CURRENT LITERATURE

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

General and Historical

Terencio de las Aguas, J. [History of leprosy.] Rev. Leprol. Fontilles **22** (1999) 19–76. (in Spanish)

The history of the disease is analyzed from ancient and medieval times in Spain and America. Also from the point of view of literature and art up to the scientific interval of the XIX century and in the Valencianne Community, finishing with a summary of the scientific and historical events of the XIX and XX centuries.—Author's English Summary

Chemotherapy

Bluhm, R. E., Adedoyin, A., McCarver, D. G. and Branch, R. A. Development of dapsone toxicity in patients with inflammatory dermatoses: activity of acetylation and hydroxylation of dapsone as risk factors. Clin. Pharmacol. Ther. **65** (1999) 598–605.

Background: Alternative independent routes of dapsone metabolism include N-hydroxylation to the hydroxylamine, a potentially toxic metabolite, by cytochrome P450 enzymes and acetylation to a nontoxic metabolite by N-acetyltransferase. Potentially, therefore, the relative extents of these two routes in an individual could determine the occurrence of adverse reaction with dapsone therapy.

Methods: Phenotypic activity of these two routes of metabolism was assessed in 18 patients receiving long-term dapsone therapy for inflammatory dermatoses and was related to the development of dapsone toxicity. N-Hydroxylation was assessed by the dapsone recovery ratio, a ratio of dapsone hydroxylamine to the sum of hydroxylamine and dapsone in 8-hour urine; whereas N-acetylation was assessed by the acetylation ratio, a ratio of monoacetyldapsone to dapsone in 8-hour plasma sample after an oral dose of dapsone.

Results: There was wide intersubject variation in both the acetylation ratio and the dapsone recovery ratio, but both phenotypic measures remained stable within individuals. The dapsone recovery ratio showed a tendency toward being lower in fast than in slow acetylators, but this was not statistically significant. There was an inverse relationship between acetylation and hydroxylation ($r = -0.69$; $p < 0.005$) at steady state that was not apparent after the first dose. Neurotoxicity developed in two subjects and hemolytic anemia developed in two subjects. Plasma levels of dapsone in these four subjects were similar to those in subjects who showed no toxicity. All four were slow acetylators and three were rapid hydroxylators, consistent with the toxic nature of dapsone hydroxylamine.

Conclusions: These observations are consistent with what is known about the toxicity profile of dapsone metabolites and suggest that assessing N-acetylation and N-hydroxylation capacities can help to identify subjects at increased risk of a toxic response. This approach of assessing the phenotypic measures of drug-metabolizing activity to predict adverse reaction may also apply to other drugs with metabolic-based adverse effects.—Authors' Abstract

Coleman, M. D., Rathbone, D. L., Abberley, L., Lambert, P. A. and Billington, D. C. Preliminary *in vitro* toxicological evaluation of a series of 2-pyridylcarboxamidrazone candidate antituberculosis compounds. Environ. Toxicol. Pharmacol. **7** (1999) 59–65.

We have investigated the toxicity of a series of 2-pyridylcarboxamidrazones *in vitro* using a rat liver metabolism system as well as human erythrocytes and monoculcear leukocytes (MNL) as target cells. Of the seven derivatives and four precursors tested, only minimal (<2.3%) metabolism-mediated methemoglobin was formed by two analogs. However, one of these, a naphthylidene 2-pyridylcarboxamidrazone derivative (compound III), was also directly toxic to human MNL. This toxicity was partially attenuated by the rat metabolizing system and incubation of diethyldithiocarbamate or cimetidine together with compound III, and the rat metabolizing system suppressed the metabolism-dependent detoxification. This indicated that cytochrome P-450-mediated biotransformation of compound III was preventing its direct toxicity to the MNL. Of the seven derivatives tested, six were low in toxicity to MNL directly and in the presence of a metabolizing system. The two compounds which were the most potent anti-mycobacterially, the dimethylpropyl and dimethylethyl benzylidene amidrazone derivatives, were also the least toxic to MNL and erythrocytes. This amidrazone series has shown promise for furture development as antituberculosis drugs.—Authors' Abstract

Harth, G. and Horwitz, M. A. An inhibitor of exported *Mycobacterium tuberculosis* glutamine synthetase selectively blocks the growth of pathogenic mycobacteria in axenic culture and in human monocytes: extracellular proteins as potential novel drug targets. J. Exp. Med. **189** (1999) 1425–1435.

Mycobacterium tuberculosis and other pathogenic mycobacteria export abundant quantities of proteins into their extracellular milieu when growing either axenically or within phagosomes of host cells. One major extracellular protein, the enzyme glutamine synthetase, is of particular interest because of its link to pathogenicity. Pathogenic mycobacteria, but not nonpathogenic mycobacteria, export large amounts of this protein. Interestingly, export of the enzyme is associated with the presence of a poly-L-glutamate/glutamine structure in the mycobacterial cell wall. In this study, we investigated the influence of glutamine synthetase inhibitors on the growth of pathogenic and nonpathogenic mycobacteria and on the poly-L-glutamate/glutamine cell wall structure.

The inhibitor L-methionine-S-sulfoximine rapidly inactivated purified *M. tuberculosis* glutamine synthetase, which was 100-fold more sensitive to this inhibitor than a representative mammalian glutamine synthetase. Added to cultures of pathogenic mycobacteria, L-methionine-S-sulfoximine rapidly inhibited extracellular glutamine synthetase in a concentration-dependent manner but had only a minimal effect on cellular glutamine synthetase, a finding consistent with failure of the drug to cross the mycobacterial cell wall. Remarkably, the inhibitor selectively blocked the growth of pathogenic mycobacteria, all of which release glutamine synthetase extracellularly, but had no effect on nonpathogenic mycobacteria or nonmycobacterial microorganisms, none of which release glutamine synthetase extracellularly. The inhibitor was also bacteriostatic for *M. tuberculosis* in human mononuclear phagocytes (THP-1 cells), the pathogen's primary host cells. Paralleling and perhaps underlying its bacteriostatic effect, the inhibitor markedly reduced the amount of poly-L-glutamate/glutamine cell wall structure in *M. tuberculosis.*

Although it is possible that glutamine synthetase inhibitors interact with additional extracellular proteins or structures, our findings support the concept that extracellular proteins of *M. tuberculosis* and other pathogenic mycobacteria are worthy targets for new antibiotics. Such proteins constitute readily accessible targets of these relatively impermeable organisms, which are rapidly developing resistance to conventional antibiotics.—Authors' Abstract

Ishida, Y., Biswas, A. K. and Guglielmelli, E. [Detection mode of leprosy and its disability grading in Khulna city, Bangladesh.] Jpn. J. Lepr. **67** (1998) 391–400.

The relationship between detection modes of leprosy and their associated disability grading was analyzed among 1115 newly registered leprosy patients in Khulna, Bangladesh, during 1995–1997 to determine the contribution of each detection mode to disability grading. Voluntary reporting patients from "out of control area" had both the high disability grading of G = 2 (38.0%) and a high MB rate (39.3%). However, voluntary reporting patients in a control area had less disability grading of G = 2 (12.2%) and less MB rate (25.3%). The number of patients referred by local doctors was still small and had both a high disability grading of G = 2 (27.0%) and a high MB rate (51.4%). Children detected by school surveillance did not have any marked disability grading of G = 2 (0%) and were mostly PB patients (88.6%). (Prevalence rate of school surveillance was low.) Patients detected under general surveillance had low disability grading of G = 2 (2.6%). The disability grading (G = 2) of voluntary patients from a control area (12.2%) was 3-fold higher than that of patients from general surveillance (2.6%). Patients of family contacts who were aware of the first symptoms had relatively less disability grading (5.9%). The small number of patients referred by local doctors (3.3% of total number) with high disability grading indicated a need for information programs on leprosy in this region.—Trop Dis. Bull. **96** (1999) 612

Patel, V. B., Misra, A. N. and Marfatia, Y. S. A topical dosage form of liposomal clofazimine: research and clinical implications. Pharmazie **54** (1999) 448–451.

A novel topical clofazimine (CLO) gel formulation containing liposomally encapsulated CLO was prepared and investigated *in vitro* followed by a clinical evaluation. CLO liposomes were prepared by the lipid Nm hydration technique. Comparative *in vitro* diffusion studies were conducted with plain and liposomal CLO in an HPMC K4M gel base (2% and 5%) using human cadaver skin (HCS). A double-blind clinical study was conducted on eight leprosy patients. The results of these studies show that the new liposomal topical gel formulation not only prolongs the drug release but also promotes drug retention by the skin. Studies further support formation of a reservoir of drug on the skin modifying therapeutic efficacy of the formulation. The new liposomal gel formulation of CLO considerably reduces the healing time of external lesions due to a significantly prolonged skin residence time compared to plain CLO gel and, hence, is expected to reduce the time needed for leprosy treatment.—Authors' Abstract

Clinical Sciences

Endoh, M., Kunishita, T. and Tabira, T. No effect of antileprosy drugs in the prevention of Alzheimer's disease and beta-amyloid neurotoxicity. J. Neurol. Sci. **165** (1999) 28–30.

There is continuing controversy as to whether or not antileprosy drugs prevent Alzheimer's disease (AD). Therefore, we examined the effect of antileprosy drugs on the prevalence of AD in leprosy patients, and also investigated the effect of antileprosy drugs on amyloid beta-protein (A beta)-induced neurotoxicity *in vitro*. The present study suggests that antileprosy treatments do not prevent the onset of AD. None of our data found antileprosy drugs (Dapsone, rifampin, clofazimine, minomycin or ofloxacin) had any effect on A beta neurotoxicity. It is now important to examine the infection of *Mycobacterium leprae* in the central nervous system to clarify the reason for the low prevalence of senile dementia, and low frequency of A beta

deposition in leprosy patients.—Authors' Abstract

Nery, J. A. C., Garcia, C. C., Wanzeller, S. H. O., Sales, A. M., Gallo, M. E. N. and Vieira, L. M. M. [Clinical and histopathological characteristics of reactional states in leprosy patients submitted to multidrug therapy.] An. Bras. Dermatol. **74** (1999) 27–33.

The clinical and histopathological aspects of the reactional states in patients submitted to multidrug therapy in Rio de Janeiro, Brazil, between 1986 and 1993, were studied. In addition, the frequency and distribution of these reactional states in 169 MB (multibacillary) patients was also evaluated. These episodes were classified based on the clinical and histopathological aspects as erythema nodosum leprosum (ENL) (51%), reversal reaction (RR) (42%) and isolated neuritis (7%). It was observed that such reactional episodes were a frequent complication (59%), occurring at any time in the course of the disease. It was observed that 77% of the patients who developed ENL and 43% of the RR patients presented systemic involvement. The RR state predominated among the BB patients (74%) while ENL predominated in the LL patients (86%). ENL was significantly more recurrent than RR. In addition RR predominated in the first year of MDT, ENL in the second year.—Trop. Dis. Bull. **96** (1999) 612

Immuno-Pathology

Bermudez, L. E., Parker, A. and Petrofsky, M. Apoptosis of *Mycobacterium avium*-infected macrophages is mediated by both tumour necrosis factor (TNF) and Fas, and involves the activation of caspases. Clin. Exp. Immunol. **116** (1999) 94–99.

Mycobacterium avium causes disseminated infection in AIDS patients and several forms of infection in immunocompetent hosts. Recent studies have shown that *M. avium* infection of macrophages *in vitro* leads to apoptosis of significant numbers of infected cells. Several strains of *M. avium* used to infect human macrophages for 5 days (multiplicity of infection of 10) triggered 28%–46% higher levels of apoptosis than observed with uninfected macrophages at the same time points. *M. avium* strains unable to replicate intracellularly resulted in a 15% rate of apoptosis, while *M. smegmatis*-infected monolayers showed the same percentage of apoptotic cells as the uninfected macrophage control. The presence of anti-TNF-alpha antibody reduced apoptosis to 17% and the presence of anti-Fas antibody reduced apoptosis to 10%. When both antibodies were used together, the apoptosis level was 5% above the control. Treatment with TGF-beta also reduced the number of apoptotic cells in infected monolayers. If intracellular growth was inhibited, apoptosis of macrophages decreased significantly. It was also shown that apoptosis was associated with IL-1 beta-converting enzyme (ICE) activation and was significantly reduced by a caspase inhibitor. Gaining understanding of the mechanisms of *M. avium*-associated apoptosis of macrophages will provide important insight into *M. avium* pathogenesis.—Authors' Abstract

Boney, M., Bouchonnet, F., Pelicic, V., Lagier, B., Grandsaigne, M., Lecossier, D., Grodet, A., Vokurka, M., Gicquel, B. and Hance, A. J. Effect of stimulation of human macrophages on intracellular survival of *Mycobacterium bovis* bacillus Calmette-Guerin—evaluation with a mycobacterial reporter strain. Am. J. Respir. Crit. Care Med. **159** (1999) 1629–1637.

The mechanisms through which immune and inflammatory responses stimulate the expression of antimycobacterial activity by human macrophages remain poorly defined. To study this question, we developed a method permitting the rapid quantification

of viable mycobacteria, based on the detection of luciferase activity expressed by a *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) reporter strain, and used this approach to evaluate mycobacterial survival in human monocyte-derived macrophages following stimulation with cytokines and through crosslinking of costimulatory molecules expressed on the cell surface. Modest proliferation, followed by persistence of mycobacteria, was observed in unpretreated macrophages as assessed both by measurement of luciferase activity and by the evaluation of colony forming units. Of the 19 cytokines tested, only granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) were found to improve the mycobactericidal activity of monocyte-derived macrophages. In both cases, this effect was observed only when macrophages were pretreated with the cytokines prior to infection. In contrast, pretreatment of human macrophages with interferon-gamma, either alone or in combination with other mediators (including tumor necrosis factor-alpha and 1,25[OH]^2-vitamin D-3), did not improve mycobacterial killing. The stimulation of macrophages through several different costimulatory molecules known to participate in macrophage-lymphocyte interactions (CD4, CD40, CD45, CD86, CD95 [Fas/Apo-1]) also failed to improve mycobactericidal activity. This study shows that GM-CSF and IL-3, cytokines whose receptors are known to share a common subunit and to use common second messengers, may contribute to the stimulation of mycobactericidal activity in humans. The ability to rapidly screen the effects of different macrophage stimuli on mycobacterial survival through the detection of luciferase activity should help define additional signals required for optimal antimycobacterial responses.—Authors' Abstract

Borelli, V., Banfi, E., Perrotta, M. G. and Zabucchi, G. Myeloperoxidase exerts microbicidal activity against *Mycobacterium tuberculosis.* Infect. Immun. **67** (1999) 4149–4152.

We investigated the antimycobacterial role of myeloperoxidase (MPO), one of the most abundant granule proteins in human neutrophils. Our data indicate that purified MPO, in the presence of hydrogen peroxide, exerts a consistent killing activity against *Mycobacterium tuberculosis* H37Rv and against a clinical isolate. The activity is time and dose dependent and requires the presence of chloride ions in the assay medium.—Authors' Abstract

Caldarelli Stefano, R., Vago, L., Bonetto, S., Nebuloni, M. and Costanzi, G. Use of magnetic beads for tissue DNA extraction and IS6110 *Mycobacterium tuberculosis* PCR. J. Clin. Pathol. Mol. Pathol. **52** (1999) 158–160.

Polymerase chain reaction (PCR) techniques are used increasingly for the diagnosis of *Mycobacterium tuberculosis* infection and can be used on the DNA obtained from both frozen and formalin-fixed, paraffin wax-embedded tissues. However, the extraction of DNA by means of the conventional phenol/chloroform method is time consuming and requires the use of potentially dangerous chemical reagents. This paper describes a method based upon the use of magnetic beads for the extraction of *M. tuberculosis* DNA from both routinely formalin-fixed, paraffin wax-embedded tissues and frozen tissues. Magnetic bead extracted DNA from brain, lymph node, and lung tissues collected from patients with human immunodeficiency virus and tuberculosis was compared with that extracted using the phenol/chloroform method. The magnetic bead extraction procedure requires less than 2 hours, including the time necessary to dewax the tissue sections. In all cases, the DNA extracted with both methods was amplified successfully by PCR for the *M. tuberculosis* IS6110 sequence. Magnetic bead DNA extraction can be used on both frozen and archival tissues: the method is reliable, simple, sensitive, and rapid; in addition, it does not use hazardous procedures or specialized laboratory equipment and can be used for routine DNA isolation from various human tissues.—Authors' Abstract

Chensue, S. W., Warmington, K. S., Allenspach, E. J., Lu, B., Gerard, C., Kunkel, S. L. and Lukacs, N. W. Differ-

ential expression and cross-regulatory function of RANTES during mycobacterial (Type 1) and schistosomal (Type 2) antigen-elicited granulomatous inflammation. J. Immunol. **163** (1999) 165–173.

The role of RANTES in Th1 and Th2 cell-mediated immune responses has been enigmatic. To approach this question, we analyzed RANTES expression and function in murine models of types 1 and 2 cell-mediated pulmonary granulomas elicited with *Mycobacterium bovis* or *Schistosoma mansoni* egg Ag-coated beads, respectively. Compared with type 2, type 1 lesions had up to 4-fold greater RANTES protein and mRNA production. Type 1 draining lymph nodes also produced up to 7-fold higher levels of RANTES. Anti-RANTES Ab treatments had opposite effects, decreasing type 1 lesion area by 25% and augmenting type 2 lesions by 50%. The latter was associated with increased IL-4, IL-5, IL-10, and IL-13 production by lymph nodes. Infusion of rRANTES (1 mg/kg/day) did not affect type 1 lesions, but reduced type 2 lesion area by 27% and eosinophils by 40%. Lymph node cultures from RANTES-treated mice had augmented type 1 and impaired type 2 responses. *In vitro,* RANTES caused selective, dose-related inhibition of IL-4 that was largely dependent on CCR1 receptors. In conclusion, RANTES plays different roles in types 1 and 2 granuloma formation, promoting the former and mediating cross-regulatory inhibition of the latter. Moreover, RANTES may have therapeutic potential in the treatment of established type 2 hypersensitivity.—Authors' Abstract

Corral, L. G., Haslett, P. A. J., Muller, G. W., Chen, R., Wong, L. M., Ocampo, C. J., Patterson, R. T., Stirling, D. I. and Kaplan, G. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. J. Immunol. **163** (1999) 380–386.

TNF-alpha mediates both protective and detrimental manifestations of the host immune response. Our previous work has shown thalidomide to be a relatively selective inhibitor of TNF-alpha production *in vivo* and *in vitro.* Additionally, we have recently reported that thalidomide exerts a costimulatory effect on T-cell responses. To develop thalidomide analogs with increased anti-TNF-alpha activity and reduced or absent toxicities, novel TNF-alpha inhibitors were designed and synthesized. When a selected group of these compounds was examined for their immunomodulatory activities, different patterns of cytokine modulation were revealed. The tested compounds segregated into two distinct classes: one class of compounds, shown to be potent phosphodiesterase 4 inhibitors, inhibited TNF-alpha production, increased IL-10 production by LPS-induced PBMC, and had little effect on T-cell activation; the other class of compounds, similar to thalidomide, were not phosphodiesterase 4 inhibitors and markedly stimulated T-cell proliferation and IL-2 and IFN-gamma production. These compounds inhibited TNF-alpha, IL-1 beta, and IL-6 and greatly increased IL-10 production by LPS-induced PBMC. Similar to thalidomide, the effect of these agents on IL-12 production was dichotomous; IL-12 was inhibited when PBMC were stimulated with LPS but increased when cells were stimulated by crosslinking the TCR. The latter effect was associated with increased T-cell CD40 ligand expression. The distinct immunomodulatory activities of these classes of thalidomide analogs may potentially allow them to be used in the clinic for the treatment of different immunopathological disorders.—Authors' Abstract

Das, G., Vohra, H., Saha, B., Agrewala, J. N. and Mishra, G. C. Apoptosis of Th1-like cells in experimental tuberculosis (TB). Clin. Exp. Immunol. **115** (1999) 324–328.

Th1 cell-induced anti-mycobacterial immunity is lost during a progressive *Mycobacterium tuberculosis* infection in a susceptible host. This study was designed to test the mechanism of the loss of anti-mycobacterial cell-mediated immune response. We demonstrate that *M. tuberculosis* infection results in increased Fas expression and decreased Bcl-2 expression in CD4+ T cells. When CD4+ T cells are stimulated *in vitro,* they show increased apoptosis and

decreased production of IL-2 and interferon-gamma but not of IL-4. These changes may result in selective apoptosis of Th1-like cells, leading to the loss of cell-mediated immune response against *M. tuberculosis.*—Authors' Abstract

Demangel, C., Bean, A. G. D., Martin, E., Feng, C. G., Kamath, A. T. and Britton, W. J. Protection against aerosol *Mycobacterium tuberculosis* infection using *Mycobacterium* bacillus Calmette Guerin-infected dendritic cells. Eur. J. Immunol. **29** (1999) 1972–1979.

In the lung, dendritic cells (DC) are key antigen-presenting cells capable of triggering specific cellular responses to inhaled pathogens and, thus, they may be important in the initiation of an early response to mycobacterial infections. The ability of DC to enhance antigen presentation to naive T cells within the lungs was characterized with respect to *Mycobacterium bovis* Bacillus Calmette Guerin (BCG) vaccination against *M. tuberculosis* infection. *In vitro*-derived DC were infected with BCG, which induced their maturation, as shown by the increased expression of MHC class II antigens, CD80 and CD86 co-stimulatory molecules. The synthesis of mRNA for IL-1, IL-6, IL-12, IL-10 and IL-1 receptor antagonists was also enhanced. When administered intratracheally in mice, infected DC induced a potent T-cell response and the production of IFN-gamma to mycobacterial antigens in the mediastinal lymph nodes, leading to a significant protection against aerosol *M. tuberculosis* infection. Intriguingly, although the vaccination schedule for BCG-infected DC was much shorter than subcutaneous BCG vaccination (7 days as compared to 100 days), both types of vaccination showed similar levels of protection. These data confirm that DC can be potent inducers of a cellular-immune response against mycobacteria and support the concept of combining DC strategies with mycobacterial vaccines for protective immunity against tuberculosis.—Authors' Abstract

Ehlers, S., Benini, J., Kutsch, S., Endres, R., Rietschel, E. T. and Pfeffer, K. Fatal granuloma necrosis without exacerbated mycobacterial growth in tumor necrosis factor receptor p55 gene-dependent mice intravenously infected with *Mycobacterium avium.* Infect. Immun. **67** (1999) 3571–3579.

The pathogenesis of mycobacterial infections is associated with the formation of granulomas in which both antibacterial protection and tissue damage take place concomitantly. We used murine *Mycobacterium avium* infection to compare the development of granulomatous lesions in intravenously infected tumor necrosis factor receptor p55 (TNFRp55) gene deficient [p55(–/–)] mice to the development of granulomatous lesions in *M. avium*-infected syngeneic C57BL/6 [p55(+/+)] mice. Up to 5 weeks after infection with either the highly virulent *M. avium* strain TMC724 or the intermediately virulent *M. avium* strain SE01, bacterial counts in the liver, spleen, and lung of p55(–/–) mice were identical to or lower than those in infected p55(+/+) mice. However, the formation of mononuclear cell foci in the liver was delayed by approximately 2 to 3 weeks in p55(–/–) mice compared to the results obtained for infected p55(+/+) mice. Despite comparable bacterial loads, granulomas in p55(–/–) mice underwent progressive necrosis, causing damage to the surrounding liver tissue. The appearance of necrotizing granulomas was associated with the death of all infected p55(–/–) mice, regardless of the virulence of the *M. avium* strain used for infection. Granulomatous lesions in the liver contained three times as many CD3+ cells in p55(–/–) mice yet appeared more diffuse than in p55(+/+) mice. Semiquantitative reverse transcription-PCR studies revealed that prior to mouse death, interleukin-12 (IL-12) and gamma interferon mRNA levels were upregulated in the livers of infected p55(–/–) mice, while mRNA levels for tumor necrosis factor, the inducible isoform of nitric-oxide synthase (iNOS), and IL-10 were similar to those found in infected p55(+/+) mice. In response to persistent mycobacterial infection, the absence of TNFRp55 causes the disregulation of T cell-macrophage interactions and results in fatal granuloma necrosis even when adequate antibacterial functions are maintained.—Authors' Abstract

Feng, C. G., Bean, A. G. D., Hooi, H., Briscoe, H. and Britton, W. J. Increase in gamma interferon-secreting CD8+, as well as CD4+, T cells in lungs following aerosol infection with *Mycobacterium tuberculosis.* Infect. Immun. **67** (1999) 3242–3247.

Although it is well established that CD4+ T cells are required for the protective immune response against tuberculosis (TB), there is some evidence that CD8+ T cells are also involved in the host response to *Mycobacterium tuberculosis.* There is, however, a paucity of information on the pulmonary CD8+ T-cell response during infection. We therefore have compared the changes in both CD8+ and CD4+ T cells following aerosol infection with *M. tuberculosis.* There was an observed delay between the peak of infection and the activated T-cell response in the lung. The kinetics of CD8+ and CD4+ T-cell responses in the lung were identical, both peaking at week 8, 4 weeks later than the peak of cellular response in draining lymph nodes. Similar changes in activation/memory phenotypes occurred on the pulmonary CD8+ and CD4+ T cells. Following *in vitro* restimulation, both subsets synthesized gamma interferon, a cytokine essential for controlling *M. tuberculosis* infection. Since lung CD8+ T cells are actively expanded during aerosol *M. tuberculosis* infection, it is important that both CD8+ and CD4+ T cells be targeted in the design of future TB vaccines.—Authors' Abstract

Ferrari, G., Langen, H., Naito, M. and Pieters, J. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. Cell **97** (1999) 435–437.

Mycobacteria are intracellular pathogens that can survive within macrophage phagosomes, thereby evading host defense strategies by largely unknown mechanisms. We have identified a WD repeat host protein that was recruited to and actively retained on phagosomes by living, but not dead, mycobacteria. This protein, termed TACO, represents a component of the phagosome coat that is normally released prior to phagosome fusion with or maturation into lysosomes. In macrophages lacking TACO, mycobacteria were readily transported to lysosomes followed by their degradation. Expression of TACO in nonmacrophages prevented lysosomal delivery of mycobacteria and prolonged their intracellular survival. Active retention of TACO on phagosomes by living mycobacteria thus represents a mechanism preventing cargo delivery to lysosomes, allowing mycobacteria to survive within macrophages.—Authors' Abstract

Florido, M., Goncalves, A. S., Silva, R. A., Ehlers, S., Cooper, A. M. and Appelberg, R. Resistance of virulent *Mycobacterium avium* to gamma interferon-mediated antimicrobial activity suggests additional signals for inductin of mycobacteriostasis. Infect. Immun. **67** (1999) 3610–3618.

The cytokine gamma interferon (IFN-γ) plays a major role in the control of *Mycobacterium avium* infections. We assessed whether the progressive growth of virulent strains of *M. avium* was associated with alterations in the production of this cytokine as evaluated by reverse transcription-PCR and detection of immunoreactive cytokine in the serum and in spleen homogenates. We found that IFN-γ was induced during infection by a virulent strain of *M. avium* to similar or even higher extents than the levels found during infections by a less virulent strain whose growth was controlled. IFN-γ produced during infection by both mycobacterial strains was partly derived from T cells and led to activation of macrophages, namely, those that were infected. Concomitant with the development of the infection with the virulent strain of *M. avium* there was an extensive depletion of lymphocytes in the spleen. Thymectomy alone promoted the proliferation of the virulent, but not of the less virulent, strain of *M. avium.* Our data indicate that virulent strains of *M. avium* resist the antimicrobial mechanisms of IFN-γ-activated macrophages and raise the possibility that a second, T cell-dependent signal is required for the effective control of mycobacterial replication inside macrophages.—Authors' Abstract

Gomes, M. S., Florido, M., Pais, T. F. and Appelberg, R. Improved clearance of *Mycobacterium avium* upon disruption of the inducible nitric oxide synthase gene. J. Immunol. **162** (1999) 6734–6739.

Mice genetically deficient in the inducible NO synthase gene (iNOS–/–) were used to study the role played by NO during infection by *Mycobacterium avium.* INOS–/– macrophages were equally able to restrict *M. avium* growth *in vitro* following stimulation by IFN-gamma and TNF-alpha as macrophages from wild-type mice. *In vivo,* the infection progressed at similar rates in wild-type and NO-deficient mice during the first 2 mos. of infection, but the latter mice were subsequently more efficient in clearing the mycobacteria than the former. The increased resistance of iNOS–/– mice was associated with higher IFN-gamma levels in the serum and following *in vitro* restimulation of spleen cells with specific antigen, increased formation of granulomas and increased survival of CD4+ T cells. We show that NO is not involved in the antimycobacterial mechanisms of *M. avium*-infected macrophages and, furthermore, that it exacerbates the infection by causing the suppression of the immune response to the pathogen.—Authors' Abstract

Gomes, M. S., Paul, S., Moreira, A. L., Appelberg, R., Rabinovitch, M. and Kaplan, G. Survival of *Mycobacterium avium* and *Mycobacterium tuberculosis* in acidified vacuoles of murine macrophages. Infect. Immun. **67** (1999) 3199–3206.

Despite the antimicrobial mechanisms of vertebrate phagocytes, mycobacteria can survive within the phagosomes of these cells. These organisms use various strategies to evade destruction, including inhibition of acidification of the phagosome and inhibition of phagosome-lysosome fusion. In contrast to mycobacteria, *Coxiella burnetii,* the etiologic agent of Q fever, inhabits a spacious acidified intracellular vacuole which is prone to fusion with other vacuoles of the host cell, including phagosomes containing mycobacteria. The *Coxiella*-infected cell thus provides a unique model for investigating the survival of mycobacteria in an acidified phagosome-like compartment. In the present study, murine bone marrow-derived macrophages were infected with either *Mycobacterium avium* or *M. tuberculosis* and then coinfected with *C. burnetii.* We observed that the majority of phagocytosed mycobacteria colocalized to the *C. burnetii*-containing vacuole, which maintained its acidic properties. In coinfected macrophages, the growth of *M. avium* was not impaired following fusion with the acidified vacuole. In contrast, the growth rate of *M. tuberculosis* was reduced in acidified vacuoles. These results suggest that although both species of mycobacteria inhibit phagosome-lysosome fusion, they may be differentially susceptible to the toxic effects of the acidic environment in the mature phagolysosome.—Authors' Abstract

Hamasur, B., Kallenius, G. and Svenson, S. B. Synthesis and immunologic characterisation of *Mycobacterium tuberculosis* lipoarabinomannan specific oligosaccharide-protein conjugates. Vaccine **17** (1999) 2853–2861.

Lipoarabinomannan (LAM) is a major structural surface component of all mycobacteria, and has been reported to have a wide range of biological effects. Immunogenic LAM-specific oligosaccharide protein conjugates were synthesized and immunologically characterized. Oligosaccharides were derived from LAM purified from *Mycobacterium tuberculosis* H37Rv and covalently conjugated to tetanus toxoid and crossreactive mutant (CRM197) diphtheria toxoid. Both types of LAM-oligosaccharide protein conjugates proved to be highly immunogenic, inducing a boosterable T-helper cell dependent-IgG response. These conjugates are currently evaluated as components in a subcellular experimental tuberculosis vaccine.—Authors' Abstract

Hamerlinck, F. F. V., Klatser, P. R., Walsh, D. S., Bos, J. D., Walsh, G. P. and Faber, W. R. Serum neopterin as a marker for reactional states in leprosy. FEMS Immunol. Med. Microbiol. **24** (1999) 405–409.

Reactions, a relatively common phenomenon among leprosy patients in treatment, require early detection and proper management to prevent serious sequelae. It is generally accepted that reactional states are immunologically mediated and, as such, usually improve with immunomodulatory treatments such as corticosteroids or thalidomide. Neopterin, a product of gamma-interferon-activated macrophages, is a marker for cell-mediated immune activation and may be useful to detect reactional states in leprosy. Here, we compared neopterin levels in single serum samples from leprosy patients with and without reaction with untreated controls and, when available, serial samples among patients with and without reaction. Levels in the single sample measurements, conducted in 22 patients with a reversal reaction (mean 14.5 nmol l^{-1}, S.D. 8.7) and 13 with erythema nodosum leprosum (mean 16.9 nmol l^{-1}, S.D. 13.6), were significantly higher ($p = 0.02$ and $p = 0.001$, respectively) than levels in 26 untreated patients (mean 9.1 nmol l^{-1}, S.D. 7.3). Values above the upper limit of normal (10 nmol l^{-1}) were found in 7 of 26 untreated patients, 14 of the 22 reversal reaction patients ($p = 0.01$) and 10 of the 13 ENL patients ($p = 0.003$). Serial serum samples, obtained from 6 patients who developed reactions and 14 who remained free of reaction, indicated that reversal reaction or erythema nodosum leprosum paralleled a concomitant increase in the serum neopterin level. Neopterin levels generally declined upon corticosteroid therapy. Neopterin may be a useful marker for reactional states in leprosy by providing a laboratory parameter to asses the onset, progression, response to therapy and resolution. — Authors' Abstract

Jouanguy, E., Doffinger, R., Dupuis, S., Pallier, A., Altare, F. and Casanova, J. L. IL-12 and IFN-gamma in host defense against mycobacteria and salmonella in mice and men. Curr. Opin. Immunol. **11** (1999) 346–351.

The development of gene-knockout mice and the identification of gene-deficient humans have improved our understanding of the role of IL-12 and IFN-gamma in host defense. Comparison of experimental and natural infections has shown that animals and humans genetically deficient in immunity mediated by IL-12 or IFN-gamma are highly susceptible to mycobacteria and salmonella. Impaired secretion of, or response to, IFN-gamma is the common pathogenic mechanism that accounts for impaired granuloma formation and uncontrolled growth of bacteria within macrophages. The axis formed between IL-12 and IFN-gamma is essential for protective immunity against mycobacteria and salmonella in mice and men.—Authors' Abstract

Juffermans, N. P., Verbon, A., van Deventer, S. J. H., van Deutekom, H., Belisle, J. T., Ellis, M. E., Speelman, P. and van der Poll, T. Elevated chemokine concentrations in sera of human immunodeficiency virus (HIV)-seropositive and HIV-seronegative patients with tuberculosis: a possible role for mycobacterial lipoarabinomannan. Infect. Immun. **67** (1999) 4295–4297.

Levels of interleukin 8 (IL-8), gamma interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein 1 beta (MIP-1 beta) were elevated in patients with tuberculosis. IP-10 and MCP-1 levels were higher in human immunodeficiency virus (HIV)-seropositive patients than in HIV-seronegative patients with tuberculosis. Lipoarabinomannan induced IL-8, MCP-1, and MIP-1 beta *in vitro,* which was partly inhibited by antitumor necrosis factor antibody.—Authors' Abstract

Levin, M. and Newport, M. Understanding the genetic basis of susceptibility to mycobacterial infection. Proc. Assoc. Am. Phys. **111** (1999) 308–312.

Genetic factors have long been suspected of determining susceptibility and resistance to mycobacterial infection. The recent identification of families with a unique susceptibility to mycobacterial infection, and the identification of mutations in the genes for either the interferon-gamma (IFN-γ) receptor or the interleukin (IL)-12 receptor as the cause of the defect, has provided an important clue to the pathways critical for resistance to mycobacterial infection in humans.

Although the genetically determined absence of key cytokines or their receptors results in susceptibility to lethal mycobacterial infections in early childhood, it is likely that more subtle mutations that result in only partial dysfunction of macrophage upregulation pathways may play a role in susceptibility to tuberculosis and leprosy in the general population.—Authors' Abstract

Logani, S., Lucas, D. R., Cheng, J. D., Ioachim, H. L. and Adsay, N. V. Spindle cell tumors associated with mycobacteria in lymph nodes of HIV-positive patients "Kaposi sarcoma with mycobacteria" and "mycobacterial pseudotumor." Am. J. Surg. Pathol. **23** (1999) 656–661.

Patients infected with HIV often have unusual manifestations of common infections and neoplasms. One such example is "mycobacterial pseudotumor," an exuberant spindle-cell lesion induced in lymph nodes by mycobacteria. Kaposi sarcoma also produces a spindle-cell proliferation in lymph nodes of HIV-positive patients. These two entities must be differentiated from one another because of differences in treatment and prognosis. We report here, however, three cases of intranodal Kaposi sarcoma with simultaneous mycobacterial infection, the occurrence of which has not been clearly documented. For comparison, we also studied three cases of mycobacterial pseudotumor, of which 14 cases have been described to date. There was considerable histologic overlap between these two lesions. Acid-fast bacilli were present in all cases, predominantly in the more epithelioid histiocytes in the cases of Kaposi sarcoma, and in spindle and epithelioid cells in the cases of mycobacterial pseudotumor. The morphologic features that favored Kaposi sarcoma over mycobacterial pseudotumor were the prominent fascicular arrangement of spindle cells and slit-like spaces, the lack of granular, acidophilic cytoplasm, and the presence of mitoses. Immunohistochemistry was a reliable adjunct study in the differential diagnosis, since the spindle cells in mycobacterial pseudotumor were positive for S-100 protein and CD68; whereas those of Kaposi sarcoma were CD31- and CD34-positive but negative for S-100 protein and CD68. Awareness that Kaposi sarcoma may coexist with mycobacterial infection in the same biopsy specimen is important because these lesions may be misdiagnosed as mycobacterial pseudotumor. The clinical impact of distinguishing between Kaposi sarcoma with mycobacteria and mycobacterial pseudotumor is significant because the presence of Kaposi sarcoma alters treatment and prognosis.—Authors' Abstract

Maes, H. H., Causse, J. E. and Maes, R. F. Tuberculosis I: a conceptual frame for the immunopathology of the disease. Med. Hypotheses **52** (1999) 583–593.

An analysis of the cellular and humoral immune responses after bacille Calmette-Guerin (BCG) vaccination and during tuberculosis treatment favors the hypothesis of an immune defense developed in four overlapping successive stages. The initial immune response is innate. The following two intermingle innate and specific responses against low molecular weight oligopeptidic and nonpetidic antigens, such as muramyldipeptide and trehalose dimycolate, and large molecular weight nonpeptidic antigens such as lipoarabinomannan. The ultimate specific response is directed against protein antigens as Antigen 60.

BCG and primary tuberculosis (TB) infections induce cellular and humoral immune responses essentially against oligopeptidic and small and large molecular weight nonpeptidic antigens. Immune responses against nonpeptidic substances contribute to the immunoprotection of the infected person who develops a primary infection. Some infected people allow the expression of the immunosuppressive activity of the pathogen. This results in the synthesis of interleukin-10 (IL-10), which suppresses the formation of interferon-gamma (INF-γ) and IL-2, and of IL-6, which suppresses T-cell responses. These patients have a skewed immune response against nonpeptidic antigens and present with symptoms. They will not recover unless responses directed against proteinic antigens occur, which restore INF-γ and IL-2 production. The formation of immunoglobulin-G

(IgG)-type antibodies and of a cellular immunity against mycobacterial peptidic antigens is essential for a good protection against a post-primary infection.—Authors' Abstract

Mustafa, A. S. *M. leprae* recombinant antigens important for T-cell reactivity. Indian J. Lepr. **71** (1999) 75–86.

Identification of *Mycobacterium leprae* antigens recognized by T cells is important for specific diagnosis, vaccine development and understanding the basic mechanisms involved in protection against and pathogenesis of leprosy. Screening of an *M. leprae* recombinant DNA library with antibody probes led to the identification of half a dozen *M. leprae* antigens recognized by B cells. When tested for T-cell reactivity, all the antigens recognized by antibodies were shown to have T-cell reactivity. However, among these antigens 18-kDa, 65-kDa and 70-kDa heat shock proteins (hsp) were most frequently recognized by T-cell lines and clones established from healthy donors vaccinated with killed *M. leprae*. A 24-kDa secreted antigen of *M. leprae* with a T-cell epitope specific for *M. leprae* and *M. tuberculosis* complex was identified by direct screening of the recombinant DNA library with T-cell clones. The recombinant T-cell antigens of *M. leprae* were recognized by memory T cells of Th1 type in association with multiple HLA-DR molecules. Epitope mapping with synthetic peptides identified *M. leprae*-specific as well as crossreactive T-cell epitopes on the 18-kDa, 65-kDa and 70-kDa hsp antigens. In conclusions, our studies suggest that the recombinant antigens of *M. leprae* could be useful as reagents for specific diagnosis as well as in subunit and recombinant vaccine design against leprosy.—Author's Abstract

Nabeshima, S., Nomoto, M., Matsuzaki, G., Kishihara, K., Taniguchi, H., Yoshida, S. I. and Nomoto, K. T-cell hyporesponsiveness induced by activated macrophages through nitric oxide production in mice infected with *Mycobacterium tuberculosis*. Infect. Immun. **67** (1999) 3221–3226.

In active tuberculosis, T-cell response to *Mycobacterium tuberculosis* is known to be reduced. In the course of *M. tuberculosis* infection in mice, we observed that T-cell proliferation in response to *M. tuberculosis* purified protein derivative (PPD) reached the maximum level on day 7, then declined to the minimal level on day 14, and persisted at a low level through day 28 postinfection. The frequency of PPD-specific CD4 T cells in the spleen on day 28 decreased to one sixth on day 7. To further investigate the mechanism of this T-cell hyporesponsiveness, we next analyzed the suppressive activity of spleen macrophages on T-cell function. The nonspecific proliferative response of naive T cells and the PPD-specific proliferative response of T cells were suppressed by day 28 macrophages, but not by day 7 macrophages or naive macrophages. This reduction of proliferative response was restored by the addition of the nitric oxide synthesis inhibitor, N-G-monoethyl-L-arginine monoacetate, but not by monoclonal antibody against interleukin-10 or transforming growth factor beta. These data indicate that the macrophages from mice chronically infected with *M. tuberculosis* suppress T-cell response through production of nitric oxide, suggesting that nitric oxide induced elimination mediated by activated macrophages may reduce the T-cell response and the number of mycobacterium-specific CD4 T cells *in vivo*.—Authors' Abstract

Nau, G. J., Liaw, L., Chupp, G. L., Berman, J. S., Hogan, B. L. M. and Young, R. A. Attenuated host resistance against *Mycobacterium bovis* BCG infection in mice lacking osteopontin. Infect. Immun. **67** (1999) 4223–4230.

Expression of the cytokine osteopontin (OPN) is elevated in granulomas caused by *Mycobacterium tuberculosis*. We tested the hypothesis that OPN contributes to host protection in a mouse model of mycobacterial infection. When infected with *M. bovis* BCG, mice lacking a functional OPN gene had more severe infections characterized by heavier bacterial loads and a delayed clearance of the bacteria. The OPN-null mice had greater granuloma burdens consistent

with the elevated bacterial load. The ability of osteopontin to facilitate the clearance of mycobacteria was most pronounced early after infection and appeared to be independent of known mediators of resistance to infection by mycobacteria: antigen-specific T-cell immunity, gamma-interferon production, and nitric oxide production. BCG grew more rapidly in macrophages derived from OPN-null mice than in those from wild-type mice, demonstrating that the null phenotype was due to an intrinsic macrophage defect. These results indicate that osteopontin augments the host response against a mycobacterial infection and that it acts independently from other antimycobacterial resistance mechanisms.—Authors' Abstract

Oftung, F., Lundin, K. E. A., Meloen, R. and Mustafa, A. S. Human T cell recognition of the *Mycobacterium leprae* LSR antigen: epitopes and HLA restriction. FEMS Immunol. Med. Microbiol. **24** (1999) 151–159.

We have in this work mapped epitopes and HLA molecules used in human T-cell recognition of the *Mycobacterium leprae* LSR protein antigen. HLA typed healthy subjects immunized with heat-killed *M. leprae* were used as donors to establish antigen-reactive CD4+ T-cell lines which were screened for proliferative responses against overlapping synthetic peptides covering the C-terminal part of the antigen sequence. By using this approach we were able to identify two epitope regions represented by peptide 2 (aa 29–40) and peptide 6 (aa 49–60), of which the former was mapped in detail by defining the N- and C-terminal amino-acid positions necessary for T-cell recognition of the core epitope. MHC restriction analysis showed that peptide 2 was presented to T cells by allogeneic cells coexpressing HLA-DR4 and DRw53 or DR7 and DRw53. In contrast, peptide 6 was presented to T cells only in the context of HLA-DR5 molecules. In conclusion, the *M. leprae* LSR protein antigen can be recognized by human T cells in the context of multiple HLA-DR molecules, of which none are reported to be associated with the susceptibility to develop leprosy. The results obtained are in support of using the LSR antigen in subunit vaccine design.—Authors' Abstract

Ruan, J., St. John, G., Ehrt, S., Riley, L. and Nathan, C. noxR3, a novel gene from *Mycobacterium tuberculosis,* protects *Salmonella typhimurium* from nitrosative and oxidative stress. Infect. Immun. **67** (1999) 3276–3283.

Reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) produced by activated macrophages participate in host defense against the facultative intracellular pathogens *Mycobacterium tuberculosis* and *Salmonella typhimurium.* To survive within macrophages, such pathogens may have evolved ROI and RNI resistance mechanisms. ROI resistance pathways have been intensively studied. Much less is known about the mechanisms of resistance to RNI. To identify possible RNI resistance genes in *M. tuberculosis,* a mycobacterial library was expressed in *S. typhimurium* and subjected to selection by exposure to the NO donor S-nitrosoglutathione (GSNO) in concentrations sufficient to kill the vast majority of nontransformed salmonellae. Among the rare surviving recombinants was a clone expressing noxR3, a novel and previously anonymous *M. tuberculosis* gene predicted to encode a small, basic protein. Expression of noxR3 protected *S. typhimurium* not only from GSNO and acidified nitrite but also from H_2O_2. NoxR3 is the third gene cloned from *M. tuberculosis* that has been shown to protect heterologous cells from both RNI and ROI. This suggests diversity in the repertoire of mechanisms that help pathogens resist the oxidative and nitrosative defenses of the host.—Authors' Abstract

Sano, K., Haneda, K., Tamura, G. and Shirato, K. Ovalbumin (OVA) and *Mycobacterium tuberculosis* bacilli cooperatively polarize anti-OVA T-helper (Th) cells toward a Th1-dominant phenotype and ameliorate murine tracheal eosinophilia. Am. J. Respir. Cell Mol. Biol. **20** (1999) 1260–1267.

A recent increase in allergic disorders has coincided with a decrease in infections,

including tuberculosis. Although an inverse association between tuberculin responses and atopic disorders was reported, it was not known how T-helper (Th)1-biased immune responses to *Mycobacterium tuberculosis* influenced Th2-dominant responses to allergens. We examined whether *M. tuberculosis* could modulate ovalbumin (OVA)-induced eosinophilic inflammation in the murine trachea in a manner that transcended the barrier of antigen specificity. We found that CD4+ T cells primed with OVA in complete Freund's adjuvant (CFA) inhibited OVA-induced tracheal eosinophilia through interferon gamma secretion. Immunization with an irrelevant antigen in CFA or with OVA in incomplete Freund's adjuvant failed to induce suppressor cells. *In vitro* experiments confirmed that both *M. tuberculosis* and OVA (as opposed to either one alone) were necessary to evoke polarized development toward a Th1-like phenotype through interleukin-12 secretion. These results indicate that exposure to an allergen along with *M. tuberculosis* switches development of allergen-specific T cells toward a Th1 phenotype which, in turn, downregulates allergic manifestations in an antigen-specific manner. The possible implications of these results are discussed in the context of the causal relationship between a decrease in tuberculosis and an increase in allergic disorders.—Authors' Abstract

Sciorati, C., Rovere, P., Ferrarini, M., Paolucci, C., Heltai, S., Vaiani, R., Clementi, E. and Manfredi, A. A. Generation of nitric oxide by the inducible nitric oxide synthase protects gamma delta T cells from *Mycobacterium tuberculosis*-induced apoptosis. J. Immunol. **163** (1999) 1570–1576.

Gamma delta T cells are early recruited into mycobacterial lesions. Upon microbial antigen recognition, gamma delta cells secrete cytokines and chemokines and undergo apoptosis via CD95/CD95 ligand (CD95L) interaction, possibly influencing the outcome of infection and the characteristics of the disease. In this paper we show that activated phagocytes acquire, upon challenge with *Mycobacterium tuberculosis,* the ability to inhibit *M. tuberculosis*-induced gamma delta cell apoptosis. Apoptosis protection was due to NO because it correlated with NO synthase (NOS)-2 induction and activity in scavenger cells and was abrogated by NOS inhibitors. Furthermore, the NO donor S-nitrosoacetylpenicillamine mimicked the effect of enzyme induction. NO left unaffected the expression of CD95 and CD95L, suggesting interference with an event ensuing CD95/CD95L interaction. NO was found to interfere with the intracellular accumulation of ceramide and the activation of caspases, which were involved in gamma delta T cells apoptosis after *M. tuberculosis* recognition. We propose that NO generated by infected macrophages determines the life span and, therefore, the function of lymphocytes at the infection site, thus linking innate and adaptive immunity.—Authors' Abstract

Serbina, N. V. and Flynn, J. L. Early emergence of CD8+ T cells primed for production of type 1 cytokines in the lungs of *Mycobacterium tuberculosis*-infected mice. Infect. Immun. **67** (1999) 3980–3988.

Several lines of evidence suggest that CD8 T cells are important in protection against tuberculosis. To understand the function of this cell population in the immune response against *Mycobacterium tuberculosis,* T cells from lungs of *M. tuberculosis*-infected mice were examined by flow cytometry. The kinetics of the appearance of CD8 T cells in lungs of infected mice closely paralleled that of CD4 T cells. Both CD4+ and CD8+ T cells displaying an activated phenotype were found in the lungs as early as 1 week postinfection. By 2 weeks, total cell numbers in the lungs had tripled and percentages of T cells were increased two- to threefold; the percentages of CD4+ T cells were ca. twofold higher than those of CD8+ T cells. Short-term stimulation with *M. tuberculosis*-infected antigen-presenting cells induced cytokine production by primed CD4+ and CD8+ T cells. Intracellular cytkokine staining revealed that 30% ± 5% of CD4+ and 23% ± 4% of CD8+ T cells were primed for production of gamma interferon (IFN-gamma).

However, a difference in *in vivo* IFN-gamma production by T cells was observed similar to 12% of CD4+ T cells and similar to 5% of CD8+ T cells secreting cytokine in the lungs at any given time during infection. The data presented indicate that although early in infection the majority of IFN-gamma is produced by CD4+ T cells, cytokine-producing CD8+ T cells are readily available when triggered by the appropriate stimuli.—Authors' Abstract

Thole, J. E. R., Janson, A. A. M., Cornelisse, Y., Schreuder, G. M. T., Wieles, B., Naafs, B., de Vries, R. R. P. and Ottenhoff, T. H. M. HLA-class II-associated control of antigen recognition by T cells in leprosy; a prominent role for the 30/31-kDa antigens. J. Immunol. **162** (1999) 6912–6918.

The recognition of 16 mycobacterial antigens (Ag) by a panel of T-cell lines from leprosy patients and healthy exposed individuals from an endemic population was examined within the context of expressed HLA-DR molecules. Although overall no significant differences were found between the frequencies of Ag recognition in the different subject groups, when Ag-specific T-cell responses were examined within the context of HLA-DR, a highly significant difference was found in the recognition of the 30/31-kDa Ag. HLA-DR3 appeared to be associated with high T-cell responsiveness to the 30/31-kDa Ag in healthy contacts ($p = 0.01$) but, conversely, with low T-cell responsiveness to this Ag in tuberculoid patients ($p = 0.005$). Within the group of HLA-DR3-positive individuals, differences in 30/31-kDa directed T-cell responsiveness were highly significant not only between healthy individuals and tuberculoid patients ($p < 0.0001$), but also between healthy individuals and lepromatous patients ($p = 0.009$) and, consequently, between healthy individuals compared with leprosy patients as a group ($p < 0.0001$). A dominant HLA-DR3-restricted epitope was recognized by healthy contacts in this population. It has been proposed that secreted Ags may dominate acquired immunity early in infection. The low T-cell response to the secreted, immunodominant 30/31-kDa Ag in HLA-DR3-positive leprosy patients in this population may result in retarded macrophage activation and delayed bacillary clearance which, in turn, may lead to an enhanced Ag load followed by T cell-mediated immunopathology.—Authors' Abstract

Verhagen, C., Faber, W., Klatser, P., Buffing, A., Naafs, B. and Das, P. Immunohistological analysis of *in situ* expression of mycobacterial antigens in skin lesions of leprosy patients across the histopathological spectrum—association of mycobacterial lipoarabinomannan (LAM) and *Mycobacterium leprae* phenolic glycolipid-I (PGL-I) with leprosy reactions. Am. J. Pathol. **154** (1999) 1793–1804.

The presence of mycobacterial antigens in leprosy skin lesions was studied by immunohistological methods using monoclonal antibodies (MAbs) to *Mycobacterium leprae*-specific phenolic glycolipid I (PGL-I) and to crossreactive mycobacterial antigens of 36 kD, 65 kD, and lipoarabinomannan (LAM). The staining patterns with MAb to 36 kD and 65 kD were heterogeneous and were also seen in the lesions of other skin diseases. The *in situ* staining of PGL-I and LAM was seen only in leprosy. Both antigens were abundantly present in infiltrating macrophages in the lesions of untreated multibacillary (MB) patients; whereas only PGL-I was occasionally seen in scattered macrophages in untreated paucibacillary lesions. During treatment, clearance of PGL-I from granulomas in MB lesions occurred before that of LAM, although the former persisted in scattered macrophages in some treated patients. This persistence of PGL-I in the lesions paralleled high serum anti-PGL-I antibody titers but was not indicative for the presence of viable bacilli in the lesions. Interestingly, we also observed a differential expression pattern of PGL-I and LAM in the lesions of MB patients with reactions during the course of the disease as compared with those without reactions. In conclusion, the *in situ* expression pattern of PGL-I and LAM in MB patients may assist in early di-

agnosis of reactions versus relapse.—Authors' Abstract

Weir, R. E., Brennan, P. J., Butlin, C. R. and Dockrell, H. M. Use of a whole blood assay to evaluate *in vitro* T cell responses to new leprosy skin test antigens in leprosy patients and healthy subjects. Clin. Exp. Immunol. **116** (1999) 263–269.

Development of an immunological tool to detect infection with *Mycobacterium leprae* would greatly benefit leprosy control programs, as demonstrated by the contribution of the tuberculin test to tuberculosis control. In a new approach to develop a "tuberculin-like" reagent for use in leprosy, two new fractions of *M. leprae* depleted of crossreactive and immunomodulatory lipids—MLSA-LAM (cytosol-derived) and MLCwA (cell wall-derived)—have been produced in a form suitable for use as skin-test reagents. T-cell responses [interferon-gamma (IFN-γ) and lymphoproliferation] to these two new fractions were evaluated in a leprosy-endemic area of Nepal using a simple *in vitro* whole blood test. The two fractions were shown to be highly potent T-cell antigens in subjects exposed to *M. leprae*—paucibacillary leprosy patients and household contacts. Responses to the fractions decreased toward the lepromatous pole of leprosy. Endemic control subjects also showed high responses to the fractions, indicating high exposure to *M. leprae,* or crossreactive mycobacterial antigens, in this Nepali population. The new fractions, depleted of lipids and lipoarabinomannan (LAM), gave enhanced responses compared with a standard *M. leprae* sonicate. The cell wall fraction appeared a more potent antigen than the cytosol fraction, which may be due to the predominance of the 65-kD GroEL antigen in the cell wall. The whole blood assay proved a robust field tool and a useful way of evaluating such reagents prior to clinical trials.—Authors' Abstract

Microbiology

Cobb, A. J. and Frothingham, R. The GroEs antigens of *Mycobacterium avium* and *Mycobacterium paratuberculosis.* Vet. Microbiol. **67** (1999) 31–35.

The GroES antigen provokes a strong immune response in human beings with tuberculosis or leprosy. We cloned and sequenced the *Mycobacterium avium* and *M. paratuberculosis* GroES genes. *M. avium* and *M. paratuberculosis* have identical GroES sequences which differ from other mycobacterial species. This supports the current formal designation of *M. paratuberculosis* as *M. avium* subsp. *paratuberculosis.* Immunodominant epitopes from *M. tuberculosis* GroES are conserved in *M. avium,* but some *M. leprae* epitopes are distinct. GroES is unlikely to be specific as a serologic or skin test reagent, but may be an appropriate component of a broad mycobacterial vaccine.—Authors' Abstract

Dhople, A. M. *In vitro* activities of phenothiazine-type calmodulin antagonists against *Mycobacterium leprae.* Microbios **98** (1999) 113–121.

Calmodulin-like protein has been established as the primary receptor for calcium in eukaryotic as well as prokaryotic cells. The calmodulin-calcium complex regulates a variety of enzymes including nucleotide phosphodiesterase. Recently, the presence of this protein in *Mycobacterium leprae* has been demonstrated and the effects of phenothiazine-type calmodulin antagonists on *in vitro* growth of *M. leprae* in a cell-free culture system were investigated. Two biochemical parameters were used to measure metabolic activity and growth of the organism. Among the six phenothiazine derivatives tested, trifluoperazine appeared to be the most potent in inhibiting the *in vitro* growth of *M. leprae,* with an MIC of 10

μg/ml. Chlorpromazine, triflupromazine and thioridazine were less active than trifluoperazine, with an MIC of 20 μg/ml each, while the other two, acetopromazine and fluphenazine, were totally ineffective even at 80 μg/ml. All four compounds inhibited the uptake of labelled acetate, glycine and thymidine by whole cells of *M. leprae.* This suggests that these phenothiazine derivatives have multiple sites of action and probably affect the synthesis of lipids, proteins and DNA.—Author's Abstract

Dong, Y., Zhao, X., Domagala, J. and Drlica, K. Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus.* Antimicrob. Agents Chemother. **43** (1999) 1756–1758.

When *Mycobacterium bovis* BCG and *Staphylococcus aureus* were plated on agar containing increasing concentrations of fluoroquinolone, colony numbers exhibited a sharp drop, followed by a plateau and a second sharp drop. The plateau region correlated with the presence of first-step resistant mutants. Mutants were not recovered at concentrations above those required for the second sharp drop, thereby defining a mutant prevention concentration (MPC). A C-8-methoxy group lowered the MPC for an *N*-1-cyclopropyl fluoroquinolone.—Authors' Abstract

Dunzendorfer, S., Herold, M. and Wiedermann, C. J. Inducer-specific bidirectional regulation of endothelial interleukin-8 production by thalidomide. Immunopharmacology **43** (1999) 59–64.

Interleukin-8 (IL-8) is a potent neutrophil chemotaxin, which can also be produced by endothelial cells to facilitate leukocyte emigration. The aim of this study was to determine the effects of the antiinflammatory drug thalidomide (THD) on chemotaxin release from endothelial cells. Human umbilical vein endothelial cells (HUVEC) were stimulated with tumor necrosis factor-alpha (TNF-α) or endotoxin (LPS) in the presence or absence of various concentrations of THD. Endothelium-derived interleukin-8 (eIL-8) in supernatants was measured using an enzyme-linked immunosorbent assay (ELISA) and biological activity of the harvested eIL-8 was tested in Boyden chamber chemotaxis assays on PMNL. THD Itself had no effect on eIL-8 release. Upon stimulation with TNF-α or LPS, HUVEC produced increased amounts of eIL-8 and THD affected this process in a bidirectional manner, with augmentation of TNF-α and inhibition of LPS-effects. Functionality of eIL-8 was confirmed in chemotaxis experiments and by inhibition of chemotactic effects of supernatants with anti-human IL-8 monoclonal antibodies. Results explain and emphasize immunomodulatory properties of THD in cytokine- and endotoxin-induced inflammation and regulation of transendothelial migration.—Authors' Abstract

Fiallo, P., Cardo, P. P. and Nunzi, E. Identification of sequence similarities between *Mycobacterium leprae* and the myelin proteolipid by computational analysis. Indian J. Lepr. **71** (1999) 1–10.

This study was undertaken under the assumption that antigenic mimicry plays a role in the pathogenesis of neuropathy in leprosy, a unique feature among mycobacterial diseases. The SWISS-PROT protein sequence databank was scanned using a computer program based on an identity matrix algorithm, to identify common amino-acid regions between human myelin and mycobacterial proteins. The highlighted motifs were back-tested against a database of MHC-binding peptides (MHCPEP). Of the 28 common sequences between mycobacterial and human myelin proteins, only two were found to yield some matches with MHC-presenting peptides. Both motifs were from *Mycobacterium leprae.* The myelin proteolipid protein was the human protein containing the identified similarities. We believe that this theoretical approach can provide a way to predict potentially "mimetic" motifs by searching for antigenic regions in protein sequence databases without screening a large number of synthetic peptides.—Authors' Abstract

Gillis, T. P. and Williams, D. L. Dapsone resistance does not appear to be associ-

ated with a mutation in the dihydropteroate synthase-2 gene of *Mycobacterium leprae.* Indian J. Lepr. **71** (1999) 11–18.

Evidence suggests that resistance to dapsone (DDS) in *Mycobacterium leprae* is related to the enzyme dihydropteroate synthase (DHPS). Two *M. leprae* genes (*folP-1* and *folP-2*) encoding DHPS-1 and DHPS-2, respectively, have been identified through the *M. leprae* genome project. We have studied DDS-susceptible and -resistant strains of *M. leprae* to determine whether the DDS-resistant phenotype is associated with a mutation(s) in *folP-2* and to establish the number of genomic copies of the gene encoding DHPS-2 (*folP-2*). RFLP analysis of genomic DNA from DDS-susceptible and -resistant strains of *M. leprae* exhibited a unique 4.2 kb restriction fragment consistent with a single genomic copy of *folP-2* in both phenotypes. DNA encoding *folP-2* was amplified by PCR and sequenced from two susceptible and two resistant strains of *M. leprae.* The *folP-2* sequences from these strains were identical, indicating that resistance to DDS was not associated with mutation(s) in the gene encoding DHPS-2.—Authors' Abstract

Hall Stoodley, L., Keevil, C. W. and Lappin Scott, H. M. *Mycobacterium fortuitum* and *Mycobacterium chelonae* biofilm formation under high and low nutrient conditions. J. Appl. Microbiol. **85** Suppl. S. (1999) 60S–69S.

The rapidly growing mycobacteria (RGM) are broadly dispersed in the environment. They have been recovered from fresh water, sea water, waste water and ewen potable water samples and are increasingly associated with nontuberculous mycobacterial disease. There is scant evidence that nontuberculous mycobacteria (NTM) and RGM form biofilms. Therefore, an experimental system was designed to assess the ability of RGM to form biofilms under controlled laboratory conditions. A flat plate reactor flow cell was attached to either a high or low nutrient reservoir and monitored by image analysis over time. Two surfaces were chosen for assessment of biofilm growth: silastic which is commonly used in medical settings and high density polyethylene (HDPE) which is prevalent in water distribution systems. The results show that *Mycobacterium fortuitum* and *M. chelonae* formed biofilms under both high and low nutrient conditions on both surfaces studied. These results suggest that RGM may form biofilms under a variety of conditions in industrial and medical environments.—Authors' Abstract

Harboe, M. and Wiker, H. G. Searching for secreted proteins of *Mycobacterium leprae.* Indian J. Lepr. **71** (1999) 19–35.

In mycobacteria secreted proteins represent a distinct group, probably of particular importance for development of immune responses following infection. Quantification of individual proteins in *Mycobacterium tuberculosis* culture fluid and corresponding disrupted bacilli permits determination of a localization index for identification of secreted proteins. This procedure cannot be applied for *M. leprae* since secreted proteins are lost during isolation of bacilli from tissues. The DNA sequences of secreted proteins of *M. tuberculosis* were compared with sequences of *M. leprae.* Genes for homologs of the 85a, 85b, 85c, mpt32 (apa), mpt51, erp, mtc28, mtb12, Rv3354 and Rv0526 genes were identified. All of these and six genes of the mcel operon contain signal sequences for secretion in *M. leprae* as well. In several instances the local distance between marker genes and occurrence on the same or the complementary DNA strand was similar in these two species. The genomic organization of genes for secreted proteins is thus very similar in *M. leprae* and *M. tuberculosis,* the homology being higher for the mature polypeptide chains than for the corresponding signal peptides.—Authors' Abstract

Hutter, B. and Dick, T. Molecular genetic characterisation of whiB3, a mycobacterial homologue of a *Streptomyces* sporulation factor. Res. Microbiol. **150** (1999) 295–301.

WhiB is an essential sporulation factor in *Streptomyces coelicolor.* We report here the molecular genetic characterization of whiB3, a whiB-like gene in the nonspore-

forming *Mycobacterium smegmatis* mc (2) 155. *M. smegmatis* whiB3 encodes a 96-amino-acid protein with 81% similarity to its *M. tuberculosis* counterpart identified in the genome project, and 35% similarity to *S. coelicolor* WhiB. In both mycobacteria, whiB3 is flanked by the same upstream gene, Rv3415c, and appears to be monocistronic. Promoter probe analyses suggest that the whiB3 gene is expressed constitutively. Disruption of whiB3 did not affect growth or the dormancy response of *M. smegmatis*.—Authors' Abstract

Kim, B. J., Lee, S. H., Lyu, M. A., Kim, S. J., Bai, G. H., Kim, S. J., Chae, G. T., Kim, E. C., Cha, C. Y. and Kook, Y. H. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (rpoB). J. Clin. Microbiol. **37** (1999) 1714–1720.

For the differentiation and identification of mycobacterial species, the rpoB gene, encoding the beta subunit of RNA polymerase, was investigated. RpoB DNAs (342 bp) were amplified from 44 reference strains of mycobacteria and clinical isolates (107 strains) by PCR. The nucleotide sequences were directly determined (306 bp) and aligned by using the multiple alignment algorithm in the MegAlign package (DNASTAR) and the MEGA program. A phylogenetic tree was constructed by the neighbor-joining method. Comparative sequence analysis of rpoB DNAs provided the basis for species differentiation within the genus *Mycobacterium.* Slowly and rapidly growing groups of mycobacteria were clearly separated, and each mycobacterial species was differentiated as a distinct entity in the phylogenetic tree. Pathogenic *Mycobacterium kansasii* was easily differentiated from nonpathogenic *M. gastri*; this differentiation cannot be achieved by using 16S rRNA gene (rDNA) sequences. By being grouped into species-specific clusters with low-level sequence divergence among strains of the same species, all of the clinical isolates could be easily identified. These results suggest that comparative sequence analysis of amplified rpoB DNAs can be used efficiently to identify clinical isolates of mycobacteria in parallel with traditional culture methods and as a supplement to 165 rDNA gene analysis. Furthermore, in the case of *M. tuberculosis,* rifampin resistance can be simultaneously determined.—Authors' Abstract

Knipfer, N., Seth, A., Roudiak, S. G. and Shrader, T. E. Species variation in ATP-dependent protein degradation: protease profiles differ between mycobacteria and protease functions differ from *Mycobacterium smegmatis* and *Escherichia coli.* Gene **231** (1999) 95–104.

We report here that the existence of the potentially broad substrate specificity protease Lon (also called La) is evolutionarily discontinuous within the order *Actinomycetales.* Lon homologs were identified in the fast-growing species *Mycobacterium smegmatis* and in the slow-growing species *M. avium* and *M. intracellulare.* However, Lon homologs were not detected in the slow-growing species *M. tuberculosis, M. bovis,* or *M. leprae*; or in the non-mycobacterial *Actinomycetale Corynebacterium glutamica.* To characterize the function of the Lon protease within the *Actinomycetales,* a viable *M. smegmatis* Delta Lon strain was constructed, demonstrating that Lon is not essential under certain conditions. Surprisingly, Lon was also dispensable in *M. smegmatis* cells already lacking intact 20S proteasome alpha- and beta-subunit genes (called prcA and prcB, respectively). Creation of the later double deletion strain (prcBA::kan Delta Lon) necessitated use of a novel gene deletion strategy that does not require an antibiotic resistance marker. The *M. smegmatis* prcBA::kan Delta Lon double mutants displayed wild type (wt) growth rates and wt stress tolerances. In addition, the *M. smegmatis* prcBA::kan Delta Lon double mutants degraded at wt rates the broad spectrum of truncated proteins induced by treating cells with puromycin. This later result was in sharp contrast to those in *Escherichia coli,* where either Eon or hslUV single mutants are strongly impaired in their degradation of puromycyl peptides (hslV is a prcB homolog). Overall these data suggested that mycobacterial species contain additional ATP-dependent proteases that have broad substrate specificity. Consistent with this suggestion, *M. smegmatis* and *M. tuberculosis* each con-

tain at least one homolog of ClpP, the proteolytic subunit common to the ClpAP and ClpXP proteases.—Authors' Abstract

Kremer, L., Besra, G. S., Brennan, P. J. and Baulard, A. R. Lipoarabinomannan: structure and functions of a glycolipid involved in tuberculosis pathogenicity. Med. Sci. **15** (1999) 842–850.

Tuberculosis is the predominant cause of morbidity and mortality worldwide, infecting 8 million and killing 3 million people annually. The current situation is exacerbated by the HIV pandemic and the increased prevalence of multiple-resistant strains of *M. tuberculosis.* While vaccine prophylaxis using BCG is unsatisfactory in many parts of the world mycobacteria have evolved many specific adaptations that enable them to infect and survive within host cells. Such host-pathogen interactions are mediated by specialized molecules, in particular those associated with the unique cellular envelope. Lipoarabinomannan (LAM) is regarded as the "lipopolysaccharide of mycobacteria" and is an important virulence factor. Its terminal mannose cars may be involved—not only in attenuating the host-immune response but also in mediating the binding of mycobacteria to, and subsequent entry into macrophages. This may be further linked to an intracellular trafficking pathway through which LAM is presented by CD1 to T-cell subsets. More systematic genome type investigations of LAM biogenesis may reveal the true significance of this macromolecule in the immunopathogenesis of tuberculosis. As a consequence, the identification of new drug targets will permit the development of novel therapies against tuberculosis and other mycobacterial-related infections which may now be visualized through the advent of the recently sequenced *M. tuberculosis* genome.—Authors' Abstract

Leite, C. Q. F., de Souza, C. W. O. and Leite, S. R. de A. Identification of mycobacteria by thin layer chromatographic analysis of mycolic acids and conventional biochemical method: four years of experience. Mem. Inst. Oswaldo Cruz **93** (1998) 801–805.

Mycolic acids analysis by thin-layer chromatography (TLC) has been employed by several laboratories worldwide as a method for fast identification of mycobacteria. This method was introduced in brazil by the authors' laboratory in 1992 as a routine identification technique. Up to the present, 861 strains isolated were identified by mycolic acids TLC and by standard biochemical tests: 61% out of these strains came as clinical samples, 4% isolated from frogs and 35% as environmental samples. *Mycobacterium tuberculosis* strains identified by classical methods were confirmed by their mycolic acids contents (I, III and IV). The method allowed earlier differentiation of *M. avium* complex (MAC) (mycolic acids I, IV and VI) from *M. simiae* (acids I, II and IV), both with similar biochemical properties. The method also permitted us to distinguish *M. fortuitum* (acids I and V) from *M. chelonae* (acids I and II), and to detect mixed mycobacterial infections cases as *M. tuberculosis* with MAC and *M. fortuitum* with MAC. In conclusion, 4 years' experience shows that mycolic acids TLC is an easy, reliable, fast and inexpensive method, an important tool to put together conventional mycobacteria identification methods.—Trop. Dis. Bull. **96** (1999) 601

Rastogi, N., Goh, K. S. and Berchel, M. Species-specific identification of *Mycobacterium leprae* by PCR-restriction fragment length polymorphism analysis of the hsp65 gene. J. Clin. Microbiol. **37** (1999) 2016–2019.

PCR-restriction fragment length polymorphism analysis (PRA) of the hsp65 gene present in all mycobacteria was used in the present investigation to characterize *Mycobacterium leprae.* Bacilli were extracted and purified from different organs from experimentally infected armadillos and nude mice (Swiss mice of nu/nu: origin). A total of 15 samples were assayed in duplicate, and the results were compared with those obtained for a total of 147 cultivable mycobacteria representing 34 species. Irrespective of its origin or viability, *M. leprae* strains from all the samples were uniformly characterized by two fragments of 315 and 135 bp upon BstEII digestion

and two fragments of 265 and 130 bp upon HaeIII digestion. PRA is a relatively simple method and permits the conclusive identification of *M. leprae* to the species level.—Authors' Abstract

Rauzier, J., Gormley, E., Gutierrez, M. C., Kassa-Kelembho, E., Sandall, L. J., Dupont, C., Gicquel, B. and Murray, A. A novel polymorphic genetic locus in members of the *Mycobacterium tuberculosis* complex. Microbiology **145** (1999) 1695–1701.

It has previously been shown that the P_{AN} promoter from *Mycobacterium paratuberculosis* can be used as a DNA probe to identify an RFLP between wild-type *M. bovis* and the vaccine strain *M. bovis* BCG. To investigate the genetic basis of this phenomenon, DNA fragments from a New Zealand *M. bovis* cattle strain and *M. bovis* BCG Pasteur, containing the P_{AN}-binding region, were isolated from gene libraries, sequenced and characterized. Sequence analysis and comparison with database sequences showed that the P_{AN} region in *M. bovis, M. bovis* BCG and *M. tuberculosis* is identical and shares 70% similarity to the P_{AN} sequence from *M. paratuberculosis.* The Shine-Dalgarno sequence and the –10 and –35 promoter regions are conserved between the different species. Analysis of the flanking sequences of the P_{AN} region revealed that less than 1 kb downstream of P_{AN} is a 2405 bp fragment that is present in *M. bovis* BCG but absent in the *M. bovis* wild-type strain. The distribution of the 2405 bp fragment in members of the *M. tuberculosis* complex was investigated and found to be present in 70 out of 70 *M. tuberculosis* strains, and 7 out of 7 *M. bovis* BCG daughter strains, 2 out of 2 *M. africanum* strains, 2 out of 2 *M. microti* strains and 7 out of 25 *M. bovis* strains. This is the first report of a genetic region of *M. bovis* BCG that is not universally present in *M. bovis* strains. The fragment does not appear to be present in any mycobacterial species outside the *M. tuberculosis* complex. It does not possess any characteristics of known transposable elements and the flanking sequences do not have any obvious features to suggest a deletion mechanism. The genetic location of this region is close to the 3′ end of the RD1 region of *M. bovis* and *M. tuberculosis.* The polymorphic nature of this locus in *M. bovis* will provide an additional genetic marker for strain differentiation.—Authors' Abstract

Raynard, C., Laneelle, M.-A., Senaratne, R. H., Draper, P., Laneelle, G. and Daffe, M. Mechanisms of pyrazinamide resistance in mycobacteria: importance of lack of uptake in addition to lack of pyrazinamidase activity. Microbiology **145** (1999) 1359–1367.

Mycobacteria are known to acquire resistance to the antituberculous drug pyrazinamide (PZA) through mutations in the gene encoding pyrazinamidase (PZase), an enzyme that converts PZA into pyrazinoic acid, the presumed active form of PZA against bacteria. Additional mechanisms of resistance to the drug are known to exist but have not been fully investigated. Among these is the non-uptake of the pro-drug, a possibility investigated in the present study. The uptake mechanism of PZA, a requisite step for the activation of the pro-drug, a possibility investigated in the present study. The uptake mechanism of PZA, a requisite step for the activation of the pro-drug, was studied in *Mycobacterium tuberculosis.* The incorporation of [^{14}C]PZA by the bacilli was followed in both neutral and acidic environments since PZA activity is known to be optimal at acidic pH. By using a protonophore (carbonyl cyanide *m*-chlorophenylhydrazone; CCCP), valinomycin, aresenate and low temperature, it was shown that an ATP-dependent transport system is involved in the uptake of PZA. While the structurally analogous compound nicotinamide inhibited the transport system of PZA, other structurally related compounds such as pyrazinoic acid, isoniazid and cytosine did not. Acidic conditions were also without effect. Based on diffusion experiments in liposomes, it was found that PZA diffuses rapidly through membrane bilayers, faster than glycerol, while the presence of OmpATb, the porin-like protein of *M. tuberculosis,* in proteoliposomes slightly increased the diffusion of the drug. This finding may explain why the cell wall mycolate hydrophobic layer does not represent the limiting step in the diffusion of PZA, as

judged from comparative experiments using an *M. tuberculosis* strain and its isogenic mutant elaborating 40% less covalently linked mycolates. PZase activity, and PZA uptake and susceptibility in different mycobacterial species were compared. *M. tuberculosis,* a naturally PZA-susceptible species, was the only species that exhibited both PZase activity and PZA uptake; no such correlation was observed with the four naturally resistant species examined. *M. smegmatis* possessed a functional PZase but did not take up PZA; the reverse was true for the PZase-negative strain of *M. avium* used, with PZA uptake comparable to that of *M. tuberculosis. M. bovis* BCG and *M. kansasii* exhibited neither a PZase activity nor PZA uptake. These data clearly demonstrate that one of the mechanisms of resistance to PZA resides in the failure of strains to take up the drug, indicating that susceptibility to PZA in mycobacteria requires both the presence of a functional PZase and a PZA transport system. No correlation was observed between the occurrence and cellular location of PZase and of nicotinamidase in the strains examined, suggesting that one or both amides can be hydrolysed by other mycobacterial amidases.—Authors' Abstract

Rozwarski, D. A., Vilcheze, C., Sugantino, M., Bittman, R. and Sacchettini, J. C. Crystal structure of the *Mycobacterium tuberculosis* enoyl-ACP reductase, InhA, in complex with NAD+ and a C16 fatty acyl substrate. J. Biol. Chem. **274** (1999) 15582–15589.

Enoyl-ACP reductases participate in fatty acid biosynthesis by utilizing NADH to reduce the trans-double bond between positions C2 and C3 of a fatty acyl chain linked to the acyl carrier protein. The enoyl-ACP reductase from *Mycobacterium tuberculosis,* known as InhA, is a member of an unusual FAS-II system that prefers longer chain fatty acyl substrates for the purpose of synthesizing mycolic acids, a major component of mycobacterial cell walls. The crystal structure of InhA in complex with NAD^+ and a C16 fatty acyl substrate, trans-2-hexadecenoyl-(N-acetylcysteamine)-thioester, reveals that the substrate binds in a general "U-shaped" conformation, with the trans-double bond positioned directly adjacent to the nicotinamide ring of NAD^+. The side chain of Tyr(158) directly interacts with the thioester carbonyl oxygen of the C16 fatty acyl substrate and, therefore, could help stabilize the enolate intermediate, proposed to form during substrate catalysis. Hydrophobic residues, primarily from the substrate binding loop (residues 196–219), engulf the fatty acyl chain portion of the substrate. The substrate binding loop of InhA is longer than that of other enoyl-ACP reductases and creates a deeper substrate binding crevice, consistent with the ability of InhA to recognize longer chain fatty acyl substrates.—Authors' Abstract

Epidemiology and Prevention

Izumi, S., Budiawan, T., Saeki, K., Matsuoka, M. and Kawatsu, K. An epidemiological study on *Mycobacterium leprae* infection and prevalence of leprosy in endemic villages by molecular biological technique. Indian J. Lepr. **71** (1999) 37–43.

One of the most important unsolved questions in the epidemiology of leprosy is the highly uneven geographic distribution of the disease. There are many hyperendemic "pockets" in endemic countries. Little is known about the reasons why leprosy is hyperendemic in these areas. We conducted, therefore, a series of epidemiological studies on *Mycobacterium leprae* infection and prevalence of leprosy in North Maluku District, Maluku Province, Indonesia, where leprosy is highly endemic. It was found that a considerable number of general inhabitants are seropositive to various mycobacterial antigens and 27% of the villagers were carrying leprosy bacilli on the surface of their nasal cavities. These results suggested the importance of *M. leprae* in

the residential environment in infection of the leprosy bacillus and the resulting transmission of the disease. Based on these observations, we conclude that new preventive measures are essential for global elimination of leprosy in addition to early diagnosis and multidrug therapy (MDT).—Authors' Abstract

Matsuoka, M., Izumi, S., Budiawan, T., Nakata, N. and Saeki, K. *Mycobacterium leprae* DNA in daily use water as a possible source of leprosy infection. Indian J. Lepr. **71** (1999) 61–67.

Some environmental factors were suspected to be sources of leprosy infection according to the results of a total survey in the highly endemic villages in Indonesia. *Mycobacterium leprae* DNA were detected by PCR from 21 out of 44 water sources used daily by villagers. Prevalence of leprosy among the people using PCR-positive water for bathing and washing was significantly higher than that among the people who used PCR-negative water. No significant difference in prevalence was, however, recognized in the case of usage of negative or positive water for drinking. Water was regarded as a reservoir and infectious source of *M. leprae*. Transmission of leprosy through the contaminated water was strongly suggested by epidemiological analysis.—Authors' Abstract

Rehabilitation

Joseph, G. A. and Sundar Rao, P. S. S. Impact of leprosy on the quality of life. Bull. WHO **77** (1999) 515–517.

Leprosy is considered by many as not merely a medical condition, but as a condition encompassing psychological, socioeconomic and spiritual dimensions that dehabilitate an individual progressively, unless properly cared for. The present study was undertaken to document the nature and extent of decreases in the quality of life (QOL) of an affected person. The World Health Organization questionnaire on QOL was given to a representative random sample of 50 leprosy-affected persons and 50 unaffected individuals in the Bommasamudram Taluk of Chittoor District, Andhra Pradesh, India. This questionnaire explores the following six domains: physical, psychological; level of independence; social relationships; spiritual; and environmental.

The mean QOL score of the cases was significantly lower than that of the controls with the exception of the spiritual domain. The mean total score for women was higher than that of males in each domain and age group. Males with deformities had a significantly lower score than those with no visible deformities. Although the scores for females with deformities were also lower than those without deformities, the differences were not statically significant. Analyses of economic status versus the QOL scores clearly showed that they were positively correlated.

The study revealed that QOL decreased progressively in leprosy-affected persons. Women had a better QOL score than men in almost every domain. Given the secondary role of women in Indian rural society, this may simply imply an acceptance of their situation. The findings are discussed in comparison with other diseases and in the context of a poor socioeconomic environment. With modern amenities, better education and higher expectations, the perception of an individual regarding his or her own quality of life is bound to change. The need for frequent assessments and further studies along these lines is emphasized.—Authors' Abstract

Other Mycobacterial Diseases and Related Entities

Adams, L. B., Sinha, I., Franzblau, S. G., Krahenbuhl, J. L. and Mehta, R. T. Effective treatment of acute and chronic murine tuberculosis with liposome-encapsulated clofazimine. Antimicrob. Agents Chemother. **43** (1999) 1638–1643.

The therapeutic efficacy of liposomal clofazimine (L-CLF) was studied in mice infected with *Mycobacterium tuberculosis* Erdman. Groups of mice were treated with either free clofazimine (F-CLF), L-CLF, or empty liposomes twice a week for five treatments beginning on day 1 (acute), day 21 (established), or day 90 (chronic) postinfection. One day after the last treatment, the numbers of CFU of *M. tuberculosis* in the spleen, liver, and lungs were determined. F-CLF at the maximum tolerated dose of 5 mg/kg of body weight was ineffective; however, 10-fold-higher doses of L-CLF demonstrated a dose response with significant CFU reduction in all tissues without any toxic effects. In acutely infected mice, 50 mg of L-CLF/kg reduced CFU 2- to 3-log units in all three organs. In established or chronic infection, treated mice showed no detectable CFU in the spleen or liver and 1- to 2-log-unit reduction in the lungs. A second series of L-CLF treatments cleared *M. tuberculosis* in all three tissues. L-CLF appears to be bactericidal in the liver and spleen, which remained negative for *M. tuberculosis* growth for 2 months. Thus, L-CLF could be useful in the treatment of tuberculosis.—Authors' Abstract

Ahmad, S., Amoudy, H. A., Thole, J. E. R., Young, D. B. and Mustafa, A. S. Identification of a novel protein antigen encoded by a *Mycobacterium tuberculosis*-specific RD1 region gene. Scand. J. Immunol. **49** (1999) 515–522.

A genomic DNA region, designated RD1, that is present in virulent and clinical strains of *Mycobacterium tuberculosis* and *M. bovis,* has been shown to be deleted in bacillus Calmette Guerin (BCG). The DNA segments corresponding to three open reading frames (ORFs: ORF-10, ORF-13 and ORF-15) of the RD1 region, that are deleted in BCG strains, were amplified from *M. tuberculosis* genomic DNA by polymerase chain reaction (PCR), subcloned into pGEX-4T vector system and expressed in *Escherichia coli* as fusion proteins with glutathione-S-transferase (GST). The recombinant proteins appeared as major cellular proteins in SDS-PAGE gels at the expected molecular mass. The identity of each fusion protein was confirmed by reactivity with anti-GST antibodies in Western immunoblots. When pooled human sera from 11 tuberculosis (TB) patients were used as the source of antibodies, only GST-ORF-14 fusion protein reacted in Western immunoblots. The protein corresponding to ORF-13 was then purified to near homogeneity and isolated free of its fusion partner (GST) by treating the purified GST-ORF-14 fusion protein with thrombin protease. In Western immunoblots, the purified ORF-13 protein reacted with antibodies in 26 of 57 human sera (46%) from TB patients while no reactivity was seen with 11 sera from *M. bovis* BCG-vaccinated healthy subjects. Interestingly, sera from 9 of 15 (60%) long-term contacts of TB patients also had antibodies reactive to the ORF-14 protein. These results suggest that the ORF-14 protein in combination with other immunodominant proteins could be useful in the serodiagnosis of individuals infected with *M. tuberculosis.*—Authors' Abstract

Amadori, M., Archetti, I. L., Scaccaglia, P., Modena, D., Fossati, G., Lucietto, P. and Mascagni, P. Chaperonin 10 of *Mycobacterium tuberculosis* induces a protective immune response to foot-and-mouth disease virus. Arch. Virol. **144** (1999) 905–919.

Chaperonin 10 of *M. tuberculosis* conferred partial or total protection against generalized foot-and-mouth disease (FMD) in guinea pigs challenged with O-1 Lausanne FMD virus. Chaperonin 10-immunized animals mounted an antibody response to the protein, one epitope of which was found in the C-terminal half. A similar recognition pattern was observed in FMD-

convalescent guinea pigs, swine and cattle. Anti-chaperonin 10 sera showed antiviral activity against FMDV-infected BHK-21 cells. There was strong evidence that early after infection these cells actively secrete their histones and that antisera to the chaperonin recognize them. The same antisera reacted with purified histones in immunoblotting. Most important, exogenously added histones abrogated the antiviral activity of the antiserum and an antihistone monoclonal antibody had strong antiviral activity against FMDV-infected BHK-21 cells. These results are consistent with previous reports on displacement of histones from the nuclear compartment and immune recognition of self-histones after viral infections. On the whole, they indicate that *M. tuberculosis* chaperonin 10 enables the immune system to react against early abnormalities of virus-infected cells; this is accomplished by antibody crossreacting with histones released during virus infection.—Authors' Abstract

Behr Perst, S. I., Munk, M. E., Schaberg, T., Ulrichs, T., Schulz, R. J. and Kaufmann, S. H. E. Phenotypically activated gamma delta T lymphocytes in the peripheral blood of patients with tuberculosis. J. Infect. Dis. **180** (1999) 141–149.

Surface molecules with the potential relevance for resistance against *Mycobacterium tuberculosis* were investigated. The expression of lymphocyte function antigen-1, very late antigen (VLA)-4, L-selectin, intercellular adhesion molecule (ICAM)-1, major histocompatibility complex class II, Fas, and CD40 on alpha beta T cells, gamma delta T cells, NK cells, and monocytes of healthy donors and patients with tuberculosis (TB) were analyzed. A high activation status of gamma delta T cells and increased levels of soluble ICAM-1 in plasma of patients with TB versus healthy individuals was detected. TB patients with and without an underlying systemic disease could be segregated by differential expression of VLA-4 and ICAM-1 on gamma delta T cells and on monocytes. The composition of peripheral blood mononuclear cells varied slightly; whereas the proportion of monocytes decreased significantly in patients with TB compared with healthy controls. The activation phenotype of peripheral gamma delta T cells in patients with TB emphasizes the role of these T cells in controlling the inflammatory process during TB and perhaps other microbial infections.—Authors' Abstract

Chaturvedi, V., Srivastava, A., Gupta, H. P. and Srivastava, B. S. Protective antigens of *Mycobacterium habana* are distributed between peripheral and integral compartments of plasma membrane: a study in experimental tuberculosis of mice. Vaccine **17** (1999) 2882–2887.

Mycobacterium habana, a cultivable nonpathogenic mycobacterium, provides appreciable resistance in the mouse against *M. tuberculosis* infection. This study is aimed at identification and characterization of protective antigens of *M. habana.* Protective potential of antigens of cell wall (CW), cell membrane (CM), cytosol (CS) and peripheral and integral compartments of the membrane fraction of *M. habana* was explored against experimental tuberculosis in mice. Peripheral and integral membrane proteins were characterized by SDS-PAGE and differential staining with silver and periodic acid. Results reveal that protective antigens are distributed in both peripheral and integral membrane compartments although such effect is dominant in the former. Polysaccharide staining showed that LAM, LM and PIMs have a preference for the detergent phase. Peripheral and integral compartments constitute, respectively, 68% and 31% of the total membrane protein.—Authors' Abstract

Cohn, D. L., Fisher, E. J., Peng, G. T., Hodges, J. S., Chesnut, J., Child, C. C., Franchino, B., Gibert, C. L., El Sadr, W., Hafner, R., Korvick, J., Ropka, M., Heifets, L., Clotfelter, J., Munroe, D. and Horsburgh, C. R. A prospective randomized trial of four three-drug regimens in the treatment of disseminated *Mycobacterium avium* complex disease in AIDS patients: excess mortality associated with high-dose clarithromycin. Clin. Infect. Dis. **29** (1999) 125–133.

The optimal regimen for treatment of *Mycobacterium avium* complex (MAC) disease has not been established. Eighty-five AIDS patients with disseminated MAC disease were randomized to receive a three-drug regimen of clarithromycin, rifabutin or clofazimine, and ethambutol. Two dosages of clarithromycin, 500 or 1000 mg twice daily (b.i.d.), were compared. The Data and Safety Monitoring Board recommended discontinuation of the clarithromycin dosage comparison and continuation of the rifabutin vs. clofazimine comparison. After a mean follow up of 4.5 months, 10 (22%) of 45 patients receiving clarithromycin at 500 mg b.i.d. had died (70 deaths per 100 person-years) compared with 17 (43%) of 30 patients receiving clarithromycin at 1000 mg b.i.d. (158 deaths per 100 person-years) (relative risk 2.43; 95% confidence interval 1.11–5.34; p = 0.02). After 10.4 months, 20 (49%) of 41 patients receiving rifabutin had died (81 deaths per 100 person-years) compared with 23 (52%) of 44 patients receiving clofazimine (94 deaths per 100 person-years) (relative risk 1.20; 95% confidence interval 0.65–2.19; p = 0.56). Bacteriologic outcomes were similar among treatment groups. In treating MAC disease in AIDS patients, the maximum dose of clarithromycin should be 500 mg b.i.d.—Authors' Abstract

Desjardin, L. E., Perkins, M. D., Wolski, K., Haun, S., Teixeira, L., Chen, Y., Johnson, J. L., Ellner, J. J., Dietze, R., Bates, J., Cave, M. D. and Eisenach, K. D. Measurement of sputum *Mycobacterium tuberculosis* messenger RNA as a surrogate for response to chemotherapy. Am. J. Respir. Dis. Crit. Care Med. **160** (1999) 203–210.

Effective treatment regimens for pulmonary tuberculosis are difficult to assess because of the slow growth rate of *Mycobacterium tuberculosis* in culture and its protracted clearance from sputum. A rapid method that reflects effective antimicrobial activity would markedly advance evaluation of treatment and promote the assessment of new antituberculosis drugs. Conventional methods measure the progressive reduction of numbers of acid-fast bacilli in the sputum smear and the clearance of organisms in sputum culture. In this study, we measured levels of *M. tuberculosis* 85B (alpha antigen) messenger RNA (mRNA), 16S ribosomal RNA (rRNA), and IS6110 DNA in patients' sputa to ascertain whether they could serve as potential surrogate markers of response to chemotherapy. Sputum specimens were sequentially collected for up to a year from 19 smear-positive, pulmonary tuberculosis patients receiving an optimal drug treatment regimen. Nucleic acids were isolated from these specimens, and two *M. tuberculosis* molecular targets (mRNA, DNA) were quantified, using the ABI Prism 7700 Sequence Detection System. The *Mycobacterium* genus-specific 165 rRNA was quantified with a limiting dilution RT-PCR assay. Results show that levels of 85B mRNA declined after initiation of therapy, as did viable *M. tuberculosis* colony counts, with 90% of patients becoming negative for both markers after 2 mos. of treatment. The rapid disappearance of *M. tuberculosis* mRNA from sputum suggests that it is a good indicator of microbial viability and a useful marker for rapid assessment of response to chemotherapy.—Authors' Abstract

Dieli, F., Friscia, G., DiSano, C., Ivanyi, J., Singh, M., Spallek, R., Sireci, G., Titone, L. and Salerno, A. Sequestration of T lymphocytes to body fluids in tuberculosis: reversal of anergy following chemotherapy. J. Infect. Dis. **180** (1999) 225–228.

The specificity of CD4 T lymphocytes was investigated in 6 patients affected by tuberculosis who had negative tuberculin purified protein derivative (PPD) skin tests at diagnosis. Polyclonal CD4 T-cell lines from the peripheral blood failed to proliferate to PPD and to the 16- or 38-kDa proteins of *Mycobacterium tuberculosis,* while CD4 cell lines from the disease site responded to PPD and to the 16- and 38-kDa proteins and derived epitopes *in vitro.* Four months after chemotherapy, the patients became responsive to PPD. The proliferative response to PPD and to the 16- or 38-kDa proteins and their derived peptides decreased in CD4 T-cell lines from the disease site and increased in lines from the peripheral blood. These results indicate that CD4

T cells recognizing a vast array of *M. tuberculosis* epitopes are compartmentalized at the site of disease in anergic patients but appear in peripheral blood after chemotherapy.—Authors' Abstract

Dillon, D. C., Alderson, M. R., Day, C. H., Lewinsohn, D. M., Coler, R., Bement, T., Campos Neto, A., Skeiky, Y. A. W., Orme, I. M., Roberts, A., Steen, S., Dalemans, W., Badaro, R. and Reed, S. G. Molecular characterization and human T-cell responses to a member of a novel *Mycobacterium tuberculosis* mtb39 gene family. Infect. Immun. **67** (1999) 2941–2950.

We have used expression screening of a genomic *Mycobacterium tuberculosis* library with tuberculosis (TB) patient sera to identify novel genes that may be used diagnostically or in the development of a TB vaccine. Using this strategy, we have cloned a novel gene, termed mtb39a, that encodes a 39-kDa protein. Molecular characterization revealed that mtb39a is a member of a family of three highly related genes that are conserved among strains of *M. tuberculosis* and *M. bovis* BCG but not in other mycobacterial species tested. Immunoblot analysis demonstrated the presence of Mtb39A in *M. tuberculosis* lysate but not in culture filtrate proteins (CFP), indicating that it is not a secreted antigen. This conclusion is strengthened by the observation that a human T-cell clone specific for purified recombinant Mtb39A protein recognized autologous dendritic cells infected with TB or pulsed with purified protein derivative (PPD) but did not respond to *M. tuberculosis* CFP. Purified recombinant Mtb39A elicited strong T-cell proliferative and gamma-interferon responses in peripheral blood mononuclear cells from 9 of 12 PPD-positive individuals tested, and overlapping peptides were used to identify a minimum of 10 distinct T-cell epitopes. Additionally, mice immunized with mtb39a DNA have shown increased protection from *M. tuberculosis* challenge, as indicated by a reduction of bacterial load. The human T-cell responses and initial animal studies provide support for further evaluation of this antigen as a possible component of a subunit vaccine for *M. tuberculosis.*—Authors' Abstract

Dobos, K. M., Quinn, F. D., Ashford, D. A., Horsburgh, C. R. and King, C. H. Emergence of a unique group of necrotizing mycobacterial diseases. Emerg. Infect. Dis. **5** (1999) 367–378.

Although most diseases due to pathogenic mycobacteria are caused by *Mycobacterium tuberculosis,* several other mycobacterial diseases—caused by *M. ulcerans* (Buruli ulcer), *M. marinum,* and *M. haemophilum*—have begun to emerge. We review the emergence of diseases caused by these three pathogens in the United States and around the world in the last decade. We examine the pathophysiologic similarities of the diseases (all three cause necrotizing skin lesions) and common reservoirs of infection (stagnant or slow-flowing water). Examination of the histologic and pathogenic characteristics of these mycobacteria suggests differences in the modes of transmission and pathogenesis, although no singular mechanisms for either characteristic has been definitively described for any of these mycobacteria.—Authors' Abstract

El Zaatari, F. A. K., Naser, S. A., Hulten, K., Burch, P. and Graham, D. Y. Characterization of *Mycobacterium paratuberculosis* p36 antigen and its seroreactivities in Crohn's disease. Curr. Microbiol. **39** (1999) 115–119.

Recent data using improved cultural, molecular, and serological techniques have strengthened the association of *Mycobacterium paratuberculosis* with Crohn's disease, an inflammatory bowel disease (IBD) with unknown etiology. To provide more evidence of an etiological association, antibody reactivities of Crohn's disease patients were tested by immunoblotting against *M. paratuberculosis*-recombinant antigens. A clone containing a 1402-bp insert and expressing a 36K-antigen (p36) was analyzed. No homology was found between the deduced amino-acid sequence of p36 and any protein sequences compiled in the GenBank, indicating that p36 is a novel mycobacterial protein. The reactivity of 199 serum samples was tested against the p36

by immunoblotting technique. Sera from 77 of 89 (86.5%) Crohn's disease patients and 16 of 18 (89%) sera from patients with tuberculosis and leprosy reacted with p36 compared to 5 of 42 (12%) ulcerative colitis and non-IBD control sera ($p < 0.0001$). In addition, p36 reacted to all sera from 10 normal controls that were Bacillus Calmette-Guerin (BCG)-immunized and only to 10% of 40 normal controls that were not BCG-immunized. The fact that sera from Crohn's disease patients reacted to p36 with the same high frequency as the sera from patients who were exposed to mycobacterial antigens further supports the hypothesis of the mycobacterial etiology in Crohn's disease.—Authors' Abstract

Gonzalez y Merchand, J. A., Colston, M. J. and Cox, R. A. Effects of growth conditions on expression of mycobacterial murA and tyrS genes and contributions of their transcripts to precursor rRNA synthesis. J. Bacteriol. **181** (1999) 4617–4627.

All mycobacteria studied to date have an rRNA operon, designated rrnA, located downstream from a single copy of the murA gene, which encodes an enzyme (EC 2.5.1.7) important for peptidoglycan synthesis. The rrnA operon has a promoter, P1(A), located within the coding region of murA near the 3′ end. Samples of RNA were isolated from *Mycobacterium tuberculosis* at different stages of the growth cycle and from *M. smegmatis* grown under different conditions. RNase protection assays were used to investigate transcripts of both murA and rrnA. Transcription of murA was found to continue into the 16S rRNA gene, as if murA and rrnA form a hybrid (protein coding-rRNA coding) operon. During the growth of *M. tuberculosis,* the hybrid operon contributed approximately 2% to total pre-rRNA. Analysis of *M. smegmatis* RNA revealed that the level of murA RNA depended on the growth rate, and that the patterns of expression during the growth cycle were different for murA and rrnA. *M. smegmatis* has a second rRNA operon, rrnB, located downstream from a single copy of the tyrS gene, encoding tyrosyl-tRNA synthetase. Transcription of tyrS was found to continue into the 16S rRNA gene rrnB. The hybrid tyrS-rrnB operon contributed 0.2% to 0.6% to rrnB transcripts. The pattern of tyrS expression during the growth cycle matched the pattern of rrnB expression, reflecting the essential role of tyrS and rRNA in protein biosynthesis.—Authors' Abstract

Howard, A. D. and Zwilling, B. S. Reactivation of tuberculosis is associated with a shift from type 1 to type 2 cytokines. Clin. Exp. Immunol. **115** (1999) 428–434.

The pattern of cytokines produced by T cells from mice with latent tuberculosis and during reactivation of tuberculosis was determined. A type 1 cytokine pattern was observed in T cells isolated from the lung of mice with latent disease. Reactivation of mycobacterial growth, by activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulted in a shift from a type 1 to a type 2 cytokine pattern in both CD4 and CD8 T cells. Classification of the T cells based on their differential expression of CD45 and CD44 showed that the phenotypically different populations of CD4 and CD8 cells exhibited a type 1 cytokine pattern at latency and that reactivation of latent tuberculosis was associated with a shift in cytokines produced by these populations to a type 2 cytokine response. Control of mycobacterial growth resulted in a return to the type 1 cytokine pattern found during latent disease.—Authors' Abstract

Hu, Y. M., Butcher, P. D., Mangan, J. A., Rajandream, M. A. and Coates, A. R. M. Regulation of hmp gene transcription in *Mycobacterium tuberculosis*: effects of oxygen limitation and nitrosative and oxidative stress. J. Bacteriol. **181** (1999) 3486–3493.

The *Mycobacterium tuberculosis* hmp gene encodes a protein which is homologous to flavohemoglobin in *Escherichia coli.* Northern blotting analysis demonstrated that hmp transcription increased when a microaerophilic culture became oxygen limited as it entered stationary phase at 20 days. There was a fivefold increase of the hmp transcripts during early stationary phase compared with the value

which was observed in the exponential growth phase. This induction of hmp transcription was not due to changes in the mRNA stability since the half-life of hmp mRNA was very short in a 20-day microaerophilic culture. No induction of hmp mRNA was observed during entry into stationary phase when the culture was continuously aerated. Hmp transcription was induced after a short exposure of a late-exponential-phase culture to anaerobic conditions. These data indicate that oxygen limitation is the trigger for hmp gene transcription. In addition, when a microaerophilic culture entered into the stationary phase at 20 days, transcription of hmp increased to a small extent after exposure to S-nitrosoglutathione [a nitric oxide (NO) releaser] and sodium nitroprusside (an NO+ donor) and decreased after exposure to paraquat (a superoxide generator) and H_2O_2. In log phase (4 days) and late stationary phase (40 days), the transcription of hmp was unaffected by nitrosative and oxidative stress. Three primer extension products were observed. The –10 region is 100% identical to that of promoter T3 in mycobacteria and shows a strong similarity to the –10 sequence of hmp and rpoS promoters in *E. coli.* These observations of hmp mRNA induction in response to O-2 limitation and nitrosative stress suggest that the hmp gene of *M. tuberculosis* may have a role in protection of the organism from NO killing under microaerophilic conditions.—Authors' Abstract

Jacobson, J. M., Spritzler, J., Fox, L., Fahey, J. L., Jackson, J. B., Chernoff, M., Wohl, D. A., Wu, A. W., Hooton, T. M., Sha, B. E., Shikuma, C. M., MacPhail, L. A., Simpson, D. M., Trapnell, C. B. and Basgoz, N. Thalidomide for the treatment of esophageal aphthous ulcers in patients with human immunodeficiency virus infection. J. Infect. Dis. **180** (1999) 61–67.

A multicenter, double-blind, randomized, placebo-controlled clinical trial was conducted to determine the safety and efficacy of thalidomide for treating esophageal aphthous ulceration in persons infected with human immunodeficiency virus (HIV). Twenty-four HIV-infected patients with biopsy-confirmed aphthous ulceration of the esophagus were randomly assigned to receive either oral thalidomide, 200 mg/day, or oral placebo daily for 4 weeks. Eight (73%) of 11 patients randomized to receive thalidomide had complete healing of aphthous ulcers at the 4-week endoscopic evaluation compared with 3 (23%) of 13 placebo-randomized patients (odds ratio 13.82; 95% confidence interval 1.16–823.75; p = 0.033). Odynophagia and impaired eating ability caused by esophageal aphthae were improved markedly by thalidomide treatment. Adverse events among patients receiving thalidomide included somnolence (4 patients), rash (2 patients), and peripheral sensory neuropathy (3 patients). Thalidomide is effective in healing aphthous ulceration of the esophagus in patients infected with HIV.—Authors' Abstract

Kang, B. Y., Chung, S. W., Lim, Y. S., Kim, E. J., Kim, S. H., Hwang, S. Y. and Kim, T. S. Interleukin-12-secreting fibroblasts are more efficient than free recombinant interleukin-12 in inducing the persistent resistance to *Mycobacterium avium* complex infection. Immunology **97** (1999) 474–480.

To determine whether the paracrine secretion of interleukin-12 (IL-12) can efficiently stimulate the resistance to *Mycobacterium avium* complex (MAC) infection, 3T3 fibroblasts were stably transfected to secrete IL-12 (480 U/10^6 cells/48 hr) and their effect on MAC infection was investigated in genetically susceptible BLAB/c mice compared with that of free recombinant IL-12 (rIL-12). Infection with IL-12-secreting fibroblasts (3T3-IL-12) during intranasal infection with MAC resulted in a significant decrease in the bacterial load of the lung during the entire 10-week observation period, while rIL-12 reduced the bacterial load initially, at 2 weeks, but not by 10 weeks postinfection. Lung CD4+ T cells in mice injected with the 3T3-IL-12 cells showed a persistent T-helper type 1 (Th1) response throughout the 10-week period. Furthermore, immunization with the 3T3-IL-12 cells induced and maintained significantly higher levels of cytotoxic activity and nitric oxide production by lung cells

than did rIL-12 immunization. This work suggests that IL-12-secreting fibroblasts may serve as a vehicle for paracrine secretion of IL-12 for immunotherapy of MAC infection.—Authors' Abstract

Lowrie, D. B., Tascon, R. E., Bonatao, V. L. D., Lima, V. M. F., Faccioli, L. H., Stavropoulos, E., Colston, M. J., Hewinson, R. G., Moelling, K. and Silva, C. L. Therapy of tuberculosis in mice by DNA vaccination. Nature **400** (1999) 269–271.

Mycobacterium tuberculosis continues to kill about 3 million people every year, more than any other single infectious agent. This is attributed primarily to an inadequate immune response toward infecting bacteria, which suffer growth inhibition rather than death and subsequently multiply catastrophically. Although the bacillus Calmette-Guerin (BCG) vaccine is widely used, it has major limitations as a preventative measure. In addition, effective treatment requires that patients take large doses of antibacterial drug combinations for at least 6 months after diagnosis, which is difficult to achieve in many parts of the world and is further restricted by the emergence of multidrug-resistant strains of *M. tuberculosis.* In these circumstances, immunotherapy to boot the efficiency of the immune system in infected patients could be a valuable adjunct to antibacterial chemotherapy. Here we show in mice that DNA vaccines, initially designed to prevent infection, can also have a pronounced therapeutic action. In heavily infected mice, DNA vaccinations can switch the immune response from one that is relatively inefficient and gives bacterial stasis to one that kills bacteria. Application of such immunotherapy in conjunction with conventional chemotherapeutic antibacterial drugs might result in faster or more certain cure of the disease in humans.—Authors' Abstract

Mansoer, J. R., Kibuga, D. K. and Borgdorff, M. W. Altitude: a determinant for tuberculosis in Kenya? Int. J. Tuberc. Lung Dis. **3** (1999) 156–161.

The objective was to determine to what extent tuberculosis incidence is associated with altitude. Notification rates were obtained from all 41 districts in Kenya during 1988–1990; the mean altitude of each district was estimated. Data on indicators of socioeconomic status such as literacy rate and infant mortality rate were obtained from the 1989 census, as well as data on other potential confounders such as urbanization and median household size. The notification rate of new smear-positive tuberculosis was 32/100,000 overall, varying between districts from 5 to 222/100,000. Notification rates steeply reduced with increasing altitude ($r = -0.71$; 95% confidence interval -0.51 to -0.83). At altitudes of 1000 m or more the notification rates were less than 30% of those in districts at altitudes below 500 m, also after adjustment for confounding. It is concluded that tuberculosis incidence in Kenya decreases strongly with increasing altitude. If the association is not due to unknown confounding factors, a range of potential biological explanations needs to be explored.—Trop. Dis. Bull. **96** (1999) 610

McCredie, J. and Willert, H. G. Longitudinal limb deficiencies and the sclerotomes—an analysis of 378 dysmelic malformations induced by thalidomide. J. Bone Joint Surg. Br. **81B** (1999) 9–23.

The pathogenesis of longitudinal reduction deformities of the limbs, or dysmelia, is still a matter of debate. Their morphological pattern was defined from a large collection of radiographs of children with dysmelia following the thalidomide disaster.

We compared radiographs of 378 of these limbs with the sclerotomes which are areas of segmental sensory innervation of the limb skeleton defined by the radiation of referred pain. The pattern of dysmelia matched the sclerotomes closely in 279 limbs (73.5%).

The principles of skeletal reduction in dysmelia are explained by the arrangement of the sclerotomes. The congruence between two separate and independent data sets shows that both patterns are expressions of the underlying segmental sensory innervation of the skeleton, and that the sensory nervous system is involved in the process of limb morphogenesis and teratogenesis.—Authors' Abstract

Meierhofer, C., Dunzendorfer, S. and Wiederman, C. J. Protein kinase C-dependent effects on leukocyte migration of thalidomide. J. Infect. Dis. **180** (1999) 216–219.

Thalidomide is effective in the treatment of some tumor necrosis factor-related diseases, but its cellular target is not known. Effects of thalidomide were investigated on lymphocytes and monocytes. Cell migration was examined in a Boyden chamber. Effects on protein kinase C (PKC) were investigated functionally by use of PKC inhibitor and in purified enzyme preparations. Thalidomide itself showed no direct chemotactic effect on lymphocytes or monocytes. Preincubation with the drug significantly enhanced random migration of both cell types. This effect was bisindolylmaleimide-reversible, suggesting involvement of PKC. Preincubation with thalidomide diminished the chemotactic response of monocytes toward formyl peptide but failed to influence lymphocyte chemotaxis toward RANTES or interleukin-8. In a cell-free assay, inhibition of PKC activation by bisindolylmaleimide could be reversed by thalidomide, indicating direct interactions of thalidomide with PKC. Results suggest that the effects of thalidomide in chronic inflammation may be related to actions on leukocyte functions.—Authors' Abstract

Petrofsky, M. and Bermudez, L. E. Neutrophils from *Mycobacterium avium*-infected mice produce TNF-alpha, IL-12 and IL-1beta and have a putative role in early host response. Clin. Immunol. **91** (1999) 354–358.

Recent evidence supports a role for neutrophils in the host defense against *Mycobacterium avium.* To determine whether the depletion of neutrophils has an effect on the outcome of infection in mice as determined by the number of bacteria in liver and spleen, we administered RB6-8C5 anti-neutrophil antibody intraperitoneally both early and late in the infection. Mice were then observed for 14 days and harvested. The number of viable bacteria in liver and spleen was determined. While administration of RB6-8C5 antibody early in infection resulted in a significant increase in the number of bacteria in organs when compared with mice receiving immunoglobulin control, administration of RB6-8C5 antibody late in infection (week 3) did not have an impact on the bacterial load in tissue. Infection of CD18 knockout mice (with impaired neutrophil function), however, did not show a significant enhancement of *M. avium* growth when compared with that of wild-type control mice. Neutrophils were found to produce increased amounts of TNF-alpha and IL-12 and IL-1 than control uninfected mice during the initial phase of infection, but not after 2 weeks following infection (although IL-1beta levels continue elevated). The results suggest that neutrophils may have a role in the early (innate) immune response against *M. avium* but it is only evident after acute depletion of neutrophils and not in mice with chronic neutrophil impairment.—Authors' Abstract

Rojas-Espinosa, O., Rangel Moreno, J., Amador Jimenez, A., Parra Maldonado, R., Arce Paredes, P. and Torres Lopez, J. Secretion antigens of *Mycobacterium tuberculosis*: a comparison between a reference strain and seven wild isolates. Arch. Med. Res. **30** (1999) 171–178.

Background. This study was carried out with the aim of detecting possible differences between proteins secreted by fresh wild isolates of *Mycobacterium tuberculosis* and from a reference strain of this microorganism, H37Rv TMCC 102.

Materials and Methods. This reference strain of *M. tuberculosis* has been in our laboratory for over 10 years, where it has been maintained by serial subcultures in PBY and Lowenstein-Jensen media. Patterns of protein secretion and recognition by sera derived from both tuberculosis patients and normal individuals were analyzed by electrophoresis and Western blotting.

Results. No major qualitative differences were observed among the several strains studied with respect to protein patterns or recognition of these proteins by test sera. Normal sera were found to react with almost all antigens recognized by tuberculosis sera, but with less intensity. However, a

small protein of 14.5 kDa, secreted by both the wild and reference strains of *M. tuberculosis,* was recognized by 32 of the 40 tuberculous patient sera tested (80%), and was not recognized by any of the 40 serum samples derived from healthy individuals.

Conclusions. This small protein seems to be a potentially important antigen for the serological diagnosis of tuberculosis and/or for use in the follow up of patients who receive treatment.—Authors' Abstract

Rowland, R. L., McHugh, S. M., Deighton, J., Ewan, P. W., Dearman, R. J. and Kimber, I. Selective downregulation of T cell- and non-T cell-derived tumour necrosis factor alpha by thalidomide: comparisons with dexamethasone. Immunol. Lett. **68** (1999) 325–332.

Both thalidomide and dexamethasone have been shown to inhibit the production of tumour necrosis factor-alpha (TNF-α), but little is known of their cellular selectivity. Inhibition of monocyte TNF-α expression has been implicated in the clinical efficacy of thalidomide, and it has been suggested that the drug modulates only monocyte-derived cytokines. Given the importance of T-lymphocyte responses in immunological disorders in which treatment with thalidomide has been successful, it is pertinent to study the effects of this drug on T cell-derived TNF-α. In the present investigations we have examined the influence of both thalidomide and dexamethasone on mitogen-induced elaboration of TNF-α by CD3+ peripheral blood mononuclear cells (PBMC) and the T-cell line MOLT-4. PBMC from healthy human volunteers were stimulated optimally with phytohemagglutinin (PHA) in the presence of varying concentrations of thalidomide or dexamethasone, and supernatants assayed for TNF-α and interleukin 2 (IL-2). Concurrently, PHA-stimulated PBMC were treated with 1×10^{-1} mM thalidomide or dexamethasone and the cells fixed, permeabilized, stained with anti-CD3 and anti-TNF-α fluorescently labelled antibodies and analyzed by flow cytometry. MOLT-4 cells were cultured in the presence or absence of the drugs following activation with phorbol myristate acetate (PMA)/ionophore, and supernatants analyzed by enzyme-linked immunosorbent assay (ELISA) for cytokine expression. Thalidomide was found to inhibit PBMC-derived TNF-α, but not IL-2. In contrast, dexamethasone downregulated both TNF-α and IL-2 in a dose-dependent manner. Thalidomide and dexamethasone both suppressed intracellular levels of TNF-α in CD3+ PBMC, reducing percentages of double-positive staining cells by 28% and 52%, respectively, compared with controls. In addition, TNF-α production by CD3– PBMC was inhibited by 31% by thalidomide and by 47% by dexamethasone. In order to determine whether thalidomide was acting directly on T cells, or indirectly through effects on accessory cells, TNF-α production in the T-cell line MOLT-4 was investigated. TNF-α secretion by PMA/ionophore-activated MOLT-4 cells was reduced by 80% following thalidomide treatment and close to background levels following dexamethasone treatment. To verify that thalidomide was acting selectively to downregulate TNF-α, IL-2 production by MOLT-4 cells was also measured and found to be unaffected by the drug. In contrast, dexamethasone reduced MOLT-4-derived IL-2 levels by 20%. These observations suggest that thalidomide, in addition to its known inhibitory effect on monocyte-derived TNF-α, is capable also of downregulating T cell-derived TNF-α in a direct and selective manner. In addition, the inhibition of intracellular levels of TNF-α strengthens the evidence that the inhibitory effect of thalidomide is at the level of transcription and/or translation and does not reduce cellular TNF-α secretion. Such effects could explain the efficacy of thalidomide treatment in various immunological disorders where T-cell activation plays an important role in the pathogenesis of the disease.—Authors' Abstract

Sadoh, D. R., Hawk, J. L. M. and Panayiotopoulos, C. P. F-chronodispersion in patients on thalidomide. Clin. Neurophysiol. **110** (1999) 735–739.

Objectives: To describe abnormalities of F-chronodispersion in patients treated with thalidomide.

Methods: We retrospectively studied F-wave latency, persistence and F-chronodispersion in 12 patients on thalidomide treatment and compared them with a control group of another 12 patients with similar dermatological conditions who did not receive thalidomide. Furthermore, we prospectively performed longitudinal neurophysiological studies in 4 patients before and during thalidomide treatment.

Results: Seven of 12 patients in the retrospective study had abnormal F-chronodispersion while this was normal in all patients of the control group ($p = 0.014$). All other neurophysiological parameters were similar in the two groups. Two of the thalidomide patients with abnormal F-chronodispersion later developed sensory neuropathy. In all 4 patients in the prospective study although F-chronodispersion was normal before thalidomide it became markedly abnormal after exposure to this drug.

Conclusions: Thalidomide may affect smaller diameter motor nerve fibers even before changes in sural sensory nerve action potentials. F-waves and F-chronodispersion should be routinely monitored in patients on thalidomide treatment.—Authors' Abstract

Schmitt, T., Schnitzler, N., Riehl, J., Adam, G., Sieberth, P. G. and Hasse, G. Successful treatment of pulmonary *Mycobacterium xenopi* infection in a natural killer cell-deficient patient with clarithromycin, rifabutin, and sparfloxacin. Clin. Infect. Dis. **29** (1999) 120–124.

Isolation of *Mycobacterium xenopi* from the respiratory tract may indicate pneumonia, often clinically indistinguishable from tuberculosis. Resistance to the classic antituberculous drugs renders the treatment of these infections problematic. We report on a case of cavernous pneumonia caused by *M. xenopi* in a 36-year-old male with natural killer cell deficiency but without severe immunodeficiency. He was successfully treated with a novel triple-drug combination comprising clarithromycin, sparfloxacin, and rifabutin. An impressive subsequent regression of pathological pulmonary changes was observed, and mycobacteria could no longer be detected. The therapeutic potential of clarithromycin and sparfloxacin in the treatment of *M. xenopi* infections is discussed.—Authors' Abstract

Senaldi, G., Shaklee, C. L., Mak, T. W. and Ulich, T. R. *Corynebacterium parvum*- and *Mycobacterium bovis* Bacillus Calmette and Guerin-induced granuloma formation in mice lacking CD4 and CD8. Cell. Immunol. **193** (1999) 155–161.

Granuloma formation is a T-cell-dependent inflammatory response that is important in the host defense against intracellular bacteria. The role of CD4 and CD8 molecules in the development of *Corynebacterium parvum*- and *Mycobacterium bovis* Bacillus Calmette and Guerin (BCG)-induced granulomas was examined in CD4/CD8 knockout (KO) mice. CD4/CD8 KO mice developed a greater granulomatous response to heat-killed *C. parvum* and heat-killed BCG than did control mice. Thus, granuloma formation is not dependent upon the presence of CD4 and CD8. On the other hand, CD4/CD8 KO mice challenged with live BCG showed initially fewer and smaller granulomas but later more and larger granulomas than control mice. The absence of CD4 and CD8 therefore impaired the host defense against infection with BCG. Alpha beta T cells were present in the granulomas of both GD4/CD8 KO and control mice in similar numbers. Also the production of IFN-gamma mRNA was similar in the two groups. In conclusion, CD4 and CD8 are not essential to the granulomatous response against *C. parvum* and BCG, but contribute to the host defense against live BCG infection.—Authors' Abstract

Silva, C. L. The potential use of heat-shock proteins to vaccinate against mycobacterial infections. Microbes Infect. **1** (1999) 429–435.

Over the last few years, some of our experiments in which mycobacterial heat-shock protein (HSP) antigens were presented to the immune system as if they were viral antigens have had a significant impact on our understanding of protective immunity against tuberculosis. They have also markedly enhanced the prospects for

new vaccines. We now know that the mycobacterial hsp65 antigen can confer protection equal to that from live BCG vaccine in mice.—Author's Abstract

Velaz Faircloth, M., Cobb, A. J., Horstman, A. L., Henry, S. C. and Frothingham, R. Protection against *Mycobacterium avium* by DNA vaccines expressing mycobacterial antigens as fusion proteins with green fluorescent protein. Infect. Immun. **67** (1999) 4243–4250.

Mycobacterium avium causes disseminated disease in humans with AIDS, paratuberculosis in ruminants, lymphadenopathy in swine, and tuberculosis in birds. We constructed DNA vaccines expressing mycobacterial antigens as fusion proteins with enhanced green fluorescent protein (EGFP). Plasmids p65K-EGFP, p85A-EGFP, and p85B-EGFP expressed the *M. avium* 65-kDa antigen, the *M. bovis* BCG 85A antigen, and the *M. avium* 85B antigen, respectively, as EGFP fusion proteins. We visualized protein expression directly in cultured murine fibroblasts and intact muscle. P65K-EGFP expressed fusion protein in a diffuse cytoplasmic pattern, and p85A-EGFP and p85B-EGFP produced a speckled pattern. We vaccinated C57BL/6 mice with three doses of plasmid DNA and then challenged them intraperitoneally with *M. avium.* Negative controls received saline, and positive controls received one dose of BCG vaccine. Mice in all groups developed disseminated infection with a high burden of organisms. Compared to negative controls, mice vaccinated with p85A-EGFP had an eightfold reduction in spleen *M. avium* CFU at 4 weeks after infection and a fourfold reduction at 8 weeks, reductions similar to those generated by BCG vaccine. Mice vaccinated with p65K-EGFP had a fourfold CFU reduction at 4 weeks and no effect at 8 weeks. This is the first report of DNA vaccines expressing foreign antigens as fusion proteins with EGFP, and the first report of successful DNA vaccination against *M. avium.*—Authors' Abstract

van der Graaf, W. T. A., Scherpbier, R. W. and van der Werf, T. S. [Buruli ulcer (*Mycobacterium ulcerans* infection); report of an international congress in Yamoussoukro, Cote d'Ivoire.] Ned. Tijdschr. Geneeskd. **143** (1999) 312–316.

A report is presented on the conference "Buruli Ulcer Control and Research" held in Yamoussoukro, Côte d'Ivoire, in July 1998. *Mycobacterium ulcerans* infection (Buruli ulcer) is the third most important mycobacterial disease worldwide in immunocompetent humans, after tuberculosis and leprosy. *M. ulcerans* is an environmental mycobacterium which has now been recovered from water and soil in swampy areas, and transmission to man occurs presumably through minor skin traumas. Endemic foci are known throughout the world, predominantly in tropical rainforest areas. The clinical presentation varies between a papule, a nodule or an ulceration with typically undermined edges. Surgery is the only effective treatment. BCG vaccination has a moderate protective effect. An association with HIV infection has not been demonstrated so far. Poor communities, with limited access to health care, and especially children are affected. The medical and socioeconomic burden imposed by the disease is tremendous. During the last decade the incidence of the disease has increased dramatically, particularly in West Africa. The Yamoussoukro declaration on Buruli ulcer, adopted 6 July 1998, is the basis of improvement of awareness, health education, treatment, and research on *M. ulcerans* infection. Support by the international community is urgently needed.—Trop Dis. Bull. **96** (1999) 613

Zumla, A., Squire, S. B., Chintu, C. and Grange, J. M. The tuberculosis pandemic: implications for health in the tropics. Trans. R. Soc. Trop. Med. Hyg. **93** (1999) 113–117.

Among infectious diseases, tuberculosis is the leading cause of death, killing around 3 million people each year. Most cases occur in young adults, but it also a major cause of illness and death in children. The problem has been exacerbated in recent years by the HIV pandemic and by the emergence of multidrug resistance. Co-infection with HIV greatly enhances the risk of overt tuberculosis, and in 1999 it is ex-

pected that tuberculosis will account for 30% of the predicted 2.5 million AIDS-related deaths. By inducing clinically and radiologically atypical forms of tuberculosis, and by increasing pressure on diagnostic facilities by sheer numbers, serious diagnostic difficulties are increasingly occurring in both adults and children in the tropics. At the present time, 2% of all cases of tuberculosis are multidrug resistant but, as the treatment of such cases is often grossly inadequate in many tropical countries, their frequency will doubtless grow. There are not simple solutions to the global emergency of tuberculosis: clearly there is a need for better use of available control measures but there is also a need to reach a much clearer understanding of the underlying immune phenomena in this disease so as to develop more effective vaccines and therapeutic agents. Finally, it cannot be ignored that tuberculosis is a disease of poverty—95% of cases and 98% of deaths due to it occur in the developing nations—and thus a major control measure is a resolution of the gross inequities in health care provision both between and within nations.—Authors' Abstract