levels were invariable findings. Raised bilirubin levels and hemolytic anemia were seen in eight cases. Apart from one case with hepatic encephalopathy, all other cases had a favorable outcome either on conservative management (eight cases) or on oral corticosteroids (eight cases). The oral provocation test was done in two cases with positive response while the intradermal test was not very reliable.—Authors' Abstract

Kumar, V., Selvakumar, N., Venkatesan, P., Chandrasekaran, V., Paramasivan, C. N. and Prabhakar, R. Bioluminescence assay of adenosine triphosphate in drug susceptibility testing of *Mycobacterium tuberculosis.* Indian J. Med. Res. **107** (1998) 75–77.

A total of 23 clinical isolates of *Mycobacterium tuberculosis* obtained from pulmonary tuberculosis patients attending the Tuberculosis Research Centre, Chennai, India, and the reference strain *M. tuberculosis* H37Rv were tested for their susceptibility to trifluoperazine (TFP) by the standard broth dilution method and the bioluminescence assay. In 15 of the 23 isolates, the minimal inhibitory concentration (MIC) was identical by both the methods and in the remaining 8 isolates the difference in the MIC values between the methods, was <2-fold and was not significant. It is suggested that the measurement of adenosine triphosphate (ATP) by bioluminescence assay can be used as an alternative method for the rapid screening of clinical isolates for their susceptibility to anitmycobacterial agents.—Authors' Abstract

Lee, J. B. and Koblenzer, P. S. Disfiguring cutaneous manifestation of sarcoidosis treated with thalidomide: a case report. J. Am. Acad. Dermatol. **39** Part 2 Suppl. (1998) 835–838.

A patient with sarcoidosis was treated with thalidomide for disfiguring and painful steroid unresponsive sarcoidal granulomas of the skin. The duration of the therapy was 14 months, during which time the skin lesions resolved almost completely. The initial dosage was 200 mg a day, which was increased to 400 mg a day after 4 months. Episodic paresthesia of the fingertips and one lower extremity was the only side effect noted, which resolved promptly after discontinuation of the drug. The dramatic response of sarcoidal granulomas of the skin to thalidomide observed in this patient demonstrates the usefulness of this drug as a possible long-term monotherapeutic or steroid-sparing agent in the treatment of sarcoidosis.—Authors' Abstract

Marriott, J. B., Westby, M., Cookson, S., Guckian, M., Goodbourn, S., Muller, G., Shire, M. G., Stirling, D. and Dalgleish, A. G. CC-3052: a water-soluble analog of thalidomide and potent inhibitor of activation-induced TNF-alpha production. J. Immunol. **161** (1998) 4236–4243.

The immunomodulatory drug thalidomide has been shown to be clinically useful in a number of situations due to its ability to inhibit TNF-alpha synthesis. However, its use is restricted by potentially serious side effects, including teratogenicity and neurotoxicity; furthermore, insolubility may present problems in terms of systemic bioavailability. Recently, structural modifications of thalidomide have been designed enabling greatly enhanced anti-TNF-alpha activity in LPS-treated mice. In contrast to thalidomide (LPS-induced TNF-alpha IC50 similar to 200 μM in DMSO) and other analogs tested, one of these compounds, CC-3052 (IC50 similar to 1 μM in water), is water soluble. Furthermore, this analog exhibits increased stability in human plasma ($t^{1/2}$ similar to 17.5 vs 1.5 hr for thalidomide) and appears to be nontoxic, nonmutagenic, and nonteratogenic. At pharmacologically active levels, cellular proliferation and LPS-induced IL-6 mRNA and IL-12p40 mRNA (as well as IL-1 beta and IL-6 protein levels) in whole blood cultures were not affected; apparent inhibition of NK activity by CC-3052 was reversed upon addition of exogenous rTNF-alpha. In addition, IL-10 mRNA and protein levels were increased. These properties are consistent with results indicating inhibition of phosphodiesterase type IV activity by CC-3052. Furthermore, CC-3052 did not increase the degradation rate of macrophage TNF-alpha transcripts nor inhibit LPS-induced primary macrophage NF-kappa B activation. Taken together, the potency of selective TNF-alpha inhibition, water solubility, and increased plasma stability make CC-3052 an excellent candidate for further development and clinical evaluation for the treatment of TNF-alpha-mediated disease.—Authors' Abstract

Menozzi, F. D., Bischoff, R., Fort, E., Brennan, M. J. and Locht, C. Molecular characterization of the mycobacterial heparin-binding hemagglutinin, a mycobacterial adhesion. Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 12625–12630.

Although it generally is accepted that the interaction of *Mycobacterium tuberculosis* with alveolar macrophages is a key step in

the pathogenesis of tuberculosis, interactions with other cell types, especially epithelial cells, also may be important. In this study we describe the molecular characterization of a mycobacterial heparin-binding hemagglutinin (HBHA), a protein that functions as an adhesin for epithelial cells. The structural gene was cloned from *M. tuberculosis* and bacillus Calmette-Guerin, and the sequence was found to be identical between the two species. The calculated M, was smaller than the observed M, when analyzed by SDS/PAGE. This difference can be attributed to the Lys/Pro-rich repeats that occur at the C-terminal end of the protein and to a putative carbohydrate moiety. Glycosylation of HBHA appears to protect the protein from proteolytic degradation, which results in the removal of the C-terminal Lys/Pro-rich region responsible for binding of HBHA to sulfated carbohydrates. Evidence suggests that glycosylation is also important for HBHA-mediated hemagglutination and for certain immunologic properties of the protein. Finally, the absence of a signal peptide in the coding region of HBHA raises the possibility that this protein is not secreted via the general secretion pathway.—Authors' Abstract

Meyers, P. R., Bourn, W. R., Steyn, L. M., Van Helden, P., Beyers, A. D. and Brown, G. D. Novel method for rapid measurement of growth of mycobacteria in detergent-free media. J. Clin. Microbiol. **36** (1998) 2752–2754.

We describe a novel, rapid, and inexpensive method for the measurement of growth of *Mycobacterium tuberculosis, M. bovis,* and *M. smegmatis* in the presence or absence of detergent. The method, which employs hot NaOH treatment of mycobacterial cells to release total cellular protein, compares favorably with other methods for monitoring mycobacterial growth but is particularly useful for heavily clumped cultures grown in defined minimal medium.—Authors' Abstract

Mitscher, L. A. and Baker W. Tuberculosis: a search for novel therapy starting with natural products. Med. Res. Rev. **18** (1998) 363–374.

The re-emergence of tuberculosis and closely related diseases as significant public health problems is briefly reviewed with particular emphasis on the exacerbating role of AIDS and multiple drug resistance. Screening methods available for discovering new chemical entities active against resistant strains are discussed and their use in screening extracts and compounds for activity is illustrated with a number of newly discovered structures being presented. In particular, the properties of the potent and structurally novel indoloquinazolinone alkaloid, tryptanthrin, is described.

Many analogs of this lead structure were synthesized by combinatorial and multiple parallel synthetic techniques and evaluated *in vitro* and *in vivo* for their potential in the chemotherapy of human infections.—Authors' Abstract

Mizrah, V. and Andersen, S. J. DNA repair in *Mycobacterium tuberculosis.* What have we learnt from the genome sequence? Mol. Microbiol. **29** (1998) 1331–1339.

The genome sequence of *Mycobacterium tuberculosis* was analyzed by searching for homologs of genes known to be involved in the reversal or repair of DNA damage in *Escherichia coli* and related organisms. Genes necessary to perform nucleotide excision repair (NER), base excision repair (BER), recombination, and SOS repair and mutagenesis were identified. In particular, all of the genes known to be directly involved in the repair of oxidative and alkylative damage are present in *M. tuberculosis.* In contrast, we failed to identify homologs of genes involved in mismatch repair. This finding has potentially significant implications with respect to genome stability, strain variability at repeat loci and the emergence of chromosomally encoded drug resistance mutations.—Authors' Summary

Mohagheghpour, N., Gammon, D., Kawamura, L. M., van Vollenhoven, A., Benike, C. J. and Engleman, E. G. CTL response to *Mycobacterium tuberculosis*: identification of an immunogenic epitope in the 19-kDa lipoprotein. J. Immunol. **161** (1998) 2400–2406.

The successful resolution of infection with *Mycobacterium tuberculosis* (M.tb) is believed to involve the induction ot CTLs that are capable of killing cells harboring this pathogen, although little information is known about the MHC restriction or fine specificity of such CTLs. In this study, we used knowledge of the HLA-A*0201-binding motif and an immunofluorescence-based peptide-binding assay to screen for potential HLA-A*0201-binding epitopes contained in the 19-kDa lipoprotein of M.tb (M.tb19). CD8+ T cells derived from HLA-A*0201+ patients with active tuberculosis (TB) as well as tuberculin skin test-positive individuals who had no history of TB were used as effector cells to determine whether these epitopes are recognized by *in vivo* primed CTLs. An *in vitro* vaccination system using HLA-A*0201+ dendritic cells (DCs) as APCs was used to determine whether these epitopes can sensitize naive CD8+ T cells *in vitro,* leading to the generation of antigen-specific CTLs. The results show that an HLA-A*0201-binding peptide comprised of residues 88 to 97 of M.tb19 (P88-97) is recognized by circulating CD8+ CTLs from both healthy tuberculin skin test-positive individuals and patients with active TB but not by tuberculin skin test-negative subjects. Moreover, dendritic cells pulsed with this peptide induced class I MHC-restricted CTLs from the T cells of healthy unsensitized persons. Finally, CTL lines that were specific for P88-97 were shown to lyse autologous monocytes that had been infected acutely with the H37Ra strain of M.tb. These results demonstrate that M.tb19 elicits HLA class I-restricted CTLs *in vitro* and *in vivo* that recognize endogenously processed antigen. Epitopes of the type identified here may prove useful in the design of an M.tb vaccine.—Authors' Abstract

Murray, C. J. L. and Salomon, J. A. Modeling the impact of global tuberculosis control strategies. Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 13881–13886.

An epidemiological model of tuberculosis has been developed and applied to five regions of the world. Globally, 6.7 million new cases of tuberculosis and 2.4 million deaths from tuberculosis are estimated for 1998. Based on current trends in uptake of the World Health Organization's strategy of directly observed treatment, short-course, we expect a total of 225 million new cases and 79 million deaths from tuberculosis between 1998 and 2030. Active case finding using mass miniature radiography could save 23 million lives over this period. A single contact treatment for tuberculosis could avert 24 million cases and 11 million deaths; combined with active screening, it could reduce mortality by nearly 40%. A new vaccine with 50% efficacy could lower incidence by 36 million cases and mortality by 9 million deaths. Support for major extensions to global tuberculosis control strategies will occur only if the size of the problem and the potential for action are recognized more widely.—Authors' Abstract

Orefice, F. and Boratto, L. M. Clinical ocular study in leprosy patients at a sanitary dermatological hospital in Brazil. Indian J. Lepr. **70** (1998) 189–195.

A total of 363 leprosy patients at a hospital in Minas Gerais, Brazil, were subjected to a thorough ophthalmic examination comprising: visual acuity, facial muscle function, eyebrows, eyelashes, lacrimal system, pupil, eye motility, corneal sensitivity, Schirmir's test and study of the anterior segment of the ocular bulb with a slit-lamp. The study patients included 275 cases of lepromatous leprosy, 57 tuberculoid, 29 indeterminate and 2 dimorphous cases. However, the eye examination was done without the knowledge of clinical diagnosis. Pupil reflexes were reduced considerably or even absent in 28.1% of all patients. Changes in the ocular adnexa occurred mainly in lepromatous leprosy patients (40%), and mostly involved the eyebrows (partial and total madarosis). There was no evidence of conjunctival involvement in any of the patients studied. Changes in the sclera (scleritis, staphyloma and corneoscleral roll) were found predominantly in lepromatous and indeterminate leprosy patients (6.9% and 6.8%, respectively). In relation to the cornea, the most important fact observed was a large number of instances of reduction of corneal sensitivity (49.9% of all

cases). The study of the iris revealed that most of the atrophies of the stroma and of the pigment cover were not caused by anterior uveal inflammatory changes, but by the mechanism of nerve fibre alterations.—Authors' Abstract

Pablos-Mendez, A., Raviglione, M. C., Laszlo, A., Binkin, N., Rieder, H. L., Bustreo, F., Cohn, D. L., Lambregts-van Weezenbeek, C. S. B., Kim, S. J., Chaulet, P. and Nunn, P. (WHO-Int. Union Against TB and Lung Dis. Working Group of Anti-tuberculosis Drug Resistance Surveillance). Global surveillance for antituberculosis-drug resistance, 1994–1997. N. Engl. J. Med. **388** (1998) 1641–1649.

This report describes the prevalence of resistance to four first-line drugs in 35 countries participating in the WHO-International Union against Tuberculosis and Lung Disease Global Project on Anti-Tuberculosis Drug Resistance Surveillance between 1994 and 1997. The data are from cross-sectional surveys and surveillance reports. The median number of patients studied in each country or region was 555 (range, 59–14,344). Among patients with no prior treatment, a median of 9.9% of *Mycobacterium tuberculosis* strains were resistant to at least one drug (range, 2.0%–41.0%); resistance to isoniazid (7.3%) or streptomycin (6.5%) was more common than resistance to rifampicin (1.8%) or ethambutol (1.0%). The prevalence of primary multidrug resistance was 1.4% (range, 0–14.4%). Among patients with histories of treatment for ≤1 month, the prevalence of resistance to any of the four drugs was 36.0% (range, 5.3%–100%), and the prevalence of multidrug resistance was 13% (range, 0–54.0%). The overall prevalences were 12.6% for single-drug resistance (range, 2.3%–42.4%) and 2.2% for multidrug resistance (range, 0–22.1 %). Particularly high prevalences of multidrug resistance were found in the former Soviet Union, Asia, the Dominican Republic, and Argentina. Resistance to antituberculosis drugs was found in all 35 countries and regions surveyed, suggesting that it is a global problem.—Authors' Abstract

Palomino, J. C., Obiang, A.M., Realini, L., Meyers, W. M. and Portaels, F. Effect of oxygen growth of *Mycobacterium ulcerans* in the BACTEC system. J. Clin. Microbiol. **36** (1998) 3420–3422.

The effect of low oxygen concentration on the growth of 15 strains of *Mycobacterium ulcerans* was evaluated in the BACTEC system. Reduced-oxygen tension enhanced the growth of *M. ulcerans,* suggesting that this organism has a preference for microaerobic environments. Application of this observation may improve rates of isolation of *M. ulcerans* in primary culture from clinical samples and promote isolation of the bacterium from environmental sources.—Authors' Abstract

Peterson, C. M., Ahkavan, M., Fernandez Soto, M. L., Rowell, C., Scott, B. K. and Peterson, K. P. Dapsone at onset of diabetes lowers glycated hemoglobin and delays death in NOD mice. Autoimmunity **28** (1998) 157–161.

Dapsone (4,4´-diaminodiphenyl sulfone) is a compound that has a large clinical experience due to its antimicrobial effects against *Mycobacterium leprae,* the causative agent of leprosy. It is increasingly used in a number of clinical situations where inflammation may play an ancillary role. An inhibitory effect of the drug or lack thereof in the cumulative incidence or propagation of diabetes mellitus in the NOD mouse has mechanistic as well as therapeutic implications. We previously showed that dapsone administered continuously as a percentage of food to NOD mice inhibits the cumulative incidence of diabetes in a dose-dependent fashion. In the present experiment, female NOD litter mates were randomized to receive dapsone (0.001% w/w as a percentage of food) at onset of diabetes. There were no differences in weight, blood glucose, or glycated hemoglobin at 10 weeks of age among the animals that were ultimately to receive dapsone (N = 10), mouse chow alone (N = 9), or those who did not develop diabetes (N = 3). The mean time to onset of diabetes, mean blood glucose at onset, and mean glycated hemoglobin at onset did not differ between animals who

did or did not receive dapsone. Animals receiving dapsone had significantly ($p \leq 0.03$) lower glycated hemoglobin at weeks 2, 3, and 4 following the onset of diabetes and lived significantly longer following diagnosis of diabetes (7 vs. 4 weeks, $p < 0.05$). In conclusion, dapsone modulates the progression of autoimmune diabetes in the NOD mouse even when administered after the initiation of hyperglycemia.—Authors' Abstract

Realini, L., DeRidder, K., Palonimo, J. C., Hirschel, B. and Portaels, F. Microaerophilic conditions promote growth of *Mycobacterium genavense.* J. Clin. Microbiol. **36** (1998) 2565–2570.

Our studies show that microaerophilic conditions promote the growth of *Mycobacterium genavense* in semisolid medium. The growth of *M. Genavense* at 2.5% or 5% oxygen was superior to that obtained at 21% oxygen in BACTEC primary cultures (Middlebrook 7H12, pH 6.0, without additives). By using nondecontaminated specimens, it was possible to detect growth with very small inocula (25 bacilli/ml) of 12 different *M. genavense* strains (from nude mice) within 6 weeks of incubation under low oxygen tension; conversely, with 21% oxygen, no growth of 8 of 12 (66.7%) *M. genavense* strains was detected (growth index, <10). The same beneficial effect of 2.5% or 5% oxygen was observed in primary cultures of a decontaminated clinical specimen. Low oxygen tension (2.5% or 5%) is recommended for the primary isolation of *M. genavense.* Microaerophilic cultivation of other atypical mycobacteria, especially slow-growing (e.g., *M. avium*) and difficult-to-grow (e.g., *M. ulcerans*) species, is discussed.—Authors' Abstract

Rosenbaum, P. S., Mbekeani, J. N. and Kress, Y. Atypical mycobacterial panophthalmitis seen with iris nodules. Arch. Ophthalmol. **116** (1998) 1524–1527.

Atypical mycobacterial infections are frequent, late complications of human immunodeficiency virus infections and may have a variety of clinical manifestations. We describe a patient with end-stage acquired immune deficiency syndrome and disseminated atypical mycobacteriosis caused by *Mycobacterium avium-intracellulare* complex with prominent iris nodules as the initial manifestation of a unilateral localized panophthalmitis. Acid-fast bacilli were identified cytologically from the iris nodule and aqueous aspirations. Topical, intracameral, and systemic treatments were used, but the infection progressed and enucleation was performed to avoid the impending scleral rupture. Histopathologic studies revealed an anterior panophthalmitis, with inferior scleral rupture due to acid-fast bacilli in the eye. *M. avium-intracellulare* complex has been described as a cause of endophthalmitis in immunocompromised patients but, to our knowledge, this is the first report of a patient with iris nodules.—Authors' Abstract

Rowland, T. L., McHugh, S. M., Deighton, J., Dearman, R. J., Ewan, P. W. and Kimber, I. Differential regulation by thalidomide and dexamethasone of cytokine expression in human peripheral blood mononuclear cells. Immunopharmacol. **40** (1998) 11–20.

Immunosuppressive drugs are used routinely to reduce the inappropriate production of cytokines in an immune response. Recent attention has focused on drugs that selectively inhibit specific cytokines. Both thalidomide and dexamethasone have been reported to exhibit immunomodulatory effects on cytokines *in vitro.* We wished to examine the effects of thalidomide and dexamethasone on the production of cytokines by peripheral blood mononuclear cells (PBMC), following mitogenic stimulation, at the level of both secreted product and mRNA production. PBMC from healthy human volunteers were stimulated optimally with phytohemagglutinin (PHA) in the presence of varying concentrations of thalidomide and dexamethasone using dimethyl sulphoxide (DMSO) as the solvent. Analysis of supernatants by enzyme-linked immunosorbent assay (ELISA) showed that thalidomide caused a dose-dependent inhi-

bition of the pro-inflammatory cytokines interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α), maximally reducing production by 20 ($p < 0.05$) and 30% ($p < 0.01$), respectively, compared with controls. However, thalidomide did not affect either proliferation or the production of IL-2, IL-4 or IL-10. A slight bell-shaped inhibition of interferon gamma (IFN-γ) was seen which was statistically significant ($p < 0.05$). In contrast, dexamethasone inhibited markedly the expression of all cytokines tested (IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α) in dose-dependent fashion, reducing levels to near to background. Reverse transcription-polymerase chain reaction (RT-PCR) analyses showed that thalidomide inhibited selectively the expression of TNF-α and IL-6 mRNA; whereas dexamethasone inhibited mRNA levels of all cytokines examined. The data indicate that dexamethasone is a broad range immunosuppressant inhibiting all cytokines tested in a dose-dependent manner at the level of both secreted product and mRNA. Conversely, thalidomide selectively inhibits the production of IL-6 and TNF-α. Due to their markedly different effects on cytokine production, and the fact that both drugs act at the level of transcription, we believe they influence separate pathways involved in cytokine gene regulation.—Authors' Abstract

Wolkenstein, P., Latarjet, J., Roujeau, J. C., Duguet, C., Boudeau, S., Vaillant, L., Maignan, M., Schuhmacher, M. H., Milpied, B., Pilorget, A., Bocquet, H., Brun Buisson, C. and Revuz, J. Randomised comparison of thalidomide versus placebo in toxic epidermal necrolysis. Lancet **352** (1998) 1586–1589.

Background. Toxic epidermal necrolysis (TEN) is associated with a 30% death rate. Tumor necrosis factor alpha (TNF-α) has been implicated in the pathogenesis of TEN. Thalidomide is a potent inhibitor of TNF-α action. We did a double-blind, randomized, placebo-controlled study of thalidomide in TEN.

Methods. The patients received a 5-day course of thalidomide 400 mg daily or placebo. The main endpoint was the progression of skin detachment after day 7. Secondary endpoints were the severity of the disease, evaluated with the simplified acute physiology score (SAPS), and the mortality. TNF-α and interleukin-6 were measured.

Findings. The study was stopped because there was excess mortality in the thalidomide group: 10 of 12 patients died compared with 3 of ten in the placebo group (Fisher's exact test with Katz's approximation, relative risk = 2.78, $p = 0.03$). After adjustment for SAPS, mortality remained significantly higher in the thalidomide group than in the placebo group (exact logistic regression mid-$p = 0.007$; 95% CI for odds ratio 2.7 to infinity). Plasma TNF-α concentration was higher in the thalidomide group than the placebo group on day 2, though the difference was not significant (Wilcoxon rank sum test $p = 0.07$).

Interpretation. Even though few patients were included, our data suggest that thalidomide is detrimental in TEN, possibly because of a paradoxical enhancement of TNF-α production.—Authors' Abstract

Zaffran, Y., Zhang, L. and Ellner, J. J. Role of CR4 in *Mycobacterium tuberculosis* human macrophages binding and signal transduction in the absence of serum. Infect. Immun. **66** (1998) 4541–4544.

The beta 2 integrin CR4 is involved in *Mycobacterium tuberculosis* phagocytosis by human mononuclear phagocytes through the opsonin C3bi. In this study, we demonstrate that *M. tuberculosis* can bind directly to monocyte-derived macrophages via CR4 in the absence of any opsonins. CR4-transfected CHO cells gave similar results, suggesting recognition by CR4 of bacterial structure. Furthermore, binding of *M. tuberculosis* transduced a potent signal, resulting in tyrosine phosphorylation of macrophage proteins, which was in part mediated by CR4.—Authors' Abstract