CURRENT LITERATURE

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

General and Historical

Kumaresan, J. A. and Maganu, E. T. Socio-cultural dimensions of leprosy in northwestern Botswana. Soc. Sci. Med. **39** (1994) 537–541.

A study to determine some socio-cultural factors influencing knowledge and attitudes of the community toward leprosy was carried out in northwestern Botswana, where cases of leprosy have been known to exist over the years. The study was largely qualitative, using ethnographic approaches. The research was tailored in a way to capture the ethnic diversity of the region, in particular two ethnic groups, namely, Bayei and Barbukushu. The name or symptom complex associated with leprosy was "ngara" or "lepero" and this was associated with bad blood. Knowledge on disease causation was lacking, which in turn influenced health seeking behavior of patients. Patients were well integrated and accepted into the social structure of communities. Women caring for these patients did experience some additional burden and identified time as their major constraint in caretaking. It was apparent that the degree of rejection correlated with seriousness of the disease and extent of disabilities and dysfunction. The present pattern of health seeking behavior needs to be altered, so that an early diagnosis can be made at the health facility. This will aid appropriate management and prevent occurrence of deformities and disabilities, which in turn will reduce rejection and isolation of patients. Education of community patients, traditional and religious healers on various aspects of the disease, especially causation, is essential to achieve a change in the health seeking behavior.—Author's Summary

Sylla, P. M. [Status of the integration of leprosy control with the general health services in Senegal.] Acta Leprol. **9** (1995) 117–125. (in French)

The survey on the integration of leprosy control in Senegal with the general health services has shown that the level of integration varies according to the services offered. Both strong and weak points have been detected and it is, therefore, advisable to reinforce the findings that are positive and to seek solutions to the problems: 82.1% of the male nurses in charge of health center included in the survey have already had to refer suspected cases of leprosy to the Leprosy Specialist for confirmation. In 85.7% of the cases, it is the Nurses-Persons in Charge who administer the supervised dose of multidrug therapy. The external validity of this study is problematic. Nevertheless, these results could still draw the attention of administrators, decision-makers, and other persons of influence to the problems that could curb the integration of leprosy control with the general health services.—Author's English Abstract

Tu, X. [Calculating the future development of leprology in China with literature statistics.] China Lepr. J. **11** (1995) 14–16. (in Chinese)

The prospects of leprology in China have been analyzed on the basis of 903 document titles in a subject index, Catalogue of Scientifical and Technical Reference in Chinese Institute of Medical Information. It showed that the number of the documents was increased by 6.5 pieces annually on an average and would continue increasing, but not so quickly, if no significant changes occur.—Author's English Abstract

Chemotherapy

Daumerie, D. and Pannikar, V. Issues in evaluating information on relapse in leprosy. Indian J. Lepr. **67** (1995) 27–33.

The concept of curing leprosy within a short period of time has only recently been introduced to the scientific community and to the world at large. Concomitantly, patients and leprosy workers need assurance that cure will not be followed by a high risk of relapse, even 20 years after completing treatment. There is sufficient evidence today that MDT cures 99% of patients and, therefore, there is no need to waste resources and expertise in actively looking for relapses. Of course, this does not mean that the scientific community should stop closely monitoring the situation because it is well known that a very effective treatment today can be rendered less effective tomorrow because of resistance, co-infections, poor compliance or even poor-quality drugs. The occurrence of relapse should be looked for through a passive surveillance system without placing unnecessary pressure on patients and health workers. Doctors would like to give to their patients an acceptable prognosis for the rest of their life. In the health sector, this situation is somehow unique, except in the field of cancer. Twelve years after the implementation of MDT, the number of relapses reported by both programs and research institutions is very low, one of the lowest in the treatment of communicable diseases. This fact led experts to make MDT implementation more flexible and to cancel the recommendation for surveillance after treatment. The term "robust" is now used to qualify MDT, meaning that even when used in difficult situations and with limited supervision and follow up, this treatment is still very effective.—Author's Conclusions

Desikan, K. V. Relapse, reactivation or reinfection? Indian J. Lepr. **67** (1995) 3–11.

The subject of relapse is much more important today than it was in earlier years when cases were continued on dapsone therapy indefinitely. With the very potent antimicrobial drugs and with the prospect of the influx of more such drugs, the natural tendency is to shorten the duration of therapy. It is absolutely essential to reduce the period of chemotherapy, but in the anxiety to do so, we cannot run the risk of precipitating a relapse. Also there is a tendency at present to cut down the period of surveillance. The risk of relapse should always be borne in mind. As such an in-depth discussion on the subject of relapse is very relevant and essential.—Author's Comment

Dhople, A. M. and Ibanez, M. A. The *in-vitro* activities of novel benzoaxinorifamycins against *Mycobacterium leprae.* J. Antimicrob. Chemother. **35** (1995) 463–471.

The activities of four newly synthesized benzoxazinorifamycin derivatives, either alone or in combination with ofloxacin, against strains of *Mycobacterium leprae* were determined by assessing their effects on two biochemical parameters of metabolic activity which served as surrogate markers for growth *in vitro.* KRM-1648 and KRM-2312 were the most active agents tested against both a rifampin-susceptible isolate (MICs of 0.05 and 0.1 mg/L, respectively) and a rifampin-resistant isolate (MICs of 0.2 and 0.3 mg/L, respectively); both compounds were more active than either rifampin or rifabutin. The activities of the two other derivatives, KRM-1657 and KRM-1668, against a rifampin-susceptible strain (MICs of 0.3 mg/L) were similar to that of rifampin, while the MIC of each of these agents for the rifampin-resistant strain was 1.0 mg/L. In common with rifabutin, both of the more active derivatives demonstrated synergy with ofloxacin against the rifampin-susceptible isolates. The results of this study suggest that these compounds, in combination with ofloxacin as part of multidrug regimens, warrant further evaluation as treatment for patients with leprosy.—Author's Summary

Doucet-Populaire, F., Truffot-Pernot, C., Grosset, J. and Jarlier, V. Acquired resistance in *Mycobacterium avium* com-

plex strains isolated from AIDS patients and beige mice during treatment with clarithromycin. J. Antimicrob. Chemother. **36** (1995) 129–136.

Clarithromycin has been reported to select clarithromycin resistant mutants of *Mycobacterium avium* complex (MAC) during treatment with clarithromycin in AIDS patients and beige mice. We selected resistant mutants *in vitro* at a frequency of 5×10^{-9}. Clarithromycin-resistant strains of MAC isolated in AIDS patients and beige mice as well as derivatives selected *in vitro* had a unique pattern of acquired crossresistance to macrolides and related antibiotics. In contrast, the pattern of resistance to nonmacrolide antibiotics remained unchanged in clarithromycin-resistant strains. A dramatic decrease in ribosome affinity for clarithromycin and erythromycin was found in clarithromycin-resistant strains, but no mutation was found in the peptidyl domain of the 23S rRNA, indicating that another ribosomal modification is involved.—Author's Abstract

Grossman, S., Budinsky, R. and Jollow, D. Dapsone-induced hemolytic anemia: role of glucose-6-phosphate dehydrogenase in the hemolytic response of rat erythrocytes to N-hydroxydapsone. J. Pharmacol. Exp. Therapeut. **273** (1995) 870–877.

Individuals deficient in erythrocytic glucose-6-phosphate dehydrogenase (G6PD) show about a twofold increase in sensitivity toward dapsone-induced hemolytic anemia. Rat studies have shown that the hemolytic activity of dapsone resides in its N-hydroxy metabolites; exposure of rat red cells to N-hydroxydapsone *in vitro* followed by readministration to isologous rats results in premature splenic sequestration of the damaged cells. This study examines the ability of the steroid, epiandrosterone, to inhibit rat red cell G6PD and the effect of such inhibition on the susceptibility of rat red cells to N-hydroxydapsone hemolytic activity. Epiandrosterone was found to inhibit rat red cell G6PD uncompetitively and to suppress red cell hexose monophosphate shunt activity by more than 95%. Epiandrosterone suppression of rat red cell G6PD activity resulted in about a twofold increase in sensitivity of the rat cells to N-hydroxydapsone hemolytic activity, and a modest but significant increase in depletion of red cell glutathione. In contrast, suppression of rat red cell catalase activity by aminotriazole had no effect on the hemotoxicity of N-hydroxydapsone. Epiandrosterone appears to be a useful tool to explore the mechanism by which G6PD deficiency enhances susceptibility to hemolytic drugs.—Author's Summary

Guebre Xabier, M., Shannon, E. J., Kazen, R., Kebret, Z. and Frommel, D. Early detection of rifampin in human nerve tissue after an oral dose of 600 milligrams. Antimicrob. Agents Chemother. **39** (1995) 1866–1870.

Rifampin in picogram quantities inhibited the ability of *Mycobacterium bovis* 44 BCG P3 to release (CO2)-C-14 from the oxidation of [C-14]palmitic acid. By using these mycobacteria in a bioassay, samples of serum and posterior tibial nerve were assayed for inhibitory concentrations of rifampin. Within 8 to 12 hr after ingestion of 600 mg of rifampin, the drug was detected in eight patients in concentrations ranging from 0.52 to 4.1 μg/ml in serum and in concentrations ranging from 0.6 to 6.3 ng/mg in posterior tibial nerve fiber tissue.—Author's Abstract

Hong, B.-Y., *et al.* [Traits of leprosy cases detected after implementation of MDT scheme.] China Lepr. J. **11** (1995) 22–23. (in Chinese)

Since 1987 Fujian Province has begun to use MDT regimen. By the end of 1991, 3307 cases of leprosy had taken or were taking it and 2428 had completed the course of treatment. The prevalence has decreased from 0.0054/10,000 in 1987 to 0.0022/10,000 in 1991; the detection rate decreased by 8.6% and the proportion of childhood cases was reduced by 1.7%. At the same time MP/PB value and early cases with single lesion and with the disease duration <2 years among newly detected patients increased by 0.23, 4% and 11.6%, respectively. The disability rate decreased by 7.2% ($p < 0.001$).—Author's English Abstract

Jacobson, R. R. Treatment of relapsed leprosy. Indian J. Lepr. **67** (1995) 99–102.

We are most fortunate in having a relatively large armamentarium of bactericidal drugs available for the treatment of leprosy today. This greatly simplifies the management of patients infected with drug resistant *Mycobacterium leprae.* Such drugs also create the possibility of developing new shorter term and/or fully supervisible regimens and increasing the efficacy of leprosy chemotherapy still further so that in the future relapses may become even less common than they are today.—Author's Conclusions

Jagannath, C., Allaudeen, H. S. and Hunter, R. L. Activities of poloxamer CRL8131 against *Mycobacterium tuberculosis in vitro* and *in vivo.* Antimicrob. Agents Chemother. **39** (1995) 1349–1354.

A poloxamer surfactant, CRL8131, was evaluated for activity against *Mycobacterium tuberculosis* (Erdman) by itself and in combination with antibiotics in broth culture, in a macrophage cell line assay, and in testing with mice. In the broth culture, CRL8131 suppressed the growth of *M. tuberculosis* and produced synergistic effects in combination with isoniazid, rifampin, and streptomycin. It also displayed synergy with isoniazid and rifampin against two drug-resistant isolates. In the macrophage cell line assay, CRL8131 produced a synergistic effect on intracellular killing of *M. tuberculosis* by isoniazid, rifampin, streptomycin, pyrazinamide, thiacetazone, D-cycloserine, ethionamide, amikacin, clindamycin, and *p*-aminosalicylic acid. It demonstrated no synergy or antagonism with ethambutol, gentamicin, kanamycin, ciprofloxacin, or nalidixic acid. Finally, with C57BL/6 mice infected with *M. tuberculosis,* a combination of CRL8131 and either thiacetazone or pyrazinamide produced 100% survival at 40 days; whereas the antibiotics produced only 33% survival and CRL8131 produced 0% survival when used as single agents. This improved survival rate was associated with a significant reduction in the number of organisms in the lungs and spleens of infected mice.—Author's Summary

Job, C. K. Histopathological features of relapsed leprosy. Indian J. Lepr. **67** (1995) 69–80.

Histopathological examination can be of great assistance in identifying and confirming relapse in both MB and PB leprosy. To get the best results, serial biopsies once every 6 months should be done preferably of the same lesion or a similar lesion. The end point of cure is recognized by the disappearance of *Mycobacterium leprae* in MB patients and by the complete resolution of granuloma in PB patients. Relapse can be identified in the MB patients by the emergence of solid-staining *M. leprae* and a bacillary load of 2+ and above in the lesion in which there was none before; and in PB patients by the reappearance of granuloma in the lesion in which no granuloma was seen during the previous examination. In routine work, regular 6 monthly skin-smear studies should be carried out to recognize relapse in MB patients. Further clinicopathological, immunological and molecular biological studies are recommended to identify relapse and to clarify and differentiate relapse from reaction in PB patients.—Author's Conclusions

Mane, I., Grauwin, M.-Y. and Cartel, J.-L. [Frequency of chronic plantar ulcers in leprosy patients according to treatment by dapsone monotherapy or multidrug therapy.] Acta Leprol. **9** (1995) 127–131. (in French)

Between 1986 and 1989, in five departments of Senegal, 436 new cases of leprosy were detected, of whom 225 were put under dapsone monotherapy and 211 under multidrug therapy (MDT). Of them, 190 could be followed up during 2 years by means of annual bacteriological and clinical examination, including neurological assessment. In 2 years, the onset of 10 (5.3%) chronic plantar ulcers (CPU) was observed: 4 (4%) among the 99 patients under dapsone monotherapy and 6 (6.6%) among the 91 under MDT (no significant difference). Of the 10 CPU, 3 (2%) appeared among the 149 patients without any disability at detection while 7 (17%) were observed among

the 41 others who presented a grade 1 disability at detection ($p < 0.01$). Of the 6 CPU appeared in the patients under MDT, 5 (22%) were observed among the 23 who presented a grade 1 disability at detection and 1 (1.5%) among the 68 who did not ($p < 0.01$). This difference was not noted in the patients under dapsone monotherapy. Our results need to be confirmed by other studies, including a higher number of patients followed up during a longer period of time. Nevertheless, they suggest that MDT could prevent the onset of CPU, but only in patients without any disability at detection. Therefore, they re-emphasize the importance of early detection of the disease in leprosy control programs.—Author's English Summary

McMillan, D. C., Simson, J. V., Budinsky, R. A. and Jollow, D. J. Dapsone-induced hemolytic anemia: effect of dapsone hydroxylamine on sulfhydryl status, membrane skeletal proteins and morphology of human and rat erythrocytes. J. Pharmacol. Exp. Therapeut. **274** (1995) 540–547.

Dapsone hydroxylamine is a direct-acting hemolytic agent responsible for dapsone-induced hemolytic anemia in the rat. In the present study, we compared the responsiveness of rat and human red cells to dapsone hydroxylamine-induced cellular changes. Dapsone hydroxylamine induced a rapid and concentration-dependent loss of erythrocytic reduced glutathione content with a concomitant increase in protein-glutathione mixed disulfide formation in both human and rat red cell suspensions. However, the rate of mixed disulfide formation in human cells was considerably slower than that in rat cells and was preceded by a transient increase in oxidized glutathione (glutathione disulfide) formation. Sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblotting analysis of membrane ghosts from human red cells revealed changes in skeletal proteins that in general were similar to those observed with rat cells, including a loss of protein band 2.1 and the appearance of membrane-bound hemoglobin. Notable differences were the resistance to loss of band 4.2 and a considerably higher amount of protein aggregation in human ghosts. Although the morphology of human red cells was altered, the incidence and degree of change were considerably less than those of rat red cells. Furthermore, the concentration of dapsone hydroxylamine required to induce damage in human red cells (175-750 μM) was significantly higher than that required for rat red cells (50–175 μM), suggesting that human cells are probably less sensitive than rat cells to dapsone hydroxylamine-induced oxidative damage.—Author's Summary

Medda, S., Das, N., Mahato, S. B., Mahadevan, P. R. and Basu, M. K. Glycoside-bearing liposomal delivery systems against macrophage-associated disorders involving *Mycobacterium leprae* and *Mycobacterium tuberculosis.* Indian J. Biochem. Biophys. **31** (1995) 147–151.

Asiaticoside, a plant glycoside with rhamnose as the end sugar and having microbicidal properties was tested against *Mycobacterium leprae* and *M. tuberculosis* both *in vivo* and *in vitro.* As rhamnose is reported to have no tissue specificity, corchorusin D having glucose as the end sugar was used for targeting with an equimolar proportion of asiaticoside in liposomal form for testing the drug value. Results showed that liposomal asiaticoside had better microbicidal property against *M. leprae* and *M. tuberculosis* when compared to that of free asiaticoside; whereas liposomes containing asiaticoside and corchorusin D were found to be equally or more active in comparison to liposomal asiaticoside alone. It is inferred that appropriate glycosides, if used in liposomal form (incorporated or covalently grafted) have enhanced drug efficacy and such glycoside bearing liposomes as targeted delivery systems could be used for chemotherapeutic control of several other diseases.—Author's Abstract

Naafs, B. Features of relapse in paucibacillary leprosy after multidrug therapy. Indian J. Lepr. **67** (1995) 61–67.

In conclusion, it is of utmost importance to assess the patient properly and carefully

at the time of withdrawal of treatment. The assessment should include careful recording of skin and nerve lesions including, if possible, sensory testing (graded bristles) and voluntary muscle testing. These parameters should then be regularly evaluated during follow up over a period of 3 to 5 years. Deterioration should invite immediate action.—From the Symposium paper

Peters, J., Kondo, K. L., Lee, R. K., Lin, C. K. and Inderlied, B. In vitro activity of oxazolidinones against *Mycobacterium avium* complex. J. Antimicrob. Chemother. **35** (1995) 675–679.

Options for treating disseminated *Mycobacterium avium* complex (MAC) disease have improved. However, efficacy is not always certain, resistance is common and rapidly bactericidal agents would improve efficacy and prevent resistance. Certain oxazolidinones were tested against MAC strains and inhibited growth at expected serum concentrations or lower. Activity correlated with hydrophobicity and one agent was bactericidal at concentrations two to five times greater than the MIC.—Author's Abstract

Ponnighaus, J. M. and Sterne, J. A. C. Epidemiological aspects of relapses in leprosy. Indian J. Lepr. **67** (1995) 35–44.

Life-table methods in which the cumulative probability of relapse in successive periods is calculated are preferable to the presentation of overall relapse rates. Their use facilitates the comparison of relapse rates and trends from different studies independent of duration of follow up. Results from various studies including data from Malawi indicate that: (1) unlike after dapsone monotherapy, the cumulative probability of relapse in multibacillary patients is near to zero after WHO/MDT if strict definitions of relapse are used and (2) the cumulative probability of relapse may approach 5% in paucibacillary patients 10 years after completion of WHO/MDT. On the whole, the epidemiological relevance of relapses is insignificant and future treatment regimens should be evaluated concerning their efficacy in preventing disabilities rather than relapses.—Author's Abstract

Qiao, J., *et al.* [The factors of impact on examination of the persons under surveillance after MDT.] China Lepr. J. **11** (1995) 24–26. (in Chinese)

By the end of March 1994 there were 587 persons cured of leprosy with dapsone (DDS) alone accumulatively in Jiaozhou City, Shandong, of which 507 were willing to be examined in the follow up. Among those who escaped or resisted the examination all were due to fear of discrimination. The authors suggest that knowledge of the disease must be widely spread if one hopes that the persons under surveillance after stopping treatment of leprosy could receive the examination without a hitch.—Author's English Abstract

Ramu, G. Clinical features and diagnosis of relapses in leprosy. Indian J. Lepr. **67** (1995) 45–59.

1. The definition of relapse as "occurrence of new signs and symptoms of the disease during the period of surveillance or thereafter in a patient who successfully completes an adequate course of multidrug therapy" accommodates the current policy of releasing patients even when there are clinical and bacteriological signs of activity after fixed duration treatment. 2. The predisposing cause of relapse is the persistence of live *Mycobacterium leprae* in various tissues in MB leprosy and in the nerve in PB leprosy. 3. The precipitating causes of relapse include (a) inadequate therapy due to miscategorization of MB cases as PB when there are solitary or few MB lesions since skin-smear examinations for AFB are not routinely done in PB cases. (b) Previously sulfhone treated LL cases inactive for more than 2 years are not included for MDT. Relapses commonly seen in NLEP units are in such cases. (c) Multiple skin and nerve lesions in PB leprosy. (d) Pregnancy and lactation. (e) Mental depression which downgrades immunity. (f) HIV infection. 4. There may be a change in type on relapsing, PB cases relapsing as MB and MB cases relapsing as PB. 5. Criteria for diagnosis of relapse are: increase in the extent of lesions, infiltration and erythema, fresh skin and nerve lesions, positive skin smears for AFB

in previously negative cases; and in bacteriologically positive cases during surveillance, an increase in BI by two logs at any site over the previous BI in two successive examinations. 6. Relapses are but too often diagnosed as reversal reactions in spite of the absence of symptoms and signs of acute inflammation to the detriment of patients; a course of steroid therapy which is administered to these patients on the diagnosis of reversal reaction does not halt the progress of the disease especially in the nerve, resulting in disability.—Author's Summary

Sengupta, U. Immunological aspects of relapse in leprosy. Indian J. Lepr. **67** (1995) 81–83.

There is an immediate need for developing a simple laboratory-based technique for detection and confirmation of cases of relapse in leprosy. There is a scope for developing assays based on immunological techniques described above. Research should be geared to this direction in various laboratories in the world so that a cheap as well as specific test could be developed that could be applied under field conditions to monitor cases who have been released from treatment or who are on chemotherapy for early diagnosis of relapse.—Author's Conclusion

Soares, D., Neupane, K. and Britton, W. J. Relapse with multibacillary leprosy caused by rifampicin sensitive organisms following paucibacillary multidrug therapy. Lepr. Rev. **66** (1995) 210–213.

Many leprosy patients treated with multidrug therapy (MDT) had previously received dapsone (DDS) monotherapy for many years. We report here 2 such patients treated with modified paucibacillary MDT composed of rifampin and DDS who subsequently relapsed with multibacillary leprosy 5 and 6 years after release from treatment. Isolates of *Mycobacterium leprae* from both patients were resistant to DDS but sensitive to rifampin, suggesting that the relapses were caused by rifampin sensitive "persister" organisms. The implications of this for surveillance of patients released from treatment (RFT) and the management of relapsed patients is discussed.—Author's Summary

Speirs, R. J., Welch, J. T. and Cynamon, M. H. Activity of *n*-propyl pyrazinoate against pyrazinamide-resistant *Mycobacterium tuberculosis:* investigations into mechanism of action of and mechanism of resistance to pyrazinamide. Antimicrob. Agents Chemother. **39** (1995) 1269–1271.

The mechanism of action of pyrazinamide (PZA) is not known. One hypothesis is that PZA functions as a prodrug of pyrazinoic acid. Susceptibility to PZA correlates with amidase activity of the *Mycobacterium tuberculosis* isolate in question. PZA-resistant isolates retain susceptibility *in vitro* to pyrazinoic acid and *n*-propyl pyrazinoate. Esters of pyrazinoic acid appear to circumvent the requirement for activation by mycobacterial amidase. The MICs of *n*-propyl pyrazinoate for *M. tuberculosis* isolates are lower than those of pyrazinoic acid. Further studies to assess the effects of modifications of the alcohol and pyrazine moieties of pyrazinoate esters on *in vitro* and *in vivo* antituberculosis activity are under way. This may lead to a candidate compound with enhanced activity against both PZA-susceptible and PZA-resistant *M. tuberculosis* isolates suitable for clinical development.—Author's Abstract

WHO Leprosy Unit. Risk of relapse in leprosy. Indian J. Lepr. **67** (1995) 13–26.

Until the introduction by WHO of the standard regimens using multidrug therapy (MDT) for the treatment of leprosy, there was a general unwillingness to release patients from treatment. This was mainly due to the high risk of relapse after dapsone monotherapy. After almost a decade of MDT implementation and after releasing more than 4 million patients, it was necessary for WHO to review the risk of relapse following WHO-recommended MDT. The results of this study, carried out on more than 20,000 MB and 50,000 PB patients, revealed that the risk of relapse is very low, 0.77% for MB and 1.07% for PB, 9 years after stopping MDT. In comparison to dap-

sone monotherapy, the risk is 10 times lower. Thus, over the last decade, MDT implementation has probably prevented close to half-a-million relapses.—Author's Abstract

Wozel, G. and Lehmann, B. Dapsone inhibits the generation of 5-lipoxygenase products in human polymorphonuclear leukocytes. Skin Pharmacol. **8** (1995) 196–202.

Dapsone (4, 4′-diaminodiphenylsulfone) is effective in treating inflammatory skin diseases. Several lines of evidence suggest that the antiinflammatory properties of this sulfone are partially due to modulation of functions of polymorphonuclear leukocytes (PMN). The goal of the present investigation is therefore to ascertain possible inhibitory effects of dapsone upon human 5-lipoxygenase (5-LOX) pathway. PMN of healthy donors were pretreated with dapsone in different concentrations (1.6-100 μM) for 5 min following by adding Ca ionophore A 23187 (10 μM) and subsequent incubation for 10 min. Thereupon the eicosanoids were assessed by reversed-phase high-performance liquid chromatography (RP-HPLC). Dapsone exhibited dose-dependent inhibitory activity showing 50% inhibition at 15 μM for leukotriene B-4 (LTB^4) with 5×10^6 PMN. The IC50 (half maximum inhibition concentration) of dapsone for 5-hydroxyeicosatetraenoic acid (5-HETE) and omega-OH-LTB^4 amounted to similar values (5-HETE: 9 μM; omega-OH-LTB^4 : 11 μM). The inhibition of the conversion of arachidonic acid to several eicosanoids mainly suggests an effect on the 5-LOX enzyme. The comparison of inhibition values between intact PMN and a cell-free system (by sonification) indicates an additional effect of dapsone upon enzymes other than 5-LOX. Since the concentrations used are comparable with therapeutic conditions, dapsone may exert part of its antiinflammatory effect by prevention of the generation of 5-LOX metabolities.—Author's Abstract

Zhou, X. [Relapse of leprosy after MDT and after DDS plus RMP.] China Lepr. J. **11** (1995) 20–22. (in Chinese)

Since 1987 the MDT regimen has been widely used in Yunnan Province. Up to now, 6117 persons under surveillance after completion of MDT (group I) and 7062 cured with DDS plus RMP (group II) are alive. The authors examined them for detection of relapse in 1993. In group I, no relapse among those who took MB: regimen and five relapses among those who had PB regimen accounting for 0.32% or 0.9/1000 PY were seen. In group II, relapse rate was 1.2%, being 0.77% in MB cases and 1.96% in PB.—Author's English Abstract

Clinical Sciences

de Carvalho, M. L. R., Araujo, M. G., Guedes, A. C. M. and Patrus, O. A. [Evolution of borderline tuberculoid hanseniasis during multidrug therapy.] An. Bras. Dermatol. **70** (1995) 201–204. (in Portuguese)

Background—There are only a few studies, in Brazilian medical literature, that provide a clinical evaluation of cases of borderline tuberculoid (BT) leprosy.

Objectives—To describe the clinical evolution of 71 cases of leprosy submitted to a multidrug therapy (MDT/WHO).

Methods—This work was carried out at the Dermatology Unit of the School of Medicine, Federal University of the State of Minas Gerais, Brazil. Seventy-one pacients with BT leprosy were evaluated from August 1989 to August 1993.

Results—The clinical picture was rated as "mild" (up to five skin lesions) or "severe" (more than five skin lesions), and, as to the histological picture, patients BT were grouped as having "high" or "low" immunity. The evolution of these two groups during multidrug therapy showed no statistically significant difference. The lepromin reaction (Mitsuda) has shown to be determinant in the evolution of BT pacients.

Conclusion—The lepromin reaction (Mitsuda) has clearly shown to be the major

determinant in the favorable evolution of BT pacients undergoing multidrug therapy.—Author's English Summary

de Carvalho, M. L. R., Araujo, M. G., Guedes, A. C. M. and Patrus, O. A. [Type 1 reactions in borderline tuberculoid hanseniasis during multidrug therapy: time of appearance of nerve involvement.] An. Bras. Dermatol. **70** (1995) 205–208. (in Portuguese)

Background—There are few studies of type 1 reaction in medical literature.

Objectives—To describe the onset of type 1 reaction and the affected nerves in patients with BT leprosy undergoing multidrug therapy.

Patients and Methods—This work was carried out at the Dermatology Unit of the School of Medicine. Federal University of the State of Minas Gerais, Brazil. Seventy-one patients with borderline tuberculoid leprosy (BT) were evaluated from August 1989 to August 1993.

Results—89.3% BT patients with type 1 reaction developed the symptoms up the sixth dose (MDT). The ulnar nerve was the more frequently affected in cases of type 1 reaction (37.0%).

Conclusion—The type 1 reaction in BT leprosy develops, most frequently, up to the sixth dose (MDT). The ulnar nerve was the more frequently affected.—Author's English Summary

Drateln, C. R. [Kaposi's sarcoma in an HIV-negative patient with lepromatous leprosy.] Rev. Med. Inst. Mex. Seguro Social **31** (1994) 19–21. (in Spanish)

Kaposi's sarcoma is considered an opportunist neoplasm and has been reported frequently in patients with AIDS. The purpose of this case review from Mexico is to consider that there are other immunosuppression factors, such as leprosy and chronic use of steroids, that may produce the sarcoma. This report presents the clinical evolution in a 53-year-old, male HIV-negative patient who suffered from lepromatous leprosy with recurrent erythema nodosum leprosum. The patient treated himself with prednisone for 18 months, then presented with Kaposi's sarcoma with visceral and cutaneous involvement and renal amyloidiasis complications.—Author's English Summary

Fiallo, P., Pesce, C., Lenti, E. and Nunzi, E. Erythema nodosum leprosum lymphadenitis. Am. J. Trop. Med. Hyg. **52** (1995) 297–298.

A case of isolated erythema nodosum leprosum lymphadenitis involving the paravertebral, intercostal, and cervical lymph nodes without concomitant skin involvement is reported in a 62-year-old male patient under treatment for lepromatous leprosy.—Author's Summary

Goto, M., Kimura, T., Hagio, S., Ueda, K., Kitajima, S. I., Tokunaga, H. and Sato, E. Neuropathological analysis of dementia in a Japanese leprosarium. Dementia **6** (1995) 157–161.

In a neuropathological study of consecutive autopsies, prevalence and cause of dementia in a Japanese leprosarium were investigated, where more than 95% of inpatients with a mean age of 70 years are now free from active leprosy. In 10 years (1983-1992), clinically overt dementia at death was 35/136 (25.7%) in the age group over 65 years (mean age 79.4). Autopsy was performed in 85 cases (mean age 81 years), and clinically overt dementia was seen in 25 subjects (29.4%). Neuropathologically, Alzheimer's disease (AD) was seen in 9 cases (10.6%), vascular dementia (VD) in 9 cases (10.6%), mixed type in 3 cases (3.5%) and unclassified in 4 cases (4.7%). In the age group of 65–84 years, AD was 5/58 (8.6%), VD was 4/58 (6.9%), mixed type was 2/58 (3.4%), and unclassified was 1/58 (1.7%). Compared with previous Japanese general population-based data, where VD was more frequent than AD, the rate of dementia in our leprosarium was high, and pathologically confirmed AD was as common as VD. Recently, a prophylactic effect of the antileprosy and antiinflammatory drug DDS (dapsone, 4, 4′-diaminodiphenyl sulfone) has been suggested. Lepromatous patients take more DDS (51.9%) than tuberculoid patients (11.5%). However, as the dementia rate of tuberculoid leprosy (17.9%) in those 65–84 years old is similar to lepromatous

leprosy (15.9%) in our study, we do not support their viewpoint.—Author's Summary

Groenen, G., Saha, N. G., Rashid, M. A., Hamid, M. A. and Pattyn, S. R. Classification of leprosy cases under field conditions in Bangladesh. I. Usefulness of skin-smear examinations. Lepr. Rev. **66** (1995) 126–133.

In two nongovernmental organization projects in Bangladesh 244 new leprosy patients were classified in the field according to clinical criteria. Skin smears were taken at four standardized sites and at the most active peripheral lesion, where a biopsy was also taken. Comparison of the clinical field classification with the results of the skin smears and biopsies gives a sensitivity of 92.1% for the clinical criteria, but a specificity of only 41.3%. The skin-smear results, on the other hand, have a sensitivity of 88.4% and a specificity of 98.1%. Thus, skin smears may contribute considerably to the operational classification of leprosy patients under field conditions. Quality control of the peripheral laboratory is essential. Appropriate site selection for the smear taking will also contribute to increased performance. Analysis of the skin-smear results suggests that the policy of taking smears at standardized sites should be abandoned in favor of the earlobes and active peripheral lesions.—Author's Summary

Groenen, G., Saha, N. G., Rashid, M. A., Hamid, M. A. and Pattyn, S. R. Classification of leprosy cases under field conditions in Bangladesh. II. Reliabilility of clinical criteria. Lepr. Rev. **66** (1995) 134–143.

In two nongovernmental organization projects 244 new leprosy patients in Bangladesh were classified in the field according to clinical criteria, i.e., number of skin lesions and number of enlarged nerves. Comparison of these classification results with the results of skin smears and biopsies yielded a sensitivity (for detection of a MB case) of 92.1%, but the "unconfirmed MB rate" amounted to 52.6%. In order to improve the reliability of the operational classification, several additional clinical criteria were investigated. It was found that neither the presence of anesthesia in the skin lesions nor the presence of grade 2 disabilities or peripheral anesthesia or voluntary muscle testing (VMT) impairment contributed to an improved classification. Counting the number of body areas showing signs of leprosy, which had proven very useful in other programs, did not result in a more reliable classification in the two projects in Bangladesh. The presence of clinical signs of lepromatous leprosy, more specifically nodules or diffuse infiltration, could be a useful addition to the classification criteria. If the sensitivity must remain higher than 90%, the lowest "unconfirmed MB rate" obtainable in Bangladesh, using clinical criteria only, is 46.4%, for a sensitivity of 91.0%. However, the inclusion of skin-smear results in the classification criteria could improve the sensitivity to 96.6% and lower the "unconfirmed MB rate" to 40.3%. A reduction in MB overclassification will result in more efficient and more cost-effective leprosy control programs.—Author's Summary

Hosokawa, A., Nonaka, S., Alava, P. J. J., Commez, L. E. A., Jurado, S. H. M. and Hashiguchi, Y. Case report of leprosy and a trial of screenings for the family members in Ecuador. Jpn. J. Trop. Med. Hyg. **22** (1994) 219–223.

Four cases of patients with leprosy were seen in an area endemic for cutaneous leishmaniasis: Los Ranchos, Department of Manabi, Ecuador. Two cases (borderline lepromatous leprosy and indeterminate leprosy) in a single family and the result of screenings of the family members are reported. It was suggested that family examination of leprosy patients might be useful for early detection of leprosy in low endemic areas for leprosy, such as Department of Manabi. A nine-banded armadillo kept by the family was examined, but no acid-fast bacilli were observed in the liver materials.—Author's Summary

Katoch, K., Natrajan, M., Yadav, V. S. and Bhatia, A. S. Response of leprosy patients with single lesions to MDT. Acta Leprol. **9** (1995) 133–137.

This study reports the clinical profile and therapeutic response of 72 monolesion leprosy cases. These 72 cases were among 578 paucibacillary (PB) cases classified according to WHO (1982) and were followed up

on multidrug therapy (MDT). Of these 72 monolesion cases, 46 (64%) were tuberculoid (TT) cases, 24 (33%) were indeterminate (Ind) cases and 2(3%) were of borderline tuberculoid (BT) types. While 37.5% of these cases presented as macular patches, the remaining 62.5% had raised erythematous lesiόns. In the majority of cases (94%), the lesions were present on the exposed parts like legs and feet, arms and hands, face; whereas only 6% presented on the covered areas of the trunk and buttocks. These cases were treated with dapsone 100 mg daily for 12 months and rifampin 600 mg once a month for 6 months. After 6 months of MDT, lesions in 81% of the patients regressed clinically and by 1 year of therapy 96% of cases had regressed. Treatment was stopped in all cases by 1 year of therapy. There were no relapses or late reactions in the 5 years of post-treatment follow up. The response of monolesion PB cases was better than the multilesion PB cases at 6 months and during the post-treatment follow up period.—Author's Summary

Martins, C. F. [Palpation of peripheral nerves (affected in hanseniasis) and their anatomical pathways.] An. Bras. Dermatol. **70** (1995) 247–250. (in Portuguese)

In the present work, the superficial pathways of nerves damaged in leprosy were revealed through dissection. The lesion of the disease, lesions occur more frequently in some nerves than in others, the former growing thicker than normal. The affected nerves run close to the surface in some places and are, thus, more easily acessible to surgical intervention in such anatomical locations.—Author's English Summary

Mizuno, K., Okamoto, H., Matsumura, Y., Fijii, K., Furukawa, F., Obara, A., Izumi, S. and Imamura, S. Neuropathy as the only sign of borderline lepromatous leprosy for 13 years. Eur. J. Dermatol. **5** (1995) 306–309.

A 57-year-old man experienced numbness in his right hand in 1975 and then noticed weakness and sensory disturbance in his legs. The neurological symptoms were suggestive of a degenerative neural disease, such as Charcot-Marie-Tooth disease. However a diagnosis of leprosy was established because of nerve enlargement and tenderness and after a nerve biopsy. The patient did not have any skin lesions. Treatment was initiated with diaminodiphenylsulfone (DDS) and discontinued 1 year later at the patient's request. Various asymmetric eruptions first appeared on his body in 1988. Based on the 5+ average bacterial index of eruptions and the negative lepromin reaction in addition to the clinical pictures, he was diagnosed as having borderline lepromatous leprosy. Our case is a very rare type of borderline lepromatous leprosy in which peripheral neuropathy preceded the onset of skin lesions by 13 years.—Author's Summary

Moran, C. A., Nelson, A. M., Tuur, S. M., Luengu, M., Fonseca, L. and Meyers, W. M. Leprosy in five human immunodeficiency virus-infected patients. Mod. Pathol. **8** (1995) 662–664.

We present five cases of leprosy in five human immunodeficiency virus-positive individuals. The patients were five men between the ages of 18 and 45 years. The five patients presented with skin lesions; three patients with hypopigmented skin lesions, one with skin macules and a history of leprosy, and one with papular lesions. In one patient, there was bilateral "claw hands." Histologically, 2 cases were categorized as lepromatous leprosy, 2 as borderline tuberculoid, and 1 as borderline lepromatous. Follow up information obtained in the 5 patients showed 1 patient had died, and the remaining 4 patients were alive and receiving antileprosy treatment.—Author's Abstract

Opromolla, D. V. A. [Relapse or reversal reaction?] Hansen. Int. **19** (1994) 10–16. (in Portuguese)

A woman, 88 years old, was admitted in the "Lauro de Souza Lima" Institute presenting acute, large, erythematous "BT" plaques over all her body. She was previously admitted at the Institute in 1935, 40 years ago, presenting similar although less intense lesions that disappeared spontaneously after 2 years. In the first reactional episode bacilli were found only in a lymph node puncture. During the second reaction bacilli could be found in all lesions. Histopathology showed tuberculoid granulo-

mas in both episodes. Usually, this sort of acute phenomenon has a duration of several months and the gap between the first and the next is from 2–3 months to several years (5 years in a case described by Wade). In our case the interval between the two eruptions was extremely large, 40 years! We may consider in this case the possibility of a reinfection but it is difficult to admit this fact due to the type of the new lesions observed and the appearance of some lesions near the site of the former ones. We believe that it is easier to accept the hypothesis of a relapse. In this sense, we believe that persisting bacilli multiply after 40 years, are destroyed, in part or totally, by the immune system and resultant antigens elicit a delayed hypersensitivity reaction presenting acute cutaneous lesions as clinical expression. If this is true, we can extrapolate this fact to explain all the reversal reactions ocurring before, during and after treatment. Thus, after completion of treatment, there is no need to differentiate between reversal reaction and relapse and, moreover, it will be necessary to reappraise the effect of chemotherapy in such cases.—Author's English Summary

Opromolla, D. V. A. and Brasil Filho, A. A. C. [Early lesions in disseminated reactional cases.] Hansen. Int. **19** (1994) 39–42. (in Portuguese)

Authors studied 50 patients with disseminated reactional lesions and classified both as reactional tuberculoid and reactional borderline (type 1 reaction of Jopling). After careful history taking and clinical and dermatological examination, it was clear that all of them had a similar clinical story as regards the beginning of the disease, regardless of the immunological grade. The initial lesions (mother lesions) are hypochromic macules or circumscribed areas of normal color skin with some sensory impairment, which should not be misinterpreted as those reported by Wade that circumscribe "immune areas." These lesions seem to precede a phase of bacillemia with destruction of bacilli, in the place they are, and the liberation of antigens with the consequent manifestation of delayed hypersensitivity, which is the reactional episode. Treatment would have some role to play only in those cases with low immunity and, thus, prone to new reactional episodes. Without treatment, these patients would continue to present new reactional episodes indefinitely.—Author's Abstract

Opromolla, D. V. A. and Fleury, R. N. [Sulfone syndrome and reversal reaction.] Hansen. Int. **19** (1994) 70–76. (in Portuguese)

A 68-year-old, black male with MB leprosy without treatment was admitted in the Institute "Lauro de Souza Lima" exhibiting diffuse infiltration and a few ENL nodules. He was treated with sulfone and thalidomide. One and a half months after the beginning of treatment he presented a rash with high fever and enlargement of lymphonodes, liver and spleen. Leukocyte count showed severe leukocytosis with increased numbers of lymphocytes, some of them with atypical features. He evolved with icterus, dehydration and mental confusion. Death occurred four days after the appearance of the rash. A diagnosis of dapsone syndrome was made. This syndrome is considered as a hypersensitivity reaction to that drug and, in some aspects, could mimic or even induce the onset of infectious mononucleosis. There are several reports on dapsone syndrome, some with fatal outcome. However, so far there are no reports on necroscopy findings.

A generalized granulomatous tuberculoid reaction mixed with lepromatous infiltration rich in bacilli was observed together with atypical lymphocytic infiltration. The authors believe that the pathologic findings suggests a reversal reaction induced by dapsone syndrome. The authors report their experience on reversal reaction arising in patients with some other conditions, such as neoplasm, hepatitis, bone tuberculosis and diabetes.—Author's English Summary

Opromolla, D. V. A., Ura, S. and Ghidella, C. [On tuberculoid reactions.] Hansen. Int. **19** (1994) 26–33. (in Portuguese)

Twenty patients with generalized type 1 reaction and positive Mitsuda test equal to 6 mm or more were studied. Clinical of baciloscopic, histopathologic and evolutionaries features were considered. Erythematous lesions, papules and plaques, with well defined edges, and little involvement of nerves were observed in all cases. Bacillary

index was negative or very low and the histopathologic findings included loose tuberculoid granuloma due to intracellular and intercellular edema and vascular congestion. Very few patients presented more than one acute episode. The authors suggest that the eruption is due to bacillary multiplication. The reactional flare would be a kind of delayed hypersensitivity to the antigens released during the destruction of bacilli. They claim that these cases exist and need to be recognized for a better understanding of the disease and this fact may have therapeutics implications since they present many lesions with few or no bacilli, and a trend to spontaneous cure.—Author's English Summary

Rao, S. P., Taori, G. M., Desikan, K. V. and Nayar, S. Clinical and electroneurophysiological assessment of leprosy patients on dapsone monotherapy—a two year follow up study. Indian J. Lepr. **67** (1995) 167–176.

Fifty-three persons with tuberculoid type of leprosy having a thickened nerve on one side and a clinically normal nerve on the contralateral side were studied before, during and after 2 years of therapy for electrophysiological abnormalities in apparently normal and in obviously thickened nerves. Twenty-seven patients had received treatment with dapsone 100 mg orally and 26 cases had received rifampin therapy. It was found that there was no extension of anesthesia or diminution of motor power over a period of 2 years. There was no significant difference between the initial and final recordings of motor and sensory nerve conductions if aggregate figures were taken. However, taking individual cases, deterioration in nerve conduction (increased latency and decreased velocity) was found in two patients, of whom one had received dapsone and the other had received rifampin.—Author's Abstract

Salafia, A. and De Geiking, I. ENL necroticans; report on 5 cases. Rev. Leprol. Fontilles **20** (1995) 645–649.

Between the years 1987–88 we have seen 18 cases of ENL necroticans and in 1993 another 4. In this paper we present 5 of these cases because of some common features and detailed records as they were hospitalized and under direct care. ENL necroticans in one of the many manifestations of type 2 reaction.—Author's Summary

Salodkar, A. D. and Kalla, G. A clinico-epidemiological study of leprosy in arid northwest Rajasthan, Jodhpur. Indian J. Lepr. **67** (1995) 161–166.

A detailed clinical, bacteriological and histopathological study of 1373 patients of leprosy who sought medical advice at the Department of Skin, STD and Leprosy of Dr. Sampurnanand Medical College, Jodhpur, during 1975–1993 is reported. The disease was observed in 154 patients per 1000 cases attending as skin department outpatients. More than 50% of them had the polar type of lepromatous leprosy. The disease was found 2.42 times more often in males than in females and was found mainly in the age group 11–70 years. Family history of leprosy was obtained in 130 (9.5%) of the cases. Lepra reactions were seen in 151 (11%) cases, of which 30 had type 1 reaction (19.2%) and 121 type 2 reaction (80.1%). The majority of leprosy cases (966 or 70.4%) were from Jodhpur district, followed by 109 (7.9%) from Nagaur district and then from, Barmer, Jaisalmer and Jalore, etc. All cases of leprosy responded well to the WHO regimens of multidrug therapy. The reactional cases were satisfactorily managed with higher doses of clofazimine along with oral prednisolone.—Author's Summary

Saporta, L. and Yuksel, A. Androgenic status in patients with lepromatous leprosy. Br. J. Urol. **74** (1994) 221–224.

Objective: to assess the incidence of testicular atrophy by evaluation of hormonal status, testicular histology and sperm production in chronic lepromatous patients. Patients and methods: 41 male patients with a mean age of 39.5 years (range 16-57) were studied at the leprosy hospital, Istanbul, Turkey, and were compared with 15 age-matched controls with proven fertility. Results: reduced testicular size was observed in 51% and gynecomastia in 27%. Of the 31 patients who were married, 15 were primarily or secondarily infertile. Twelve of 16 patients had oligospermia or azoospermia.—Author's Summary

Immuno-Pathology

Appelberg, R., Sarmento, A., and Castro, A. G. Tumour necrosis factor-alpha (TNF-α) in the host resistance to mycobacteria of distinct virulence. Clin. Exp. Immunol. **101** (1995) 308–313.

The relative virulence of different isolates of *Mycobacterium avium* has been linked to their capacity to trigger the secretion of TNF from the macrophages they infect. Smooth opaque (SmOp) variants of *M. avium* have been shown to trigger higher expression of TNF-alpha by macrophages *in vitro* than the smooth transparent (SmTr) variants. To analyse the role of TNF in resistance to infection by *M. avium,* we studied the infection by two different morphotypes of strain 2.151 of *M. avium* both *in vitro* and *in vivo* in the presence or absence of neutralizing antibodies to TNF. No effects were found *in vitro* regarding the growth of either isolate of *M. avium. In vivo,* only the virulent SmTr morphotype showed enhanced growth in the presence of the neutralizing antibodies. This enhancement occurred relatively late when priming for TNF secretion *in vivo* was evident. Among four isolates of *M. avium,* three virulent ones induced a marked priming for TNF release and one avirulent strain did not. *M. tuberculosis* H37Ra, which is very active in inducing TNF release due to its lipoarabinomannan moiety, was used to compare with the previous results. The growth of H37Ra in macrophages was increased *in vitro* by the neutralization of TNF and neutralization of either TNF and/or interferon-gamma (IFN-γ) enhanced the *in vivo* proliferation of this microbe in the spleen and liver of infected animals; whereas only the combination of both anti-TNF and anti-IFN-enhanced bacterial proliferation in the lung. We conclude that resistance to the avirulent strains of *M. avium* did not involve TNF, but rather antimicrobial mechanisms expressed consitutively in the mononuclear phagocytes. In contrast, TNF plays an important role in the control of *M. tuberculosis* H37Ra infection.—Author's Summary

Castro, A. G., Silva, R. A. and Appelberg, R. Endogenously produced IL-12 is required for the induction of protective T cells during *Mycobacterium avium* infections in mice. J. Immunol. **155** (1995) 2013–2019.

Immunity to *Mycobacterium avium* depends on the induction of protective $CD4^+$ T cells. In mice, *M. avium* induces a Th1 response leading to protective immunity dependent on IFN-gamma and TNF. In this study, we analyzed whether endogenously produced IL-12 was involved in the generation of such protective T cells. We found that the neutralization of IL-12 with the administration of specific monoclonal antibodies (mAb) throughout the course of the infection led to the inability of BALB/c mice to control the infection by *M. avium,* strain 2447. On the contrary, the late neutralization of IL-12, with the administration of the mAB starting only at the third week of infection, did not affect the growth of *M. avium.* The neutralization of IL-12 blocked the induction of protective T cells detected upon adoptive transfer to sublethally irradiated recipient mice. The neutralization of IL-12 in the recipient mice did not affect the protective activity of immune cells, showing that IL-12 is involved mainly in the induction, and not the expression, of acquired cell-mediated immunity. IL-12 was also shown to be required for a T cell-independent pathway of resistance present in T cell-deficient severe combined immunodeficient (SCID) mice. Finally, animals whose IL-12 was blocked expressed heightened levels of IL-4 and IL-10 message and reduced expression of IFN-gamma as compared with control mice.—Author's Abstract

Cestari, T. F., Kripke, M. L., Baptista, P. L., Bakos, L. and Bucana, C. D. Ultraviolet radiation decreases the granulomatous response to lepromin in humans. J. Invest. Dermatol. **105** (1995) 8–13.

Ultraviolet radiation (UVR) modulates cellular immunity in humans and experimental animals and can interfere with immune responses against infectious agents in animal models. We used the lepromin reaction, a cell-mediated immune response to antigens of *Mycobacterium leprae,* to determine whether UVR affects the cellular im-

mune response to an infectious agent in humans. We selected 29 healthy, lepromin-positive contacts of leprosy patients and determined their minimal erythema dose (MED) of UVR. Immediately afterward, each subject was injected with 0.1 ml of lepromin in two areas of the buttocks: one at the site that had received twice the MED of UVR and the other on the contralateral, unirradiated site. The irradiated site was given twice the MED every 4 days for a total of five treatments. One week after the last irradiation, both lepromin reactions were measured and biopsied. The size of the lepromin-induced granulomas was significantly reduced in the irradiated site, as was the number of lymphocytes. Immunohistochemical analysis showed a depletion in the number of infiltrating cells and a lower percentage of T cells, particularly the $CD4^+$ subpopulation, in granulomas formed in UV-irradiated skin. This study demonstrates that local UV irradiation reduces the granulomatous reaction to lepromin in sensitized individuals. These findings are of clinical relevance because of the fundamental role played by the delayed-type hypersensitivity response in defense against intracellular pathogens and because of potential increases in the amount of UVR in sunlight reaching the earth's surface.—Author's Abstract

Conradt, P. and Kaufmann, S. H. E. Impact of antigen-presenting cells on cytokine profiles of human Th clones established after stimulation of *Mycobacterium tuberculosis* antigens. Infect. Immun. **63** (1995) 2079–2081.

Human T cells reactive with mycobacterial antigens are generally considered to correlate with a Th1 cytokine profile. Our data show that, in addition, Th0 and Th2 clones develop in bulk culture with appropriate antigen-presenting cells before cloning. $CD4^+$ blasts activated by mycobacterial antigens were cloned, and their mRNA patterns for the interleukins IL-2, IL-4, IL-5, IL-6, and IL-10 and gamma-interferon were characterized by reverse-transcribed PCR. Nonadherent, nonrosetting, enriched peripheral blood mononuclear cells promoted development of Th0; after further depletion of monocytes and natural killer cells? Th2 clones were also found; Epstein-Barr virus-transformed B cells, with specificity for the stimulating antigen, increased the proportion of Th2 clones.—Author's Abstract

Daugelat, S., Ladel, C. H. and Kaufmann, S. H. E. Influence of mouse strain and vaccine viability on T-cell responses induced by *Mycobacterium bovis* bacillus Calmette-Guerin. Infect. Immun. **63** (1995) 2033–2040.

C57BL/6 and BALB/c mice were vaccinated with either live or heat-killed *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) organisms, and splenic T cells were used to screen the stimulatory potential of fractionated somatic and secreted mycobacterial proteins by production of gamma interferon (IFN-γ). Maximum responses were obtained with fractionated secreted proteins of *M. tuberculosis*. There was no single dominant antigen, but five regions of mycobacterial proteins induced high concentrations of IFN-γ. However, only two of the five regions stimulated T cells from both mouse strains: two were exclusively recognized by T cells from BALB/c mice, and one was exclusively recognized by T cells from C57BL/6 mice. T cells from mice vaccinated with heat-killed *M. bovis* BCG organisms failed to respond to fractionated secreted proteins but recognized several somatic antigen fractions. As late as 1 year after primary vaccination, memory T cells responded to similar protein regions, and IFN-γ production was intensified by secondary infection. Our data confirm a central role for secreted proteins in immunity to mycobacteria. Moreover, we demonstrate that a major set of mycobacterium-reactive T cells is stimulated only by vaccination with live but not with heat-killed *M. bovis* BCG organisms. Because a major impact of genetic host factors on antigen recognition was observed, we favor the use of live carrier organisms which secrete mycobacterial proteins over subunit vaccines as an improved antituberculosis vaccine.—Author's Abstract

Flesch, I. E. A., Hess, J. H., Huang, S., Aguet, M., Rothe, J., Bluethmann, H. and Kaufmann, S. H. E. Early interleukin 12 production by macrophages in response to mycobacterial infection depends on in-

terferon gamma and tumor necrosis factor alpha. J. Exp. Med. **181** (1995) 1615–1621.

Interleukin 12 (IL-12) produced by macrophages immediately after infection is considered essential for activation of a protective immune response against intracellular pathogens. In the murine *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) model we assessed whether early IL-12 production by macrophages depends on other cytokines. *In vitro,* murine bone marrow-derived macrophages produced IL-12 after infection with viable *M. bovis* BCG or stimulation with LPS. However, priming with recombinant interferon gamma (rIFN-γ) was necessary. In addition, IL-12 production by these macrophages was blocked by specific anti tumor necrosis factor alpha (TNF-α) antiserum. Macrophages from gene deletion mutant mice lacking either the IFN-γ receptor or the TNF receptor 1 (p55) failed to produce IL-12 *in vitro* after stimulation with rIFN-γ and mycobacterial infection. *In vivo,* IL-12 production was induced in spleens of immunocompetent mice early during *M. bovis* BCG infection but not in those of mutant mice lacking the receptors for IFN-γ or TNF. Our results show that IL-12 production by macrophages in response to mycobacterial infection depends on IFN-γ and TNF. Hence, IL-12 is not the first cytokine produced in mycobacterial infections.—Author's Abstract

Flynn, J. L., Goldstein, M. M., Triebold, K. J., Sypek, J., Wolf, S. and Bloom, B. R. IL-12 increases resistance of BALB/c mice to *Mycobacterium tuberculosis* infection. J. Immunol. **155** (1995) 2515–2524.

IL-12, a cytokine produced by macrophages and B cells, has recently been found to exert pleiotropic effects on the immune system. When BALB/c mice, a strain highly susceptible to virulent *Mycobacterium tuberculosis* infection, were given IL-12 at the initiation of infection with M. tuberculosis, their mean survival time doubled from 58 to 112 days. IL-12-treated mice had diminished bacterial burdens; whereas treatment with exogenous IFN-gamma had no effect on survival or bacterial burden. IL-12 treatment also delayed lung pathology in BALB/c mice. In contrast with the findings in the BALB/c model, IL-12 did not increase survival of *M. tuberculosis*-infected gko mice, transgenic mice in which the IFN-gamma gene has been disrupted, indicating that IL-12 does not induce protection against tuberculosis in mice in the absence of IFN-gamma.—Author's Summary

Goodall, J. C., Henwood, J., Bacon, P. A. and Gaston, J. S. H. Marked conservation of complementarity-determining region 3 of the beta-chain of TCRs recognizing a mycobacterial heat shock protein 60-derived peptide with strong sequence similarity to human heat shock protein 60. J. Immunol. **60** (1995) 2329–2338.

The variable gene usage and sequence of human TCRs specific for a particular MHC/peptide combination have been investigated. The peptide comprises amino acids 456-466 of the 65-kDa *Mycobacterium tuberculosis* heat-shock protein (hsp60), and is recognized in the context of HLA-DP. TCRs from both synovial fluid and peripheral blood (PB)-derived T-cell clones used only five different V beta genes, three of which are closely related (V beta 6.7a, V beta 6.7b, and V beta 21.3). Among TCRs using these three genes there was marked conservation of the beta-chain sequence, whereby complementarity determining region 3 (CDR3) contained an amino acid motif (*R*G*, amino acids 96-100) in association with either J beta 1.4 or J beta 2.5. These conclusions were strengthened by analysis of peptide-stimulated T-cell lines that revealed not only TCR beta-chain sequences identical with those seen in T-cell clones, but also additional beta-chains with similar CDR3 region sequences and gene usage. In contrast, T-cell lines derived by using IL-2 or a control peptide revealed variable usage of V beta and J beta genes; V beta 6.7a/b sequences from these lines and from freshly isolated PB did not contain the CDR3 motif noted in TCRs from Antigen-specific T cells. We suggest that the remarkably limited diversity of TCRs noted in this study is a consequence of the similarity between the mycobacterial hsp60 peptide and the equivalent peptide from human hsp60, and reflects the trimming of the TCR repertoire required to maintain self-tolerance.—Author's Summary

Howe, R. C., Wondimu, A., Demissee, A. and Frommel, D. Functional heterogeneity among CD4+ T-cell clones from blood and skin lesions of leprosy patients, identification of T-cell clones distinct from Th0, Th1 and Th2. Immunology **84** (1995) 585–594.

In the present study we examined the functional properties of T-cell clones reactive with *Mycobacterium leprae* and other mycobacterial antigens. Clones isolated from the skin lesions and blood of leprosy patients across the spectrum were exclusively $CD4^+$ $CD8^-$ and expressed the alpha beta T-cell receptor. Substantial heterogeneity in the production of cytokines, in particular interleukin-4 (IL-4), was observed, although no striking correlation with clinical status was apparent. A variety of patterns of cytokine secretion distinct from those of T-helper type-1 (Th1) Th2 or Th0, as defined in murine studies, was evident. Most noteworthy was a large number of clones from skin which secreted neither IL-2 nor IL-4, but large amounts of tumor necrosis factor (TNF) and interferon-gamma (IFN-γ). Clones isolated from the blood of leprosy patients had a more restricted cytokine secretion profile, and appeared to resemble more closely previously described patterns, including those of high level production of IL-2 and/or IL-4. Virtually all clones, from either skin or blood, produced high levels of IFN-γ, and thus many clones were IL-4 and IFN-γ co-producers. The pattern of cytokine production by skin-derived T-cell clones was significantly affected by the *in vitro* activation status of the cells. Cells enriched in activated blasts tended to produce more IL-4 than small resting cells. In addition, the production of IFN-γ by skin T-cell clones after ≤ 10 weeks of culture was strikingly distinct from that of these clones after 5 months of culture. IL-4 and IFN-γ co-producing clones shifted to a Th2-like pattern with much less IFN-γ secretion; whereas non-IL-4-producing clones secreted much higher levels of IFN-γ after prolonged culture, and became much more Th1-like. However, there was still no correlation between clinical status and pattern of cytokines produced. These results imply that a high fraction of T cells exists in leprosy lesions that is distinct from or that has not yet fully matured into Th1 or Th2 cells.—Author's Abstract

Ladel, C. H., Daugelat, S. and Kaufmann, S. H. E. Immune response to *Mycobacterium bovis* bacille Calmette Guerin infection in major histocompatibility complex class I- and II-deficient knock-out mice: contribution of CD4 and CD8 T cells to acquired resistance. Eur. J. Immunol. **25** (1995) 377–384.

Knock-out mice with defined major histocompatibility complex (MHC) deficiencies were infected intravenously with *Mycobacterium bovis* bacille Calmette Guerin (*M. bovis* BCG) to assess the relative impact of MHC class I-/and II-dependent immune responses. Heterozygous control mice were capable of controlling growth of *M. bovis* BCG, although infection progressed chronically, as assessed by determination of colony-forming units. Furthermore, infected controls developed granulomatous lesions at the site of mycobacterial growth and delayed-type hypersensitivity (DTH) reactions after challenge with purified protein derivative of tuberculin. *In vitro,* spleen cells from heterozygous control mice produced high concentrations of interferon-gamma (IFN-γ) after restimulation with mycobacterial antigens. In contrast, the MHC class II-deficient A beta (-/-) mice, which are virtually devoid of functional CD4 T cells, succumbed to *M. bovis* BCG infection. Furthermore, A beta (-/-) mice lacked DTH reactions to tuberculin and only few minute picnotic lesions were formed in the livers of infected mice. Finally, spleen cells from infected A beta (-/-) mice failed to produce measurable IFN-γ concentrations after restimulation *in vitro,* with various mycobacterial antigen preparations. The capacity of beta Z-microglobulin (beta 2m)-deficient mice, which are devoid of CD8 alpha/beta T cells, to inhibit growth of *M. bovis* BCG was only slightly affected at low inocula, although significantly higher colony-forming units were detected in spleens. These knock-out mice developed strong DTH responses to tuberculin and their spleen cells produced high levels of IFN-γ once reactivated by mycobacterial antigens. Furthermore, in livers of infected beta 2m-deficient mice, extravascular infiltrates developed

which were more diffuse than those in infected control littermates. Remarkably, the beta 2m-deficient mice were substantially more susceptible to higher inocula of *M. bovis* BCG than their control littermates. Our data formally prove the essential role of MHC class II-dependent immune mechanisms in all relevant aspects of immunity to *M. bovis* BCG. In addition, our findings emphasize an important contribution of MNC class I-dependent immunity to effective antimycobacterial protection. We assume that CD4 T cells are highly effective in containing *M. bovis* BCG within distinct granulomatous lesions, but fail to eradicate their intracellular pathogens. It appears most likely that CD8 T cells are also required to achieve this goal.—Author's Summary

Ladel, C. H., Hess, J., Daugelat, S., Mombaerts, P., Tonegawa, S. and Kaufmann, S. H. E. Contribution of alpha/beta and gamma/delta T lymphocytes to immunity against *Mycobacterium bovis* bacillus Calmette Guerin: studies with T cell receptor-deficient mutant mice. Eur. J. Immunol. **15** (1995) 838–846.

Mutant mice with defined T-cell deficiencies were infected with *Mycobacterium bovis* bacillus Calmette Guerin (BCG) and the relative contribution of alpha/beta T cells and gamma/delta T cells to the host immune response was assessed. Recombinase activating gene (RAG-1) (-/-) mutants as well as T-cell receptor (TcR) beta (-/-), but not TcR-delta (-/-), mutants succumbed to *M. bovis* BCG infection and failed to develop granulomatous lesions. Antigen-induced IFN-gamma production by spleen cells *in vitro* was abrogated in RAG-1 (-/-) mutants and markedly diminished in TcR-beta (-/-) and TcR-delta (-/-) mice. Reconstitution experiments suggest that both alpha/beta and gamma/delta T cells are essential for antigen-specific IFN-gamma secretion. Our data formally prove the crucial role of alpha/beta T cells and reveal accessory functions of gamma/delta T cells in optimum immunity against *M. bovis* BCG.—Author's Summary

Larsen, C. G., Thomsen, M. K., Gesser, B., Thomsen, P. D., Deleuran, B. W., Nowak, J., Skodt, V., Thomsen, H. K., Deleuran, M., Thestrup Pederson, K., Harada, A., Matsushima, K. and Menne, T. The delayed-type hypersensitivity reaction is dependent on IL-8; inhibition of a tuberculin skin reaction by an anti-IL-8 monoclonal antibody. J. Immunol. **155** (1995) 2151–2157.

Cell-mediated immune reactions are essential to our immune response toward foreign organisms such as microorganisms, or in the response toward foreign tissue antigens, as seen in the rejection of allogeneic transplanted organs. Similar reactions form the basis for the development and the progression of delayed-type hypersensitivity (DTH) reactions. We found that the alpha-chemokine IL-8 plays an important pathophysiologic role for the development of a DTH reaction because infusion of a neutralizing anti-IL-8 monoclonal antibody (WS-4) was able to suppress the development of a tuberculin skin reaction in rabbits, as judged by histologic, biochemical, and clinical examinations. Thus, the number of neutrophil granulocytes and lymphocytes at the site of tuberculin injection was decreased considerably, and the clinical signs of inflammation were suppressed almost completely at 24 hr after intracutaneous injection of tuberculin, as judged by the size of the infiltrates. In contrast, we did not see any effect on the visible erythema of the skin. We found that the tissue content of myeloperoxidase (MPO), reflecting the number of infiltrating neutrophils, was lowered significantly. Furthermore, immunohistochemical analysis confirmed that IL-8 immunoreactivity is actually enhanced in the skin of positive tuberculin reactions. The results indicate that IL-8 plays an important role for the early accumulation of leukocytes in the skin and for the clinical signs of a DTH reaction.—Author's Summary

Muller, D., Pakpreo, P., Filla, J., Pederson, K., Cigel, F. and Malkovska, V. Increased gamma-delta T-lymphocyte response to *Mycobacterium bovis* BCG in major histocompatibility complex class I-deficient mice. Infect. Immun. **63** (1995) 2361–2366.

Mice with a homologous deletion of the beta(2)-microglobulin gene (beta(2)m(−)) are deficient in class I major histocompatibility complex molecules (MHC) and con-

sequently are deficient in CD8(+) T cells. These beta 2m(−) mutant mice control the intraperitoneal growth of an avirulent vaccine strain of mycobacteria, *Mycobacterium bovis* BCG, after intraperitoneal infection similarly to normal mice. We show that beta(2)m(−) mice have an increased gamma-delta (gamma delta) T-cell response after infection with live avirulent mycobacteria. beta(2)m(−) mice have an earlier and more sustained rise in the proportion of intraperitoneal gamma delta T cells, averaging 17% of T cells, compared with 6% in normal mice, at 28 days after infection. Compared with the population in normal mice, gamma delta T cells in the spleens of beta(2)m(−) mice averaged a higher proportion of the total T-cell population of the spleen on days 5, 8, and 14 after intraperitoneal infection. These data document the kinetics of gamma delta T cells reactive to mycobacterial antigens *in vivo* without class I MHC restriction and support a role for class I MHC and CD8(+) T cells in the *in vivo* regulation of gamma delta T cells.—Author's Summary

Patil, S. A., Katoch, K., Ramu, G. and Sengupta, U. Detection of antibodies against phenolic glycolipid-I (PGL-I), 35-kDa and 30-40-kDa components of *Mycobacterium leprae* on the cerebrospinal fluid of leprosy patients. J. Med. Microbiol. **43** (1995) 115–119.

The involvement of the central nervous system (CNS) in lepromatous (LL) leprosy patients was investigated; 33 patients were examined clinically in detail and upper motor neuron involvement was observed in eight and lower motor neuron in three of these patients. Anti-*Mycobacterium leprae* antibodies could be detected in the CSF by PGL-I enzyme-linked immunosorbent assay (ELISA) and monoclonal antibody (MAb) based competitive assays against defined epitopes on the 35-kDa protein and 30-40-kDa polysaccharide (lipo-arabinomannan) antigens with MAbs ML04 and ML34, respectively. Antibodies against PGL-I and 35-kDa protein were observed in more subjects than antibodies against the 30-40-kDa antigen. Some correlation was observed between the upper motor neuron signs and antibody positivity for 35-kDa and PGL-I antigens in the CSF of these patients.—Author's Abstract

Peetermans, W. E., Raats, C. J. I., van Furth, R. and Langermans, J. A. M. Mycobacterial 65-kilodalton heat shock protein induces tumor necrosis factor alpha and interleukin 6, reactive nitrogen intermediates, and toxoplasmastatic activity in murine peritoneal macrophages. Infect. Immun. **63** (1995) 3454–3458.

The 65-kDa heat shock protein (hsp65) is supposed to play a role in host defense against infections with various microbial pathogens and in autoimmune inflammatory disorders, These effects are thought to result mainly from an hsp65-specific T-lymphocyte-mediated immune response that recognizes conserved epitopes. The aim of the present study was to assess whether mycobacterial hsp65 has a direct effect on resident murine peritoneal macrophages, independent of hsp65-sensitized T lymphocytes. Exposure of peritoneal macrophages from naive C57BL/6 mice to the mycobacterial hsp65 *in vitro* induced an enhanced release of tumor necrosis factor-alpha (TNF-α) and interleukin 6. These cells also produced large amounts of reactive nitrogen intermediates (RNI) and inhibited the intracellular proliferation of *Toxoplasma gondii*. Small amounts of gamma interferon acted synergistically with hsp65. Thus, exposure of murine macrophages to hsp65 results in activation of these cells. The acquisition of these characteristics by peritoneal macrophages occurred in the absence of sensitized T lymphocytes. Addition of anti-TNF-α antiserum resulted in an attenuation of the hsp65-induced release of RNI and toxoplasmastatic activity, indicating that endogenous TNF-α is involved in the hsp65-induced macrophage activation. The conclusion of this study is that *in vitro* exposure of peritoneal macrophages to the mycobacterial hsp65 induces the release of pro-inflammatory cytokines and RNI and results in inhibition of the intracellular proliferation of *T. gondii*. These effects on murine macrophages occur independently of hsp65-specific T lymphocytes. The pro-inflammatory effect of hsp65 demonstrated in this study suggests that this heat shock protein may play a role in the initiation of inflammation that adds to a nonspecies-spe-

cific resistance in the early stages of infections.—Author's Abstract

Roxo, E., Sinhorini, I. L. and Mariano, M. [Evolution of lesions induced by mycobacteria, BCG and H37Rv in hampsters.] Hansen. Int. **19** (1994) 19–25. (in Portuguese)

Some aspects of the pathogenesis of tuberculosis was experimentally investigated by inoculating either an attenuated mycobacterial strain—BCG—or a virulent strain—*Mycobacterium tuberculosis.* $H_{37}Rv$ into the foot pad or the cheek pouch of hamsters. The inoculation of these agents caused different patterns of lesions. The BCG-induced lesions in the foot pad had a tendency to decrease in size. On the other hand, the lesions by $H_{37}Rv$ showed a progressive evolution. The same pattern of evolution was observed with the lesions induced in the cheek pouch. However, the $H_{37}Rv$ disseminated to internal organs and the BCG did not.—Author's English Summary

Sampaio, E. P., Caneshi, J. R. T., Nery, J. A. C., Duppre, N. C., Pereira, G. M. B., Vieira, L. M. M., Moreira, A. L., Kaplan, G. and Sarno, E. N. Cellular immune responses to *Mycobacterium leprae* infection in human immunodeficiency virus-infected individuals. Infect. Immun. **63** (1995) 1848–1854.

The immune responses to *Mycobacterium leprae* and other mycobacterial antigens were studied in 11 leprosy patients with concurrent human immunodeficiency virus type 1 (HIV-1) infection. Three patients manifested borderline lepromatous leprosy, and eight patients had borderline tuberculoid (BT) leprosy. Despite the low CD4+ T-cell count in the peripheral blood, no histologic or phenotypic change in the cellular infiltrate in either the lepromatous or tuberculoid lesions was observed when compared with HIV-1-negative patients. Lepromatous lesions contained heavily parasitized macrophages and few CD8+ T cells. Lesions from the patients with BT leprosy showed extensive CD4+ T-cell infiltration despite a significant reduction in CD4+ T-cell counts in the peripheral blood. No acid-fast bacilli were detected in the tuberculoid lesions. HIV-1 infection did not alter the lack of response in lepromatous leprosy to *M. leprae* antigens either *in vitro* or *in vivo.* In contrast, the skin test response to *M. leprae* antigens as well as the *in vitro* lymphoproliferative responses to mycobacterial antigens that are usually seen in patients with tuberculoid leprosy were abrogated in the BT HIV-1+ patients. However, production of gamma interferon in response to the same stimuli was preserved in most of the patients. Analysis of cytokine gene expression showed activation of additional cytokine genes in the unstimulated peripheral blood cells of patients with both leprosy and HIV-1 infections as compared with cells from patients with leprosy alone. These results suggest that granuloma formation in leprosy can be independent of the impaired CD4+ T-cell response of the HIV-1 infection. Furthermore, in HIV-1+ individuals with *M. leprae* infection, activation of cytokine genes is observed even when the circulating CD4+ T-cell count is significantly reduced.—Author's Summary

Thomssen, H., Ivanyi, J., Espitia, C., Arya, A. and Londei, M. Human $CD4^-$, $CD8^-$, alpha $beta^+$ T-cell receptor T cells recognize different mycobacteria strains in the context of CD1b. Immunology **85** (1995) 33–40.

Double-negative alpha $beta^+$ T-cell receptor (TCR) human T cells have been reported to recognize antigen in the context of the HLA class I-like (Ib) CD1 complex. In particular, the CD1b molecule has been shown to act as the element of genetic restriction for antigens derived from *Mycobacterium tuberculosis.* The stenotopic nature of these major histocompatibility complex (MHC) class Ib molecules raised the question of whether the antigenic moiety recognized by $CD4^-$ $CD8^-$ alpha $beta^+$ TCR T cells was shared by different mycobacteria. We demonstrate here that a $CD4^-$ $CD8^-$ alpha $beta^+$ TCR T-cell line and three clones raised against *M. tuberculosis* proliferated following stimulation with soluble extracts from organisms of the *M. tuberculosis* complex, *M. leprae,* and 10 out of 16 tested isolates of *M. avium* complex; however, four species of weakly or non-pathogenic my-

cobacteria were not stimulatory. Furthermore, the *M. tuberculosis* soluble extract (MTSE) -derived, recognized antigenic moiety proved to be proteinase K resistant and to have a molecular weight greater than 5000 MW. Thus it differed from the reported antigenic moiety recognized by $CD4^-$ $CD8^-$ gamma $delta^+$ TCR cells. Our results suggest that a common antigenic moiety, presented by CD1b molecules to $CD4^-$ $CD8^-$ alpha $beta^+$ TCR T cells, is shared by many mycobacterial species. Therefore they raise interest in the question of whether $CD4^-$ $CD8^-$ alpha $beta^+$ TCR T cells, elicited by *M. tuberculosis,* may play a role in the natural history of other mycobacterial infections.—Author's Abstract

Torrella, A., Solis, R. L., Rodriguez, N., Medina, Y., Pita, M., Perez, I. and Licourt, N. [Ultramicro-ELISA (UMELISA) for the detection of IgM antibodies to *Mycobacterium leprae* using eluates of dried blood spots.] Rev. Inst. Med. Trop. São Paulo **36** (1994) 131–138. (in Spanish)

The study established the conditions needed for the detection of IgM antibodies to PGL-I in eluates from dried blood spots using UMELISA Hansen and SUMA technology. Samples from 300 blood donors and 58 leprosy patients in Cuba were studied. For both populations, with the previously established conditions, the authors compared the results for the eluates from dried blood spots with those for the serum samples, and obtained a correlation of 0.919 in blood donors, 0.969 in patients, and 0.954 for the total of both populations. The level of agreement was 98% in patients and 96% in blood donors. In the patient population the sensitivity was 93% and the specificity 100% for eluates of dried blood spots evaluated by the UMELISA Hansen compared with serum samples analysed by the same assay.—Author's English Summary

Zugel, U., Schoel, B., Yamamoto, S., Hengel, H., Morein, B. and Kaufmann, S. H. E. Crossrecognition by CD8 T cell receptor alpha beta cytotoxic T lymphocytes of peptides in the self and the mycobacterial hsp60 with share intermediate sequence homology. Eur. J. Immunol. **25** (1995) 451–458.

Immunization of C57BL/6 mice with the mycobacterial heat shock protein (hsp) 60 in immunostimulating complexes caused the *in vivo* activation of autoreactive histocompatibility complex class I (H-2D(b))-restricted CD8 T-cell receptor (TcR) alpha/beta cells. A CD8 TcR alpha/beta clone with specificity for the mycobacterial hsp60 peptide (499-508) was derived from this immunization, which, in addition, recognized syngeneic macrophages which had been stressed by interferon-gamma (IFN-γ) stimulation. The stress-induced, self peptide could be extracted from IFN-γ-stressed macrophages by acid elution, suggesting that the IFN-γ-induced self peptide is derived from an endogenous protein. Based on our observation that lysis of stressed target cells by this cytotoxic T lymphocyte (CTL) clone was specifically inhibited by hsp60-specific antisense oligonucleotides, we used synthetic peptides representing amino acid sequences of the murine hsp60 for target cell sensitization and identification of the relevant self peptide. Synthetic peptides representing 9-mer to 11-mer amino acid sequences of the murine hsp60 with asparagine in anchor position 4 or 5 as the minimal requirement for $H\text{-}2D^b$ binding were tested in CTL assays. The overlapping murine hsp60 peptides (162-170/171) were stimulatory at a concentration as low as 10-100 pM. Seven other peptides of the murine hsp60 required intermediate peptide concentrations of 10-100 nM for recognition by the CTL clone. Although the murine and mycobacterial hsp60 peptides recognized by this CTL clone showed only intermediate homology (3 identical and 3 similar amino acids), our data suggest that endogenous hsp60 itself is the source of self peptide(s) presented by IFN-γ-stressed macrophages to the crossreactive CTL clone with promiscuous specificity. This notion is consistent with the idea of hsp as a link between infection and autoimmunity.—Author's Abstract

Microbiology

Basak, P. and Banerjee, P. P. Culture of nocardioform bacilli from leprosy patients and clinical evaluation of nocardioform bacilli-derived antigen. Indian J. Med. Res. **101** (1995) 150–153.

An antigen derived from cultured nocardioform bacilli was compared with Mitsuda lepromin in intradermal skin-test reactions. Nocardioform bacilli were cultured in gelatin minimal medium from the tissue fluid of 85 lepromatous patients (56 M, 29 F). Of these, 65 samples showed uncontaminated growth of the organism, which were pooled for the manufacture of the test antigen. This antigen was intradermally tested in 50 untreated leprosy patients irrespective of the type, together with Mitsuda lepromin and sterile gelatin minimal media, which served as a control. No early reaction was observed at 72 hr, while the late reaction at 28 days was positive in all patients in the tuberculoid (TT) group with both antigens. Eighteen patients (81.8%) in the borderline tuberculoid (BT) group reacted strongly to Mitsuda lepromin at 28 days, while 21 patients (95.5%) in this group showed a strong late reaction with the test antigen. The lepromatous (LL) group did not show any reaction with the two antigens. It is inferred that nocardioform bacilli are easy to cultivate, and that the test antigen compares well with Mitsuda lepromin.—Author's Summary

Baulard, A., Escuyer, V., Haddad, N., Kremer, L., Locht, C. and Berche, P. Mercury resistance as a selective marker for recombinant mycobacteria. Microbiology **141** (1995) 1045–1050.

The use of antibiotic-resistance markers for the selection of recombinant mycobacteria is widespread but questionable considering the development of live recombinant BCG vaccines. In contrast, vector-encoded resistance to heavy metals such as mercury may represent an interesting alternative for the development of live vaccines compatible with use in humans and in animals. The mercury resistance genes (mer) from *Pseudomonas aeruginosa* and from *Serratia marcescens* were cloned into the *Escherichia coli-Mycobacterium* shuttle vector pRR3, The resulting vectors, designated pMR001 and pVN2, were introduced by electroporation into *Mycobacterium smegmatis, M. bovis* BCC and *M. tuberculosis.* The recombinant mycobacteria were stable *in vitro* and *in vivo,* and had high-level mercury resistance, thus indicating that the mer genes can be useful as selective markers in mycobacteria.—Author's Summary

Brennan, P. J. and Nikaido, H. The envelope of mycobacteria. Ann. Rev. Biochem. **64** (1995) 29–63.

Mycobacteria, members of which cause tuberculosis and leprosy, produce cell walls of unusually low permeability, which contribute to their resistance to therapeutic agents. Their cell walls contain large amounts of C-60-C-90 fatty acids, mycolic acids, that are covalently linked to arabinogalactan. Recent studies clarified the unusual structures of arabinogalactan as well as of extractable cell wall lipids, such as trehalose-based lipooligosaccharides, phenolic glycolipids, and glycopeptidolipids. Most of the hydrocarbon chains of these lipids assemble to produce an asymmetric bilayer of exceptional thickness. Structural considerations suggest that the fluidity is exceptionally low in the innermost part of bilayer, gradually increasing toward the outer surface. Differences in mycolic acid structure may affect the fluidity and permeability of the bilayer, and may explain the different sensitivity levels of various mycobacterial species to lipophilic inhibitors. Hydrophilic nutrients and inhibitors, in contrast, traverse the cell wall presumably through channels of recently discovered porins.—Author's Summary

Britschgi, T. B. and Cangelosi, G. A. Detection of rifampin-resistant bacteria using DNA probes for precursor rRNA. Mol. Cell. Probes **9** (1995) 19–24.

Ribosomal RNA precursor (pre-rRNA) molecules have terminal domains (fails) which are removed during late steps in rRNA processing to yield the mature rRNA subunits. Transcriptional inhibitors such as

rifampin can deplete pre-rRNA in sensitive cells by inhibiting *de novo* pre-rRNA synthesis while allowing maturation to proceed. We developed direct DNA probe assays for pre-rRNA tail sequences of *Escherichia coli,* and evaluated their ability to rapidly distinguish rifampin-resistant from rifampin-sensitive strains in cultures treated with the drug. Pre-rRNA became undetectable in sensitive cells less than a generation time after rifampin exposure, but remained abundant in resistant cells. Resistant cells were detectable by this method against a 100-fold excess of sensitive cells, showing that this method can detect resistant mutants even when present as a small percentage of a pathogen population. Our data indicate that the response of pre-rRNA to antibiotic treatment is sufficient in rate and magnitude to make it a useful metabolic marker for antibiotic sensitivity.—Author's Abstract

Dellagostin, O. A., Esposito, G., Eales, L. J., Dale, J. W. and McFadden, J. Activity of mycobacterial promoters during intracellular and extracellular growth. Microbiology **141** (1995) 1785–1792.

pUS933, a bifunctional *Mycobacterium-Escherichia coli* translational fusion vector, containing an amino-terminally truncated *E. coli* lacZ reporter gene, was constructed. Derivatives of pUS933, containing the promoter, RBS and start codon of the *Mycobacterium bovis* BCG hsp60 gene, the *M. leprae* 28-kDa gene and the *M. leprae* 18-kDa gene were constructed and introduced into *E. coli, M. smegmatis* and *M. bovis* BCG. beta-Galactosidase activity was measured for mycobacteria grown in liquid culture. Primer-extension analysis was used to determine the transcriptional start point for the 18-kDa promoter in *M. smegmatis.* Murine macrophages were infected with recombinant BCC containing the pUS933 derivatives and expression levels were examined, by fluorescence microscopy and fluorometry, during intracellular growth of BCG. Both the BCG hsp60 gene promoter and the *M. leprae* 28-kDa gene promoter gave high levels of beta-galactosidase expression in all situations examined. In contrast, the *M. leprae* 18-kDa promoter fragment gave very low levels of expression in *M. smegmatis* and BCG grown in liquid culture, but in BCG growing within macrophages it was induced to levels almost as high as the other promoters. This indicated that the 18-kDa gene is specifically activated during intracellular growth and may, therefore, be involved in survival of *M. leprae* within macrophages. This pattern of regulation may be useful for controlling expression of foreign genes in recombinant BCG strains.—Author's Abstract

Deshpande, R. G., Khan, M. B., Bhat, D. A. and Navalkar, R. G. Immunoaffinity chromatographic isolation of a high molecular weight seroreactive protein from *Mycobacterium leprae* cell sonicate. FEMS Immunol. Med. Microbiol. **11** (1995) 163–169.

The purpose of this study was to isolate *Mycobacterium leprae* antigen(s) by immunoaffinity chromatography using immunoglobulins from leprosy patients and from rabbit anti-*M. leprae* hyperimmune serum coupled to CNBr-Sepharose 4B. A high molecular weight (M^r) *M. leprae* protein (MLP) with a subunit M^r of 22,000 was isolated. MLP was recognized by monoclonal antibody MMPII1G4 which is known to react with MMPII, a 22-kDa protein of *M. leprae.* The *N*-terminal sequence of the 22-kDa subunit (Met-gln-gly-asp-pro-asp-val-leu-arg-leu-leu-asn-glu-gln-leu-thr) was identical to MMPII and to antigen D (bacterioferritin) of *M. paratuberculosis.* It showed 44% homology with the *N*-terminal end of *E. coli* bacterioferritin. In ELISA, MLP showed 100% and 60% positivity with leprosy and TB sera, respectively, as compared to normal healthy sera. The role of bacterioferritin in *M. leprae* and the importance of MLP as an immunogen has been discussed.—Author's Abstract

Dhople, A. M. and Ibanez, M. A. *In vitro* activity of levofloxacin, singly and in combination with rifamycin analogs, against *Mycobacterium leprae.* Antimicrob. Agents Chemother. **39** (1995) 2116–2119.

The *in vitro* susceptibility of *Mycobacterium leprae* to levofloxacin was studied by using two biochemical parameters to measure the metabolic activity of the organism.

Levofloxacin consistently exhibited twofold greater bactericidal activity than ofloxacin, with the MIC being 0.75 μg/ml. When combined with one of the three rifamycin analogs, synergism was obtained with KRM-1648 and rifabutin but not with rifampin.—Author's Summary

Falcone, V., Bassey, E., Jacobs, W., Jr. and Collins, F. The immunogenicity of recombinant *Mycobacterium smegmatis*-bearing BCG genes. Microbiology **141** (1995) 1239–1245.

Specific pathogen-free C57BL/6 mice infected with recombinant *Mycobacterium smegmatis* (r*M. smegmatis*) bearing BCG genes showed increased splenic survival compared to those receiving the vector control (plasmid DNA only). The mouse-passaged r*M. smegmatis* (J3R) survived in peritoneal macrophages better than the vector control, regardless of whether the macrophages were infected *in vivo* or *in vitro*. When r*M. smegmatis* J3R was cultured in synthetic Proskauer-Beck-Tween medium, protein bands characteristic of BCG culture filtrates and not present in the vector control preparation were observed. Mice immunized with two doses of heat-killed J3R suspended in Freund's adjuvant were able to limit the growth of virulent *M. tuberculosis* within the lung and spleen compared to that observed in control mice receiving adjuvanted vector control or Freund's adjuvant alone.—Author's Abstract

Felmlee, T. A., Liu, Q., Whelen, A. C., Williams, D., Sommer, S. S. and Persing, D. H. Genotypic detection of *Mycobacterium tuberculosis* rifampin resistance: comparison of single-strand conformation polymorphism and dideoxy fingerprinting. J. Clin. Microbiol. **33** (1995) 1617–1623.

Detection of mutations in the *rpoB* gene of *Mycobacterium tuberculosis* can be used as an accurate predictor of rifampin resistance in the majority of strains tested. Simple but highly accurate screening methods must be developed for the detection of these mutations. Either DNA sequence analysis or single-strand conformation polymorphism (SSCP) screening can be used to detect *rpoB* mutations, but these techniques either are expensive or yield results that may prove difficult to interpret when used in a clinical setting. This report describes the use of dideoxy fingerprinting (ddF) as a postamplification screening method to identify rifampin-resistant genotypes. The ddF protocol was performed on the amplified *rpoB* fragment with no preparatory steps, thus making ddF practical for laboratories equipped for polyacrylamide gel electrophoresis. When compared with the results of SSCP analysis, ddF results were more easily interpreted and contained more sequence-dependent information that facilitated differentiation of functionally significant and silent mutations. The ddF method was used for genotypic determination of rifampin susceptibility of 20 multidrug-resistant strains of *M. tuberculosis*. The results of this analysis were concordant with DNA sequence analysis and conventional clinical laboratory methods.—Author's Summary

Fsihi, H. and Cole, S. T. The *Mycobacterium leprae* genome: systematic sequence analysis identifies key catabolic enzymes, ATP-dependent transport systems and a novel polA locus associated with genomic variability. Mol. Microbiol. **16** (1995) 909–919.

In the framework of the mycobacterial genome sequencing project, a continuous 37,049 bp sequence from the *Mycobacterium leprae* chromosome has been determined, Computer analysis revealed 10 complete open reading frames, and nine of their products show similarity to known proteins. Seven of these were identified as the enzyme isocitrate lyase, two P-type ATPase cation transporters, two AMP-binding proteins, the ribosomal protein S1, and DNA polymerase I. Interestingly, the polA gene, encoding DNA polymerase, is flanked by two inverted copies of a new class of the *M. leprae*-specific repetitive sequence, RLEP, and this structure resembles a transposable element. A second copy of this element was found at another locus in the genome, but the two copies were not present in equal amounts and could not be found in all isolates of *M. leprae*. This is the first evidence for genomic variability in the leprosy bacillus and might ultimately be useful for developing a molecular test ca-

pable of distinguishing between strains of *M. leprae.*—Author's Abstract

Guillemin, I., Cambau, E. and Jarlier, V. Sequences of conserved region in the A subunit of DNA gyrase from nine species of the genus *Mycobacterium:* phylogenetic analysis and implication for intrinsic susceptibility to quinolones. Antimicrob. Agents Chemother. **39** (1995) 2145–2149.

The sequences of a conserved region in the A subunit of DNA gyrase corresponding to the quinolone resistance-determining region were determined for nine mycobacterial species and were compared. Although the nucleotide sequences were highly conserved, they clearly differentiated one species from another. The results of the phylogenetic analysis based on the sequences of the quinolone resistance-determining regions were compared with those provided by the 16S rRNA sequences. Deduced amino acid sequences were identical within the nine species except for amino acid 83, which was frequently involved in acquired resistance to quinolones in many genera, including mycobacteria. The presence at position 83 of an alanine for seven mycobacterial species (*Mycobacterium tuberculosis, M. bovis* BCG, *M. leprae, M. avium, M. kansasii, M. chelonae,* and *M. smegmatis*) and of a serine for the two remaining mycobacterial species (*M. fortuitum* and *M. aurum*) correlated well with the MICs of ofloxacin for both groups of species, suggesting the role of this residue in intrinsic susceptibility to quinolones in mycobacteria.—Author's Summary

Iivanainen, E. Isolation of mycobacteria from acidic forest soil samples: comparison of culture methods. J. Appl. Bacteriol. **78** (1995) 663–668.

To evaluate which combination of decontamination method and medium is most reliable when examining acidic, organic forest soils for mycobacteria, three decontamination methods and five media supplemented with cycloheximide were compared. Before decontamination, the samples were incubated at 37°C for 5 hr to allow germination of microbial spores. The recovery of mycobacteria was significantly influenced both by method and by medium. Decontamination with NaOH or H_2SO_4 both combined with malachite green and cycloheximide yielded higher viable counts of mycobacteria than decontamination with NaOH followed by oxalic acid. Egg media at pH 5.5 resulted in lower mycobacterial counts than egg media at pH 6.5 or mycobacteria 7H11 agar. The numbers of slopes totally free of contaminants revealed mycobacteria 7H11 agar medium to be more prone to contamination than the four egg media tested. The highest counts of mycobacteria and a low rate of contamination were obtained when decontamination with NaOH-malachite green-cycloheximide was combined with culture on glycerol and cycloheximide supplemented egg medium at pH 6.5.—Author's Summary

Ilangumaran, S., Arni, S., Poincelet, M., Theler, J. M., Brennan, P. J., Nasiruddin and Hoessli, D. C. Integration of mycobacterial lipoarabinomannans into glycosylphosphatidylinositol-rich domains of lymphomonocytic cell plasma membranes. J. Immunol. **155** (1995) 1334–1342.

Lipoarabinomannans (LAMs) are major antigens of the mycobacterial cell envelope where they apparently insert through a glycosylphosphatidylinositol (GPI) anchoring structure. LAMs induce host macrophages to secrete TNF-alpha, IL-1, and IL-6 and inhibit T-cell proliferative responses. The mechanisms by which LAMs mediate these effects remain poorly understood. We show that LAMs were efficiently inserted into the plasma membranes of human and murine lymphomonocytic cells through their GPI anchor. Prior deacylation of LAMs abrogated this event. Phosphatidylinositol hexamannoside (PIM^6), the GPI anchor of all LAMs, competitively inhibited LAM insertion. Deacylated PIM^6 was not inhibitory. The hexamannoside glycan of PIM^6 appears to be important for LAM insertion, since phosphatidylinositol from soybean, lacking the glycan core, was not as efficient an inhibitor. Interaction of LAM with target cells was influenced by the gel/fluid phase distribution of membrane lipids, suggesting a direct interaction of the LAM-GPI anchor with the membrane bilayer. The inserted LAMs were mobile in the plane of the mem-

brane and interfered with Ab-mediated mobilization of the GPI-anchored Thy-1 molecules. Further, LAMs were preferentially incorporated into isolated plasma membrane vesicles enriched in Thy-1. Our results strongly suggest that 1) interaction of LAMs with host lymphomonocytic cells is mediated through a preferential integration of LAM-GPI anchor into specialized plasma membrane domains enriched in endogenous GPI-anchored molecules, and 2) both the acyl chains and the mannoside core glycan of the LAM-CPI anchor contribute to the specificity of integration.—Author's Summary

Kato, L. Water soluble palmitic acid in media for cultivation of leprosy derived psychrophilic mycobacteria from *Mycobacterium leprae* infected tissues. Hansen. Int. **19** (1994) 17–27.

Cultivation trials for *Mycobacterium leprae* resulted in growth of *M. psychrophilum* (L). Media were inoculated with host grown *M. leprae* cells from armadillo tissues, nu mice foot pads or human lepromata. Cultures were obtained in liquid and on semisolid multifactorial media containing water soluble palmitic acid or its salts. Ammonium thioglycolate and Na palmitate served as carbon and energy sources. The water-soluble palmitic acid remained in perfect solution following sterilization in the autoclave, thus easily accessible to the cells. The cyclodextrin-Fe complex served as a siderophore to grow the obtained leprosy-derived psychrophilic cells. The leprosy-derived cultures and subcultures grew optimally at +10°C, but deteriorated rapidly at +32°C in the multifactorial media. No growth occurred in 7H9 media. Cultures were not identified for classification.—Author's Summary

Katoch, V. M. Microbiological aspects of relapse in leprosy. Indian J. Lepr. **67** (1995) 85–98.

Several bacteriological parameters to monitor the chemotherapy response and to confirm the diagnosis of leprosy or its relapse are now available. Most of these methods are, however, applicable to MB cases only. The ongoing technique development programs, particularly in the area of gene probe technology, are very promising and are likely to provide methods for PB leprosy cases. Better characterization of histological markers by immunological and *in situ* hybridization techniques as well as development of sero-assays by using enzymes as antigens are other important areas to explore.—From the Symposium paper

Khoo, K. H., Dell, A., Morris, H. R., Brennan, P. J. and Chatterjee, D. Inositol phosphate capping of the nonreducing termini of lipoarabinomannan from rapidly growing strains of *Mycobacterium*. J. Biol. Chem. **270** (1995) 12380–12389.

Previous studies have demonstrated that the nonreducing termini of the lipoarabinomannan (LAM) from *Mycobacterium tuberculosis* are extensively capped with mannose residues, whereas those from a fast growing *Mycobacterium sp.*, once thought to be an attenuated strain of *M. tuberculosis*, are not. The noncapped LAM, termed AraLAM, is known to be more potent than the mannose capped LAM (ManLAM) in inducing functions associated with macrophage activation. Using a combination of chemical and enzymatic approaches coupled with fast atom bombardment-mass spectrometry analysis, we demonstrated that LAMs from all *M. tuberculosis* strains examined (Erdman, H37Ra, and H37Rv), as well as the attenuated *M. bovis* BCG strain, are mannose-capped with the extent of capping varying between 40% and 70%. The nonreducing termini of LAM from *M. leprae* were also found to be capped with mannoses but at a significantly lower level. A novel inositol phosphate capping motif was identified on a minor portion of the otherwise uncapped arabinan termini of LAMs from the fast-growing *Mycobacterium sp.* and *M. smegmatis* ATCC 14468 and mc(2)155. In addition, an inositol phosphate tetra-arabinoside was isolated from among endoarabinase digestion products of AraLAM and was shown to induce tumor necrosis factor-alpha production. Accordingly, we concluded that AraLAM is characteristic of some rapidly growing *Mycobacterium spp.* It is distinct from ManLAMs of *M. tuberculosis, M. bovis* BCG, and *M. leprae* not only in the absence of mannose-capping but also in containing some terminal inositol phosphate substituents which

may account for its particular potency in inducing macrophage activation.—Author's Abstract

Klatser, P. R. Amplification reactions in mycobacteriology. J. Microbiol. Methods **23** (1995) 75–87.

Tuberculosis control programs are faced with an increased burden of cases, a shift toward diagnostically more difficult categories of patients, such as extrapulmonary and smear-negative cases, and the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis.* Improved diagnosis would be a valuable contribution in the struggle to solve this global public health emergency. Nucleic acid amplification reactions promise to reduce the time for diagnosis from weeks to hours, while surpassing the sensitivity and specificity of the classical methods. Besides their potential value in diagnosis, amplification reactions offer the possibility of a rapid identification and drug susceptibility determination. Their application in other fields, such as epidemiology, could benefit the control of mycobacteriosis indirectly. Although critical assessment is warranted, several features, such as the availibility of standardized kits, strongly justify the evaluation of amplification reactions for the detection of mycobacteria for use in the clinical laboratory. In fact, evaluation of the technology now, at its present level of development, even in an endemic setting in a developing country, would yield useful recommendations toward its improvement for application in different settings, in terms of efficiency, simplicity and cost.—Author's Summary

Lane, S. J., Marshall, P. S., Upton, R. J., Ratledge, C. and Ewing, M. Novel extracellular mycobactins, the carboxymycobactins from *Mycobacterium avium.* Tetrahedron Lett. **36** (1995) 4129–4132.

A series of novel extracellular siderophores, termed carboxymycobactins, have been isolated from *Mycobacterium avium* as iron complexes and their structures determined. They are the first extracellular siderophores to be characterized from pathogenic mycobacteria and are analogous to their intracellular mycobactin counterparts, except that their R(1) alkyl sidechain is shorter and terminates in a carboxylic acid, resulting in much greater hydrophilicity and explaining their occurrence in the aqueous extracellular medium.—Author's Summary

Lee, B.-Y. and Horwitz, M. A. Identification of macrophage and stress-induced proteins of *Mycobacterium tuberculosis.* J. Clin. Invest. **96** (1955) 245–249.

Using phosphorimager technology to quantitate differences in protein expression, we have investigated the modulation of protein synthesis by *Mycobacterium tuberculosis* in response to intracellular residence in human macrophages and, for comparison, in response to various stress conditions during extracellular growth. Proteins of *M. tuberculosis* growing intracellularly in human THP-1 cells and extracellularly in broth were labeled with [^{35}S]methionine; during intracellular growth, host cell protein synthesis was inhibited with cycloheximide. The metabolically labeled proteins were separated by two-dimensional gel electrophoresis and quantitatively analyzed. Intracellular residence in macrophages induced a profound change in the overall phenotype of *M. tuberculosis.* The expression of at least 16 *M. tuberculosis* proteins was induced (at least a twofold increase compared with growth in broth) and 28 proteins repressed (at least a twofold decrease). Many of the phenotypic changes in protein expression induced during intracellular growth occurred during extracellular growth in response to stress conditions, including heat-shock, low pH, and H_2O_2. However, the pattern of induced and repressed proteins was unique to each stress condition. Of the 16 macrophage-induced proteins, 6 were absent during extracellular growth under both normal and stress conditions. Such proteins are potential virulence determinants and/or they may be important in the cell-mediated and protective immune response to *M. tuberculosis* infection.—Author's Summary

Li, T., *et al.* [Establishment of a PCR method by using the terminal repeated sequence of the gene coded for 65 KD antigen of *M. leprae.*] China Lepr. J. **11** (1995) 12–14. (in Chinese)

A PCR method for detection of *Mycobacterium leprae* has been established by using the terminal repeated sequence of the gene coded for its 65-kD antigen as the amplification target. It can detect 20 *M. leprae*/μl, and has very strong specificity which had been proved in examination of 13 different strains of mycobacteria.—Author's English Abstract

Oskam, L., Hartskeerl, R. A., Hermans, C. J., de Wit, M. Y. L., Jarings, G. H., Nicholls, R. D. and Klatser, P. R. A 46 kDa integral membrane protein from *Mycobacterium leprae* resembles a number of bacterial and mammalian membrane transport proteins. Microbiology **141** (1995) 1963–1968.

In this paper we describe the nucleotide sequence of a 3.4 kbp region of the *Mycobacterium leprae* genome. This region contains an open reading frame of 1290 bp with a coding capacity for a protein of 46, 179 Da, designated the 38L protein. Using antibodies against part of the 38L protein, we were able to demonstrate that the 38L protein is present in the membrane protein fraction of *M. leprae*. The 38L protein showed significant matches with a number of integral membrane proteins involved in the transport of small molecules through the cellular membrane. Among these are a human and a murine protein involved in melanin biosynthesis. The 38L protein might play a role in the hypopigmentation observed in leprosy patients.—Author's Summary

Radzioch, D., Kramnik, I. and Skamene, E. Molecular mechanisms of natural resistance to mycobacterial infections. Circ. Shock **44** (1994) 115–120.

Natural resistance to infection with intracellular parasites is controlled by a dominant gene on mouse chromosome 1, called *Bcg* *Bcg* affects the capacity of macrophages to destroy ingested intracellular parasites early during infection. Reactive nitrogen intermediates (RNI) have been implicated in the interferon-gamma (IFN-γ)-induced antimicrobial action of macrophages against a wide variety of pathogens. To determine whether *Bcg*(*Nramp*) is involved in the production of RNI, these studies have taken advantage of the recent cloning of the *Bcg* candidate gene, designated *Nramp*. The expression of *Bcg* has been down-regulated in the B10R (*Bcg*^{r}) macrophage cell line using a ribozyme hybrid to site-specifically cleave the *Nramp* mRNA. Following activation with IFN-γ, the secretory activity [nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α)] and surface marker expression (Ia antigen) of these *Bcg*(*Nramp*) ribozyme-transfected macrophages were markedly lower than in activated control mock-transfected macrophages (B10R-CTL). However, there was no difference in NO production of B10R-*Bcg*(*Nramp*) Rb and B10R-CTL macrophages if the treatment with IFN-γ occurred in the presence of lipopolysaccharide (LPS). These studies support the hypothesis that the *Bcg*(*Nramp*) gene is involved in the regulation of early signaling that occurs in macrophages activated with IFN-γ. Furthermore, it seems that IFN-γ, but not LPS-induced activation, is affected by the inhibition of *Bcg*(*Nramp*) gene expression. Definitive evidence will be provided by transfection experiments that will show whether the *Bcg*^{r} allele of *Bcg*(*Nramp*) can restore NO production of the *Bcg*^{s} macrophage.—Author's Summary

Santos, A. R., Filho, J. T. G., Nery, J. A. C., Duppre, N. C., Gallo, M. E. N., Suffys, P. N. and Degrave, W. M. Evaluation of PCR mediated DNA amplification in non-invasive biological specimens for subclinical detection of *Mycobacterium leprae*. FEMS Immunol. Med. Microbiol. **11** (1995) 113–120.

DNA from *Mycobacterium leprae*, present in non invasive clinical samples from leprosy patients, such as nasal secretion and hair bulbs, was submitted to amplification by the polymerase chain reaction using a *M. leprae*-specific repetitive sequence as a target. After optimization of sample processing and of the PCR conditions, we were able to detect DNA from *M. leprae* in both types of clinical samples, even from paucibacillary leprosy patients. The use of hair bulbs and nasal secretion as clinical samples for screening of household contacts and for the evaluation of a risk population, or for the follow up of patients under chemotherapy, and monitoring of bacterial load is discussed.—Author's Summary

Schorey, J. S., Li, Q. L., McCourt, D. W., Bong Mastek, M., Clark-Curtiss, J. E., Ratliff, T. L. and Brown, E. J. A *Mycobacterium leprae* gene encoding a fibronectin binding protein is used for efficient invasion of epithelial cells and Schwann cells. Infect. Immun. **63** (1995) 2652–2657.

Mycobacterium leprae, the causative agent of leprosy, is an obligate intracellular pathogen. *M. leprae* can infect a variety of cells *in vivo,* including epithelial cells, muscle cells, and Schwann cells, in addition to macrophages. The ligand-receptor interactions important in the attachment and ingestion of *M. leprae* by these nonmacrophage cells remains unknown. Fibronectin (FN) significantly enhances both attachment and ingestion of *M. leprae* by epithelial and Schwann cell lines. We cloned an *M. leprae* FN binding protein (FN attachment protein [FAP]) distinct from the 85ABC complex which has been shown previously to bind FN. The FAP open reading frame predicts a protein of 29.5 kDa with a 39-amino-acid signal peptide and was previously described as an antigen in leprosy patients. *M. leprae* FAP has homologies in *M. vaccae, M. avium,* and *M. tuberculosis,* as determined by Southern blotting and direct peptide analysis. Both anti-FAP antibodies and an *Escherichia coli*-expressed recombinant protein significantly blocked *M. leprae* attachment and internalization by T-24, an epithelial cell line, and JS1, a Schwann cell line. These data suggest that FN can be a bridging opsonic ligand for attachment of mycobacteria to nonphagocytes and that FAP plays an important role in this process. This may be an important step in the initiation of *M. leprae* infection *in vivo.*—Author's Summary

Sharman, G. J., Williams, D. H., Ewing, D. R. and Ratledge, C. Determination of the structure of exochelin MN, the extracellular siderophore from *Mycobacterium neoaurum.* Chem. Biol. **2** (1995) 553–561.

Background: Siderophores are compounds produced by bacteria to acquire iron. Exochchelin MN, the extracellular siderophore from *Mycobacterium neoaurum,* is of particular interest because it has been shown to transport iron into *M. leprae,* which is responsible for the disease leprosy. Exochelins from other species cannot mediate iron transport in *M. leprae,* suggesting a specific uptake mechanism involving exochelin MN. We set out to determine the structure of exochelin MN and identify the features of the molecule that may account for this specificity.

Results: The structure of exochelin MN was elucidated by a combination of techniques including nuclear magnetic resonance, mass spectrometry, derivatization and gas chromatography. Exochelin MN is a peptide, containing the unusual amino acid beta-hydroxyhistidine and an unusual N-methyl group. The peptide coordinates iron(III) octahedrally using its two cis-hydroxamate groups plus the hydroxyl and imidazole nitrogen of the beta-hydroxyhistidine. The three-dimensional structure of the hexadentate exochelin/gallium complex was deduced from NMR data.

Conclusions: Exochelin MN has some structural features in common with other siderophores but has a unique three-dimensional structure, which is presumably important for its specific activity in *M. leprae.* Exochelin MN may be a target for drug design in the fight against infection with this pathogen.—Author's Abstract

Venisse, A., Riviere, M., Vercauteren, J. and Puzo, G. Structural analysis of the mannan region of lipoarabinomannan from *Mycobacterium bovis* BCG. J. Biol. Chem. **270** (1995) 15012–15021.

Lipoarabinomannan (LAM) is a major antigen of mycobacterial cell walls, involved in host-*Mycobacterium* interactions. In a previous work, LAM from the vaccine strain, *Mycobacterium bovis* BCG, was found to exhibit mannooligosaccharides at its arabinan nonreducing ends (ManLAM). The present report concerns the mannan core structure of this ManLAM. After partial hydrolysis of ManLAM, two populations of mannans (Ma1 and Ma2) were obtained by gel filtration chromatography. Their structural features were defined by means of two-dimensional homo-/and heteronuclear (^1H-^{13}C) NMR sequences and methylation analysis. They were both found to be composed of an α-(1 → 6)-linked mannan backbone with α-(1 → 2)-Man*p*-linked side chains. They are highly branched, and

Ma2 presents a higher frequency of branching than Ma1. Moreover, chemical analysis indicates that only Ma1 is phosphorylated. By a two-dimensional heteronuclear ^{1}H-^{31}P total correlation experiment, the phosphate was found to be involved in a phosphodiester bond between inositol C-1 and glycerol C-3. Then, the molecular mass of mannan was established by mass spectrometry, which revealed a molecular mass of 3517 Da for the major molecular species of Ma1. Likewise, analysis of unfractionated mannans showed the occurrence of other, quantitatively minor molecular species, endowed with two phosphates.

This study clearly indicates that the mannan region of *M. bovis* BCG ManLAM exists as a heterogeneous population of molecules whose structures differ in their degree of glycosylation, level of branching, and phosphorylation state. The hypothesis that the relative abundance of these different molecules modulates the biological functions of LAM is discussed.—Author's Abstract

Wieles, B., van Soolingen, D., Holmgren, A., Offringa, R., Ottenhoff, T. and Thole, J. Unique gene organization of thioredoxin and thioredoxin reductase in *Mycobacterium leprae.* Mol. Microbiol. **16** (1995) 921–929.

The thioredoxin system comprising thioredoxin (Trx), thioredoxin reductase (TR) and NADPH operates via redox-active disulfides and provides electrons for a wide variety of different metabolic processes in prokaryotic and eukaryotic cells. Thioredoxin is also a general protein disulfide reductase involved in redox regulation. In bacteria, the Trx and TR proteins previously identified were encoded by separate genes (trxA and trxB). In this study, we report a novel genomic organization of Tn and Trx in mycobacteria and show that at least three modes of organization of Tn and Trx genes can exist within a single bacterial genus: (i) in the majority of mycobacterial strains the genes coding for Tn and Trx are located on separate sites of the genome; (ii) interestingly, in all pathogenic *Mycobacterium tuberculosis* complex mycobacteria both genes are found on the same locus, overlapping in one nucleotide; (iii) in the pathogen *M. leprae,* TR and Trx are encoded by a single gene. Sequence analysis of the *M. leprae* gene demonstrated that the *N*-terminal part of the protein corresponds to Tn and the *C*-terminal part to Trx. A corresponding single protein product of approximately 49 kDa was detected in cell extracts of *M. leprae.* These findings demonstrate the very unusual phenomenon of a single gene coding for both the substrate (thioredoxin) and the enzyme (thioredoxin reductase), which seems to be unique to *M. leprae.*—Author's Abstract

Williams, D. L., Gillis, T. P. and Dupree, W. G. Ethanol fixation of sputum sediments for DNA-based detection of *Mycobacterium tuberculosis.* J. Clin. Microbiol. **33** (1995) 1558–1561.

The effect of ethanol fixation on PCR detection and viability of *Mycobacterium tuberculosis* in human sputum sediments was evaluated. *M. tuberculosis* seeded into sputum sediments was efficiently killed when treated for 1 hr with 50%, 70%, or 95% ethanol. PCR amplification of a 123-bp fragment of the *M. tuberculosis*-specific IS*6110* was not affected in ethanol-treated samples even when fixation was extended to 24 hr Ethanol fixation of sputum sediments did not affect the PCR detection of *M. tuberculosis* in clinical samples. PCR results from ethanol-treated clinical samples containing *M. tuberculosis* (smear positive and smear negative) or other respiratory pathogens correlated directly with the results by conventional detection methods for *M. tuberculosis.* Our results show that ethanol fixation of human sputum sediments containing *M. tuberculosis* significantly reduces the potential exposure of workers to viable *M. tuberculosis* without affecting DNA analysis by PCR. Also, ethanol fixation of sputum sediments provides a simple and inexpensive way to store and transport clinical specimens identified for DNA-based diagnostics without refrigeration.—Author's Abstract

Winter, N., Triccas, J. A., Rivoire, B., Pessolani, M. C. V., Eiglmeier, K., Lim, E. M., Hunter, S. W., Brennan, P. J. and Britton, W. J. Characterization of the gene encoding the immunodominant 35 kDa protein of *Mycobacterium leprae.* Mol. Microbiol. **16** (1995) 865–876.

Analysis of the interaction between the host immune system and the intracellular parasite *Mycobacterium leprae* has identified a 35-kDa protein as a dominant antigen, The native 35-kDa protein was purified from the membrane fraction of *M. leprae* and termed MMPI (major membrane protein I). As the purified protein was not amenable to *N*-terminal sequencing, partial proteolysis was used to establish the sequences of 21 peptides. A fragment of the 35-kDa proteinen-coding gene was amplified by the polymerase chain reaction from *M. leprae* chromosomal DNA with oligonucleotide primers derived from internal peptide sequences and the whole gene was subsequently isolated from a *M. leprae* cosmid library. The nucleotide sequence of the gene revealed an open reading frame of 307 amino acids containing most of the peptide sequences derived from the native 35-kDa protein. The calculated subunit mass was 33.7 kDa, but the native protein exists as a multimer of 950 kDa. Database searches revealed no identity between the 35-kDa antigen and known protein sequences. The gene was expressed in *M. smegmatis* under the control of its own promoter or at a higher level using an "up-regulated" promoter derived from *M. fortuitum*. The gene product reacted with monoclonal antibodies raised to the native protein. Using the bacterial alkaline phosphatase reporter system, we observed that the 35-kDa protein was unable to be exported across the membrane of recombinant *M. smegmatis*. The 35-kDa protein-encoding gene is absent from members of the *M. tuberculosis* complex, but homologous sequences were detected in *M. avium, M. haemophilum* and *M. smegmatis*. The avaibility of the recombinant 35-kDa protein will permit dissection of both antibody-/and T-cell-mediated immune responses in leprosy patients.—Author's Abstract

Experimental Infections

Gormus, B. J., Xu, K., Baskin, G. B., Martin, L. N., Bohm, R. P., Blanchard, J. L., Mack, P. A., Ratterree, M. S., McClure, H. M., Meyers, W. M. and Walsh, G. P. Experimental leprosy in monkeys. I. Sooty mangabey monkeys: transmission, susceptibility, clinical and pathological findings. Lepr. Rev. **66** (1995) 96–104.

A total of 31 sooty mangabey monkeys (SMM) (*Cercocebus torquatus atys*) inoculated by various routes with differing numbers of SMM-origin *Mycobacterium leprae* (ML) and 4 SMM inoculated with human-origin ML were observed for 4–12 years. SMM-origin ML was more pathogenic in SMM than human-origin ML. The spectrum of disease ranged from indeterminate to borderline and lepromatous in different animals. Some animals developed pure neural leprosy. Erythema nodosum leprosum (SNL) was also observed.

Combined intravenous/intracutaneous (IV/IC) routes of inoculation more effectively induced advancing, disseminated lepromatous forms of leprosy; IV or IC routes alone were less effective at comparable doses. Total IV/IC doses of SMM-origin ML $\geq 5 \times 10^8$, with morphologic indices (MIs) ranging from 5% to 10%, produced advancing, disseminated LL leprosy in 92% of SMM. Lower IV/IC doses and inoculations by a single IV or IC route produced fewer leprosy infections and more spontaneous regressions. As a species, captive SMM are highly susceptible to experimental leprosy and provide an excellent model for the longitudinal study of leprosy.—Author's Summary

Gormus, B. J., Xu, K., Cho, S.-N., Baskin, G. B., Bohm, R. P., Martin, L. N., Blanchard, J. L., Mack, P. A., Ratterree, M. S., Meyers, W. M. and Walsh, G. P. Experimental leprosy in monkeys. II. Longitudinal serological observations in sooty mangabey monkeys. Lepr. Rev. **66** (1995) 105–125.

In this study, 11 SMM were grouped and inoculated with differing doses of SMM-origin *Mycobacterium leprae* (ML) between

4.5×10^8 and 1×10^9 by either combined IV/IC routes or by IV or IC route alone. The combined route was the most effective in eliciting progressive, disseminated LL leprosy. In all, 6 of 7 SMM inoculated by the combined routes developed leprosy requiring treatment at some point. Only 1 of 4 inoculated by a single route developed persisting leprosy requiring chemotherapy. Either no disease or spontaneous regression of initial disease occurred in the other 3 animals inoculated by a single route. Doses in excess of 1×10^9 ML were more effective than lesser doses.

An association was observed between the development of IgG anti-PGL-I ELISA OD values and resistance to leprosy and between IgM anti-PGL-I and leprosy progression or susceptibility. Serum PGL-I antigen levels, determined by dot ELISA, paralleled disease severity longitudinally. High positive OD values of anti-LAM IgG prior to ML inoculation were observed in the majority of leprosy-susceptible SMM in contrast to negative levels in more resistant animals. Anti-LAM IgG OD values exceeded the positive cut-off point after inoculation in 5 of 11 SMM; 3 of these 5 had concurrent detectable serum levels of PGL-I antigen.—Author's Summary

Epidemiology and Prevention

Awofeso, N. AIDS and tuberculosis/leprosy in Nigeria: the urbanisation factor. Acta Leprol. **9** (1995) 149–151.

A study was conducted between February and June 1994 on the influence of urbanization on the seroprevalence of human immunodeficiency virus (HIV) among tuberculosis (TB) and leprosy patients in the our Primary Health Care Zones in Nigeria. Results indicate that 71.4% of all smear-positive TB patients and 75% of all multibacillary (MB) leprosy patients that are HIV seropositive in this study are resident in the urban areas. This study emphasizes the need for careful sample selection in studies involving HIV and tuberculosis/leprosy, and for careful monitoring of the HIV/leprosy interactions.—Author's Summary

Bonilha, V. F., Dalpino, D., Fleury, R. N., Garbino, J. A., Goncalves, A., Goncalves, R. P., Marques, F., Nakandakari, S., Opromolla, D. V., Tonelo, C. and Ura, S. [Experimental project for the control of hanseniasis in the city of Bauru, São Paulo, Brazil; evaluation after the first year of implementation.] Hansen. Int. **19** (1994) 11–18. (in Portuguese)

Bauru, a city in the State of São Paulo, Brazil, presents interesting reasons to develop studies regarding hanseniasis endemics: concerning health services, World Health Organization has elected a specialized local hospital as Reference Center for Portuguese Speaking Countries; concerning the epidemiological point of view, local incidence and prevalence rates are peculiarly lower than Brazilian figures. An intervention experimental project was developed integrating 19 public health agencies, according to national and international recommendation toward integration of services and intercoordination of measures. This paper presents assessment of the first year of interventions, based on nine technical areas: clinics, therapeutics, human resources, bacteriology, histology, public health, rehabilitation and coordination. The most important results were: i) there is an epidemiological "iceberg," even bigger than currently admitted, between the finding in the population and data from official files; ii) an institutional reconstruction should be considered as a possible solution to improve the health conditions in the region.—Author's English Summary

Feitosa, M. F., Borecki, I., Krieger, H., Beiguelman, B. and Rao, D. C. The genetic epidemiology of leprosy in a Brazilian population. Am. J. Hum. Genet. **56** (1995) 1179–1185.

Data on leprosy patients have been obtained from the Dispensary of Leprosy of

Campinas, São Paulo, Brazil. Where records on practically all cases of leprosy in the Campinas area during the period 1960–70 are filed. The whole sample comprises 10, 886 individuals, distributed among 1568 families. Complex segregation analysis was utilized to determine the nature of the genetic factors that may operate on leprosy and its subtypes. The results suggest the presence of a recessive major gene controlling susceptibility to leprosy *per se*, with frequency of similar to 0.05, although there are deviations from the expected Mendelian segregation proportions. Possible etiologic heterogeneity was examined by considering two subtypes separately: for lepromatous leprosy and tuberculoid leprosy there are suggestions for a segregating major effect; however, Mendelian transmission could not be demonstrated in either case. Therefore, there is no evidence to suggest unique genetic determinants for leprosy subtypes.—Author's Abstract

Ferra Torres, T. M. and Carrazana Hernandez, G. B. [Incidence of leprosy in the city of Camaguey, Cuba, in the years 1978–1993.] Rev. Leprol. Fontilles **20** (1995) 603–611. (in Spanish)

A study was carried out on the incidence of leprosy in the city of Camaguey, Cuba, during 1978–1993. The diagnosis was based on five main criteria: immunology, histopathology, bacteriology, clinical examination and epidemiology. It utilized the classification of Madrid for the classification of the cases. The incidence was of 411 patients. The incidence rates varied between 22.5 and 2.7 per 100,000 inhabitants. The average was about 26 new patients each year. It is a tendency toward a decrease in the index of detection of new cases. There was no significant difference between the multibacillary forms (50.6%) and the paucibacillary forms (49.4%). In a decreasing order, the clinical forms percentages were as follows: LL (28.5%), LT (26.3%), paucibacillary LI (23.1%), and LD (21.9%). There was a slight predominance of females (54.0%) over males (46.0%). Nine cases under 15 years of age were reported (2.2%) and 402 of 15 years and older. In adults the greater number of new cases presented after 35 years of age.—Author's English Summary

Li, X., *et al.* [Aggregation property of leprosy in distribution.] China Lepr. J. **11** (1995) 17–18. (in Chinese)

The accumulative number of leprosy cases detected in Langzhong City, taking an area of four square kilometers as a unit, was analyzed with Poisson and negative binomial distributions. It showed that leprosy patients were clustered in distribution. The author suggested that the focal point of leprosy control should be put in small areas with the patients in the past and at present.—Author's English Abstract

Liu, C., *et al.* [Following up of 363 healthy children living in colonies and taking DDS for prevention of leprosy.] China Lepr. J. **11** (1995) 23–24. (in Chinese)

The effect of taking dapsone (DDS) for prevention of leprosy in 363 healthy children accompanying their parents suffering from the disease and living in leprosaria or colonies for 4 months to 16 years, being 5.5 years on the average, has been observed for 8 to 20 years; 1822 children of the parents with leprosy at home in the endemic areas who did not take the medicine were regarded as control. No leprosy was seen in the DDS- taking group, but 26 cases of leprosy have been found in the controls, of whom 23 were less than 14 years old when detected. It shows that preventive medicine-taking might be effective.—Author's English Abstract

Lopez Sifontes, M. E., Carrazana Hernandez, G. B. and Castano Hernandez, S. [Epidemiologic indicators of the incidence of leprosy in a health district.] Rev. Leprol. Fontilles **20** (1995) 625–643. (in Spanish)

During the years 1989–1993 a descriptive survey of the incidence of leprosy in the District of Joaquín de Agüero, Camagüey, Cuba, was performed. The following aspects were analyzed; incidence of leprosy and its rates, sex distribution, age groups, clinical forms, detection methods, source of infection, early or late diagnosis, results of the epidemiological survey, first symptoms of the disease and their localization, disabilities present in the moment of diagnosis and treatment compliance. The incidence

of leprosy during the 5 years was 32 cases with rates of 10.7 and 2.7 per 100.000 with a decreasing tendency in both cases. A slight predominance in the female group could be detected (56.2%). The most frequent age group was between 35–44 and over 65 years old. The percentage of multibacillary leprosy was 62.5. The most frequent clinical forms were LL (37.5%) and LT (28.1%). The clinical exam of contacts and risk population were satisfactory in the incidence detection. The indicators related with determinate of the source of infection, early diagnosis of leprosy and results of the epidemiological survey were unsatisfactory. The anesthetic macules are the first and major symptoms mentioned by patients (56.3%). The first symptoms are localized on the face and limbs. The percentage of patients with disabilities at the moment of diagnosis was (28.1%), with the highest percentage in the hands. The treatment compliance rate was 100%.—Author's English Summary

Martelli, C. M. T., Moraes Neto, O. L., Andrade, A. L. S. S., Silva, S. A., Silva, I. M. and Zicker, F. Spatial patterns of leprosy in an urban area of central Brazil. Bull. WHO **73** (1995) 315–319.

Reported is the spatial variation of leprosy in an urban area of Brazil and its correlation with socioeconomic indicators. From November 1991 to October 1992 a total of 752 newly diagnosed leprosy patients who were attending all outpatient clinics in Goiânia city, central Brazil, were identified. A database of leprosy cases was set up linking patients' addresses to 64 urban districts. Leprosy cases were detected in 86% of the districts and three risk strata were identified. The highest-risk area for leprosy was in the outskirts of the city and detection rates increased on moving from more developed to poorer areas. The risk of detecting leprosy cases was 5.3-fold greater (95% CI: 3.8–7.4) in the outskirts of the town than in the central zone. Discussed are the methodological issues related to leprosy case ascertainment, completeness and reliability of information, and the interpretation of the spatial distribution of leprosy per unit area. High lighted also are the lack of leprosy control activities in primary health care units and the usefulness of geographical analysis in planning health services.—Author's Summary

Monchy, D., Huerre, M. Crouzat, M. Dubourdieu, D., Duval, P. and Sottil, J. P. [Leprosy in New Caledonia from 1983 to 1992; histopathological and epidemiological data.] Bull. Soc. Pathol. Exot. **87** (1994) 28–32. (in French)

New Caledonia is a South Pacific Island inhabited by more than 170,000 people: most of them are Melanesians or Europeans. Multidrug therapy for Hansens disease has been employed since 1983; so the authors made an epidemiological and histopathological study of the new cases diagnosed for 10 years, from 1983 to 1992. Local (clinical, histological, microbiological and immunological) means of diagnosis are described. In New Caledonia, the endemic level is lower than in the small neighboring Pacific islands but there is still a native reservoir of *Mycobacterium leprae* with 40% multibacillary types. Nearly half of the new patients are less than 25 years old. They are often male Melanesians. The diagnosis of indeterminate forms is debated when there is no acid-fast bacillus. Borderline forms are rare. Tuberculoid leprosy poses many differential diagnosis problems. Despite available multidrug therapy, it cannot yet be considered that incidence is decreasing significantly.—Author's English Summary

Richardus, J. H. and Croft, R. P. Estimating the size of the leprosy problem: the Bangladesh experience. Lepr. Rev. **66** (1995) 158–164.

Assessing the size of the leprosy problem in a country is an important but difficult issue for the purpose of program planning. Different methods have been proposed but often estimates have proved to be very different from reality. We have attempted to address this issue in Bangladesh, a country where official estimates are more than five times greater than the registered number of leprosy cases. A combination of methods, including surveys, data from leprosy control programs and local knowledge based on the Delphi technique have been combined to construct an estimate of the total number

of cases in Bangladesh. This figure (173, 196) is only 10% greater than the official estimate (136,000). It will be possible over the next few years to see how close this figure is to reality through data obtained from the National Leprosy Control Programme which is now rapidly developing to cover the whole country.—Author's Summary

Rodrigues, S., Becaro, E., Koizumi, F. and Alchorne, M. M. A. [Armadillos and leprosy.] An. Bras. Dermatol. **68** (1993) 340–345. (in Portuguese)

Focusing on 127 leprosy patients (out of 205 studied in São Paulo, Brazil) who had eaten the flesh of armadillos, the authors discuss the role of these animals in the transmission of leprosy to the human population.—Author's English Summary

Sterne, J. A. C., Turner, A. C., Fine, P. E. M., Parry, J. V., Lucas, S. B., Ponnighaus, J. M., Mkandwire, P. K., Nyasulu, S. and Warndorff, D. K. Testing for antibody to human immunodeficiency virus type 1 in a population in which mycobacterial diseases are endemic. J. Infect. Dis. **172** (1995) 543–546.

During a large epidemiologic study in the Karonga District of northern Malawi, serum samples from 139 patients with incident leprosy, 124 with newly diagnosed leprosy, 277 patients with incident tuberculosis, and 2296 controls were tested for antibodies to human immunodeficiency virus. Sera were tested according to a four-test protocol using two ELISAs and two particle agglutination assays. Overall, 188 samples were considered positive, 2634 were considered negative, and 14 were indeterminate. All 18 available positive specimens from leprosy patients, a random sample of 14 positive specimens from tuberculosis patients, and 15 positive specimens from controls were tested by Western blot. There was no evidence of substantial numbers of ELISA false-positives in any patient group or among controls.—Author's Abstract

Tiendrebeogo, A., Blanc, L. and Sylla, P. M. [MDT for leprosy in Member States of OCCGE: a ten-year implementation (1983–1993)]. Acta Leprol. **9** (1995) 139–147. (in French)

MDT for leprosy recommended by WHO in 1981 has been introduced and implemented in 8 Member States of OCCGE (an organization for leprosy control in francophone West Africa). This implementation from 1983 to 1993 can be divided in two phases: 1983–1987: introduction phase by pilot projects: and 1988–1993: extension phase by national leprosy control programs. During the 10 years, MDT coverage rose to 68%, leprosy prevalence rate widely decreased (40.71 to 6.56 per 10,000), while annual detection rate weakly varied (1.89 to 1.26 per 10,000). Factors influencing this evolution of leprosy are brought out and recommendations are made about strategies to be developed for leprosy control up to year 2000.—Author's English Summary

Yang, J., *et al.* [Analysis of 90 children with leprosy.] China Lepr. J. **11** (1995) 5–7. (in Chinese)

Ninety children with leprosy have been detected during the period of 1983 to 1992 in Wenshan Prefecture, Yunnan. Among them, those who have been householdly infected make up 63.2%, the source of infection was lepromatous case in 84.2%, the disease duration is less than 2 years in 91.9% and shorter than that of adult cases (45.3%), the disability rate and the MB/PB value are 4.4% and 0.45, respectively, and lower than 28.0% and 1.29 in the adult; 70.2% of householdly infected children were detected at the time of examining household contacts but over 80% of those who have been infected outside the household were passively detected as adult cases. In the last 10 years the prevalence and the incidence of leprosy have been decreasing but the childhood patients showed an increasing trend, indicating that the endemicity of leprosy is not yet controlled.—Author's English Abstract

Zhao, T., *et al.* [The experience obtained in basic eradication of leprosy in Shandong Province.] China Lepr. J. **11** (1995) 19–20. (in Chinese).

Shandong Province has jurisdiction over 135 counties (or cities) with a population of 86 million. From 1955 to 1993, 53, 305

cases of leprosy have been detected accumulatively, of which 39, 654 have been cured. By the end of 1993, there remained 255 active cases, the prevalence was 0.029/10,000 and the mean incidence for the last 5 years was 0.003/10,000. So, after every effort of 37 years the goal of basic eradication of leprosy, taking county as a unit, has finally been reached with known and existing control measures. The experience in this process was discussed.—Author's English Abstract

Rehabilitation

Anandaraj, H. Measurement of dehabilitation in patients of leprosy; a scale. Indian J. Lepr. **67** (1995) 153–160.

Leprosy interferes with the psychological and social life of the patients leading to their "dehabilitation." Therefore, it is necessary to assess the extent and direction of dehabilitation in order to make the treatment plan comprehensive and effective. The objective of this work was to: (a) construct a scale for measuring dehabilitation and (b) to standardize it. The methodology included preparation of 52 statements (in English) spread over four subareas of life, namely, family relations, vocational condition, social interaction and self-esteem. It was administered to 122 randomly selected respondents. Scores were awarded by summing up the weights of each statement, a high score indicating low dehabilitation. Statistical tests were applied for standardizing the scale. To establish reliability, split-half reliability test and item discriminant analysis were used. Factor analysis was used to test the validity. The results show, that the split-half reliability coefficient ranged high (from 0.64 to 0.83) in all four subareas. The item discriminant analysis had a level of significance of 0.001 for 42 statements while the factor analysis explained variance covered over 70%. Hence the scale can be an useful intervention strategy for counselling, case work or rehabilitation.—Author's Summary

Cross, H., Sane, S., Dey, A. and Kulkarni, V. N. The efficacy of podiatric orthoses as an adjunct to the treatment of plantar ulceration in leprosy. Lepr. Rev. **66** (1995) 144–157.

This study examines the outcome of a mangement approach to plantar ulceration secondary to leprotic neuropathy. Locally available resources were used to produce podiatric orthoses which were supplied to an experimental group. The effects on healing time and quality of healing were compared with a control group. Both groups were ambulant (the program sought not to interfere with socioeconomic independence). Using standard nonparametric methods of analysis, it was demonstrated that the effects on healing rate, attributed to the experimental intervention, were highly significant. Over a 7-month period, 57% of the experimental group ulcers healed, while only 12.5% of the control group ulcers healed. The experimental intervention also demonstrated a positive effect on the quality of ulcer healing. This effect was not reflected in the control group.—Author's Summary

Kopparty, S. N. M., Kurup, A. M. and Sivaram, M. Problems and coping strategies of families having patients with and without deformities. Indian J. Lepr. **67** (1995) 133–152.

Deformity in leprosy is a major problem causing serious socioeconomic and psychological consequences to the patients and their families, as well as for the programmers. This paper examines the nature and extent of social and economic problems of leprosy-affected families having patients with and without deformities and their strategy to cope with those problems. The data were collected from 500 sampled families in two monotherapy districts in Tamil Nadu in 1989–1990. About 20% of the families reported facing socioeconomic problems. The proportion of families having patients with deformities facing problems was ten times higher (57.3%) than those having patients with no deformities (5.7%). The majority of the problems of the affected families were

economic. The major strategy adopted to deal with economic problems was to adjust within the earnings of other family members to make up the loss or reduction in income from the patient. The major social problem faced was denial of participation in the community. While families with deformed patients adopted "acceptance of their existing situation," families with nondeformed patients adopted "avoidance" as their coping strategy. Appropriate rehabilitation programs to restore economic security to the patients and their families is called for. There is also the need to educate the community about the disease in order to dispel the myths and fears associated with leprosy.—Author's Summary

Long, P., *et al.* [The effect of a modified temporal muscle bundle transposition on lagophthalmos in leprosy.] China Lepr. J. **11** (1995) 10–11. (in Chinese)

Twelve cases of lagophthalmos with leprosy corrected by using a modified temporal muscle bundle transposition have been evaluated, of which three eyes in three cases showed the width of the palpebral fissures to be <1 mm at closing the eye and nine eyes in six cases to be 1–3 mm after the operation, making up 66.7%. In six eyes in four cases with the palpebral fissure of 4–5 mm had some correction as compared with that before the operations. The difference between the modified and original methods is that the upper strand of the fascia strip is fixed on the tarsus at the junction of medial and middle thirds but not on the medial canthus ligament through a tunnel in the upper palpebral margin, so as to modulate the tension of the fascia strip for averting possible difficulty in opening the eye because of coincidence of the points of traction and catching-force of the two strands of the fascia strip.—Author's English Abstract

Malaviya, G. N. Toward restoring sensibility in anesthetic extremities of leprosy patients. Acta Leprol. **9** (1995) 111–115.

In leprosy patients, the problems arising due to anesthesia in the extremities have been outlined. The available modalities, some of them still experimental, for overcoming the handicap have been discussed.—Author's Summary

Marciano, L. H. S. C. and Garbino, J. A. [Comparison of techniques for monitoring the neuropathy of hanseniasis; tests of sensibilities and nerve conduction studies.] Hansen. Int. **19** (1994) 5–10. (in Portuguese)

This paper compares the nerve conduction studies with light touch-deep pressure evaluation in upper extremities of Hansen's disease patients. Thirty nerves were studied; 6 median and 24 ulnar. The equipment used in this study was 5 monofilament Semmes-Weinstein pocket-portable field kit and an electromyograph. The first assessment served to establish the presence or absence of nerve disfunction. The follow up evaluation showed whether the nerve conduction was improved, deteriorated or stable. Statistical studies showed significant similarity between the test results of the two procedures when used for the objectives discribed above.—Author's English Summary

Narayanakumar, T. S., Subramanian, A. and Manivannan, K. A method for texture discrimination in the sole of the foot; a preliminary communication. Lepr. Rev. **66** (1995) 165–168.

A new method for testing sensibility in the sole of the foot is described. In this method the ability to distinguish different surfaces while walking is assessed. This dynamic, functional and more objective test is recommended as an additional tool to evaluate sensibility in the sole of the foot.—Author's Summary

Soares, D. Tibialis posterior transfer in the correction of footdrop due to leprosy. Lepr. Rev. **66** (1995) 229–234.

In the correction of footdrop due to leprosy neuritis the tibialis posterior muscle is re-routed and used to provide dorsiflexion of the foot. This study of tibialis posterior transfer was carried out to compare the results of the circumtibial and interosseous routes. There is no significant difference in the range of motion between either route although the range of the interosseous route is more functional (better dorsiflexion). The interosseous route is preferable as this results in a significantly lower incidence of recurrent inversion deformity of the foot at

long-term follow up when compared with the circumtibial route.—Author's Summary

Soares, D. and Desar, N. Hand wounds in leprosy patients. Lepr. Rev. **66** (1995) 235–238.

This study assessed the causes, duration and site of hand wounds seen among patients in order to try and improve the delivery of self-care teaching to patients with anesthetic hands. Seventy-seven patients with 102 affected hand surfaces were assessed. The commonest cause was a burn from a tea glass. The average duration of the wound was 2 weeks. Most patients had a single current wound and 62% of wounds were on the palmar surface.—Author's Summary

Virmond, M., Camargo Marciano, L. H. S. and Almedia, S. N. [The results of neurolysis of the ulnar nerve in the neuritis of hanseniasis.] Hansen Int. **19** (1994) 5–9. (in Portuguese)

A group of 33 leprosy patients with ulnar nerve neuritis surgical release and anterior subcutaneous transposition of this nerve. Surgery was indicated due to progressive deterioration of neural function despite clinical treatment. Semmes-Weinstein monofilaments and VMT were used to assess nerve function before and 12 months after surgery, 39.3% showed improvement of nerve function, 39.3% remained stable and 21.4% developed further deterioration.—Author's English Abstract

Wang, X., *et al.* [The effect of eyebrow transposition with single hairs in 274 cases of leprosy.] China Lepr. J. **11** (1995) 26–27. (in Chinese)

The effect of eyebrow grafting with single hairs in 274 cases of MB leprosy, no matter how many their BI was, has been observed since 1980. They all expressed satisfaction with some improvement in their appearance. The operation is simple and safe. A piece of the scalp behind the ear is taken and separated into single hairs with intact bulbus pili. Some 350 to 400 hairs on one side for a man are needed and 300 to 350 for a woman.—Author's English Abstract

Xu, S. [The effect of a comprehensive protection measure on the feet with plantar ulcer and numbness.] China Lepr. J. **11** (1995) 8–10. (in Chinese)

The effect of a comprehensive protection measure on 47 cases with 64 plantar ulcers and on 48 cases of leprosy with insensive feet for 3 years was reported. Among those with the ulcer, 54 ulcers healed and 2 improved and the ulcer relapsed in 2 cases. The incidence of new ulcer and the relapse rate were 4.3% and zero in the first year, 2.1% and 7.8% in the 2nd year, and 2.1% and 3.1% in the 3rd year, respectively. Among those with numb feet, the ulcers occurred in two feet only in the first year but then no one.—Author's English Abstract

Yu, B., *et al.* [Following up of 80 amputees of leprosy after fitting with artificial limbs.] China Lepr. J. **11** (1995) 27–28. (in Chinese)

By June 1992, 80 amputees cured of leprosy have been fitted with artificial limbs for 18 thighs and 67 legs in Fujian Province, including 71 men and 9 women with a mean age of 56 years. So they all have obtained the ability to live independently and to labor.—Author's English Abstract

Other Mycobacterial Diseases and Related Entities

Adhikari, N. and Menzies, R. Community-based tuberculin screening in Montreal: a cost-outcome description. Am. J. Public Health **85** (1995) 786–790.

This study describes the costs and outcomes of community-based tuberculin screening programs conducted between 1987 and 1991 in Montreal, Quebec, Canada.

Follow up information was abstracted from hospital records of all reactors detected in tuberculin screening of students in grades 6 and 10, of first-year health professional students, and of workers aged 18 to 25 in a number of workforces. Screening costs were estimated directly from survey records, and follow up costs were estimated from the annual financial report of the Montreal Chest Hospital for 1989/90. Of 7669 persons tested, 782 (10.2%) had positive results and 757 (9.9%) were referred to a clinic. Of those, 525 (6.8% of the original 7669) reported, 293 (3.8%) were prescribed therapy, and 154 (2.0%) were compliant. In Canadian dollars, screening cost $5.70 per person tested and $56 per tuberculin reactor detected, but follow up of reactors accounted for 73% of the total program cost of $13, 455 to $18, 753 per case of tuberculosis prevented. Because of high rates of patient and provider noncompliance, a tuberculin screening program was much less cost-effective than anticipated. Screening costs must be targeted to the highest risk populations, and compliance with recommendations for preventive therapy must be maximized.—Author's Summary

Alangaden, G. J., Manavathu, E. K., Vakulenko, S. B., Zvonok, N. M. and Lerner, S. A. Characterization of fluoroquinolone-resistant mutant strains of *Mycobacterium tuberculosis* selected in the laboratory and isolated from patients. Antimicrob. Agents Chemother. **39** (1995) 1700–1703.

To examine the mechanism of resistance to fluoroquinolones in *Mycobacterium tuberculosis,* we selected spontaneous fluoroquinolone-resistant mutants from a susceptible strain, H37Rv, and studied the susceptibilities of these mutants and two fluoroquinolone-resistant clinical isolates (A-382, A-564) to various fluoroquinolones and to isoniazid and rifampin. Furthermore, since mutations within the quinolone resistance-determining region of the structural gene encoding the A subunit of DNA gyrase are the most common mechanism of acquired resistance, we amplified this region by PCR and compared the nucleotide sequences of the fluoroquinolone-resistant strains with that of the susceptible strain. Fluoroquinolone-resistant mutants of H37Rv appeared at frequencies of 2×10^{-6} to 1×10^{-8}. For three mutants selected on ciprofloxacin, ofloxacin, and sparfloxacin, respectively, and the two clinical isolates, MICs of ciprofloxacin and ofloxacin were as high as 16 μg/ml, and those of sparfloxacin were 4 to 8 μg/ml. They displayed cross-resistance to all fluoroquinolones tested but not to isoniazid or rifampin. Sparfloxacin and FQ-A (PD 127391-0002) were the most potent fluoroquinolones. All of the fluoroquinolone-resistant strains (MICs, ≥4 μg/ml) had mutations in the quinolone resistance-determining region which led to substitution of the Asp residue at position 87 (Asp-87) by Asn or Ala or the substitution of Ala-83 by Val in the A subunit of DNA gyrase. Similar mutations have been noted in other bacterial species and recently in mycobacteria. The broad resistance to fluoroquinolones that arose readily by point mutation in the laboratory and apparently during inadequate therapy, as was the case in the clinical isolates, may ultimately lead to serious restriction of the use of these drugs in the treatment of tuberculosis.—Author's Summary

Appelberg, R., Castro, A. G., Gomes, S., Pedrosa, J. and Silva, M. T. Susceptibility of beige mice to *Mycobacterium avium*; role of neutrophils. Infect. Immun. **63** (1995) 3381–3387.

The beige mutation in C57BL/6 mice has been shown to increase the susceptibility to infection by *Mycobacterium avium.* In this study, we confirmed those results and showed that the effect of the beige mutation was most obvious after infection with a strain of lower virulence than with a highly virulent isolate of *M. avium.* The dissemination of *M. avium* from the gut was observed with both C57BL/6 and beige mice but was faster in the latter. The expression of gamma interferon (IFN-γ) and the priming for tumor necrosis factor production during an *in vivo* infection were similar between beige and immunocompetent C57BL/6 mice. IFN-γ produced during the infection of beige mice was protective in the spleen, and the administration of recombinant IFN-γ restored the resistance in the spleen to levels similar to those found in

control mice. There were no histological differences between wild-type and beige mice with respect to granuloma formation in the liver. The increased susceptibility of beige mice to *M. avium* as manifested in the liver was reduced by transfusing neutrophils from wild-type C57BL/6 mice. Likewise, depletion of neutrophils from C57BL/6 mice rendered them as susceptible to *M. avium* infection of the liver as beige mice. Our results point to the participation of neutrophils in the defect of beige mice in addition to other defects. Furthermore, these results show that neutrophils play a significant role in the defense mechanisms against mycobacterial infections and that beige animals may be a useful model for study of the role of neutrophils in mycobacteriosis.—Author's Abstract

Berning, S. E., Madsen, L., Iseman, M. D. and Peloquin, C. A. Long-term safety of ofloxacin and ciprofloxacin in the treatment of mycobacterial infections. Am. J. Respir. Crit. Care Med. **151** (1995) 2006–2009.

Ofloxacin and ciprofloxacin are potentially useful agents for treating mycobacterial infections. We retrospectively reviewed 7 years' experience with these agents in 103 patients. Ofloxacin was used primarily to treat tuberculosis (TB), dosed to achieve 2-hour postdose serum concentrations of 8–12 μg/ml. Ciprofloxacin was used primarily to treat *Mycobacterium avium* complex (MAC) infection, dosed to achieve 2-hour postdose serum concentrations of 4–6 μg/ml. Despite differences in patient characteristics, underlying disease, and concurrent medications, ofloxacin and ciprofloxacin were associated with a similar spectrum and incidence of adverse reactions. Both drugs were generally well tolerated. Adverse effects led to an ofloxacin dosage change in 1 patient (3%) and discontinuation of ofloxacin in 2 patients (6%). Adverse effects led to a ciprofloxacin dosage change in 2 patients (3%) and discontinuation of ciprofloxacin in 5 patients (7%). Ofloxacin and ciprofloxacin appear to be tolerated as well as or better than other "second-line" antimycobacterial drugs.—Author's Summary

Brown, D. H., Miles, B. A. and Zwilling, B. S. Growth of *Mycobacterium tuberculosis* in BCG-resistant and -susceptible mice: establishment of latency and reactivation. Infect. Immun. **63** (1995) 2243–2247.

Growth of mycobacterial species is controlled by a gene, *Bcg* (candidate *Nramp*). *Bcg* acts at the macrophage level and is thought to control some aspect of macrophage priming for activation. Infection of *Mycobacterium bovis* BCG-susceptible (Bcg^s) mice with several different mycobacterial species results in the growth of the microorganisms, while the growth of the same organisms is controlled in BCG-resistant (Bcg^r) mice. The capacity of *Bcg* to control the growth of *M. tuberculosis* has not been extensively explored. The purpose of this investigation, therefore, was to compare the growth of *M. tuberculosis* in Bcg^r and Bcg^s mice. We found that the growth of tubercule bacilli was different in the lungs and spleens of Bcg^r, and Bcg^s mice when they were inoculated with fewer then 10^3 CFU of the mycobacterium. The differences in growth were more easily distinguished in the lungs then in the spleens. The growth of the microorganisms in both strains of mice peaked between 35 and 43 days, and a latent infection was established by 65 days after initial infection. Activation of the hypothalamic-pituitary-adrenal axis resulted in reactivation of the growth of *M. tuberculosis* in both Bcg^r and Bcg^s mice. Greater numbers of tubercule bacilli were isolated from lungs than from spleens following reactivation. The utility of this mouse model in the study of the establishment of latency and reactivation of *M. tuberculosis* is discussed.—Author's Abstract

Burwen, D. R., Bloch, A. B., Griffin, D., Ciesielski, C. A., Stern, H. A. and Onorato, I. M. National trends in the concurrence of tuberculosis and acquired immunodeficiency syndrome. Arch. Intern. Med. **155** (1995) 1281–1286.

Background: Elucidation of the relationship between tuberculosis (TB) and the acquired immunodeficiency syndrome (AIDS) is needed to help predict the future course of these two epidemics. We examined na-

tionwide trends in TB and AIDS occurring in the same individual.

Methods: Health departments in the 50 states, District of Columbia, Puerto Rico, and Guam matched their TB and AIDS case registries to determine the number of persons diagnosed with both TB and AIDS. The number of AIDS cases, TB cases, AIDS cases that matched with a TB case on the TB registry, and TB cases that matched with an AIDS case on the AIDS registry were reported to the Centers for Disease Control and Prevention, Atlanta, Ga. Data were analyzed for the period from 1981 through 1991. The number of matched TB-AIDS cases was compared with a modeled estimate of excess TB cases during the period from 1985 through 1990.

Results: From 1981 through 1991 there were 11, 299 AIDS cases that matched with a TB case on the TB registry, representing 5.1% (geographic variation, 0% to 9.3%) of AIDS cases. The TB cases that matched with an AIDS case on the AIDS registry represent 4.3% (geographic variation, 0% to 15.1%) of TB cases from 1981 through 1991. Since 1981, matched TB and AIDS cases increased yearly through 1990. When examined by year of AIDS report, the percentage of AIDS cases that matched with a TB case increased from 1981 to 1982 (1.9% to 5.1%), remained fairly constant from 1983 through 1987 (range, 4.0% to 4.7%), increased in 1988 (5.4%) after extrapulmonary TB was added to the AIDS case definition, and increased slightly through 1990 (5.8%). When examined by year of TB report, the percentage of TB cases that matched with an AIDS case increased steadily from 1981 through 1990 (0.1% to 9.5%). The calculated fraction of excess TB cases during the period from 1985 through 1990 that could be accounted for by identified TB-AIDS cases was 30%.

Conclusion: The risk of TB or AIDS among persons already diagnosed with one disease is much higher than among the general population. The percentage of persons with TB who are also diagnosed with AIDS has been increasing rapidly. Human immunodeficiency virus-induced immunosuppression is an important contributor to the TB epidemic and probably accounts for a minimum of 30% of excess TB cases during the period from 1985 through 1990.—Author's Abstract

Carlesimo, M., Guistini, S., Rossi, A., Bonaccorsi, P. and Calvieri, S. Treatment of cutaneous and pulmonary sarcoidosis with thalidomide. J. Am. Acad. Dermatol. **32** (1995) 866–869.

Many therapeutic agents have been proposed for treatment of steroid-resistant sarcoidosis. Because administration of low doses of thalidomide has been successful in treating other inflammatory diseases, it was used in a patient with systemic sarcoidosis who was unresponsive to corticosteroids and in a patient with pulmonary sarcoidosis, in whom Kaposi's sarcoma developed after a course of corticosteroid therapy. Thalidomide, 200 mg/day for 2 weeks followed by 100 mg/day for 11 weeks, was given. This treatment was effective in both patients. No adverse reactions were observed. Thalidomide, 100 mg on alternate days, is still being administered. No relapse has occurred. Thalidomide, particularly because of its inhibition of the macrophage function, may be a useful alternative therapy in steroid-resistant cases. In addition, the correlation between the angiotensin-converting enzyme level and the clinical improvement observed in our patients suggests a direct parallel between angiotensin-converting enzyme and the activity of the granulomatous process.—Author's Abstract

Cavalieri, S. J., Biehle, J. R. and Sanders, W. E. Synergistic activities of clarithromycin and antituberculous drugs against multidrug-resistant *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **39** (1995) 1542–1545.

The rise of multidrug-resistant *Mycobacterium tuberculosis* has complicated therapy for tuberculosis and led us to search for a potentially active combination of drugs against these strains. The susceptibilities of 12 strains of multidrug-resistant *M. tuberculosis* to standard antituberculous drugs (isoniazid, rifampin, ethambutol, and pyrazinamide), clarithromycin, and its metabolite, 14-hydroxyclarithromycin, were determined by use of the BACTEC radiometric method. All strains were resistant to at

least two of the antituberculous drugs. Clarithromycin and 14-hydroxyclarithromycin MICs were in the range indicating resistance at ≥8.0 μg/ml for all strains. Combination testing by the BACTEC method was performed with various concentrations of isoniazid, rifampin, and ethambutol, and with clarithromycin/14-hydroxyclarithromycin at fixed concentrations of 2.0/0.5 μg/ml, respectively. Addition of clarithromycin/14-hydroxyclarithromycin to these antituberculous drug mixtures resulted in a 4- to 32-fold reduction in MICs of isoniazid, rifampin, and ethambutol and made resistant strains susceptible. Fractional inhibitory concentrations ranged from 0.23 to 0.50 for all strains, suggesting a synergistic interaction between standard antituberculous drugs and clarithromycin/14-hydroxyclarithromycin. The ability of clarithromycin/14-hydroxyclarithromycin to enhance the activities of isoniazid, ethambutol, and rifampin *in vitro* suggests that this combination may be efficacious in the treatment of multidrug-resistant *M. tuberculosis* infections.—Author's Abstract

Ching, L. M., Xu, Z. F., Gummer, B. H., Palmer, B. D., Joseph, W. R. and Baguley, B. C. Effect of thalidomide on tumour necrosis factor production and anti-tumour activity induced by 5, 6-dimethylxanthenone-4-acetic acid. Br. J. Cancer **72** (1995) 339–343.

The investigational antitumor agent, 5, 6-dimethylxanthenone-4-acetic acid (5, 6-MeXAA), an analog of flavone acetic acid (FAA), has been scheduled for clinical evaluation. Like FAA, 5, 6-MeXAA exhibits excellent experimental antitumor activity and is an efficient inducer of cytokines in mice. We have examined the effect of pharmacological suppression of tumor necrosis factor (TNF) production on the antitumor activity of 5, 6-MeXAA, taking advantage of previous observations that TNF production in response to endotoxin *in vitro* is inhibited by thalidomide. Thalidomide at doses of between 8 and 250 mg kg efficiently suppressed serum TNF activity in response to 5, 6-MeXAA at its optimal TNF inducing dose of 55 mg/kg. Suppression was achieved when thalidomide was administered at the same time as, or up to 4 hr before, 5, 6-MeXAA. Under conditions in which TNF activity was suppressed, the degree of tumor hemorrhagic necrosis and the proportion of cures in the subcutaneous colon 38 tumors were increased. In mice administered thalidomide (100 mg/kg) together with 5, 6-MeXAA (30 mg/kg) complete tumor regression was obtained in 100% of mice, as compared with 67% in mice receiving 5, 6-MeXAA alone. The results suggest a possible new application for thalidomide and pose new questions about the action of 5, 6-MeXAA and related compounds.—Author's Abstract

Chow, C. C., Mak, T. W. L., Chan, C. H. S. and Cockram, C. S. Euthyroid sick syndrome in pulmonary tuberculosis before and after treatment. Ann. Clin. Biochem. **32** (1995) 385–391.

Alterations of circulating thyroid hormones are frequently present in chronic nonthyroidal illnesses and may predict prognosis. Pulmonary tuberculosis, a common treatable debilitating disease, may provide a useful model for detailed evaluation of changes of thyroid hormones in relation to subsequent recovery or mortality. Over a period of 12 months, we performed a prospective study of 40 consecutive Chinese patients aged over 50 years and admitted with newly diagnosed pulmonary tuberculosis. Blood samples were drawn for serial thyroid function tests [free thyroxine (T-4), free triiodothyronine (T-3) and thyroid-stimulating hormone] before treatment and at 1, 2 and 4 months afterward. Mortality was determined up to 12 months of follow up. The euthyroid sick syndrome occurred in 63% of patients at presentation. Twelve of 25 euthyroid sick patients died as compared to one of 15 patients with normal baseline thyroid function tests ($p < 0.02$). Among euthyroid sick patients, those who died had significantly lower free T-3 concentration at presentation than those who survived ($p < 0.05$). An undetectable free T-3 concentration at presentation was associated with a subsequent mortality of 75% (9 of 12). Of the survivors, all patients demonstrated a significant rise in serum free T-4 concentrations following treatment, which was apparent by 1 month. These data suggest that an undetectable free T-3 concen-

tration at presentation reflects severity of illness and predicts a subsequent high mortality.—Author's Abstract

Collins, D. M., Kawakami, R. P., Delisle, G. W., Pascopella, L., Bloom, B. R. and Jacobs, W. R. Mutation of the principal sigma factor causes loss of virulence in a strain of the *Mycobacterium tuberculosis* complex. Proc. Natl. Acad. Sci. U.S.A. **92** (1995) 8036–8040.

Tuberculosis continues to be responsible for the deaths of millions of people, yet the virulence factors of the causative pathogens remain unknown. Genetic complementation experiments with strains of the *Mycobacterium tuberculosis* complex have identified a gene from a virulent strain that restores virulence to an attenuated strain. The gene, designated *rpoV,* has a high degree of homology with principal transcription or sigma factors from other bacteria, particularly *M. smegmatis* and *Streptomyces griseus.* The homologous *rpoV* gene of the attenuated strain has a point mutation causing an arginine → histidine change in a domain known to interact with promoters. To our knowledge, association of loss of bacterial virulence with a mutation in the principal sigma factor has not been previously reported. The results indicate either that tuberculosis organisms have an alternative principal sigma factor that promotes virulence genes or, more probably, that this particular mutant principal sigma factor is unable to promote expression of one or more genes required for virulence. Study of genes and proteins differentially regulated by the mutant transcription factor should facilitate identification of further virulence factors.—Author's Summary

Cooksey, R. C., Morlock, G. P., Beggs, M. and Crawford, J. T. Bioluminescence method to evaluate antimicrobial agents against *Mycobacterium avium.* Antimicrob. Agents Chemother. **39** (1995) 754–756.

Plasmid pLUC10, carrying the firefly luciferase gene, was transformed by electroporation into *Mycobacterium avium* A5. Bioluminescence production by strain A5(pLUC10), as measured in a microdilution plate luminometer, was ~ 1 relative light unit per 2×10^6 viable bacilli; whereas it was 0.0005 relative light unit for an equal number of parental cells. The susceptibility of strain A5(pLUC10) to eight concentrations of each of eight antimicrobial agents was evaluated by the luciferase microplate assay in parallel with a conventional broth macrodilution method with antimicrobial agents. Decreases in bioluminescence to levels that were ≤ 10% of those of drug-free controls were observed in microplate wells containing inhibitory concentrations of drugs in as few as 3 days. The close correlation of these inhibitory concentrations with the MICs determined by a conventional broth macrodilution method suggests that the luciferase microplate method may offer a convenient and reliable means of evaluating the *in vitro* activities of antimicrobial agents against the *M. avium* complex.—Author's Abstract

Dlugovitzky, D., Luchesi, S., Torres Morales, A., Ruiz Silva, J., Canosa, B., Valentini, E. and Bottasso, O. Circulating immune complexes in patients with advanced tuberculosis and their association with autoantibodies and reduced CD4+ lymphocytes. Braz. J. Med. Biol. Res. **28** (1995) 331–335.

We investigated the presence of circulating immune complexes (CICs) in serum from tuberculosis (TB) patients with different degrees of pulmonary involvement. Patients were classified into four groups according to the extent of lung involvement: mild (N = 9), moderate (N = 12), moderate plus (N = 16), and severe cases (N = 10). A search for CICs by the 3.5% PEG precipitation test showed that the CIC values of patients with the moderate plus or severe form of pulmonary TB were significantly higher compared to healthy controls and to mild and moderate cases ($p < 0.01$ and $p < 0.001$, respectively). Further analysis demonstrated that increased CIC levels were associated with increased autoantibody production, since this abnormality was more prevalent in patients with advanced disease ($p < 0.01$), who also showed a significant reduction of CD4+ T. lymphocytes. The immunoregulatory and pathogenetic implications of these findings are discussed.—Author's Abstract

Dobos, K. M., Swiderek, K., Khoo, K. H., Brennan, P. J. and Belisle, J. T. Evidence for glycosylations sites on the 45-kilodalton glycoprotein of *Mycobacterium tuberculosis*. Infect. Immun. **63** (1995) 2846–2853.

The occurrence of glycosylated proteins in *Mycobacterium tuberculosis* has been widely reported. However, unequivocal proof for the presence of true glycosylated amino acids within these proteins has not been demonstrated, and such evidence is essential because of the predominance of soluble lipoglycans and glycolipids in all mycobacterial extracts. We have confirmed the presence of several putative glycoproteins in subcellular fractions of *M. tuberculosis* by reaction with the lectin concanavalin A (ConA). One such product, with a molecular mass of 45 kDa, was purified from the culture filtrate. Compositional analysis demonstrated that the protein was rich in proline and that mannose, galactose, glucose, and arabinose together represented about 4% of the total mass. The 45-kDa glycoprotein was subjected to proteolytic digestion with either the Asp-N or the Glu-C endopeptidase or subtilisin, peptides were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and glycopeptides were identified by reaction with ConA. Peptides were further separated, and when they were analyzed by liquid chromatography-electrospray mass spectrometry for neutral losses of hexoses (162 mass units), four peptides were identified, indicating that these were glycosylated with hexose residues. One peptide, with an average molecular mass of 1516 atomic mass units (AMU), exhibited a loss of two hexose units. The *N*-terminal sequence of the 1516-AMU glycopeptide was determined to be DPEPAPPVP, which was identical to the sequence of the amino terminus of the mature protein, DPEPAP PVPXTA. Furthermore, analysis of the glycopeptide by secondary ion mass spectrometry demonstrated that the complete sequence of the glycopeptide was DPEPAPPVPTTA. From this, it was determined that the 10th amino acid, threonine, was O-glycosicically linked to a disaccharide composed of two hexose residues, probably mannose. This report establishes that true, O-glycosylated proteins exist in mycobacteria.—Author's Abstract

Flynn, J. L., Goldstein, M. M., Chan, J., Triebold, K. J., Pfeffer, K., Lowenstein, C. J., Schreiber, R., Mak, T. W. and Bloom, B. R. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. Immunity **2** (1995) 561–572.

Understanding the immunological mechanisms of protection and pathogenesis in tuberculosis remains problematic. We have examined the extent to which tumor necrosis factor-alpha (TNF-α) contributes to this disease using murine models in which the action of TNF-α is inhibited. TNF-α was neutralized *in vivo* by monoclonal antibody; in addition, a mouse strain with a disruption in the gene for the 55-kDa TNF receptor was used. The data from both models established that TNF-α and the 55-kDa TNF receptor are essential for protection against tuberculosis in mice, and for reactive nitrogen production by macrophages early in infection. Granulomas were formed in equal numbers in control and experimental mice, but necrosis was observed only in mice deficient in TNF-α or TNF receptor. TNF-α and the 55-kDa TNF receptor are necessary conditions for protection against murine *Mycobacterium tuberculosis* infection, but are not solely responsible for the tissue damage observed.—Author's Abstract

Gan, H. X., Newman, G. W. and Remold, H. G. Plasminogen activator inhibitor type 2 prevents programmed cell death of human macrophages infected with *Mycobacterium avium,* serovar 4. J. Immunol. **155** (1995) 1304–1315.

Although *Mycobacterium avium* is usually nonpathogen ic in healthy individuals, *in vitro* infection of macrophages from the majority of healthy donors induces death of the cells 2 wk after infection; this effect is in contrast to noninfected macrophages, which survive for months in culture. We demonstrate here that treatment of normal macrophages with indomethacin further shortens the life of these cells to 48 hr after infection with *M. avium.* Indomethacin treatment of the macrophages also prevents *M. avium*-dependent accumulation of mRNA-encoding plasminogen activator inhibitor type-2 (PAI-2), an inhibitor of uro-

kinase-type plasminogen activator. Occurrence of nuclear condensation and DNA fragmentation in macrophages pretreated with indomethacin and infected with *M. avium* indicates that the early death of these cells is caused by apoptosis. In contrast, priming of macrophages with CM-CSF significantly prolongs their survival after *M. avium* infection and enhances *M. avium*-induced accumulation of PAI-2 mRNA. Most importantly, addition of PAI-2 is sufficient to prevent apoptosis of macrophages infected with *M. avium* in the presence of indomethacin. Finally, macrophages not treated with indomethacin also die of apoptosis 7 to 10 days after *M. avium* infection and can be rescued by PAI-2. These studies indicate that production of PAI-2 by normal macrophages as a consequence of *M. avium* infection inhibits programmed cell death, a mechanism that might serve to prevent the spread of the infection.—Author's Abstract

Gevaudan, M. J. and Demicco, P. Extra and intracellular activities of dirithromycin against *Mycobacterium avium.* Pathol. Biol. **43** (1995) 284–288.

The *in vitro* activity of dirithromycin alone and in combination with clofazimine, ethambutol and rifabutin was tested against 30 strains of *Mycobacterium avium* isolated from patients. Extracellular activity of dirithromycin was assessed by determining MICs using the radiometric methodology in 7H(12) broth at two pHs 6.8 and 7.4. The MICs obtained at pH 7.4 were 3 to 4 more dilutions lower than those obtained at pH 6.8. Activity of pairs of antibiotics was measured using the FIC indices. Dirithromycin-clofazimine combination demonstrated the most important additive effects and even produced synergic effect against 5 of 30 strains. Studies of intracellular bacteria showed that the most effective bactericidal combination was dirithromycin, clofazimine and ethambutol together.—Author's Abstract

Gobin, J., Moore, C. H., Reeve, J. R., Wong, D. K., Gibson, B. W. and Morwitz, M. A. Iron acquisition by *Mycobacterium tuberculosis:* isolation and characterization of a family of iron-binding exochelins. Proc. Natl. Acad. Sci. U.S.A. **92** (1995) 5189–5193.

Mycobacterium tuberculosis, the primary agent of tuberculosis, must acquire iron from the host to cause infection. To do so, it releases high-affinity iron-binding siderophores called exochelins. Exochelins are thought to transfer iron to another type of high-affinity iron-binding molecule in the bacterial cell wall, mycobactins, for subsequent utilization by the bacterium. In this paper, we describe the purification of exochelins of *M. tuberculosis* and their characterization by mass spectrometry. Exochelins comprise a family of molecules whose most abundant species range in mass from 744 to 800 Da in the neutral Fe3+-loaded state. The molecules form two 14-Da-increment series, one saturated and the other unsaturated, with the increments reflecting different numbers of CH2 groups on a side chain. These series further subdivide into serine-/or threonine-containing species. The virulent *M. tuberculosis* Erdman strain and the avirulent *M. tuberculosis* H37Ra strain produce a similar set of exochelins. Based on a comparison of their tandem mass spectra, exochelins share a common core structure with mycobactins. However, exochelins are smaller than mycobactins due to a shorter alkyl side chain, and the side chain of exochelins terminates in a methyl ester. These differences render exochelins more polar than the lipophilic mycobactins and, hence, soluble in the aqueous extracellular milieu of the bacterium in which they bind iron in the host.—Author's Abstract

Gomez-Flores, R., Gupta, S., Tamez-Guerra, R. and Mehta, R. T. Determination of MICs for *Mycobacterium avium-M. intracellulare* complex in liquid medium by a colorimetric method. J. Clin. Microbiol. **33** (1995) 1842–1846.

We investigated the potential of a rapid colorimetric microassay based on the reduction of dimethylthiazol-diphenyltetrazolium bromide (MTT) for determining the growth of *Mycobacterium avium-M. intracellulare* complex (MAC) and MICs of clofazimine, resorcinomycin A, and the quinolone PD 127391 against MAC. The reduction of MTT was directly proportional

to the number of viable bacteria. A comparison of the MTT reduction test with the [^{3}H]glycerol uptake assay showed the former to possess higher analytical sensitivity for detecting MAC growth in microtiter plates. The MTT reduction test avoids the use of radioisotopes and costly material and equipment; it is reliable, reproducible, and convenient for rapid routine susceptibility testing of MAC.—Author's Summary

Gonzalez-Montaner, L. J., Natal, S., Yongchaiyud, P. and Olliaro, P. (Rifabutin Study Group). Rifabutin for the treatment of newly diagnosed pulmonary tuberculosis: a multinational, randomized, comparative study versus rifampicin. Tuber. Lung Dis. **75** (1994) 341–347.

The efficacy, tolerability and toxicity of two regimens containing different daily dosages of rifabutin in comparison with rifampin were analyzed. The patients were recruited from 6 centers in Argentina, Brazil and Thailand, and had newly diagnosed drug-sensitive, radiographically active and bacteriologically confirmed pulmonary tuberculosis. A total of 520 patients were enrolled and randomly assigned to receive rifampin (N = 175), rifabutin 150 mg (N = 174), or rifabutin 300 mg (N = 171). Considering all patients with positive baseline culture, the success rates at the last valid observation for each patient were 89%, 94% and 92% in the rifampin, rifabutin 150 mg, and rifabutin 300 mg groups, respectively. The median time to culture conversion was comparable in the 3 groups and was 34 days for rifampin and 37 days for each of the rifabutin groups. During the drug-free follow up period, one relapse occurred in the rifampin group, and two in each of the rifabutin groups. The 3 treatment schedules appeared well tolerated. No patients had to discontinue therapy because of an adverse event in the rifabutin 150 mg group, compared to one in the rifampin and 5 in the rifabutin 300 mg group. All 3 regimens proved effective and well tolerated. Rifabutin at 150 mg/d showed the best risk-to-benefit ratio, in that this group had the highest proportion of patients completing treatment, the highest bacteriological conversion rates and the lowest incidence of adverse events.—Author's Summary

Hermans, P. W. M., Messadi, F., Guebre Xabier, H., Van Soolingen, D., Dehaas, P. E. W., Heersma, H., Deneeling, H., Ayoub, A., Portaels, F., Frommel, D., Zribi, M., and van Embden, J. D. A. Analysis of the population structure of *Mycobacterium tuberculosis* in Ethiopia, Tunisia, and The Netherlands: usefulness of DNA typing for global tuberculosis epidemiology. J. Infect. Dis. **171** (1955) 1504–1513.

The genetic heterogeneity among *Mycobacterium tuberculosis* isolates from 501 patients in Ethiopia, Tunisia, and The Netherlands was compared by analysis of DNA polymorphism driven by insertion element IS6110. The percentage of isolates displaying two or more identical patterns differed greatly in the three countries: it was highest among Tunisian isolates and lowest in Dutch isolates. In contrast to isolates from Dutch subjects infected with *M. tuberculosis,* the majority of strains from Ethiopia and Tunisia were from a few families of genetically highly related strains. Furthermore, little overlap was observed among isolates from the three countries, indicating strict isolation of the bacterial reservoirs in the countries. A few strains from The Netherlands matched strains from Ethiopia and Tunisia. Those strains were invariably isolated from refugees, immigrants, or persons who visited Ethiopia or Tunisia.—Author's Abstract

Hetherington, S. V., Watson, A. S. and Patrick, C. C. Sequence and analysis of the *rpoB* gene of *Mycobacterium smegmatis.* Antimicrob. Agents Chemother. **39** (1995) 2164–2166.

The *rpoB* gene encodes the beta subunit of the DNA-dependent RNA polymerase of bacteria. Mutations in defined areas result in resistance to rifampin. *Mycobacterium smegmatis* is naturally resistant to rifampin, but analysis of the *rpoB* gene revealed no identifiable rifampin-resistance mutations. Another mechanism of resistance may be present.—Author's Summary

Ji, B., Lounis, N., Truffot-Pernot, C. and Grosset, J. *In vitro* and *in vivo* activities of levofloxacin against *Mycobacterium*

tuberculosis. Antimicrob. Agents Chemother. **39** (1995) 1341–1344.

In tests with 18 drug-susceptible strains of *Mycobacterium tuberculosis,* the MIC at which 50% of the strains are inhibited by levofloxacin (LVFX) was one dilution less than that at which 50% of the strains are inhibited by ofloxacin (OFLO), but the MICs at which 90% of the strains are inhibited were similar. The *in vivo* activity of LVFX against *M. tuberculosis* was compared with the activities of isoniazid, OFLO, and sparfloxacin (SPFX). Mice were inoculated intravenously with 1.74×10^6 CFU of H37Rv, and treatments began the next day and were carried out six times weekly for 4 weeks. The severity of infection and effectiveness of treatment were assessed by survival rate, spleen weights, gross lung lesions, and enumeration of CFU in the spleen. In terms of CFU counts, the ranking of the anti-*M. tuberculosis* activities of the treatments used ran in the following order: LVFX (300 mg/kg of body weight) = SPFX (100 mg/kg) > isoniazid > SPFX (50 mg/kg) > OFLO (300 mg/kg) = LVFX (150 mg/kg) > OFLO (150 mg/kg) = LVFX (50 mg/kg). It seems, therefore, that the *in vivo* activity of LVFX is comparable to that produced by a twofold-greater dosage of OFLO. It is assumed that the maximal clinically tolerated dosage of LVFX is similar to that of OFLO, i.e., 800 mg daily, which is equivalent to 300 mg of LVFX per kg in mice. Because LVFX displayed powerful bactericidal activity, promising effects against human tuberculosis may be achieved if patients are treated with the maximal clinically tolerated dosage of LVFX.—Author's Summary

Kroger, H., Klewer, M., Gratz, R., Dietrich, A., Ockenfels, H. and Miesel, R. Exacerbation of acetaminophen hepatotoxicity by thalidomide and protection by nicotinic acid amide. Gen. Pharmacol. **26** (1995) 1243–1247.

The effects of racemic thalidomide (D[+]/L[−] alpha-phthalimido-glutarimide) on acetaminophen (AAP)-induced hepatitis were tested in male NMRI mice (N = 133) and quantified as serum activities of glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT). A 2.1-fold increase of GOT and a 1.9-fold increase of GPT activities ($p < 0.001$) were observed in mice treated perorally with 500 mg/kg of AAP plus 150 mg/kg of thalidomide (Thal). In the absence of AAP, Thal did not display any detectable hepatotoxic effects. The Thal-induced exacerbation of AAP hepatotoxicity was completely inhibited by nicotinic acid amide, a selective inhibitor of poly(ADP-ribose) polymerase (PARP) ($p < 0.0001$), suggesting a possible influence of Thal on the hepatic metabolism of NAD-adenoribosylation. We see the main application of nicotinic acid amide as for the combinational use in pharmaceutical preparations of AAP in order to avoid hepatic damage in patients treated with AAP and Thal.—Author's Summary

Leitch, A. G., Rubilar, M., Watt, B., Laing, R., Willcocks, L., Brettle, R. P. and Leen, C. L. S. Why disease due to *Mycobacterium tuberculosis* is less common than expected in HIV-positive patients in Edinburgh. Respir. Med. **89** (1995) 495–497.

By December 1993, only five cases of tuberculosis were observed in the 1030 HIV-positive patients in Edinburgh, U.K., although, on the basis of historical tuberculin skin-test data, between four and eight new cases of tuberculosis were expected per year. Of 310 HIV-positive patients, none of the 19 (6.1%) who were tuberculin skin-test positive had developed tuberculosis after 87 months (average) of follow up. It is suggested that new or re-infection is a more common cause of tuberculosis in HIV-positive patients than reactivation. Restriction fragment length polymorphism typing of *Mycobacterium tuberculosis* strains could confirm this hypothesis and support currently suggested additional infection control procedures.—Author's Summary

Manders, S. M., Kostman, J. R., Mendez, L. and Russin, V. L. Thalidomide-resistant HIV-associated aphthae successfully treated with granulocyte colony-stimulating factor. J. Am. Acad. Dermatol. **33** (1995) 380–382.

Thalidomide has been advocated as the treatment of choice for recalcitrant aphthae.

We describe the case of patient with HIV infection and extensive aphthae whose condition failed to respond to corticosteroids, cyclosporine, and thalidomide. The patient's course was complicated by colonic aphthae. Rapid and sustained resolution was achieved through treatment with granulocyte colony-stimulating factor, a previously unreported therapeutic option.—Author's Summary

Meister, G. E., Roberts, C. G. P., Berzofsky, J. A. and Degroot, A. S. Two novel T cell epitope prediction algorithms based on MHC-binding motifs: comparison of predicted and published epitopes from *Mycobacterium tuberculosis* and HIV protein sequences. Vaccine **13** (1995) 581–591.

We have designed two computer-based algorithms for T-cell epitope prediction, OptiMer and EpiMer, which incorporate current knowledge of MHC-binding motifs. OptiMer locates amphipathic segments of protein antigens with a high density of MHC-binding motifs. EpiMer identifies peptides with a high density of MHC-binding motifs alone. These algorithms exploit the striking tendency for MHC-binding motifs to cluster within short segments of each protein. Putative epitopes predicted by these algorithms contain motifs corresponding to many different MHC alleles, and may contain both class I and class II motifs, features thought to be ideal for the peptide components of synthetic subunit vaccines. In this study, we describe the use of OptiMer and EpiMer for the prediction of putative T-cell epitopes from *Mycobacterium tuberculosis* and human immunodeficiency virus protein antigens, and demonstrate that these two algorithms may provide sensitive and efficient means for the prediction of promiscuous T-cell epitopes that may be critical to the development of vaccines against these and other pathogens.—Author's Summary

Mor, N., Simon, B., Mezo, N. and Heifets, L. Comparison of activities of rifapentine and rifampin against *Mycobacterium tuberculosis* residing in human macrophages. Antimicrob. Agents Chemother. **39** (1995) 2073–2077.

The activities of rifapentine and rifampin against *Mycobacterium tuberculosis* residing in human monocyte-derived macrophages were determined. The MICs and MBCs of rifapentine for intracellular bacteria were two-/to fourfold lower than those of rifampin. For extracellular bacteria, this difference was less noticeable. Nevertheless, the more favorable pharmacokinetics of rifapentine over rifampin was addressed in other experimental models. These models showed substantial differences after short pulsed exposures of the infected macrophages to the drugs and when the infected macrophages were exposed to changing drug concentrations that imitated the pharmacokinetic curves observed in blood. Once-a-week exposures to rifapentine concentrations equivalent to those attained in blood after one 600-mg dose resulted during the first week in a dramatic decline in the number of bacteria, and this decline was maintained at a minimal level for a period of 4 weeks. The results of this study have shown the suitability of rifapentine for intermittent-treatment regimens. The prolonged effect of rifapentine found in this study may be associated with high ratios of intracellular accumulation, which were four-/to fivefold higher than those found for rifampin. Further studies on the intracellular distribution of rifamycins and on the sites of actual interaction between the drugs and bacteria residing in macrophages are necessary.—Author's Summary

Ortalo-Magne, A., Dupont, M.-A., Lemassu, A., Andersen, A. B., Gounon, P. and Daffe, M. Molecular composition of the outermost capsular material of the tubercle bacillus. Microbiology **141** (1995) 1609–1620.

To gain insight into the pathogenesis of tuberculosis, a molecular definition of the tubercle bacillus cell envelope, which is involved in the early stages of the infection, is required. The cell-surface-exposed material of the pathogen was isolated by mechanical means and chemically analyzed. It was shown by scanning electron microscopy that the method used for extracting the surface-covering material preserves the integrity of the bacilli. Surprisingly, in view of the current opinion, only small amounts of

lipids (1%–6%) were present. Polysaccharides and proteins were the main components of the material. The polysaccharides were neutral and lipid-free D-glucan, D-arabino-D-mannan and D-mannan, which were eluted from gel-filtration columns in positions corresponding to molecular masses of 120, 13 and 4 kDa, respectively. Based on NMR spectroscopy and conventional chemical analyses, the major structural motifs of the purified polysaccharides were established as being identical to those of the polysaccharides we previously isolated from the culture filtrate of the tubercle bacillus. Immunocytochemical studies showed that these compounds were not only surface-located but were also present in the inner capsular compartment. The major protein constituents exhibited the same mobilities on SDS-PAGE as those of the culture filtrate of the tubercle bacillus and readily reacted with the monoclonal antibodies directed against these molecules. These proteins included the 19 and 38 kDa lipoproteins, the 30/31 kDa fibronectin-binding proteins and the 40 kDa L-alanine dehydrogenase. These findings suggest that the culture filtrate material represents part of the capsule which, in an *in vivo* context, could contribute to the electron transparent zone surrounding the tubercle bacillus. The 24 kDa (MPB/T64) protein was found to be a secreted protein, as it was detected almost exclusively in the culture filtrate. Taken together, the data give a new insight into the surface-exposed compounds of the tubercle bacillus and may explain part of the nature and limitation of the host immunity toward the pathogen.—Author's Abstract

Prabhakaran, K., Harris, E. B., Randhawa, B. and Hastings, R. C. β-Lactamase activity in mycobacteria including *Mycobacterium avium* and suppression of their growth by a β-lactamase-stable antibiotic. Microbios **81** (1995) 177–185.

It is widely assumed that *Mycobacterium avium* strains do not contain the enzyme, but earlier assays were done using insensitive methods. Thus the beta-lactamase activity in cell-free extracts of ten selected strains of mycobacteria, including four strains of *M. avium,* was determined using a highly sensitive spectrophotometric method. The results showed that all of the mycobacteria tested possess the enzyme, which explains their resistance to beta-lactam antibiotics. However, some of the bacteria differed from others in the action of the inhibitors, clavulanate, sulbactam and tazobactam against their beta-lactamases. Growth of the mycobacteria was suppressed by novel combinations of the beta-lactam/beta-lactamase-inhibitors, and by a new beta-lactamase-stable cephalosporin, Cefepime® (aminothiazolyl methoxyimino cephalosporin). The results presented, as well as reports of previous studies *in vivo,* suggest that the intracellular growth of the bacilli or the high partition coefficient of a beta-lactamase inhibitor such as sulbactam does not impede the antimycobacterial action of these compounds.—Author's Abstract

Raja, A., Narayanan, P. R., Mathew, R. and Prabhakar, R. Characterization of mycobacterial antigens and antibodies in circulating immune complexes from pulmonary tuberculosis. J. Lab. Clin. Med. **125** (1995) 581–587.

Circulating immune complexes (CICs) in serum samples from patients with pulmonary tuberculosis (bacteriologically positive [S+C+] and bacteriologically negative [S−C−]) and controls (NHC) have been measured by using Clq binding assay (ClqBA) and 3.5% polyethylene glycol precipitation and measurement of absorbance at 280 nm (PEG-OD 280). Although ClqBA did not show any difference between tuberculous and normal serum samples, PEG-OD 280 was significantly elevated in tuberculous samples. The effect of chemotherapy on CIC levels was studied. During the treatment, initially (for up to 2 months) there was a rise in CIC levels and later a fall, coinciding with bacterial clearance. Anti-purified protein derivative antibodies of class immunoglobulin G (IgG) and immunoglobulin M were measured in the serum samples and PEG precipitates. Antimycobacterial antibody measurement in CICs was more discriminatory between the groups than serum antibody. For characterization of the complexed antibody, the PEG precipitates were used in the Western blot and the patterns were compared. S+C+

CICs contained antibodies for a wide range of antigens ranging from 100 kDa to 10 kDa. However, none of the NHC-CICs contained antibodies for antigens <70 kDa. Thus, when using the criterion of positivity for antibodies to antigens <70 kDa as a marker for pulmonary tuberculosis, 24 of 24 (100%) of the S+C+ CICs were positive. Similarly, 11 of 16 (70%) of the S−C− CICs contained antibodies for antigens <70 kDa. The results are promising that measurement of complexed IgG for mycobacterial antigens of molecular weight <70 kDa might prove to be useful in accurately discriminating between the tuberculous patients and endemic normal subjects (100% sensitivity and 100% specificity). Moreover, the test can also be very useful in borderline positive (smear-negative) cases for which group diagnosis is very difficult. Such a test will be extremely useful in extrapulmonary and childhood tuberculosis where early diagnosis is needed.—Author's Abstract

Renau, T. E., Sanchez, J. P., Shapiro, M. A., Dever, J. A., Gracheck, S. J. and Domagala, J. M. Effect of lipophilicity at N-1 on activity of fluoroquinolones against mycobacteria. J. Med. Chem. **38** (1995) 2974–2977.

The dramatic increase in drug-resistant *Mycobacterium tuberculosis* has caused a resurgence in research targeted toward these organisms. As part of a systematic study to optimize the quinolone antibacterials against mycobacteria, we have prepared a series of N-1-phenylsubstituted derivatives to explore the effect of increasing lipophilicity on potency at this position. The compounds, synthesized by the modification of a literature procedure, were evaluated for activity against gram-negative and gram-positive bacteria, *M. fortuitum* and *M. smegmatis,* and the results correlated with log P, pK_a, and other attributes. The activity of the compounds against the rapidly growing, less hazardous organism *M. fortuitum* was used as a measure of *M. tuberculosis* activity. The results demonstrate that increasing lipophilic character by itself does not correlate with increased potency against mycobacteria. Rather, intrinsic activity against gram-negative and/or gram-positive bacteria is the governing factor for corresponding activity against mycobacteria.—Author's Abstract

Roberts, A. D., Sonnenberg, M. G., Ordway, D. J., Furney, S. K., Brennan, P. J., Belisle, J. T. and Orme, I. M. Characteristics of protective immunity engendered by vaccination of mice with purified culture filtrate protein antigens of *Mycobacterium tuberculosis.* Immunology **85** (1995) 502–508.

In this study highly purified culture filtrate proteins obtained from *Mycobacterium tuberculosis* strains Erdman and H37Rv were tested for their capacity to stimulate immune T cells *in vitro,* and to immunize mice *in vivo.* Analysis of the culture filtrate antigen pool revealed a complex mixture of proteins; after separation of this pool into fractions of defined molecular size using an electrophoretic method, it was found that multiple fractions strongly stimulated interferon-gamma (IFN-γ) secretion by immune CD4 T cells *in vitro.* In a further series of experiments mice were given multiple immunizations with the culture filtrate protein pool suspended in emulsions of incomplete Freund's adjuvant. Such mice were as resistant as mice given live bacillus Calmette-Guerin (BCG) vaccine to a low dose aerosol challenge infection with *M. tuberculosis,* but this resistance waned to low levels by 5 months post-vaccination. Furthermore, experiments using the filtrate antigens to boost or augment immunity induced by the BCG vaccination itself were unsuccessful. These data therefore support the hypothesis that the culture filtrate proteins of *M. tuberculosis* contain multiple antigens that are strongly recognized by T cells acquired during the initial expression of protective immunity to tuberculosis. Conventional immunization with these purified protein antigens can engender a strong degree of protective immunity, but this immunity is apparently not sustained at the same level as that induced by the live vaccine, perhaps suggesting a lack of suitable stimulation of memory immunity.—Author's Summary

Ryan, C., Nguyen, B.-T. and Sullivan, S. J. Rapid assay for mycobacterial growth and antibiotic susceptibility using gel micro-

drop encapsulation. J. Clin. Microbiol. **33** (1995) 1720–1726.

Effective control of tuberculosis transmission in vulnerable population groups is dependent on rapid identification of the infectious agent and its drug susceptibility. However, the slow growth rate of mycobacteria has undermined the ability to quickly identify antimicrobial resistance. These studies describe a mycobacterial growth assay based on microencapsulation technology used in conjunction with flow cytometric analysis. Mycobacteria were encapsulated in agarose gel microdrops approximately 25 μm in diameter, and colony growth was monitored by using flow cytometry to evaluate the intensity of auramine staining after culture for various times at 37°C. By this method, colony growth of *Mycobacterium bovis* and *M. smegmatis* could be quantified within 1 to 3 days after encapsulation. Inhibition of growth by rifampin and isoniazid was also evaluated in this time period, and the presence of an isoniazid-resistant subpopulation representing 3% of the total microorganisms could be detected. This use of encapsulation and flow cytometry has the potential to facilitate rapid and automated evaluation of inhibition of growth by antimicrobial agents and shorten the time frame for analysis of clinical specimens.—Author's Summary

Smithwick, R. W., Bigbie, M. R., Jr., Ferguson, R. B., Karlix, M. A. and Wallis, C. K. Phenolic acridine orange fluorescent stain for mycobacteria. J. Clin. Microbiol. **33** (1995) 2763–2764.

A new fluorescence acid-fast staining method with acridine orange as the specific stain is presented. Only two reagents are required: the acridine orange-specific stain and a destaining-counterstaining reagent. Compared with auramine fluorescence acid-fast staining, there was less nonspecific staining of non-acid-fast debris which fluoresced a pale green contrasting color to provide a background in which to search for the red-to-orange fluorescing acid-fast bacilli. The results of the study indicate that the acridine orange method is superior to the auramine method in detecting acid-fast bacilli in specimen smears.—Author's Abstract

Stokes, R. W. and Speert, D. P. Lipoarabinomannan inhibits nonopsonic binding of *Mycobacterium tuberculosis* to murine macrophages. J. Immunol. **155** (1995) 1361–1369.

The initial phagocytic interaction between *Mycobacterium tuberculosis* and macrophages in the lung is probably nonopsonic, which would mean that macrophage receptors will bind directly to bacterial ligands without the involvement of serum opsonins. Lipoarabinomannan (LAM) is a major component of the cell wall of mycobacteria. The possibility that LAM is involved in the nonopsonic binding of *M. tuberculosis* to macrophages was investigated by using competitive inhibition assays. LAM inhibited binding of *M. tuberculosis* to murine peritoneal macrophages in a dose-dependent manner. LAM also inhibited the binding of *M. avium* and *M. bovis* BCG to macrophages. Phosphatidyl inositol mannoside and lipomannan have the same phosphatidyl inositol (PI) moiety as LAM, but differ in their glycosylation patterns. Both molecules inhibited binding of *M. tuberculosis* to macrophages. Deacylation of LAM abrogated its capacity to inhibit binding of *M. tuberculosis* to macrophages. These observations indicated that it was the PI moiety of LAM that was important in mediating its inhibitory properties. In support of this hypothesis, commercial PI was shown to inhibit the binding of *M. tuberculosis* to macrophages. Our results suggest that cell-free LAM is able to inhibit the binding of mycobacterial to macrophages, but that it does not do so by competing with any interaction between macrophage receptors and cell-associated LAM because the PI end of the molecule is believed to be anchored in the bacterial plasma membrane and, therefore, not available as a ligand on the cell surface. However, LAM that is released from *M. tuberculosis* in the course of its active replication during infection may be able to interfere with the phagocytic clearance of mycobacteria.—Author's Abstract

Sueoka, E., Nishiwaki, S., Okabe, S., Iida, N., Suganuma, M., Yano, I., Aoki, K. and

Fujiki, H. Activation of protein kinase C by mycobacterial cord factor, trehalose 6-monomycolate, resulting in tumor necrosis factor-alpha release in mouse lung tissues. Jpn. J. Cancer Res. **86** (1995) 749–755.

Cord factors are mycoloyl glycolipids in cell walls of bacteria belonging to *Actinomgcetales,* such as *Mycobacterium, Nocardia* and *Rhodococcus.* They induce granuloma formation in the lung and interstitial pneumonitis, associated with production of macrophage-derived cytokines. We studied how cord factors induce biological activities in the cells. Cord factors isolated from *M. tuberculosis,* trehalose 6-monomycolate (mTMM) and trehalose 6, 6/-dimycolate (mTDM), enhanced protein kinase C (PKC) activation in the presence of phosphatidylserine (PtdSer), diacylglycerol and Ca2+, and mTMM activated PKC alpha more strongly than PKC beta or gamma under the same assay conditions. Kinetic studies of mTMM in response to PKC activation revealed that mTMM increased the apparent affinity of PKC to Ca2+ in the presence of both PtdSer and diolein. Although this is similar to observations with unsaturated fatty acids, such as arachidonic acid, mTMM was synergistic with PtdSer for PKC activation, but arachidonic acid was not. mTMM was also different as regards PKC activation, as phorbol ester was. A single i.p. administration of mTMM to mouse induced tumor necrosis factor-alpha (TNF-α) in serum and in the lung, which is a unique target tissue of cord factors. Based on our recent finding that TNF-α is an endogenous tumor promoter, the correlation between lung cancer and pulmonary tuberculosis is discussed.—Author's Abstract

Sullivan, E. A., Kreiswirth, B. N., Palumbo, L., Kapur, V., Musser, J. M., Ebrahimzadeh, A. and Frieden, T. R. Emergence of fluoroquinolone-resistant tuberculosis in New York City. Lancet **345** (1995) 1148–1150.

Twenty-two patients infected with fluoroquinolone-resistant *Mycobacterium tuberculosis* in New York City were identified between January 1991 and November 1993. In 16 patients resistance arose as a result of inadequate or inappropriate treatment; 6 patients had primary infection with fluoroquinolone-resistant organisms; 5 acquired the organisms nosocomially. Seven distinct patterns of restriction-fragment length polymorphism were identified in isolates from 21 patients. Fluoroquinolones should be restricted to patients with multidrug-resistant disease or intolerance to other antituberculosis drugs. All patients with multidrug-resistant tuberculosis should be on directly observed therapy.—Author's Abstract

Torrea, G., Levee, G., Grimont, P., Martin, C., Chanteau, S. and Gicquel, B. Chromosomal DNA fingerprinting analysis using the insertion sequence IS6110 and the repetitive element DR as strain-specific markers for epidemiological study of tuberculosis in French Polynesia. J. Clin. Microbiol. **33** (1995) 1899–1904.

The polymorphism of *Mycobacterium tuberculosis* strains was evaluated in French Polynesia, an area with a low incidence of tuberculosis and a population which has been geographically stable during recent decades. Nonrepetitive strains isolated from 64 patients during 1991 and 1992 were subjected to DNA restriction fragment length polymorphism (RFLP) analysis, using the insertion sequence IS6110 and the repetitive element DR as probes. Thirty-eight different IS6110 RFLP types were identified. They could be clustered in 11 groups. All members of each group are identical or differ by one to three bands. All the other strains are gathered in the miscellaneous group. In some cases, transmission of strains with identical RFLP types between patients of the same family or between patients living in the same area was identified. Strains exhibiting similar IS6110 RFLP types also exhibited identical DR RFLP patterns, confirming that strains with similar types were genetically linked. Strains belonging to two different IS6110 clusters exhibited the same DR RFLP type. These data may also indicate a common origin for these strains and evolution to new IS6110 types. The results obtained in this study suggest that not only reactivation of latent tuberculous infections but also active transmissions are still oc-

curring in French Polynesia.—Author's Abstract

Tramontana, J. M., Utaipat, U., Molloy, A., Akarasewi, P., Burroughs, M., Makonkawkeyoon, S., Johnson, B., Klausner, J. D., Rom, W. and Kaplan, G. Thalidomide treatment reduces tumor necrosis factor-alpha production and enhances weight gain in patients with pulmonary tuberculosis. Mol. Med. **1** (1995) 384–397.

Background: The monocyte-derived cytokine, tumor necrosis factor alpha (TNF-α), is essential for host immunity, but overproduction of this cytokine may have serious pathologic consequences. Excess TNF-α produced in pulmonary tuberculosis may cause fevers, weakness, night sweats, necrosis, and progressive weight loss. Thalidomide (alpha-N-phthalimidoglutarimide) has recently been shown to suppress TNF-α production by human monocytes *in vitro* and to reduce serum TNF-α in leprosy patients. We have therefore conducted a two-part placebo-controlled pilot study of thalidomide in patients with active tuberculosis to determine its effects on clinical response, immune reactivity, TNF-α levels, and weight.

Materials and Methods: 30 male patients with active tuberculosis, either human immunodeficiency virus type 1 positive (HIV-1 +) or HIV-1 −, received thalidomide or placebo for single or multiple 14 day cycles. Toxicity of the study drug, delayed-type hypersensitivity (DTH), cytokine production, and weight gain were evaluated.

Results: Thalidomide treatment was well tolerated, without serious adverse events. The drug did not adversely affect the DTH response to purified protein derivative (PPD), total leukocyte, or differential cell counts. TNF-α production was significantly reduced during thalidomide treatment while interferon-gamma production was enhanced. Daily administration of thalidomide resulted in a significant enhancement of weight gain.

Conclusions: The results indicate that thalidomide is well tolerated by patients receiving antituberculosis therapy. Thalidomide treatment reduces TNF-α production both *in vivo* and *in vitro* and is associated with an accelerated weight gain during the study period.—Author's Abstract

Utrup, L. J., Moore, T. D., Actor, P. and Poupard, J. A. Susceptibilities of nontuberculosis mycobacterial species to amoxicillin-clavulanic acid alone and in combination with antimycobacterial agents. Antimicrob. Agents Chemother. **39** (1995) 1454–1457.

Neither amoxicillin nor clavulanic acid used alone was active at the highest level tested, i.e., 256.0 μg/ml, *in vitro* against 24 isolates of *Mycobacterium fortuitum, M. kansasii,* and *M. marinum.* However, the MIC of an amoxicillin-clavulanic acid combination of 2:1 was $\leq$8.0/4.0 μg/ml for 50% of the isolates tested, with all isolates being inhibited in the range of 4.0/2.0 to 32.0/16.0 μg/ml, respectively. Titration of amoxicillin-clavulanic acid with a fixed 2 μg/ml concentration of ethambutol resulted in synergistic activity against 3 of 9 isolates of *M. fortuitum,* 10 of 10 isolates of *M. kansasii,* and 5 of 5 isolates of *M. marinum.* This observation was confirmed in a checkerboard analysis in which fractional inhibitory concentrations were $\leq$0.5 for 20 of the 24 isolates. Synergistic activity was observed against the other four isolates in one of two trials. On the other hand, titration of amoxicillin-clavulanic acid in the presence of either one or two fixed concentrations of isoniazid, rifampin, cycloserine, tetracycline, or amikacin failed to result in synergism.—Author's Abstract

Veasey, R. S., Taylor, H. W., Horohov, D. W., Krahenbuhl, J. L., Oliver, J. L. and Snider, T. G. Histopathology of C57BL/6 mice inoculated orally with *Mycobacterium paratuberculosis.* J. Compar. Pathol. **113** (1995) 75–80.

The susceptibility of C57BL/6 mice to oral inoculation with *Mycobacterium paratuberculosis* was evaluated histopathologically. Granulomatous lesions containing acid-fast bacteria developed in the mesenteric lymph nodes in over 50% of the mice by 11 months after inoculation. The results suggest that C57BL/6 mice may be useful for studying infection, pathogenesis, and

other aspects of paratuberculosis.—Author's Abstract

Wadee, A. A., Kuschke, R. H. and Dooms, T. G. The inhibitory effects of *Mycobacterium tuberculosis* on MHC class II expression of monocytes activated with riminophenazines and phagocyte stimulants. Clin. Exp. Immunol. **100** (1995) 434–439.

The expression of MHC class II antigens by peripheral blood monocytes from normal individuals was investigated. Class II expression as determined by a cell ELISA was effectively induced by various phagocyte stimulants. A further aspect of our study investigated the effects of clofazimine, a riminophenazine antimicrobial agent and its analogue, B669, on class II expression. Both agents at concentrations attainable *in vivo* increased the expression of MHC class II antigens. A 25-kDa glycolipoprotein derived from *Mycobacterium tuberculosis* that inhibits phagocyte functions has previously been described. This component significantly reduced the expression of MHC class II antigens induced by the riminophenazines, clofazimine and B669, interferon-gamma or opsonised yeast when added at the initiation of experiments. The riminophenazines could not restore the decrease in class II antigen expression previously inhibited by the 25-kDa mycobacterial fraction. However, cultures prestimulated with the riminophenazines or phagocyte stimulants were unaffected by the 25-kDa mycobacterial fraction. The results suggest the potential use of these agents as modulators of phagocyte function.—Author's Abstract

Yajko, D. M., Chin, D. P., Gonzalez, P. C., Nassos, P. S., Hopewell, P. C., Reingold, A. L., Horsburgh, C. R., Yakrus, M. A. and Ostroff, S. M. *Mycobacterium avium* complex in water, food, and soil samples collected from the environment of HIV-infected individuals. J. AIDS Hum. Retrovirol. **9** (1995) 176–182.

As part of an epidemiologic study of *Mycobacterium avium* complex (MAC) infection in San Francisco, water, food and soil samples were collected from the home environment of 290 persons with human immunodeficiency virus (HIV) infection and cultured for mycobacteria. Isolates recovered from the environment were compared with isolates cultured from study patients. Although mycobacteria were recovered from numerous environmental samples, isolates reactive with MAC-specific DNA probes were recovered from only four of 528 (0.76%) water samples and one of 397 (0.25%) food samples. The species *M. avium* was recovered from one water (0.19%) and one food sample. In contrast, MAC was recovered from 55% and *M. avium* from 27% of soil samples taken from potted plants in patients' homes. Speciation of 76 MAC isolates from study patients showed all isolates belonged to the species *M. avium*. With use of serotype and multilocus enzyme electrophoresis analysis, some of the soil isolates were found to be similar to isolates recovered from study patients. The results of this study suggest that soil, rather than water, may be a significant reservoir of organisms causing MAC infection in San Francisco.—Author's Abstract

Yajko, D. M., Madej, J. J., Lancaster, M. V., Sanders, C. A., Cawthon, V. L., Gee, B., Babst, A. and Hadley, W. K. Colorimetric method for determing MICs of antimicrobial agents for *Mycobacterium tuberculosis*. J. Clin. Microbiol. **33** (1995) 2324–2327.

A colorimetric method for quantitative measurement of the susceptibility of *Mycobacterium tuberculosis* to antimicrobial agents is described. The method utilizes an oxidation-reduction dye, Alamar blue, as an indicator of growth. By this method, MICs of isoniazid, rifampin, streptomycin, and ethambutol were determined for 50 strains of *M. tuberculosis*. Colorimetric MIC results were available on the 7th, 10th, or 14th day of incubation for 29 (58%), 14 (28%), and 7 (14%) of the 50 strains, respectively. When MIC susceptibility results were compared with results obtained by the agar proportion method, increased levels of resistance detected by agar proportion were associated with higher MICs obtained by the colorimetric method. Tentative interpretive criteria for colorimetric MIC results which showed good agreement with results obtained by the agar proportion method were established. Interpretive agreement be-

tween the two methods was 98% for isoniazid, rifampin, and ethambutol and 94% for streptomycin. Overall, there was agreement between the two methods for 194 of 200 test results (97%). The colorimetric method is a rapid, quantitative, nonradiometric method for determining the antimicrobial susceptibility of *M. tuberculosis.*—Author's Abstract

Yamomoto, S., Toida, I., Watanabe, N. and Ura, T. *In vitro* antimycobacterial activities of pyrazinamide analogs. Antimicrob. Agents Chemother. **39** (1995) 2088–2091.

We synthesized various pyrazine derivatives and pyrazinamide analogs and assayed their antimycobacterial activities *in vitro* in order to find new drugs which are more active against *Mycobacterium tuberculosis* than pyrazinamide and also active against *M. avium* and *M. intracellulare.* Of the drugs synthesized, four drugs, namely, pyrazine thiocarboxamide, *N*-hydroxymethyl pyrazine thiocarboxamide, pyrazinoic acid *n*-octyl ester, and pyrazinoic acid pivaloyloxymethyl ester, were not only bacteriostatic but also bacteriocidal against these three species of mycobacteria *in vitro* under conditions in which pyrazinamide showed no or little activity. In conclusion, these four drugs are possible candidates for new antimycobacterial agents, and animal experiments are now under way.—Author's Abstract

Zhang, Y. H., Nakata, K., Weiden, M. and Rom, W. N. *Mycobacterium tuberculosis* enhances human immunodeficiency virus-1 replication of transcriptional activation at the long terminal repeat. J. Clin. Invest. **95** (1995) 2324–2331.

Tuberculosis has emerged as an epidemic fueled by the large number of individuals infected with the human immunodeficiency virus, especially those who are injecting drug users. We found a striking increase from 4- to 208-fold in p24 levels in bronchoalveolar lavage fluid from involved sites of *Mycobacterium tuberculosis* infection vs uninvolved sites in three HIV+ patients. We used an *in vitro* cell culture model to determine if tuberculosis could activate replication of HIV-1. Mononuclear phagocyte cell lines U937 and THP-1 infected with HIV-1 (JR-CSF) *in vitro* and stimulated with live *M. tuberculosis.* H37Ra had a threefold increase in p24 in culture supernatants. Using the HIV-1 long terminal repeat with a chloramphenicol acetyltransferase (CAT) reporter construct, live *M. tuberculosis* increased transcription 20-fold in THP-1 cells, and cell wall components stimulated CAT expression to a lesser extent. The nuclear factor-kappa B enhancer element was responsible for the majority of the increased CAT activity although two upstream nuclear factor-IL6 sites may also contribute to enhanced transcription. Antibodies to TNF-α and IL-1 inhibited the increase in CAT activity of the HIV-1 long terminal repeat by *M. tuberculosis* from 21-fold to 8-fold. Stimulation of HIV-1 replication by *M. tuberculosis* may exacerbate dysfunction of the host immune response in dually infected individuals.—Author's Abstract