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CURRENT LITERATURE

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

General and Historical

Andrade, V. G. [Urbanization of the treatment for hanseniasis.] Hansen. Int. **21** (1996) 51–59. (in Portuguese)

In Brazil, the leprosy control program is presently showing some loss of quality in the care of patients of urban areas. In this regard, the study of the endemic in these areas is of importance, being of value the integration of all aspects of the control program that have some connection to the understanding of the deterioration of the endemic and its social consequences. The purpose of this paper is to present some suggestions to the formulation of interventions to each condition. Taking into consideration that decentralization of the knowledge of the object to be studied is prerequisite to an adequate structure of the leprosy control program in urban areas, the author discusses the decentralization of the data analysis with improvement of its analysis in the local level to a better monitoring of treatment in view of the alarming number of defaulters which is the main cause of the failure of the leprosy control program; the adoption of a concept of "areas and communities with differential risk" and the concept of prevalence which, in the author's point of view, should consider only patients effectively under treatment and, additionally, that this is the best indicator to the efficiency of leprosy control programs. To the process of urbanization of the treatment the author suggests: at the individual level: to adapt WHO/MDT treatment to fit each particular situation (flexible supervised doses); in the district level: to take maximum advantage of the existing basic health network for diagnosis and treatment with WHO/MDT; to identify nearby referral centers to cope with reactional cases and other ailments; to assure the participation of the general clinicians and other health personnel in the diagnosis and treatment of leprosy cases; to look for resources (knowledge and materials) in order that all new cases are properly treated with WHO/MDT; to guarantee referral for patients with disabilities; to develop an adequate and efficient information system in each area; to develop joint action with the state level; at the state level: to define focal points of coordination; to guarantee resources and drugs for treatment with WHO/MDT of all new cases; to stimulate by mass media the completion of treatment, mainly to MB cases; to guarantee the adequate treatment of disabilities; to support the development of an information system adequate to each specific area of actuation; to guarantee training and continuing education of human resources; to ascertain that the information processed in the central level reach the district level (feedback); to stimulate the delegation of responsibility and the participation of technical personnel in the field level; to support the local level during the transition process offering knowledge and improving practical abilities.—Author's English Abstract

Awofeso, N. Stigma and socio-economic reintegration of leprosy sufferers in Nigeria. Acta Leprol. **10** (1996) 89–91.

Leprosy is the commonest cause of peripheral neuropathy in the world. This feature causes gross deformities of the face and limbs of infected individuals as well as crippling disabilities involving sight, touch and manual dexterity. Such stigmata intensified the social and economic isolation of patients. Although concerted efforts by national governments and international organizations have made leprosy cease to be a medical problem in most parts of the world,

leprosy still remains a "human problem": about 30% of past or present leprosy sufferers in Nigeria are disabled and/or handicapped as a result of the disease. This paper reviews the various factors contributing to leprosy stigma in Nigeria and proposes ways of minimizing it.—Author's Abstract

Gala Leon, F. J., Lupiani Gimenez, M., Bas Sarmiento, P., Paublete Herrera, M. C., Martinez Nieto, J. M., Diaz Rodriguez, M., Guillen Gestoso, R. and Cano Valero, M. [Leprosy versus AIDS: comparison of the attitudes generated by both concepts using a semantic differential.] Rev. Leprol. Fontilles **20** (1996) 1057–1075. (in Spanish)

The semantic differential is used to measure the connotative profile of words and to analyze the attitudes that words evoke. The semantic differential is very useful in health psychology since it allows us to analyze the connotations of words referring to disease.

We have analyzed and contrasted the connotative-attitudinal profile that "leprosy" and "AIDS" evoke. We have used our own semantic differential according to variables such as sex, age, health professionals and non-professionals, university graduates and non-graduates. The sample was formed by 144 subjects of both sexes, between 21 and 60 years, from Cádiz and province.

After analyzing the data we could draw several conclusions: a) AIDS is occupying the negative and ignominious semantic spectrum that leprosy used to have, b) There are moral and negative prejudices towards both concepts, and c) Prevention is unknown or ignored.—Authors' English Summary

Gil Suarez, R. E., Ramirez Fernandez, R., Santin Pena, M. and Lombardi, C. [Leprosy situation on in Cuba. Is it feasible to interrupt transmission?] Hansen. Int. **21** (1996) 34–45. (in Spanish)

The current leprosy epidemiological situation in Cuba is described and analyzed, as well as the results of the control program and the perspective toward further steps of the control, aiming at the interruption of transmission. During the period 1988–1994, it has been observed that since 1993 the leprosy prevalence rate in Cuba is below 1 case per 10,000, although it seems that this indicator is tending to stabilize in recent years. The detection data studied during the 1978–1998 period show a decreasing trend, leading us to think about the possibility of a transmission decline, but according to a very slow pattern. The operational aspects presented reveal a very high coverage (95%) with MDT treatment, besides a good accessibility to health services; nevertheless, around 30% of the new cases are detected later. The data show also that even in the favorable conditions of the Cuban health system leprosy has characteristics which make it difficult to make the diagnosis at a sufficiently early stage to impact transmission. The authors conclude that, in the absence of immunization tools and in order to try to interrupt leprosy transmission, it is necessary to continue studying the incorporation of new technologies which could allow the identification of asymptomatic sources of infection, as well as the deflection of leprosy cases at a very early stage of the disease.—Authors' English Abstract

Chemotherapy

Gallo, M. E. N., Nery, J. A. C. and Garcia, C. C. [Side effects of drugs used in multidrug therapy for hanseniasis.] Hansen. Int. **21** (1996) 46–50. (in Portuguese)

The authors present the clinical side effects produced by drugs used in multidrug therapy scheme standardized by World Health Organization for leprosy. The study encompasses the experience in 8 years with a total of 980 patients, 496 (50%) of which were allocated in the paucibacillary leprosy scheme and 484 (49.3%) in the multibacillary scheme. Side effects were observed which were attributed to the drugs that were used: rifampin, dapsone and clofazimine in 18 (1.8%) of the cases. Among the paucibacillary patients the side effects were observed in 10 (2%) and among the multi-

bacillary patients in 8 (1.6%). In all the cases the standard scheme was adapted thus allowing a continuation of the treatment.—Authors' English Summary

Jing, Z., *et al.* [Effect of MDT on MB case resistant to DDS.] China Lepr. J. **12** (1996) 162–164. (in Chinese)

Thirty-three multibacillary cases of leprosy with secondary or primary resistance to dapsone (DDS) were treated with MDT. In comparison with non-resistant cases, the effect is the same in clinical improvement and for leprosy reaction. After treatment of at least 2 years, all of them have been inactive clinically and negative bacteriologically, and no relapse for 7 ± 2.1 years of follow up. It is interesting that two cases with secondary resistance to DDS have been cured with DDS monotherapy, because they refused to take MDT.—Authors' English Abstract

Li, Y., *et al.* [A comparison between DDS monotherapy and MDT in leprosy.] China Lepr. J. **12** (1996) 186–187. (in Chinese)

Sixty multibacillary (MB) leprosy patients were treated with dapsone (DDS) alone in 1974–1984 and another 60 MB cases with WHO/MDT in 1984 to 1994. Bacteriological negativity has come in the 54th month of DDS monotherapy, but in the 30th month of MDT on the average. In addition, ENL reaction was less in the latter, being 31 to 43 cases.—Authors' English Abstract

Meier, A., Sander, P., Schaper, K.-J., Scholz, M. and Bottger, E. C. Correlation of molecular resistance mechanisms and phenotypic resistance levels in streptomycin-resistant *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **40** (1996) 2452–2454.

Quantitative susceptibility testing of clinical isolates of streptomycin-resistant *Mycobacterium tuberculosis* demonstrated that there is a close correlation between the molecular resistance mechanism and the *in vitro* activity of streptomycin: mutations in *rpsL* were mainly associated with high-level resistance, mutations in *rrs* were associated with an intermediate level of resistance, and streptomycin-resistant isolates with wild-type *rpsL* and *rrs* exhibited a low-level resistance phenotype. Investigations of streptomycin-resistant isolates with wild-type *rpsL* and *rrs* revealed that (i) there is no crossresistance to other drugs and (ii) a permeability barrier may contribute to resistance, because resistance was significantly lowered in the presence of a membrane-active agent.—Authors' Summary

Moghazeh, S. L., Pan, X., Arain, T., Stover, C. K., Musser, J. M. and Kreiswirth, B. N. Comparative antimycobacterial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. Antimicrob. Agents Chemother. **40** (1996) 2655–2657.

A collection of 24 rifampin-resistant clinical isolates of *Mycobacterium tuberculosis* with characterized RNA polymerase β-subunit (*rpoB*) gene mutations was tested against the antimycobacterial agents rifampin, rifapentine, and KRM-1648 to correlate levels of resistance with specific *rpoB* genotypes. The results indicate that KRM-1648 is more active *in vitro* than rifampin and rifapentine, and its ability to overcome rifampin resistance in strains with four different genetic alterations may prove to be useful in understanding structure-function relationships.—Authors' Abstract

Selwyn, G. J., Jayakumar, A. and Samson, P. D. Thrombocytopenic purpura with blood blisters on once a month rifampicin. Indian J. Lepr. **68** (1996) 371–372.

Toxicity of rifampin depends both on the dosage and on the interval between the doses. Toxic effects with monthly doses of rifampin have been rare. In view of this, we report here a case of rifampin-induced thrombocytopenic purpura with blood blisters (hemorrhagic bullae) in a young male

who was taking rifampin 600 mg once a month as supervised dose.—From the Article

Shah, L. M., DeStefano, M. S. and Cynamon, M. H. Enhanced *in vitro* activity of WR99210 in combination with dapsone against *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **40** (1996) 2644–2645.

WK99210, a dihydrofolate reductase inhibitor, has promising *in vitro* activity against *Mycobacterium avium* complex (MAC). The *in vitro* activities of WR99210 alone and in combination with a fixed concentration of dapsone (0.5 μg/ml) were evaluated against 35 clinical MAC isolates by a broth dilution method. The MIC at which 50% of isolates were inhibited (MIC_{50}) and MIC_{90} of WR99210 alone were 2 and 8 μg/ml, respectively. The MIC_{50} and MIC_{90} of WR99210 in combination with dapsone were 0.25 and 4 μg/ml, respectively. Overall, 75% of the MAC isolates displayed enhanced susceptibility to the combination.—Authors' Abstract

Wnendt, S., Finkam, M., Winter, W., Ossig, J., Raabe, G. and Zwingenberger, K. Enantioselective inhibition of TNF-alpha release by thalidomide and thàlidomide-analogues. Chirality **8** (1996) 390–396.

The question whether the immunomodulating activity of rac-thalidomide resides in either the (–)-(S)- or the (+)-(R)-enantiomer was addressed by synthesis and separation of pure enantiomers of thalidomide-analogs which carry a methyl-group at the asymmetric carbon atom and are thus prevented from racemization. The effect of the pure enantiomers of the thalidomide-analogs and also of the enantiomers of thalidomide on release of TNF-alpha was tested *in vitro* by using stimulated peripheral mononuclear blood cells. Both enantiomers of thalidomide inhibited the release of TNF-alpha equally well at low concentrations (5 and 12.5 μg/ml) but at higher concentrations (25 and 50 μg/ml) there was a weak but statistically significant selectivity towards the (–)-(S)-enantiomer. In the case of the configuration-stable thalidomide-analogs there was a very pronounced and statistically significant enantioselectivity toward the (S)-form even at lower concentrations (greater than or equal to 5 μg/ml). The (S)-enantiomers of the thalidomide-analogs differed in their inhibitory potency from (–)-(S)-thalidomide, suggesting that the introduction of the methyl-group increases the TNF-alpha-inhibitory activity while the reduction of one of the carbonyl-functions in the glutarimide moiety to a methylene-group decreases activity. The effect of these small molecular alterations on activity and the enantioselectivity toward the (S)-enantiomers may indicate that thalidomide and its analogs directly interact with one or several cellular target-proteins.—Authors' Abstract

Yu, J., *et al.* [Effects of MDT regimens with different doses of RMP in leprosy.] China Lepr. J. **12** (1996) 180–181. (in Chinese)

Ninety cases of MB leprosy had taken a regimen containing various doses of rifampin (RMP) for 6 to 14 months, and the decrease in BI was by 0.6 and 1.6 at the sixth and fourteenth month, respectively, and faster than that among those taking dapsone (DDS) monotherapy ($p < 0.01$). Clinical improvement was 83%. The doses of RMP were 450 to 600 mg a day, 1200 to 1500 mg a week and 600 mg a month, respectively, adding DDS 100 mg a day or DDS 100 mg plus prothionamide (PTH) 300 to 500 mg a day. The regimen containing PTH caused hepatic damage in 30%, including one case of hepatodystrophy who died.—Authors' English Abstract

Clinical Sciences

Andrade, V., Moreira Alves, T., Regazzi Avelleira, J. C. and Bayona, M. Prevalence of HIV1 in leprosy patients in Rio de Janeiro, Brazil. Hansen. Int. **21** (1996) 26–33.

The purpose of this study was to learn if HIV1 infection was associated with leprosy in South America (Rio de Janeiro, Brazil) by comparing the prevalence rates of 1016 leprosy patients tested on a voluntary basis

and 78,482 blood donors. A cross-sectional survey of anti-HIV1 antibodies was conducted in Rio de Janeiro from 1990 to 1992 for this purpose. HIV1 prevalence found among leprosy patients was (three cases) 2.9 per 1000, and among blood donors was (282 cases) 3.8 per 1000. Such difference was not significant (OR = 0.79, p = 0.69). Since HIV1 cases were only found among male leprosy patients, further analysis excluded females. Male leprosy patients showed a slightly higher prevalence of HIV1 than blood donors before and after age adjustment. However, this result was not statistically significant (adjusted odds ratio = 1.38, 95% CI 0.35–4.5, p = 0.83). These data do not provide evidence that leprosy and HIV1 infection are associated in the state of Rio de Janeiro. This is consistent with similar investigations conducted elsewhere.—Authors' Abstract

de Freitas, T. and Fleury, R. N. Hematologic profile of leprosy patients in reactional episode of erythema nodosum leprosum. Hansen. Int. **21** (1996) 58–66.

In this work, 18 patients in a reactional episode of erythema nodosum leprosum (ENL) had their hematologic parameters evaluated. Eight of these patients had intense and moderate ENL, and 10 patients had mild ENL. The results showed that patients with intense and moderate ENL had significant alterations in the hematology findings: iron deficiency, an increased euglobulin lysis time and hyperfibrinogenemia. Nevertheless, no alteration was observed in the other parameters studied (prothrombin time, Heinz body and fibrinogen degradation products), in ENL.—Authors' Abstract

de Oliveira, M. L. W., Gomes, M. K., Pimental, M. I. F. and Castro, M. C. R. [Macular reversal reaction after MB MDT.] Hansen. Int. **21** (1996) 46–51. (in Portuguese)

This report shows three cases of multibacillary (MB) leprosy, who presented hypochromic macules, by 6 months after treatment release from multidrug therapy for MB patients. Based on bacteriological and histopathological exams, and on good clinical resolution, with or without steroid therapy, they received a diagnosis of atypical macular reversal reaction. We discuss this point of view, opposing it to the hypothesis of relapse of MB form as PB form. We emphasize the need for a clear characterization of the reversal reaction lesions, after stopping drug therapy to avoid that they will be re-introduced into the multidrug therapy regimen unnecessarily.—Authors' English Abstract

Faye, O., Mahe, A., Jamet, P., Huerre, M. and Bobin, P. [Anatomopathological study of five cases of leprosy in human immunodeficiency virus (HIV) seropositive patients.] Acta Leprol. **10** (1996) 93–99. (in French)

In an effort to establish whether the human immunodeficiency virus (HIV) modifies the histological image of a lepromatous skin lesion, a comparative study was conducted in 1994 at the Marchoux Institute in Bamako, Mali, on persons newly suffering from leprosy who had been tested seropositive and seronegative for the HIV virus. These new leprosy patients had never been treated and could be grouped as follows: 5 HIV-positive (1 TT, 1 BT, 1 BL, 2 LL) and 10 controls testing HIV-negative, selected according to the following criteria: each seropositive leprosy subject was matched with two seronegative controls having the same clinical features, same stage under the Ridley classification system, same age and sex. No discordance between the clinical classifications and the histological features in the subjects testing HIV-positive has been observed. They display features similar to those testing negative, with the presence of histiocytes, in particular epithelioid cells and giant cells in normal proportion depending on the form of leprosy. The only remarkable difference was a greater incidence of edema in the subjects testing seropositive, compared with patients testing seronegative. In conclusion, HIV infection does not appear to cause major modifications in cellular response to *Mycobacterium leprae*, and no changes should be made in leprosy control programs.—Author's English Summary

Fleury, R. N., Ura, S. and Opromolla, D. V. A. [Lucio phenomenon (erythema necrotisans).] Hansen. Int. **21** (1996) 60–65. (in Portuguese)

A 36-year-old woman with lepromatous leprosy has presented characteristic lesions of Lucio's phenomenon that evolved with necrosis and large ulcerations on her limbs and face. There was secondary infection, sepsis, shock, and she died the 10th day after admission. Several venous thromboses, bronchopneumonia, foci of necrosis, and neutrophilic exudation in lepromatous granuloma in lymph nodes and liver were observed. Findings of this necropsy and the data from the literature suggest that Lucio's phenomenon is a hemorrhagic infection of cutaneous areas caused by occlusion of veins of the vascular plexus of the deep dermis and subcutis previously involved by lepromatous infiltration and bacilli. Exudative foci, observed in lepromatous granuloma in the lymph nodes and liver, suggest a type 2 reaction with a subclinical evolution and the exudative alterations may be accounted for by full venous occlusion. A patient with Lucio's phenomenon is one that is similar to a patient with large burns all over his body; he has loss of water, salts, and proteins and is prone to develop a bacterial infection in the cutaneous ulcers. Several areas of necrosis in the dermis and subcutis may release coagulation factors that cause thrombosis found in several venous territories. There is no relation between foci of necrosis in the gastrointestinal mucous membranes and Hansen's disease and they are certainly caused by shock.—Authors' English Abstract

Fu, J., *et al.* [Trace elements in the sera of leprosy patients.] China Lepr. J. **12** (1996) 185–186. (in Chinese)

Contents of iron, copper, zinc and manganese in the sera of 85 leprosy patients were determined taking tuberculosis patients and healthy people as controls. The results showed that between leprosy patients and healthy people contents of iron and copper were significantly different ($p < 0.01$) and no difference in zinc and manganese ($p > 0.05$). Between leprosy and tuberculosis patients, contents of copper and zinc were different ($p < 0.05$) and no difference in iron and manganese. Between the forms of leprosy the differences were significant.—Authors' English Abstract

Ginestar, W., Terencio de las Aguas, J. and Torres Peris, V. [The incidence of epitheliomas among leprosy patients in the Fontilles sanatorium (Spain) between 1985 and 1995.] Rev. Leprol. Fontilles **20** (1996) 1083–1099.

A survey was conducted on the incidence of epitheliomas on 290 leprosy patients attended at the Sanatorium of Fontilles. Several parameters, age, sex, type of leprosy and site of appearance are studied in relation with association with epitheliomas. The conclusion is that it is a pathology frequently associated with these patients, with a similar appearance in nonleprosy patients. A special mention is that the relapses are more frequent probably due to the residual lesions of Hansen's disease.—Authors' English Summary

Goncalves, A., Pedroso, M., Bacarelli, R. and De Oliveira, S. Assessment of the courses of prevention of disabilities in Hansen's disease national reference center in Brazil—a national investigation. Acta Leprol. **10** (1996) 111–116.

Among the efforts endeavored for inclusion of prevention of disabilities in programs of control of Hansen's disease in Brazil, "Lauro de Sousa Lima" sanitary dermatology hospital, Bauru, São Paulo state, performed the role of the main center of formation of human resources as the national reference center and as a reference center for Portuguese-speaking countries by World Health Organization. In this communication, results obtained are presented on a study performed with the aim of knowing judgments of ex-students about this process. Four-hundred-sixteen professionals have been investigated, with a final amount of 151 respondents.

Data obtained, besides distinguishing them in conformity with their basic formation and geographic area, quantify their appreciations on degree of learning, distribution according to the scheme of work in re-

spective sanitary units and contributions of the course for their activities, not only in service level, but also as multipliers. Frequency and quantity of co-workers in the same unit, period of performance, as well as relationship between training and existence of systematic activities of prevention of disabilities in their place of work are also inquired.

In conclusion, maintenance of the existing course is strongly recommended, since case studies of Brazilian sanitary realities were taken as models of discussion, with emphasis on primary attention and integration of prevention of disabilities to general conditions of treatment.—Authors' Summary

Grauwin, M. Y., Mane, I. and Cartel, J. L. [Cauliflower growths in chronic plantar ulcer: how to act?] Acta Leprol. **10** (1996) 101–104. (in French)

Between 1983 and 1994, 66 Senegalese leprosy patients were seen for cauliflower growths developed in chronic plantar ulcer (CPU), (2 patients each had 2 tumors); 68 biopsies for pathological examination were taken: the diagnosis of squamous cell carcinoma was effectively made in 39 cases (38 patients) and that of pseudo-epitheliomatous hyperplasia in the remaining 29 cases (28 patients). The mean annual frequency of cauliflower growths was 0.45 per 100 CPU. Among these tumors, the percentage of carcinoma was 57%. Of the 38 patients with a carcinoma, 5 refused amputation and all of them died. The 33 others were amputated and of these 8 died as a direct result of their carcinoma (24%). In the case of the 28 patients with hyperplasia, amputation was carried out on 18 patients and local excision on 10. In the months following the operation 8 recurrences were observed in 10 of the patients on whom excision had been carried out. These recurrences were treated by amputation. This gives a total of 93% of amputations in the cases of hyperplasia. These facts lead us to conclude that at least in countries where pathological examination is not available below knee amputation is the most reasonable action to take in the proliferative tumors developed on a CPU. —Authors' English Summary

Kelleher, P., Helbert, M., Sweeney, J., Anderson, J., Parkin, J. and Pinching, A. Uveitis associated with rifabutin and macrolide therapy for *Mycobacterium avium intracellulare* infection in AIDS patients. Genitourin. Med. **72** (1996) 419–421.

Objective: Uveitis has been increasingly recognized as a side effect of rifabutin regimens in the prophylaxis and treatment of *Mycobacterium avium intracellulare* (MAI) infection. This study describes the clinical features and analyzes the factors associated with rifabutin induced uveitis.

Design: Retrospective observational study.

Setting: Tertiary care institution, The Royal Hospitals NHS Trust, London.

Patients: 68 HIV-seropositive individuals receiving rifabutin for prophylaxis or treatment of MAI infection.

Results: 11 episodes of uveitis occurred in 10 different individuals at a median of 62 days. The disease was bilateral in 4 and unilateral in the remainder. All subjects experienced ocular pain and photophobia and 9 individuals had a significant reduction in visual acuity. Uveitis was treated with mydriatics and topical steroids and resolved in all cases when rifabutin was stopped. The risk of uveitis was significantly increased with concurrent clarithromycin therapy (odds ratio 13.08, 95% Confidence Interval 1.98 to 83.12).

Conclusion: Rifabutin can cause a reversible uveitis. This risk is increased with concurrent clarithromycin therapy. Adverse drug interactions can be an important cause of morbidity in patients with advanced HIV disease.—Authors' Abstract

Li, Y., *et al.* [Comparison of dietetic states of leprosy patients in 1987 and 1994.] China Lepr. J. **12** (1996) 154–156. (in Chinese)

Two surveys of diet and nutrition in leprosy patients in 1987 and 1994 showed that in this period of time both the amount and quality of diet for them have bettered with increase of their income, i.e., the calories taken from animal foods has increased from 7.9% in 1987 to 8.6% in 1994 and intake of calories, protein, and fat increased by

20.43%, 14.08% and 27.59%, respectively.—Authors' English Abstract

Lin, Y., *et al.* [Deficiency disease in leprosy patients.] China Lepr. J. **12** (1996) 149–151. (in Chinese)

An examination of 328 leprosy patients found 70 of them with deficiency disease, of which 80% were vitamin A and riboflavin deficiency, being in 29 and 27, respectively, and 13 persons have suffered from scurvy. Its cause was mainly lack of these nutrients in their foods as a result of low income. The majority of those who had deficiency disease are severely disabled old people with age over 60 years.—Authors' English Abstract

Lin, Y., *et al.* [Diet and nutrition of leprosy patients.] China Lepr. J. **12** (1996) 152–154. (in Chinese)

A survey of diet and nutrition in 328 cases of leprosy in eight counties of Weifang City, Shandong Province, showed that the staple foods for them are grains and vegetables with few fruits, fish, meats, eggs and milk, and intake of retinol equivalent, calcium and riboflavin only just reached 36.98% 43.5% and 78.5% of the requirements, respectively.—Authors' English Abstract

Luo, Y., *et al.* [On six cases misdiagnosed as leprosy.] China Lepr. J. **12** (1996) 188–189. (in Chinese)

During a re-examination of leprosy patients diagnosed by medical workers at the county level, the author found that six persons suffered from varicose syndrome, chronic rheumatoid arthritis, hyperplastic scar, disuse unilateral faciomuscular hypotonia, injury of brachial plexus and keloid, respectively. These misdiagnoses were corrected.—Authors' English Abstract

Mahe, A. [Differential diagnosis of leprosy in 1996.] Acta Leprol. **10** (1996) 69–77. (in French)

Due to a decrease in the prevalence of leprosy, discussion of its differential diagnosis assumes increasing importance. Research on the early diagnosis of leprosy, before the onset of nerve lesions, the appearance (or improved definition) of certain diseases and the expansion of intercontinental migration are some of the factors which have induced changes in the conditions of leprosy diagnosis The most frequent and most sensitive differential diagnosis of leprosy have been reviewed: hypochromic diseases, tropical infections diseases, systemic diseases, deforming diseases, cutaneous lymphoma and AIDS. The difficulties posed by forms of leprosy revealed by reactional manifestations are stressed. Today, as in previous times, positive and differential diagnosis of leprosy is based on the following simple practices: study of lesion, sensitivity, bacilloscopy and the examination of the peripheral nervous system. Cutaneous histology is the complementary reference examination.—Author's English Summary

Mishra, B., Mukherjee, A., Girdhar, A., Husain, S., Malaviya, G. N. and Girdhar, B. K. Involvement of lips and gums in a borderline tuberculoid leprosy patient. Indian J. Lepr. **68** (1996) 367–370.

Recently, a borderline tuberculoid patient was seen in the outpatient department of CJIL Agra, with a facial lesion around the mouth including the lips and involving the gums. Because of the rarity of the lip and gingival lesions in this type of leprosy, the case is being reported.—From the Article

Opromolla, D. V. A., Fleury, R. N. and Taborda, P. R. O. [A case of subpolar virchovian hanseniasis treated with WHO/MDT.] Hansen. Int. **21** (1996) 67–74. (in Portuguese)

The authors present a case of sub-polar virchovian leprosy treated with WHO/MDT. After release from treatment (2 years MDT) showed with intense ENL reaction, articular pain, edema of extremities and multiple bone lesions. They analyze the literature and discuss the low incidence of these specific bony lesions, the radiographic and histopathological findings and the rarity of these disseminated lesions. In this case, a borderline infiltrate was found

in the bone biopsy with suppurative foci characteristic of type 2 reaction which helped to classify this case as a sub-polar virchovian and to justify the onset of lesions. They discuss the cause for the bone destruction during the reactional episode and regret the lack of consistent studies about bony lesions in leprosy which could help in the understanding of the pathology of this disease.—Authors' English Abstract

Sethi, N. C., Madadi, A. J. and Bhandari, S. Serum zinc, copper, magnesium, proteins and superoxide dismutase in leprosy patients on multidrug therapy—a follow-up study. Indian J. Lepr. **68** (1996) 325–333.

Serum zinc, copper, magnesium, total proteins and albumin-globulin fractions and superoxide dismutase (SOD) were estimated in 80 untreated patients with TT/BT/BL/LL type of leprosy and in 40 controls. The investigations were repeated on days 30, 60 and 120 after starting multidrug therapy (MDT-WHO) on the patients. Serum zinc was significantly lowered in all types of leprosy on days 0 and 30. Serum copper was significantly raised in all types of leprosy. This was not significant in BT/TT cases on 60, 120 days. There was a correlation between serum zinc and copper levels and the severity and type of leprosy. The lowering of serum magnesium values were not significant. With therapy, there was a shift of all the three elements toward normal values. Serum total proteins reduction was not significant. Serum albumin was significantly lowered in all types of leprosy. Serum globulin was significantly raised in all types of leprosy. This rise in TT/BT was not significant on day 60 and 120 after starting treatment. Serum SOD was significantly reduced in all the untreated cases. It gradually increased with the clinical improvement under MDT.—Authors' Abstract

Terencio de las Aguas, J. [Diagnosis of leprosy, its clinical forms and differential diagnosis.] Rev. Leprol. Fontilles **20** (1996) 1115–1138. (in Spanish)

The diagnosis of leprosy is based mainly on sensibility alterations, neural enlargement and the presence of *M. leprae*. The diagnosis of the different types of clinical leprosy, differences with other dermatosis and neurological processes are studied.—Author's English Abstract

Terencio de las Aguas, J. [Visceral and endocrine leprosy.] Rev. Leprol. Fontilles **20** (1996) 1101–1114. (in Spanish)

The different visceras affected by leprosy mainly in the multicbacillary types with frequent leprosy reactions are basically the liver, spleen, kidney, suprarenal, lymphatic system, and the male genital organ. The frequency of amyloidosis is discussed. Kidney affection is the most frequent cause of mortality.—Authors' English Summary

Immuno-Pathology

Gupte, M. D., Vallishayee, R. S., Anantharaman, D. S., Britto, R. L. J. and Nagaraju, B. Sensitization and reactogenicity of two doses of candidate antileprosy vaccine *Mycobacterium w*. Indian J. Lepr. **68** (1996) 315–324.

Mycobacterium w vaccine is one of the antileprosy vaccines under test in an ongoing comparative vaccine trial in South India. The objective of the present study was to examine the sensitizing ability, as measured by skin test reactions to Rees' MLSA and lepromin, and reactogenicity of *Mycobacterium w* vaccine in the local population. Two doses of *Mycobacterium w*, 1×10^9 bacilli and 5×10^9 bacilli, were used in two separate studies of 395 and 400 "healthy" individuals aged 1–65 years. In each study, the study subjects received ei-

ther *Mycobacterium w* vaccine or normal saline (control) by random allocation. The results showed that healing of vaccination lesions was uneventful although the healing process was somewhat prolonged with the higher dose. The mean size of lesions was 7.0 mm and 9.5 mm with the low and high doses of the vaccine, respectively. The results also showed that *Mycobacterium w* vaccine in a dose of 1×10^9 bacilli, failed to induce post-vaccination sensitization as measured by reactions to Rees' MLSA and by Fernandez and Mitsuda reactions to lepromin-A. However, when the dose of the vaccine was increased to 5×10^9 bacilli the mean sizes of post-vaccination reactions to Rees' MLSA and lepromin-A (both early and late) were significantly larger in the vaccine group compared to that in the control group. The sensitizing effect attributable to the vaccine was of the order of 1.5 mm to 1.8 mm.—Authors' Abstract

Katoch, K. Immunotherapy of leprosy. Indian J. Lepr. **68** (1996) 349–361.

Immunotherapy aims to modify the defective cell-mediated immune response in a section of leprosy cases. This presentation reviews the various immunomodulators developed/investigated for this purpose. Among the various mycobacterial agents, BCG, BCG + *Mycobacterium leprae, Mycobacterium w,* ICRC bacillus and *M. vaccae* have been tried in leprosy patients and varying degree of beneficial effects on bacterial killing and clearance have been observed. Studies carried out at CJIL, Agra, and elsewhere suggest an important role for these mycobacteria as immunotherapeutic agents. Other mycobacteria—*M. habana, M. phlei, M. gordonae*—have also been reported to be promising experimentally. In addition, various drugs such as levamisole, zinc and RACA 854 have been observed to have an immunomodulatory role in leprosy cases. Other promising immunomodulators include transfer factor, interferon-γ, interleukin 2 and acetoacetylated *M. leprae.* The progress achieved shows that immunotherapy may be considered as an adjunct to chemotherapy to enhance bacterial killing as well as bacterial clearance and thus may be recommended to shorten the treatment period, especially in bacilliferous leprosy cases.—Authors' Abstract

Kifayet, A., Shahid, F., Lucas, S. and Hussain, R. Erythema nodosum leprosum is associated with upregulation of polyclonal IgG1 antibody synthesis. Clin. Exp. Immunol. **106** (1996) 447–453.

Erythema nodosum leprosum (ENL) is a serious complication of lepromatous (L) disease in leprosy. We have previously shown that of the four IgG subclasses, IgG1 and IgG3 *Mycobacterium leprae*-specific antibodies are significantly lower in leprosy patients during ENL reaction compared with untreated L patients. To see if this decrease results from a down-regulation of antibody synthesis during ENL, the frequency of antibody-secreting B cells (ABSC) in the blood compartment was determined by ELISPOT and related to serum immunoglobulin concentrations (μg/ABSC). Control groups consisted of 16 patients with stable L disease and 32 healthy endemic controls (EC). Paired samples were analyzed during acute ENL (N = 13) and after the reaction had subsided to identify changes associated with ENL. Polyclonal (PC) IgG1 was elevated in L patients compared with EC (325 μg versus 180 μg). Interestingly, patients during acute ENL showed concentrations higher than L patients (419 μg) which decreased after the reaction had subsided (260 μg), indicating the transient nature of the antibody response. IgG2 antibodies showed the reverse trend and were lower during ENL and increased after the reaction had subsided. The mean concentrations for PC IgG3 and IgG4 antibodies were similar during ENL and after the reaction had subsided. Thus, decrease in *M. leprae*-specific IgG1 and IgG3 antibodies is not related to downregulation of B-cell responses. Identification of factors which regulate PC IgG1 antibody synthesis may provide additional insights into determinants of ENL reactions.—Authors' Abstract

Miles, B. A., Lafuse, W. P. and Zwilling, B. S. Binding of alpha-adrenergic receptors stimulates the anti-mycobacterial ac-

tivity of murine peritoneal macrophages. J. Neuroimmunol. **71** (1996) 19–24.

The effects of adrenergic stimulation of the antimycobacterial activity of peritoneal macrophages was investigated. We found that epinephrine and norepinephrine stimulated macrophages to suppress the growth of *Mycobacterium avium*. Stimulation was mediated by binding to the alpha 2 adrenergic receptor. The addition of the alpha 2 agonist clonidine to cultures resulted in an inhibition of mycobacterial growth and the effect of epinephrine was blocked by the alpha-antagonist phentolamine. Treatment of the macrophages with propranolol, a beta-antagonist, potentiated the effect of epinephrine. Epinephrine mediates its effect by stimulating the expression of macrophage activation genes.—Authors' Abstract

Moura, A. C. N. and Mariano, M. Lipids from *Mycobacterium leprae* cell wall are endowed with an anti-inflammatory property and inhibit macrophage function *in vivo*. Immunology **89** (1996) 613–618.

In general, the majority of bacteria are pro-inflammatory when injected in experimental animals. However, *Mycobacterium leprae* has no inflammatory effect when injected into mouse foot pad, but using the delipidated mycobacteria we observed a mild significant increase in foot pad edema. Other mycobacteria, *M. bovis*-BCG or *M. tuberculosis* induce a strong paw edema. Furthermore, *M. leprae* reduced locally the BCG-induced inflammatory reaction in mouse foot pad, whereas delipidated *M. leprae* did not influence this reaction. Both *M. leprae* and *M. leprae* cell-wall lipids blocked immune phagocytosis *in vivo* by inflammatory macrophages (from an induced focus). In contrast delipidated *M. leprae* stimulated the phagocytosis reaction. Neither intact *M. leprae,* delipidated *M. leprae,* nor its lipids had any toxic effect on macrophages or on cell migration. Although *M. leprae* did not interfere on cell influx and cell type in an induced-inflammatory site, this mycobacterium led to the appearance of a distinct cell population *in vivo*. The hypothesis is that *M. leprae* would transform macrophages in epithelioid cells, suggested by morphology analysis of cells by fluorescence-activated cell sorter and observed under optic microscopy.—Authors' Abstract

Murray, P. J., Wang, L., Onufryk, C., Tepper, R. I. and Young, R. A. T cell-derived IL-10 antagonizes macrophage function in mycobacterial infection. J. Immunol. **158** (1996) 315–321.

Pathogenic mycobacteria survive within macrophages despite T-cell responses that activate host defenses against most pathogens. Among cytokines produced by T cells, IL-10 is known to negatively regulate Th1 cells as well as macrophages. IL-10 has been shown to inhibit the antimycobacterial activity of macrophages *in vitro* and could account for the ability of mycobacteria to survive intracellularly. To test the inhibitory functions of IL-10 *in vivo,* transgenic mice that secrete IL-10 from the T-cell compartment were constructed and infected with Calmette-Guerin bacillus (*Mycobacterium bovis*). These mice were unable to clear the infection and developed large bacterial burdens. Nonetheless, their T cells produced abundant amounts of IFN-gamma and IL-2 in response to antigen challenge. These results indicate that the presence of excess IL-10 had little, if any, effect on T-cell function or development during the immune response to Calmette-Guerin bacillus. Rather, the data suggest that IL-10 helps maintain mycobacterial infections by acting primarily at the level of the macrophage, overriding antimycobacterial signals delivered by IFN-gamma.—Authors' Abstract

Rojas, R. E. and Segal Eiras, A. Immunoglobulin G response against 10-kDa and 65-kDa heat-shock proteins in leprosy patients and their household contacts. FEMS Immunol. Med. Microbiol. **15** (1996) 189–198.

We measured antibody responses to recombinant *Mycobacterium leprae* 65-kDa (rML65) and 10-kDa (rML10) by indirect ELISA in sera from leprosy patients, household contacts and healthy controls in a leprosy-endemic area in the northeast of Argentina. Serum antibody levels to those

antigens were correlated with IgM anti-phenolic glycolipid I (PGL-I) levels, with bacterial index and the period of time under chemotherapy. Bacterial index positive (BI+) patients showed higher mean values when compared with BI negatives (BI–). Among lepromatous patients a positive correlation was observed between IgG antibody responses to both recombinant antigens and IgM antibody response to PGL-I. The anti-rML10 test detected a higher percentage of positives/total than anti-rML65 in all leprosy groups and healthy contacts. Bacterial load, leprosy clinical form and the time under chemotherapy were factors which could influence levels of the antibody response. The contribution of these antibody studies for a precise and early diagnosis in leprosy is discussed.—Authors' Summary

Shen, J., *et al.* [Second follow-up of household contacts with seropositivity to leprosy antigens.] China Lepr. J. **12** (1996) 168–171. (in Chinese)

Two-hundred healthy controls and 119 leprosy household contacts who were of antibody positivity in 1991 have been followed up with a serological method once a year for an additional 2 years. Two-hundred-twenty-two healthy controls and 71 leprosy household contacts who were antibody negative in 1991 have also been followed up at the same time. The results showed that 26.5% and 23.5% of healthy controls and household contacts with antibody positivity in 1992 were still positive in 1993. The rates of converting to antibody positivity in healthy controls and the contacts with antibody negativity were 20.1% to 33.0% and 13.8% to 36.0%, respectively. The mean antibody titers of healthy controls and household contacts with persistent antibody positivity were the highest in 1993. Five MB leprosy patients were detected in the second follow up. The study suggested that youngsters with persistent antibody positivity and lepromin negativity have a high risk of developing clinical leprosy, and the risk in household contacts was higher than that in the healthy controls. Trial skin smear in individuals with the high risk is very helpful for the early detection of MB leprosy.—Authors' English Abstract

Singh, N., Bhatia, A., Gupta, K. and Ramam, M. Cytomorphology of leprosy across the Ridley-Jopling spectrum. Acta Cytol. **40** (1996) 719–723.

Objective: To evaluate the possible role of cytology in classifying leprosy lesions on the Ridley-Jopling scale.

Study design: A double-blind, prospective study comparing cytologic assessment of 30 clinically diagnosed cases of leprosy with their histopathology. May-Grunwald-Giemsa and Ziehl-Neelsen stains were done on slit-skin smears and fine needle aspiration material.

Results: Cytologic subclassification was possible in 23 cases as tuberculoid leprosy (11), midborderline (3), borderline lepromatous (5) and lepromatous leprosy (4). These correlated with histologic subtypes.

Conclusion: May-Grunwald-Giemsa complements Ziehl-Neelsen stain, yielding information almost comparable to that from histologic examination of skin biopsies.—Authors' Abstract

Triccas, J. A., Roche, P. W., Winter, N., Feng, C. G., Butlin, C. R. and Britton, W. J. A 35-kilodalton protein is a major target of the human immune response to *Mycobacterium leprae*. Infect. Immun. **64** (1996) 5171–5177.

The control of leprosy will be facilitated by the identification of major *Mycobacterium leprae*-specific antigens which mirror the immune response to the organism across the leprosy spectrum. We have investigated the host response to a 35-kDa protein of *M. leprae*. Recombinant 35-kDa protein purified from *M. smegmatis* resembled the native antigen in the formation of multimeric complexes and binding by monoclonal antibodies and sera from leprosy patients. These properties were not shared by two forms of 35-kDa protein purified from *Escherichia coli*. The *M. smegmatis*-derived 35-kDa protean stimulated a gamma interferon-secreting T-cell proliferative response in the majority of paucibacillary leprosy patients and healthy contacts of leprosy patients tested. Cellular responses to the protein in patients with multibacillary leprosy were weak or absent, consistent with hyporesponsiveness to *M. leprae* char-

acteristic of this form of the disease. Almost all leprosy patients and contacts recognized the 35-kDa protein by either a T-cell proliferative or an immunoglobulin G antibody response; whereas few tuberculosis patients recognized the antigen. This specificity was confirmed in guinea pigs, with the 35-kDa protein eliciting strong delayed-type hypersensitivity in *M. leprae*-sensitized animals but not in those sensitized with *M. tuberculosis* or *M. bovis* BCG. Therefore, the *M. leprae* 35-kDa protein appears to be a major and relatively specific target of the human immune response to *M. leprae* and is a potential component of a diagnostic test to detect exposure to leprosy or a vaccine to combat the disease.—Authors' Abstract

Van Heyningen, T. K., Collins, H. L. and Russell, D. G. IL-6 produced by macrophages infected with *Mycobacterium* species suppresses T cell responses. J. Immunol. **158** (1997) 330–337.

The ability of *Mycobacterium bovis* Calmette-Guerin bacillus-infected bone marrow-derived macrophages to process and present exogenously added antigens to T cells and stimulate their growth and production of IL-2 was examined. The infected macrophages were inhibited in their ability to activate T cells, and this inhibition could be transferred to uninfected macrophages with filtered supernatants from mycobacteria-infected macrophages. The inhibition was not due to decreases in macrophage viability, antigen uptake, or cell surface expression of MHC class II or other accessory molecules necessary for antigen presentation. Other intracellular pathogens such as *Listeria monocytogenes* and *Leishmania mexicana* did not induce the soluble inhibitory factor, while *M. avium* strain 101 did, suggesting the factor is specific to infection with mycobacteria. The inhibitory effect was reversed completely by preincubation with neutralizing antibodies against IL-6, and rIL-6 partially restored the effect. Approximately 10,000-fold more IL-6 was produced by mycobacteria-infected macrophages compared with uninfected controls. Such sustained levels of IL-6 may account for the immune unresponsiveness apparent in both human and murine mycobacterial disease.—Authors' Abstract

Vieira, L. M. M., Sampaio, E. P., Nery, J. A. C., Duppre, N. C., Albuquerque, E. C. A., Scheinberg, M. A. and Sarno, E. N. Immunological status of ENL (erythema nodosum leprosum) patients: its relationship to bacterial load and levels of circulating IL-2R. Rev. Inst. Med. Trop. Sao Paulo **38** (1996) 103–111.

Recent data suggest that the clinical course of reactional states in leprosy is closely related to the cytokine profile released locally or systemically by the patients. In the present study, patients from Rio de Janeiro, Brazil, with erythema nodosum leprosum (ENL) were grouped according to the intensity of their clinical symptoms. Clinical and immunological aspects of ENL and the impact of these parameters on bacterial load were assessed in conjunction with patients' *in vitro* immune responses to mycobacterial antigens. In 10 out of the 17 patients tested, BI (bacterial index) was reduced by at least 1 log from leprosy diagnosis to the onset of their first reactional episode (ENL), as compared to an expected 0.3 log reduction in the unreactional group for the same MDT (multidrug therapy) period. However, no difference in the rate of BI reduction was noted at the end of MDT among ENL and unreactional lepromatous patients. Accordingly, although TNF-α (tumour necrosis factor) levels were enhanced in the sera of 70.6% of the ENL patients tested, no relationship was noted between circulating TNF-α levels and the decrease in the BI detected at the onset of the reactional episode. Evaluation of bacterial viability of *Mycobacterium leprae* isolated from the reactional lesions showed no growth in the mouse foot pads. Only 20% of the patients demonstrated specific immune response to *M. leprae* during ENL. Moreover, high levels of soluble IL-2R (interleukin-2 receptor) were present in 78% of the patients. Circulating anti-neural (anti-ceramide and anti-galactocerebroside antibodies) and anti-mycobacterial antibodies were detected in ENL patients' sera as well, which were not related to the clinical course of disease. The authors conclude

that their data suggest that bacterial killing is enhanced during reactions. Emergence of specific immune response to *M. leprae* and the effective role of TNF-α in mediating fragmentation of bacteria still need to be clarified.—Trop. Dis. Bull.

Vordermeier, H. M., Venkatprasad, N., Harris, D. P. and Ivanyi, J. Increase of tuberculous infection in the organs of B cell-deficient mice. Clin. Exp. Immunol. **106** (1996) 312–316.

Protective immunity against infection with *Mycobacterium tuberculosis* is imparted by T cells rather than antibodies, but B cells can play a role as antigen-presenting cells and in granuloma formation. We re-evaluated the role of B cells in the course of tuberculous infection in μ-chain knock-out (Ig^-) mice. Surprisingly, the organs of *M. tuberculosis*-infected Ig^- mice were found to have three- to eightfold elevated counts of viable bacilli compared with normal littermates at 3–6 weeks postinfection. Splenic interferon-gamma responses to whole antigen were unimpaired, while proliferation to certain mycobacterial peptides was found to be diminished. However, bacille Calmette-Guerin (BCG) vaccination significantly reduced the infection in Ig^- mice. The mechanisms by which B cells can influence primary tuberculous infection need further study.—Authors' Abstract

Microbiology

Baulard, A., Kremer, L., Supply, P., Vidaud, D., Bidart, J. M., Bellet, D. and Locht, C. A new series of mycobacterial expression vectors for the development of live recombinant vaccines. Gene **176** (1996) 149–154.

Recombinant BCG (bacillus Calmette-Guerin) is a promising candidate as a live vaccine delivery system. Thus far, however, only autoreplicative plasmids carrying the heterologous genes to be expressed in BCG, together with antibiotic-resistance genes, have been successfully used. This could potentially lead to the spreading of antibiotic resistance among other bacteria, and might therefore be unsafe for the environment. In this study, we present a series of three *Escherichia coli-Mycobacteria* shuttle vectors which enable expression and secretion of antigens without the use of antibiotic-resistance markers. All these plasmids confer mercury resistance to the host bacteria as the only selectable marker and contain a unique restriction site to allow for single-step in-frame cloning of open reading frames downstream from the *Mycobacterium tuberculosis* 85A antigen promoter export signal. The system was used to express the free beta-subunit of human chorionic gonadotropin (hCG beta), a potential target of an immunotherapeutic vaccine.—Authors' Abstract

Beggs, M. L., Cave, M. D. and Eisenach, K. D. Isolation and sequence of a *Mycobacterium tuberculosis* sigma factor. Gene **174** (1996) 285–287.

A DNA segment from *Mycobacterium tuberculosis* containing a gene for a putative sigma factor was isolated and sequenced. The protein encoded by this gene in 92% similar to the *M. smegmatis* sigma factor MysB, and has been designated Mtu SigB. A *M. leprae* homolog of mysB and into sigB was identified in the database.—Authors' Abstract

Fsihi, H., De Rossi, E., Salazar, L., Cantoni, R., Lobo, M., Riccardi, G., Takiff, H. E., Eiglmeier, K., Bergh, S. and Cole, S. T. Gene arrangement and organization in a ~76 kb fragment encompassing the *oriC* region of the chromosome of *Mycobacterium leprae.* Microbiology **142** (1996) 3147–3161.

A continuous 75,627 bp segment of the *Mycobacterium leprae* chromosome spanning the *oriC* region was sequenced. The gene order at this locus was similar to that found in the replication origin region of many other prokaryotes, particularly *M. tuberculosis* and *Streptomyces coelicolor.* As in the case of several gram-positive bacteria, essential genes involved in basic cellu-

lar functions, such as DNA or RNA metabolism (*dnaA, dnaB, dnaN, gyrB, gyrA, pcnB, recF, rnpA, ssb*), cell wall synthesis (*ponA, pbpA*) and probably cell division (*gidB, rodA*) were found. Strikingly, the *gidA* gene was absent from this part of the genome and there was no rRNA operon near *oriC*. The *gyrA* gene harbors an intein coding sequence indicating that protein splicing is required to produce the mature A subunit of DNA gyrase. Among the many other noteworthy features were ORFs encoding putative serine/threonine protein kinases and a protein phosphatase, three tRNA genes, one *M. leprae*-specific repetitive element and a *glnQ* pseudogene.—Authors' Abstract

Mizrahi, V. and Huberts, P. Deoxy- and dideoxynucleotide discrimination and identification of critical 5′ nuclease domain residues of the DNA polymerase I from *Mycobacterium tuberculosis*. Nucleic Acids Res. **24** (1996) 4845–4852.

The DNA polymerase I (PolI) from *Mycobacterium tuberculosis* (Mtb) was overproduced in *Escherichia coli* as an enzymatically active, recombinant protein with or without an N-terminal His-tag. The proteins catalyzed both the DNA polymerization of home- and heteropolymer template-primers and the 5′-3′ exonucleolytic hydrolysis of gapped and nicked substrates but lacked an associated proofreading activity. In accordance with recent predictions, both recombinant forms of the *M. tuberculosis* enzyme were unable to discriminate against dideoxynucleotide B-triphosphates and were thus efficiently inhibited by these chain-terminating nucleotide analogs during DNA synthesis. This unusual property might be potentially exploitable in terms of novel antimycobacterial drug design. A mutational analysis of 5′ nuclease domain residues allowed the roles of nine invariant acidic residues to be evaluated. Acidic side chain neutralization resulted in a greater than or equal to 20-fold reduction in activity, with the most profound reduction (greater than or equal to 10^4-fold) being caused by neutralization of the Asp(125), Asp(148) and Asp(150) residues.—Authors' Abstract

Pearson, R. E., Jurgensen, S., Sarkis, G. J., Hatfull, G. F. and Jacobs, W. R. Construction of D29 shuttle phasmids and luciferase reporter phages for detection of mycobacteria. Gene **183** (1996) 129–136.

Diseases caused by *Mycobacterium tuberculosis, M. leprae* and *M. avium* cause significant morbidity and mortality worldwide. Effective treatments require that the organisms be speciated and that drug susceptibilities for the causative organisms be characterized. Reporter phage technology has been developed as a rapid and convenient method for identifying mycobacterial species and evaluating drug resistance. In this report we describe the construction of luciferase reporter phages from mycobacteriophage D29 DNA. Shuttle phasmids were first constructed with D29 in order to identify nonessential regions of the D29 genomes and to introduce unique cloning sites within that region. Using this approach, we observed that all of the D29 shuttle phasmids had the cosmid vector localized to one area of the phage genome near one cohesive end. These shuttle phasmids had been constructed with a cosmid that could be readily excised from the D29 genome with different sets of restriction enzymes. Luciferase reporter phages were made by substituting the luciferase cassette for the cosmid vector. Recombinant phages with the luciferase cassette fall into two groups. One group produced light and had the expression cassette oriented with the promoter directing transcription away from the cohesive end. In contrast, the other group had the expression cassette in the opposite orientation and failed to produce light during lytic infection, but did produce light in L5 lysogens which are known to repress D29 promoters. These results suggest that a phage promoter of the D29 phage can occlude the expression of a promoter introduced into this region. D29 luciferase reporter phages are capable of detecting low numbers of L5 lysogens like L5 luciferase phages. However, unlike L5 luciferase phages, D29 luciferase phages can readily infect *M. tuberculosis* and *M. bovis* BCG, demonstrating that these phages can be used to evaluate drug susceptibilities of many types of mycobacteria.—Authors' Abstract

Pessolani, M. C. V. and Brennan, P. J. Molecular definition and identification of new proteins of *Mycobacterium leprae.* Infect. Immun. **64** (1996) 5425–5427.

This report describes N-terminal group analysis of six new proteins isolated from by *in vivo*-grown *Mycobacterium leprae,* three of which correspond to products of the cysA, ahpC, and rplL genes, which were recently defined through the *M. leprae* genome project and which encode a putative sulfate sulfurtransferase, an antioxidant enzyme, and the L7/L12 ribosomal protein, respectively.—Authors' Summary

Philipp, W. J., Nair, S., Guglielmi, G., Lagranderie, M., Gicquel, B. and Cole, S. T. Physical mapping of *Mycobacterium bovis* BCG Pasteur reveals differences from the genome map of *Mycobacterium tuberculosis* H37Rv and from *M. bovis.* Microbiology **142** (1996) 3135–3145.

A *Dra*I restriction map of the ~4.35 Mb circular chromosome of the vaccine strain *Mycobacterium bovis* BCG Pasteur was constructed by linking all 21 *Dra*I fragments, ranging in size from 6 to 820 kb, using specific clones that spanned the *Dra*I recognition sites as hybridization probes. The positions of 20 known genes were also established. Comparison of the resultant genome map with that of the virulent tubercle bacillus *M. tuberculosis* H37Rv revealed extensive global conservation of the genomes of these two members of the *M. tuberculosis* complex. Possible sites of evolutionary rearrangements were localized on the chromosome of *M. bovis* BCG Pasteur by comparing the *Asn*I restriction profile with that of *M. tuberculosis* H37RV. When selected cosmids from the corresponding areas of the genome of *M. tuberculosis* H37Rv were used as hybridization probes to examine different BCG strains, wild-type *M. bovis* and *M. tuberculosis* H37Rv, a number of deletions up to 10 kb in size, insertions and other polymorphisms were detected. In addition to the known deletions covering the genes for the protein antigens ESAT-6 and mpt64, other genetic loci exhibiting polymorphisms or rearrangements were detected in *M. bovis* BCG Pasteur.—Authors' Abstract

Prabhakaran, K., Harris, E. B. and Randhawa, B. Properties of lysophospholipase in *Mycobacterium leprae.* J. Basic Microbiol. **36** (1996) 341–349.

Lysophospholipids are key intermediates in the metabolism of phospholipids. Cytoplasmic membranes of both eukaryotes and prokaryotes are made of phospholipid bilayers. Phospholipases are activated during phagocytosis. Lysophospholipids generated by phospholipase A(2) or A(1) degrade cell membranes and can cause cell lysis. An active lysophospholipase, that hydrolyzes lysophospholipids, was detected by the radioisotope technique in *Mycobacterium leprae.* About two-thirds of the enzyme was particulate and one-third cytoplasmic. Optimum activity was at 37°C and at pH 6.0. Temperatures above 70°C completely inactivated the enzyme. The compound AACOCF3, a trifluromethylketone analog of arachiodonic acid, inhibited the activity; the inhibition appeared to be of the uncompetetive type. The K-m of the enzyme was 2.5×10^{-4} M, suggesting a fairly strong affinity for the substrate. Lysophospholipids have been shown to be microbicidal to invading organisms. Possession of lysophospholipase by *M. leprae* is apparently one of the methods by which the bacilli overcome the defense mechanisms of the host.—Authors' Summary

Ratnakar, P., Rao, S. P. and Catanzaro, A. Isolation and characterization of a 70 kDa protein from *Mycobacterium avium.* Microb. Pathogen. **21** (1996) 471–486.

Mycobacterium avium complex (MAC) is an intracellular pathogen which causes disseminated bacterial infection in immunocompromised individuals. This organism predominantly infects macrophages. Attachment of MAC to macrophages is the first step prior to invasion. We have previously shown that a 70-kDa protein of *M. avium* (Ma) is one of nine monocyte-binding proteins. In the present study, we have purified this protein from sonic extracts of Ma and studied some of its properties. The N-terminal sequence of this protein was identified and found to exhibit a strong homology to the 70-kDa heat shock protein (hsp) of *M. leprae* (Ml) and *M. tuberculosis* (Mtb). This protean was found to be present

on the surface of the organism and was able to inhibit the attachment of intact Ma to human monocyte-derived macrophages (MDM) up to 49% in an *in vitro* attachment assay using intact fluorescein isothiocyanate (FITC)-labeled Ma. Bovine serum albumin (BSA) and recombinant 70-kDa hsp from Mtb, which were used as controls, inhibited this attachment by 9.8% and 18%, respectively. These results suggest that the 70-kDa protein may have a role in the attachment of intact Ma to MDM. When tested in lymphocyte activation assays, this protein did not appear to significantly stimulate proliferation. However, it was found to stimulate the production of tumor necrosis factor (TNF)-alpha by MDM. This protein may be one of several Ma antigens that trigger host immune response by binding to MDM and stimulating the production of inflammatory cytokines such as TNF-alpha by these cells.—Authors' Abstract

Wu, Q., *et al.* [A comparison between several PCRs for *M. leprae*.] China Lepr. J. **12** (1996) 164–167. (in Chinese)

A study on comparison between several PCRs for detection of *M. leprae* was done. The PCRs were established on the basis of *M. leprae* genes coding for the 65-kDa, 36-kDa, 18-kDa, groEL antigens and 16s rRNA. The indicators for the comparison are sensitivity, specificity, simplicity, speed, economic cost and so on. The results indicated that the PCR with *M. leprae* gene coding for the 65-kDa antigen, which was developed by Woods, *et al.* and then modified by Wu, *et al.* was the PCR of choice. The lowest limit of its detection is 10 to 100 AFBs: there was no crossreaction with other mycobacteria used in the test, and it only needs one pair of primers. The optimum number of cycles are 30 which consume 2.5 to 3 hrs, and the volume of the reaction system is 50 μl which saves 50% of the materials in comparison with the other PCRs. The authors consider that this PCR is the most valuable to be popularized for the detection of *M. leprae*.—Authors' English Abstract

Wu, Q., *et al.* [Preliminary study on Western blotting test and serological activity of *M. leprae* antigens.] China Lepr. J. **12** (1996) 172–176. (in Chinese)

SDS-polyacrylamide electrophoresis (SDS-PAGE) and Western blotting (WB) based on SDS-PAGE were established and used for analyzing and characterizing the antigenic components of *M. leprae* (ML) and some mycobacteria (AFB) and their serological activities by using leprosy patients' sera. This technique involved the separation of sonicates from ML and AFBs on SDS-PAGE, and the separated bands were electropheretically transferred to nitrocellulose (NC) and the NC incubated with patients' sera. The visualization of the antigen-antibody complexes was done by an indirect immunoperoxidase technique. The preliminary results showed that ML and the AFBs used in the test all have characteristic separation bands' spectra on SDS-PAGE and their sum of the bands were different, and that the positive reactional bands of the ML and AFBs all showed on WB but there were no crossreactional bands among them. The sum of positive bands in ML were significantly more than other known AFBs, which were 70-kDa, 68-kDa, 65, 55, 42/43, 36, 22, 18, 14 and 10-kDa and all have potential for immunodiagnosis. The optimum conditions for the use of SDS-PAGE and WB jointly or separately, purification, identification of ML and AFBs as well as for selecting absorbents of serological tests and so on are all discussed in detail.—Authors' English Abstract

Experimental Infections

Garbino, J. A., Virmond, M. and Almeida, J. A. Nerve conduction study technique in the armadillo. Hansen. Int. **21** (1996) 10–13.

The authors present a technique to perform nerve conduction study in armadillos (*Dasypus novemcinctus*) and suggest that 66.17 m/s could be the mean normal nerve

conduction velocity for the sciatic nerve, a parameter that could be used for assessment of the peripheral nervous system in this animal, considered the choice for experimental development of leprosy neuropathy and even in other peripheral neuropathies.—Authors' Summary

Lathrop, G., Scollard, D. M. and Dietrich, M. Reactivity of a population of lymphocytes with an antibody to human γ,δ T-cells. Clin. Immunol. Immunopathol. **82** (1996) 68–72.

Reactivity of lymphocytes from the nine-banded armadillo (*Dasypus novemcinctus*) was examined by flow cytometry using a panel of 16 commercially available fluorochrome-conjugated monoclonal antibodies raised against human or marine leukocyte antigens. The only reactivity observed was with antibody TCRδ1, directed against a common determinant on the δ chain of the human γ,δ T-cell receptor. Using this antibody, a distinct, bright population of lymphocytes was seen in the peripheral blood in all of 47 animals examined, accounting for 2.0%–47.1% of lymphocytes (median, 10.6%). The γ,δ-reactive lymphocyte population comprised a greater percentage of intra-epithelial lymphocytes in the small intestine than in the blood; variable percentages of γ,δ-reactive cells were also observed in the spleen, thymus, lymph nodes, and bone marrow, and in cutaneous lepromas. In armadillos with disseminated *Mycobacterium leprae* infection, a significantly greater percentage of circulating lymphocytes reacted with the anti-γ,δ antibody. This is the first described reactivity of armadillo lymphocytes with a monoclonal antibody to a lymphocyte antigen, and it may offer a useful tool in disease models involving the armadillo.—Authors' Abstract

Epidemiology and Prevention

Andrade, V., Moreira Alves, T., Tebaldi Tardin, R. and Werneck de Castro, A. J. [Model of the campaign for the elimination of hanseniasis combined with an antipolio vaccine—municipality of Rio de Janeiro, Brazil.] Hansen. Int. **21** (1996) 14–21. (in Portuguese)

Taking in consideration that the municipality of Rio de Janeiro has a reasonable infrastructure of health services, the Secretary of Health decided to implement a Leprosy Elimination Campaign (LEC) as recommended by WHO, an initiative to be developed from 16 June to 12 August, 1996. The thrust of this campaign is the promotion of awareness regarding leprosy in order to stimulate individuals with suspected signs and symptoms of leprosy and living in areas not covered by leprosy control services, to show for diagnosis and treatment in health units. After analysis of the epidemiological and operational picture of leprosy in the municipality of Rio de Janeiro, the authors present their proposal to develop a LEC jointly with the standard second dose of the anti-polio vaccination. It is hoped that this initiative, fully discussed with social movements and clubs of services causes an important mobilization of the community and that the motivation of health personnel increases the process of integration of leprosy control in the general health services. Finally, it is hoped that this initiative, developed jointly with technical personnel of the leprosy control program, personnel from the vaccination campaign and the community, will promote the exclusion of some stigmatizing concepts such as the non-curability of leprosy, despite being a unique opportunity to learn from the antipolio campaigns, worldwide recognized due to the success of its strategy.—Authors' English Abstract

Carranzana Hernandez, G. B. and Ferra Torres, T. M. [Incidence of leprosy according to sex, age, and clinical form; city of Camagüey, Cuba in the years 1984–1994.] Rev. Leprol. Fontilles **20** (1996) 1051–1056. (in Spanish)

In order to determine the relation between sex, age and clinical forms a study was carried out on 214 cases of leprosy in

Camagüey City, Cuba, from 1984 to 1994. In females the greater incidence in the paucibacillary (PB) group starts at an age of 30 years and an age of 45 years in the multibacillary (MB) group; the most frequent clinical form was TL. In males the greatest incidence was observed starting at the age of 30 years both for the PB and the MB leprosy; the most found clinical form in males was LL.—Authors' English Summary

Kaur, M. and Bharti, R. Leprosy among circus workers. Indian J. Lepr. **68** (1996) 363–366.

Seven (2.31%) circus workers of two circus companies visiting Amritsar were detected to be suffering from leprosy. Whether or not they acquired the disease during their stay in the circus is not so important, but it is necessary to detect and treat these itinerant persons. Our experience clearly underlines the necessity for undertaking surveys of various workers living in crowded conditions (like a circus) because of their occupation.—Authors' Abstract

Vazquez, F. A., Varela, N. N., Antola, M. C., Wand-del-Ray, M. L. and Leguizamon, O. R. [Hansen's disease in MERCOSUR.] Acta Leprol. **10** (1996) 79–84. (in Spanish)

In the context of the important changes of a political economic, social and health order upon which Argentina, Brazil, Paraguay and Uruguay have now embarked in the framework of the commitment undertaken to create instruments for the Common Market of the South America (MERCOSUR), the importance of leprosy for public health in this new region is analyzed. In this connection a description is given of the background and reasons which led to the creation of the MERCOSUR Committee for technical cooperation on leprosy, composed of the heads of the national leprosy control programs of the countries in question for the purpose of implementing the Protocol of Intention signed by the representatives of the ministers of member countries, with the object of establishing policies of technical cooperation in activities to control this disease, with a view to attaining the goal proposed by WHO of eliminating leprosy as a public health problem by the year 2000. Using the data contributed by the different programs, the epidemiological situation existing in this region in December 1995 is outlined, with analysis of certain epidemiological, demographic and operational variables, showing that MERCOSUR has a prevalence of 6.03 per 10,000 population, one of the highest rates in comparison with other WHO regions; a high percentage of cases lost to view (abandons); and a low rate of coverage with multidrug therapy. In this region a total of 33,654 new cases were detected during 1995, of which more than 50% were multibacillary forms, while nearly 10% of them were youngsters of less of 15 years of age. The action carried out jointly among the four countries, the successes achieved and the results to be achieved in the short term are also described.—Authors' English Summary

Wang, S. [Surveillance of three years following basic eradication of leprosy.] China Lepr. J. **12** (1996) 187–188. (in Chinese)

Since 1957 to 1994 Tianchang City, Anhui Province, with a population of 570,627, has accumulatively found 135 leprosy patients, including 94 men and 41 women, 94 PB and 41 MB, of which 114 have been cured, 15 died, 15 moved away and only 1 is active at present. The prevalence decreased from 0.084‰ (1966) to 0.0017‰ (1994) and detection rate from 2.03/100,000 to 0.13/100,000. The proportion of childhood patients was reduced from 13.8% (1967–1971) to 0 (1982–1986). In the last 3 years examination of the cures and household contacts and clue survey yearly found no relapse or new patients.—Authors' English Summary

Rehabilitation

Jiang, J., *et al.* [Silent neuritis in leprosy.] China Lepr. J. **12** (1996) 159–162. (in Chinese)

As the part of an extensive study of rehabilitation for leprosy patients, a pilot survey was carried out, including detection and treatment of neuritis. Eight areas with different leprosy prevalences were involved, 3571 patients were examined and 151 of them were found to have silent neuritis, accounting for 4.2%; 330 nerves were involved, including 15 facial, 98 ulnar, 36 median, 148 posterior tibial and 33 peroneal nerves. After a standard prednisone regimen, 71.2% of the nerves have showed improvement in their function. The treatment with prednisone is simple and effective in reducing nerve damage.—Authors' English Abstract

Ju, J., *et al.* [Comprehensive treatment for plantar ulcer in 51 leprosy patients.] China Lepr. J. **12** (1996) 182–183. (in Chinese)

Sixty-eight complicated plantar ulcers were treated with a comprehensive regimen in 51 leprosy patients, of which 66 (97.1%) have healed within 5 months. During 4 years of follow up, in the first year 30 ulcers relapsed (78.9%) because of overwalking. To teach patients to do self-care and to avoid overwalking are essential for preventing relapse of plantar ulcer.—Authors' English Abstract

Mehta, R., Husain, S., Malaviya, G. N., Goel, R. K. and Shrivastava, K. P. Single tendon-two insertion versus two tendon transfers for reablement of ulnar-median palsied thumb in leprosy. Acta Leprol. **10** (1996) 105–109.

Twenty-three opponensplasties for ulnar median paralysis in 20 leprosy patients were performed by two different procedures, i.e., extensor indicis proprius and flexor digitorum superficialis transfers using standard techniques. The results were evaluated using various objective and subjective, anatomical and functional parameters. Two tendon transfers appeared to be superior to single tendon two insertion transfers. It was concluded that if the situation permits, two tendon transfers should be performed for combined ulnar median paralysis.—Authors' Summary

Smith, W. C. S. [Prevention of disability in leprosy.] Acta Leprol. **10** (1996) 85–88. (in French)

A survey of the prevention of disability policy and activities in a random sample of 200 ILEP-assisted projects was conducted in 1995. This was followed by a workshop of field experts in different aspects of prevention of disability who work in different geographical regions. The survey findings and state of current knowledge on prevention of disability were reviewed during the workshop and recommendations on the planning, implementation, and evaluation of simple and effective prevention of disability developed. Prevention of disability includes complex activities, such as nerve decompression and reconstructive surgery. However these recommendations focus on the simple techniques and approaches which can be implemented through leprosy control programs, primary health care and community-based rehabilitation. These recommendations have been approved by the ILEP Medical Commission, and reported in the ILEP Medical Bulletin (No. 8, December 1995).—Author's English Summary

Wei, X., *et al.* [Follow-up of persons using artificial limbs who have and had leprosy.] China Lepr. J. **12** (1996) 183–185. (in Chinese)

After fitting artificial limbs under support of the PRC MOPH/TLMI rehabilitation program, 392 people who had or have leprosy in five provinces of China were followed up once a half year, and the follow up was done four times in 2 years (1990 to 1992). The intact data collected in 256 people, including 198 men and 58 women with a mean age of 55 years, 236 uni- and 18 bilateral ones, and 244 for the leg, 19 for the

thigh and 6 and 5 for under knee and ankle joints, respectively, showed that in four follow ups there were ulcers on the tip of the limbs in 27%, 22%, 19% and 21%, respectively. The causes of ulceration were mainly lack of fit between the receptive cavity and the tip of the limb and lack of maintenance of the prostheses. But there only were 5.1% to 6.2% of the users who expressed dissatisfaction with the prostheses.—Authors' English Abstract

Yan, L., *et al.* [Progress of China MOPH-TLMI Program II for leprosy rehabilitation.] China Lepr. J. **12** (1996) 177–179. (in Chinese)

After first cooperation program for leprosy rehabilitation between PRC MOPH and TLMI had been smoothly completed, and since May of 1995 a second 3-year program on it started, which covered 73 units in 13 provinces and cities. In the first year, following the preparatory works which had been accomplished, early detection of neuritis; self-care on eye, hand, foot; wearing protective footwear; management of complicated plantar ulcer and fitting artificial limbs were already carried out in over 87.64% of the program. To complete the task successfully, it has yet to strengthen administrative and economical management and technical guidance.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Andersen, S. J., Quan, S., Gowan, B. and Dabbs, E. R. Monooxygenase-like sequence of a *Rhodococcus equi* gene conferring increased resistance to rifampin by inactivating this antibiotic. Antimicrob. Agents Chemother. **41** (1997) 218–221.

A DNA clone from *Rhodococcus equi* conferring a low-level rifampin resistance through the ability to inactivate this antibiotic via its decomposition was identified. The *iri* (inactivation of rifampin) gene consisted of an open reading frame of 1437 bp encoding a 479-amino-acid sequence strongly resembling those of monooxygenases acting upon phenolic compounds or involved in polyketide antibiotic synthesis. When expressed in *Escherichia coli,* the gene conferred resistance to a >50-μg/ml concentration of the drug.—Authors' Abstract

Anton, V., Rouge, P. and Daffe, M. Identification of the sugars involved in mycobacterial cell aggregation. FEMS Microbiol. Lett. **144** (1996) 167–170.

Incubation of *Mycobacterium tuberculosis* and *M. smegmatis* cell with the sugar components of their surface-exposed glycans demonstrated that D-arabinose, but not alpha-D-glucose or D-mannose, led to the dispersion of the large clumps formed by the bacilli in stationary liquid cultures. These results confirm the presence of arabinose-containing glycans on the mycobacterial cell surface and demonstrate the implication of selective sugars in cell aggregation, suggesting that the clumping of mycobacterial cells is probably mediated by lectin-carbohydrate interactions.—Authors' Abstract

Aranaz, A., Liebana, E., Mateos, A., Dominguez, L., Vidal, D., Domingo, M., Gonzalez, O., Rodriguez Ferri, E. F., Bunschoten, A. E., van Embden, J. D. A. and Cousins, D. Spacer oligonucleotide typing of *Mycobacterium bovis* strains from cattle and other animals: a tool for studying epidemiology of tuberculosis. J. Clin. Microbiol. **34** (1996) 2734–2740.

The spacer oligonucleotide typing (spoligotyping) method was evaluated for its ability to differentiate *Mycobacterium bovis* strains. This method detects the presence or absence of spacers of the direct repeat locus of the *M. bovis* genome. The spacers in the direct repeat locus are ampli-

fied by polymerase chain reaction (PCR) and are detected by hybridization of the biotin-labeled PCR product with a membrane containing oligonucleotides derived from spacer sequences that have previously been bound to a membrane. One-hundred-eighty-two *M. bovis* isolates from domestic animals (cattle, goat, sheep, and cats) and wild animals (deer and wild boar) were spoligotyped, and the results were compared with those obtained by IS6110 restriction fragment length polymorphism analysis. Two rather homogeneous clusters of isolates containing 20 and 4 types, respectively, were identified by spoligotyping. The first cluster included isolates from cattle, cats, and feral animals. By spoligotyping, isolates from the Spanish wild boar and deer had the same pattern as some bovine isolates, suggesting transmission between these animals and cattle and highlighting the importance of the study of these reservoirs. The second cluster included all the caprine and ovine isolates. Within each cluster, the patterns of the different strains differed only slightly, suggesting that the spoligotypes may be characteristic of strains from particular animal species. Spoligotyping proved to be useful for studying the epidemiology of bovine *M. bovis* isolates, especially of those isolates containing only a single copy of IS6110. In view of our results, we suggest fingerprinting all *M. bovis* strains by the spoligotyping method initially and then by IS6110 restriction fragment length polymorphism typing of the strains belonging to the most common spoligotypes.—Authors' Abstract

Belanger, A. E., Besra, G. S., Ford, M. E., Mikusova, K., Belisle, J. T., Brennan, P. J. and Inamine, J. M. The embAB genes of *Mycobacterium avium* encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 11919–11924.

The antimycobacterial compound ethambutol [Emb; dextro-2,2′-(ethylenediimino)-di-1-butanol] is used to treat tuberculosis as well as disseminated infections caused by *Mycobacterium avium*. The critical target for Emb lies in the pathway for the biosynthesis of cell wall arabinogalactan, but the molecular mechanisms for drug action and resistance are unknown. The cellular target for Emb was sought using drug resistance, via target overexpression by a plasmid vector, as a selection tool. This strategy led to the cloning of the *M. avium* emb region which rendered the otherwise susceptible *M. smegmatis* host resistant to Emb. This region contains three complete open reading frames (ORFs), embR, embA, and embB. The translationally coupled embA and embB genes are necessary and sufficient for an Emb-resistant phenotype which depends on gene copy number, and their putative novel membrane proteins are homologous to each other. The predicted protein encoded by embR, which is related to known transcriptional activators from *Streptomyces*, is expendable for the phenotypic expression of Emb resistance, but an intact divergent promoter region between embR and embAB is required. An Emb-sensitive cell-free assay for arabinan biosynthesis shows that overexpression of embAB is associated with high-level Emb-resistant arabinosyl transferase activity, and that embR appears to modulate the *in vitro* level of this activity. These data suggest that embAB encode the drug target of Emb, the arabinosyl transferase responsible for the polymerization of arabinose into the arabinan of arabinogalactan, and that overproduction of this Emb-sensitive target leads to Emb resistance.—Authors' Abstract

Chakrabarti, A., Bhattacharya, C. P., Acharya, D. P., Chakrabarty, A. N., Ghosh, K. and Dastidar, S. G. *In vitro* and *in vivo* experimental susceptibility of *Mycobacterium marinum* to Augmentin. Indian J. Med. Res. **104** (1996) 281–283.

The effect of Augmentin alone and in combination with various beta-lactam antibiotics was studied against a pathogenic mycobacterium, *Mycobacterium marinum*. The *in vitro* studies did not reveal any additional advantage over that found with Augmentin alone and this antibiotic seemed considerably inhibitory to *M. marinum* at <1 μg/ml concentration. *In vivo*, the effects of Augmentin on experimentally produced

lesions in the mouse foot pads (MFPs) showed a significant regression of the lesions, which was compatible with an early disappearance of *M. marinum* from the MFP, in contrast with those of the untreated, control animals.—Authors' Abstract

Chan, J., Tian, Y., Tanaka, K. E., Tsang, M. S., Yu, K. M., Salgame, P., Carroll, D., Kress, Y., Teitelbaum, R. and Bloom, B. R. Effects of protein calorie malnutrition on tuberculosis in mice. Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 14857–14861.

Infectious diseases and malnutrition represent major burdens afflicting millions of people in developing countries. Both conditions affect individuals in industrialized nations, particularly the aged, the HIV-infected, and people with chronic diseases. While malnutrition is known to induce a state of immunodeficiency, the mechanisms responsible for compromised antimicrobial resistance in malnourished hosts remain obscured. In the present study, mice fed a 2% protein diet and developing protein calorie malnutrition, in contrast to cell-nourished controls receiving a 20% protein diet, rapidly succumbed to infection with *Mycobacterium tuberculosis*. Malnourished mice exhibited a tissue-specific-diminution in the expression of interferon gamma, tumor necrosis factor alpha and the inducible form of nitric oxide synthase in the lungs, but not the liver. The expression of these molecules critical to the production of mycobactericidal nitrogen oxides was depressed in malnourished animals in the lungs specifically at early times (<14 days) after infection. At later times, levels of expression became comparable to those in well-nourished controls, although the bacillary burden in the malnourished animals continued to rise. Nevertheless, urinary and serum nitrate contents, an index of total nitric oxide (NO) production *in vivo*, were not detectably diminished in malnourished, mycobacteria-infected mice. In contrast to the selective and early reduction of lymphokines and the inducible form of nitric oxide synthase in the lung, a marked diminution of the granulomatous reaction was observed in malnourished mice throughout the entire course of infection in all tissues examined (lungs, liver, and spleen). Remarkably, the progressively fatal course of tuberculosis observed in the malnourished mice could be reversed by restoring a full protein (20%) diet. The results indicate that protein calorie malnutrition selectively compromises several components of the cellular-immune response that are important for containing and restricting tuberculous infection, and suggest that malnutrition-induced susceptibility to some infectious diseases can be reversed or ameliorated by nutritional intervention.—Authors' Abstract

Clemens, D. L. and Horwitz, M. A. The *Mycobacterium tuberculosis* phagosome interacts with early endosomes and is accessible to exogenously administered transferrin. J. Exp. Med. **184** (1996) 1349–1355.

Previous studies have demonstrated that the *Mycobacterium tuberculosis* phagosome in human monocyte-derived macrophages acquires markers of early and late endosomes, but direct evidence of interaction of the *M. tuberculosis* phagosome with the endosomal compartment has been lacking. Using the cryosection immunogold technique, we have found that the *M. tuberculosis* phagosome acquires exogenously added transferrin in a time-dependent fashion. Near-maximal acquisition of transferrin occurs within 15 min, kinetics of acquisition consistent with interaction of the *M. tuberculosis* phagosome with early endosomes. Transferrin is chased out of the *M. tuberculosis* phagosome by incubation of the infected macrophages in culture medium lacking human transferrin. Phagosomes containing latex beads or heat-killed *M. tuberculosis*, on the other hand, do not acquire staining for transferrin. These and other findings demonstrate that *M. tuberculosis* arrests the maturation of its phagosome at a stage which the phagosome interacts with early and late endosomes, but not with lysosomes. The transferrin endocytic pathway potentially provides a novel route for targeting antimicrobials to the *M. tuberculosis* phagosome.—Authors' Abstract

Coleman, M. D., Thorpe, S., Lewis, S., Buck, N. S., Perris, A. D. and Seydel, J. K. Preliminary evaluation of the toxicity and efficacy of novel 2,4-diamino-5-benzylpyrimidine-sulphone derivatives using rat and human tissue *in vitro*. Environ. Toxicol. Pharmacol. **2** (1996) 389–395.

Four novel combined dapsone and trimethoprim analogs, K-120, K-150, K-138 and DRS-506, have been compared with dapsone in their methemoglobin forming abilities as well as their anti-inflammatory properties using rat and human tissues *in vitro*. All four compounds formed consistently less methemoglobin compared with dapsone in both the rat and human microsomes. Using human microsomes from five livers, K-120 was significantly less toxic than the other analogs in three of the five livers ($p < 0.01$). DRS-506 and K-138 both inhibited the human neutrophil respiratory burst to a significantly greater degree compared with dapsone at 0.5 mM ($p < 0.01$), while K-120 and K-150 showed no significant effect at 0.5 mM. At 1 mM, DRS-506, K-120 and K-138 were more potent than dapsone ($p < 0.01$), although K-150 appeared to increase the neutrophil activation. All four analogs caused a significant reduction in neutrophil adhesion to human umbilical vein cells at 0.1 mM. In view of its efficacy and low toxicity, K-120 shows considerable promise for future clinical evaluation.—Authors' Abstract

Cywes, C., Godenir, N. L., Hoppe, H. C., Scholle, R. R., Steyn, L. M., Kirsch, R. E. and Ehlers, M. R. W. Nonopsonic bindng of *Mycobacterium tuberculosis* to human complement receptor type 3 expressed in Chinese hamster ovary cells. Infect. Immun. **64** (1996) 5373–5383.

Nonopsonic invasion of mononuclear phagocytes by *Mycobacterium tuberculosis* is likely important in the establishment of a primary infection in the lung. *M. tuberculosis* binds to a variety of phagocyte receptors, of which the mannose receptor and complement receptor type 3 (CR3) may support nonopsonic binding. CR3, a beta(2) integrin, is a target for diverse intracellular pathogens, but its role in nonopsonic binding remains uncertain. We have examined the binding of *M. tuberculosis* H37Rv to human CR3 heterologously expressed in Chinese hamster ovary (CHO) cells, thereby circumventing the problems of competing receptors and endogenously synthesized complement, which are inherent in studies with mononuclear phagocytes. The surface expression of CD11b and CD18 was assessed by immunofluorescence, immunobead binding, flow cytometry, and immunoprecipitation with anti-CD11b and anti-CD18 monoclonal antibodies (MAbs). The functional activity of the surface-expressed CD11b/CD18 (CR3) heterodimer was confirmed by resetting with C3bi-coated microspheres. We found that *M. tuberculosis* bound four- to fivefold more avidly to CR3-expressing CHO cells than to wild-type cells and, importantly, that this binding was at similar levels in the presence of fresh or heat-inactivated human or bovine serum or no serum. In contrast, *M. smegmatis* bound poorly to CR3-expressing CHO cells in the absence of serum, but after opsonization in serum, binding was comparable to that of *M. tuberculosis*. The binding of *M. tuberculosis* to the transfected CHO cells was CR3 specific, as it was inhibited by anti-CR3 MAbs, particularly the anti-CD11b MAbs LM2/1 (I domain epitope) and OKM1 (C-terminal epitope), neither of which inhibit C3bi binding. MAb 2LPM19c, which recognizes the C3bi-binding site on CD11b, had little or no effect on *M. tuberculosis* binding. The converse was found for the binding of opsonized *M. smegmatis*, which was strongly inhibited by 2LPM19c but unaffected by LM2/1 or OKM1. CR3-specific binding was also evidence by the failure of *M. tuberculosis* to bind to CHO cells transfected with an irrelevant surface protein (angiotensin-converting enzyme) in the presence or absence of serum. We conclude that the binding of *M. tuberculosis* H37Rv to CR3 expressed in CHO cells is predominantly nonopsonic and that the organism likely expresses a ligand that binds directly to CR3.—Authors' Abstract

Griffith, D. E., Brown, B. A., Girard, W. M., Murphy, D. T. and Wallace, R. J. Azithromycin activity against *Mycobacterium avium* complex lung disease in pa-

tients who were not infected with human immunodeficiency virus. Clin. Infect. Dis. **23** (1996) 983–989.

We initiated a prospective trial of an azithromycin-containing regimen for the treatment of human immunodeficiency virus-negative patients with *Mycobacterium avium* complex (MAC) lung disease; the initial 4 months of therapy were with azithromycin (600 mg/d) alone. The primary study endpoint was microbiological response measured at 4 and 6 months of therapy. Of 29 patients enrolled in the study, 23 completed therapy. Fifty-two percent of these 23 patients were male, and 65% were smokers. All 23 patients were older than 45 years of age; 83% had bilateral disease, and 48% had fibrocavitary disease. Macrolide (clarithromycin)-susceptible MAC isolates were recovered from these 23 patients before treatment. Cultures of sputum from 38% of these patients became negative, and the positivity of cultures of sputum from 76% of these patients was significantly reduced. Sixty-eight percent of sputum cultures were strongly positive (>200 colonies) before therapy, while only 27% were strongly positive after therapy. Although most patients continued to receive 600 mg of azithromycin/d, the high incidence of gastrointestinal side effects (76%) and altered hearing (41%) suggests the need for lower or less frequent dosing. Macrolide (clarithromycin) resistance did not develop in any MAC isolates during monotherapy. These results, which demonstrate that azithromycin is active against MAC pulmonary disease, provide a rationale to include this drug in the initial multidrug regimens recommended for the treatment of this disease.—Authors' Abstract

Haque, M. A., Yoshino, S., Inada, S., Nomaguchi, H., Tokunaga, O. and Kohashi, O. Suppression of adjuvant arthritis in rats by induction of oral tolerance to mycobacterial 65-kDa heat shock protein. Eur. J. Immunol. **26** (1996) 2650–2656.

Oral administration of mycobacterial 65-kDa heat-shock protein (hsp) given daily for 5 days prior to immunization with *Mycobacterium tuberculosis* (Mt) suppressed the development of adjuvant arthritis (AA) in rats. AA was significantly suppressed by 30 and 300 μg hsp and variably by 0.3, 3 μg or 1 mg. Histological analysis of joint samples obtained from control and test rats confirmed the suppression of AA in the fed group. Feeding Mt or hen egg lysozyme (HEL) failed to affect AA, indicating that the suppression was hsp specific. The oral administration of 30 μg hsp decreased both delayed-type hypersensitivity (DTH) reactions and proliferative responses to hsp and Mt. In addition, the proliferation of lymph node cells (LNC) from Mt-sensitized rats was inhibited by the addition of spleen cells (SPC) from hsp-fed animals, possibly by the secretion of transforming growth factor (TGF)-beta. Spleen cells obtained from tolerized donors were capable of transferring the tolerance to naive recipients. These results demonstrate that feeding hsp is an effective way to suppress AA and that the suppression of AA may be mediated by regulatory T cells generated following oral administration of mycobacterial 65-kDa hsp.—Authors' Abstract

Keane, J., Balcewicz Sablinska, M. K., Remold, H. G., Chupp, G. L., Meek, B. B., Fenton, M. J. and Kornfeld, H. Infection by *Mycobacterium tuberculosis* promotes human alveolar macrophage apoptosis. Infect. Immun. **65** (1996) 298–304.

The effect of *Mycobacterium tuberculosis* infection on the viability of healthy (control) human alveolar macrophages was evaluated by staining with ethidium homodimer and calcein to discriminate live from dead cells. Infection with *M. tuberculosis* H37Ra or H37Rv increased macrophage mortality at 6 days from the control level of 3.8% ± 0.7% to 28.7% ± 6.9% or 12.6% ± 3.1%, respectively ($p < 0.001$ for comparisons of all conditions). A role for tumor necrosis factor-alpha (TNF-alpha) in the *M. tuberculosis*-induced cytolysis of alveolar macrophages was demonstrated by increased cytotoxicity following the addition of exogenous TNF-alpha to the cultures and by enhancement of macrophage survival when *M. tuberculosis*-infected alveolar macrophages were treated with pentoxifylline or anti-TNF-alpha antibody. The cytolytic mechanism was determined to be apoptosis by the demonstration of a characteristic internucleosomal ladder of genomic

DNA by agarose gel electrophoresis, by finding nuclear fragmentation and condensation by electron microscopy, and by *in situ* terminal transferase-mediated nick end labeling of fragmented DNA in alveolar macrophages infected with *M. tuberculosis in vitro.* The latter technique was employed to reveal extensive apoptosis within caseating granulomas from lung tissue samples from clinical tuberculosis cases. The induction of apoptosis in alveolar macrophages by *M. tuberculosis* may play a role in the macrophage-pathogen interaction of tuberculosis *in vivo.*—Authors' Abstract

Kelly, B. P., Furney, S. K., Jessen, M. T. and Orme, I. M. Low-dose aerosol infection model for testing drugs for efficacy against *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **40** (1996) 2809–2812.

As a paradigm for chronic infectious diseases, tuberculosis exhibits a variety of clinical presentations, ranging from primary pulmonary tuberculosis to reactivation tuberculosis and cavitary disease. To date, the animal models used in evaluating chemotherapy of tuberculosis have been high-dose intravenous models that mimic the disseminated forms of the disease. In the present study, we have used a low-dose aerosol exposure model which we feel better reflects newly diagnosed tuberculosis in patients converting to tuberculin positivity. As appropriate examples of chemotherapy, four rifamycins (rifampin, rifabutin, rifapentine, and KRM-1648) were tested, first in an *in vitro* murine macrophage model and then in the low-dose aerosol infection model, for their activity against *Mycobacterium tuberculosis.* In both models, KRM-1648 had the highest level of activity of the four compounds. In the infected-lung model, rifabutin, rifapentine, and KRM-1648 all had sterilizing activity when given orally at 5 mg/kg of body weight per day. When given at 2.5 mg/kg/day, KRM-1648 had the highest level of activity of the four drugs, reducing the bacterial load by 2.7 logs over 35 days of therapy.—Authors' Abstract

Klopman, G., Fercu, D., Renau, T. E. and Jacobs, M. R. N-1-tert- butyl-substituted quinolones: *in vitro* anti-*Mycobacterium avium* activities and structure-activity relationship studies. Antimicrob. Agents Chemother. **40** (1996) 2637–2643.

We determined the MICs of 63 quinolones against 14 selected reference and clinical strains of the *Mycobacterium avium-M. intracellulare* complex. Sixty-one of the compounds were selected from the quinolone library at Parke-Davis, Ann Arbor, Michigan, U.S.A., including N-1-tert-butyl-substituted agents. T 3761 and tosufloxacin were also tested. The activities of all 63 compounds were compared with those of ciprofloxacin and sparfloxacin. The results showed 45 of the quinolones to be active against the *M. avium-M. intracellulare* complex, with MICs at which 50% of the strains were inhibited (MIC(50)s) of less than 32 μg/ml. Twenty-four of these quinolones had activities equivalent to or greater than that of ciprofloxacin, and nine of them had activities equivalent to or greater than that of sparfloxacin. The most active compounds were the N-1-tert-butyl-substituted quinolones, PD 161315 and PD 161314, with MIC(50)s of 0.25 μg/ml and MIC(50)s of 1 μg/ml; comparable values for ciprofloxacin were 2 and 4 μg/ml, respectively, while for sparfloxacin they were 1 and 2 μg/ml, respectively. The next most active compounds, with MIC(50)s of 0.5 μg/ml and MIC(50)s of 1 μg/ml, were the N-1-cyclopropyl-substituted quinolones, PD 138926 and PD 158804. These values show that the tert-butyl substituent is at least as good as cyclopropyl in rendering high levels of antimycobacterial activity. However, none of the quinolones showed activity against cyprofloxacin-resistant laboratory-derived *M. avium-M. intracellulare* complex strains. A MULTICASE program-based structure-activity relationship analysis of the inhibitory activities of these 63 quinolones and 109 quinolones previously studied against the most resistant clinical strain of *M. avium* was also performed and led to the identification of two major biophores and two biophobes.—Authors' Abstract

Li, B. W., Rossman, M. D., Imir, T., Oner Eyuboglu, A. F., Lee, C. W., Biancaniello, R. and Carding, S. R. Disease-specific changes in gamma delta T cell

repertoire and function in patients with pulmonary tuberculosis. J. Immunol. **157** (1996) 4222–4229.

Although gamma delta T cells are known to contain the highest frequency of mycobacteria-reactive cells in humans and numerous studies have suggested that they play an important role in the initial immune response to *Mycobacterium tuberculosis* (Mtb), very few studies have attempted to analyze these cells in patients with active pulmonary tuberculosis. The aim of the present study was, therefore, to evaluate the consequences of infection on the number and activity of mycobacteria-reactive gamma delta T cells. Three-color flow cytometric analysis of blood and bronchoalveolar lavage gamma delta T cells of patients diagnosed with active pulmonary tuberculosis showed that compared with normal healthy subjects and patients with the unrelated pulmonary granulomatous diseases sarcoidosis and berylliosis the size of the mycobacteria-reactive V gamma 9^+/V delta 2^+ gamma delta T cell subset in both the blood and lung was dramatically reduced. In addition, the V gamma 9^+/V delta 2^+ cells left intact in patients with tuberculosis were refractory to *in vitro* stimulation by Mtb antigens, which are potent stimuli for these cells in normal subjects. Our results demonstrate for the first time a strong correlation between the absence or loss of the major V gamma 9^+/V delta 2^+ Mtb-reactive subset of gamma delta T cells and manifestations of disease, consistent with the hypothesis that these gamma delta T cells play a role in the protective immune response to Mtb infection.—Authors' Abstract

Mazzaccaro, R. J., Gedde, M., Jensen, E. R., van Santen, H. M., Ploegh, H. L., Rock, K. L. and Bloom, B. R. Major histocompatibility class I presentation of soluble antigen facilitated by *Mycobacterium tuberculosis* infection. Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 11786–11791.

Cell-mediated immune responses are essential for protection against many intracellular pathogens. For *Mycobacterium tuberculosis* (MTB), protection requires the activity of T cells that recognize antigens presented in the context of both major histocompatibility complex (MHC) class II and I molecules. Since MHC class I presentation generally requires antigen to be localized to the cytoplasmic compartment of antigen-presenting cells, it remains unclear how pathogens that reside primarily within endocytic vesicles of infected macrophages, such as MTB, can elicit specific MHC class I-restricted T cells. A mechanism is described for virulent MTB that allows soluble antigens ordinarily unable to enter the cytoplasm, such as ovalbumin, to be presented through the MHC class I pathway to T cells. The mechanism is selective for MHC class I presentation, since MTB infection inhibited MHC class II presentation of ovalbumin. The MHC class I presentation requires the tubercle bacilli to be viable, and it is dependent upon the transporter associated with antigen processing (TAP), which translocates antigenic peptides from the cytoplasm into the endoplasmic reticulum. The process is mimicked by *Listeria monocytogenes* and soluble listeriolysin, a pore-forming hemolysin derived from it, suggesting that virulent MTB may have evolved a comparable mechanism that allows molecules in a vacuolar compartment to enter the cytoplasmic presentation pathway for the generation of protective MHC class I-restricted T cells.—Authors' Abstract

Mukherjee, T., Basu, O., Mahapatra, S., Goffin, C., van Beeumen, J. and Basu, J. Biochemical characterization of the 49 kDa penicillin-binding protein of *Mycobacterium smegmatis*. Biochem. J. **320** (1996) 197–200.

The 49-kDa penicillin-binding protein (PBP) of *Mycobacterium smegmatis* catalyzes the hydrolysis of the peptide or S-ester bond of carbonyl donors R(1)-CONH-CHR(2)-COX-CHR(3)-COO- (where X is NH or S). In the presence of a suitable amino acceptor, the reaction partitions between the transpeptidation and hydrolysis pathways, with the amino acceptor behaving as a simple alternative nucleophile at the level of the acyl-enzyme. By virtue of its N-terminal sequence similarity, the 49-kDa PBP represents one of the class of monofunctional low-molecular-mass PBPs.

An immunologically related protein of M(r) 52,000 is present in *M. tuberculosis*. The 49-kDa PBP is sensitive toward amoxycillin, imipenem, flomoxef and cefoxitin.—Authors' Summary

Norton, G. R., Sweeney, J., Marriott, D., Law, M. G. and Brew, B. J. Association between HIV distal symmetric polyneuropathy and *Mycobacterium avium* complex infection. J. Neurol. Neurosurg. Psychiatry **61** (1996) 606–609.

Objectives—Pronounced infiltration activated macrophages occurs in peripheral nerves of patients with distal symmetric polyneuropathy (DSPN). *Mycobacterium avium* complex (MAC) is a common facultative intracellular parasite of the macrophage in advanced HIV disease and may induce macrophage activation. Whether MAC disease is associated with DSPN was examined prospectively.

Methods—One-hundred-fifty consecutive patients with HIV infection were assessed for the probability of DSPN. Blood cultures for MAC were performed, independently of neurological assessment, as part of the investigation of unexplained fever, anemia, weight loss, or, less commonly, diarrhea.

Results—There were 20 patients with possible, 14 with probable, and 22 with definite HIV DSPN. Blood cultures for MAC were performed on 80 patients, of whom 39 were positive and 41 negative. The test for trend, when corrected for CD4 count, disclosed a significant association ($p = 0.01$). There was no statistically significant association between DSPN and cytomegalovirus (CMV) disease.

Conclusion—Co-infection of the macrophage by MAC may further activate the HIV-infected macrophage, thereby accelerating the elaboration of neural toxins, or MAC infection of the macrophage itself may lead to the production of neural toxins.—Authors' Abstract

Ozanne, V., Ortalo Magne, A., Vercellone, A., Fournie, J. J. and Daffe, M. Cytometric detection of mycobacterial surface antigens: exposure of mannosyl epitopes and of the arabinan segment of arabinomannans. J. Bacteriol. **178** (1996) 7254–7259.

The physical arrangement of cell envelope components leads to the exposure of selected structural motifs which, in turn, may influence host-parasite interactions. To gain insight into the exposed epitopes, the present study describes a flow cytometric method designed to probe defined molecules on dispersed mycobacteria. The hydrophobic fluorophore N-hexadecanoyl aminofluorescein inserted in the mycobacterial cell envelope permitted focusing of fluorescence-activated cell sorter analysis on cells that were further labeled with defined monoclonal antibodies and fluorochrome-coupled streptavidin. The use of antibodies directed against the lipo-oligosaccharide of *Mycobacterium tuberculosis* demonstrated the specific detection of the antigen on the cell surface of a Canetti-like strain of *M. tuberculosis*, and not on those of mycobacterial strains that were devoid of the glycolipid. Thus, the method was applied to investigate the relative amounts of surface-exposed mannosylated compounds and D-arabinan-containing substances of different strains of the tubercle bacillus and a strain of the rapidly growing nonpathogenic species *M. smegmatis*. Both *M. tuberculosis* and *M. smegmatis* are endowed with mannosyl and arabinan epitopes on their surfaces, although there are many differences in terms of exposed mannosyl epitopes between the various strains of the tubercle bacillus examined. These differences are correlated with the amounts of terminal mannosyl residues that cap the surface-exposed arabinomannans but not with the degrees of virulence of the strains. This novel approach could provide new insights into the distribution of defined surface-exposed antigens and thereby into the architecture of the cell envelopes.—Authors' Abstract

Quiros, E., Maroto, M. C., Bettinardi, A., Gonzalez, I. and Piedrola, G. Diagnosis of cutaneous tuberculosis in biopsy specimens by PCR and Southern blotting. J. Clin. Pathol. **49** (1996) 889–891.

Aims—To evaluate the use of a gene amplification and hybridization method for de-

tecting mycobacterial nucleic acid as a possible diagnostic method for cutaneous tuberculosis infection.

Methods—Biopsy specimens from 20 patients with various skin conditions of possible tuberculous etiology were studied. Six patients had ulcerative nodules, seven lupiform lesions, two non-necrotic granulomas, one scrofulous lichen, one impetigo, one erythematosus lesions, one warty lesions, and one suspected tuberculous lipoma. Biopsy specimens were stained using Ziehl-Neelsen stain and cultured in Lowenstein-Jensen medium. DNA was extracted and then amplified by PCR using primers specific for the *Mycobacterium tuberculosis* complex. Specificity was confirmed by Southern blotting.

Results—Of the specimens, 30% were positive for mycobacteria on staining with Ziehl-Neelsen stain, 60% were culture positive and 85% PCR positive. Only 35.2% of specimens were positive with all three techniques. A further 32.5% were both culture and PCR positive. All PCR-negative samples were also negative when cultured or stained with Ziehl-Neelsen stain. Of the PCR-positive specimens, 29.4% were negative when cultured or stained.

Conclusions—PCR, using suitable primers, is an efficient and sensitive method for the diagnosis of cutaneous tuberculosis.—Authors' Summary

Reyes-Teran, G., Sierra Madero, J. G., del Cerro, V. M., Arroyo Figueroa, H., Pasquetti, A., Calva, J. J. and Ruiz Palacios, G. M. Effects of thalidomide on HIV-associated wasting syndrome: a randomized, double-blind, placebo-controlled clinical trial. AIDS **10** (1996) 1501–1507.

Objective: To evaluate the efficacy of thalidomide in treating wasting syndrome in patients with advanced HIV disease, and to assess the effects of thalidomide on circulating CD4+ T cells, and on HIV viral burden in peripheral blood mononuclear cells (PBMC).

Design: Randomized, double-blind placebo-controlled clinical trial.

Setting: Public tertiary care hospital in Mexico City.

Patients: Twenty-eight adults with advanced HIV disease being treated with antiretroviral therapy, and who had received antiretrovirals for at least 6 months, who did not have an active opportunistic infection, and who had 10% weight loss in the previous 6 months.

Interventions: Patients received thalidomide (100 mg by mouth, four times daily) or a matching placebo for the duration of the study (12 weeks).

Main outcome measures: The main clinical endpoint for efficacy of thalidomide was weight gain or no progression of wasting. Secondary endpoints were Karnosfsky performance status, CD4+ cell counts, and HIV viral burden in PBMC.

Results: Both groups were comparable in their baseline status. Therapeutic failure occurred in 10 out of 14 patients from the placebo group and in 3 out of 14 from the thalidomide group ($p = 0.021$). Weight gain occurred in one patient on placebo and in eight given thalidomide. The Karnofsky index was significantly higher by the end of the study in the thalidomide group ($p = 0.003$). Mild and transient somnolence and erythematous macular skin lesions were significantly more common in the thalidomide group. CD4+ T-cell counts and HIV viral burden in PBMC did not change in either group.

Conclusions: Results suggest that thalidomide not only impeded but also reverted the wasting syndrome, preserving the Karnofsky index in patients with advanced HIV disease. Thalidomide, at the dosage used in this study, had no effect on peripheral CD4+ T cells nor on HIV viral burden in PBMC.—Authors' Abstract

Schlesinger, L. S., Kaufman, T. M., Iyer, S., Hull, S. R. and Marchiando, L. K. Differences in mannose receptor-mediated uptake of lipoarabinomannan from virulent and attenuated strains of *Mycobacterium tuberculosis* by human macrophages. J. Immunol. **157** (1996) 4568–4575.

Phagocytosis of the virulent Erdman and H37Rv strains of *Mycobacterium tuberculosis*, but not that of the attenuated H37Ra strain, by human macrophages is mediated

by the mannose receptor (MR) in addition to complement receptors. We have recently determined that a major capsular lipoglycan, lipoarabinomannan (LAM), from the Erdman strain serves as a ligand for the MR during phagocytosis of bacteria. In this study we directly compare uptake of Erdman, H37Rv, and H37Ra LAM by human macrophages and assess the relative contribution of the MR in this process. Microspheres coated with LAM served as model phagocytic particles for studies of LAM as a capsular ligand. Uptake (37°C) of LAM microspheres by monocyte-derived macrophages was greatest for Erdman LAM and intermediate for H37Rv and H37Ra LAM compared with that of buffer microspheres or microspheres coated with LAM from a nontuberculosis strain of mycobacterium (AraLAM). Inhibition of microsphere uptake in the presence of mannan or mannose-BSA was highest for Erdman LAM (75 ± 8 and 50 ± 7%, respectively) and H37Rv LAM (57 ± 13 and 21 ± 5%, respectively) relative to H37Ra LAM (36 ± 16 and 22 ± 11%, respectively). Inhibition of microsphere uptake in the presence of anti-MR Ab followed a similar pattern: Erdman LAM (80 ± 9%) > H37Rv LAM (53 ± 1%) > H37Ra LAM (26 ± 12%). Attachment (4°C) of microspheres coated with Erdman LAM, H37Rv LAM, and H37Ra LAM was enhanced 12-, 5-, and 4-fold, respectively, compared with that of microspheres coated with AraLAM, and mannose-BSA inhibited attachment of these microspheres by 82 ± 7, 69 ± 8, and 12 ± 17%. Galactose-BSA did not inhibit attachment of any LAM microsphere groups. Chromatographic analyses of mild acid hydrolysates of LAM from Erdman, H37Rv, and H37Ra all revealed the major terminal dimannosyl units. These studies demonstrate differences in the ability of LAM from different *M. tuberculosis* strains to mediate adherence to macrophages and to serve as ligands for the macrophage MR despite the presence of terminal dimannosyl units. Thus, these studies point toward other subtle structural alterations in LAM among strains that influence initial interactions with human phagocytes.—Authors' Abstract

Senaldi, G., Yin, S. M., Shaklee, C. L., Piguet, P. F., Mak, T. W. and Ulich, T. R. *Corynebacterium parvum*- and *Mycobacterium bovis* bacillus Calmette-Guerin-induced granuloma formation is inhibited in TNF receptor I (TNF-RI) knockout mice and by treatment with soluble TNF-RI. J. Immunol. **157** (1996) 5022–5026.

The aim of this study was to examine the role of tumor necrosis factor receptor I (TNF-RI) in the pathogenesis of heat-killed *Corynebacterium parvum*- and live bacillus Calmette-Guerin (BCG)-induced granulomas. Granuloma formation was analyzed in TNF-RI knockout mice and after treatment with soluble TNF-RI (sTNF-RI). TNF-RI knockout mice injected with *C. parvum* or ECG developed fewer and smaller granulomas than wild-type control mice. Mice treated with sTNF-RI from days 7 to 13 after injection of *C. parvum* or BCG developed fewer and smaller granulomas than saline-treated control mice. Established granulomas regressed in rats treated with sTNF-RI from days 10 to 13 after injection of *C. parvum*. In conclusion, TNF signaling via TNF-RI contributes to the pathogenesis of *C. parvum*- and BCG-induced granulomas. STNF-RI inhibits the development of granulomas and can cause the regression of established granulomas.—Authors' Abstract

Shirakawa, T., Enomoto, T., Shimazu, S. and Hopkin, J. M. The inverse association between tuberculin responses and atopic disorder. Science **275** (1997) 77–79.

Human immune responses are heterogeneous and may involve antagonism between T-helper (T-H) lymphocyte subsets and their cytokines. Atopy is characterized by immediate immunoglobulin E (IgE)-mediated hypersensitivity to agents such as dust mites and pollen, and it underlies the increasingly prevalent disorder asthma. Among Japanese school children, there was a strong inverse association between delayed hypersensitivity to *Mycobacterium tuberculosis* and atopy. Positive tuberculin responses predicted a lower incidence of asthma, lower serum IgE levels, and cytokine profiles biased toward T-H 1 type. Exposure and response to *M. tuberculosis* may, by modification of immune profiles, inhibit atopic disorder.—Authors' Abstract

Smith, D. M., Torres, R. D. and Stephens, T. D. Mesonephros has a role in limb development and is related to thalidomide embryopathy. Teratology **54** (1996) 126–134.

Recent studies have demonstrated a link between limb reduction defects and mesonephros removal. However, there is some question as to whether the limb-reduction defects seen in that study resulted from the removal of mesonephros or from the formation of scar tissue medial to the limb territory. The current study was conducted to test the hypothesis that elimination of the mesonephros without producing scar tissue adjacent to the limb will adversely affect limb morphogenesis. The hypothesis was tested by the insertion of tantalum foil barriers into various levels of the intermediate mesoderm of developing chick embryos to prevent the caudal elongation of the mesonephros. Limb reduction defects were obtained when the mesonephros was prevented from forming caudal to somite 14. No limb defects were seen when a foil barrier was placed into the intermediate mesoderm at the level of somite 21 or 25. Our results support the notion that a signal from the mesonephros is necessary for normal limb development. In addition, it appears that a craniocaudal factor emanating from the mesonephros plays a role in limb development. The limb reduction defects obtained in this study were also compared to the pattern of thalidomide embryopathy in humans. There is a close correspondence between the types of limb reduction anomalies seen with thalidomide and mesonephric blocks and between the severity of defects vs. the timing of thalidomide intake on mesonephric blockage. A model for possible thalidomide embryopathy is presented.—Authors' Abstract

Thangaraj, H. S., Bull, T. J., DeSmet, K. A. L., Hill, M. K., Rouse, D. A., Moreno, C. and Ivanyi, J. Duplication of genes encoding the immunodominant 38 kDa antigen in *Mycobacterium intracellulare.* FEMS Microbiol. Lett **144** (1996) 235–240.

Mycobacterium avium is a causative agent of mycobacterioses in systemically immunocompromised individuals; whereas *M. intracellulare* is responsible for causing infections in relatively immunocompetent hosts. In an attempt to identify components that could be involved in virulence, we characterized the 38-kDa-encoding gene of *M. intracellulare* that is absent in *M. avium.* This antigen crossreacts immunologically with a major 38-kDa antigen of *M. tuberculosis,* and both antigens are homologs of the phosphate transport subunit S (PstS) of the pst complex of *Escherichia coli.* Unlike the *M. tuberculosis* complex, the *M. intracellulare* coding gene was found to be duplicated. We also identified and characterized other pst genes that may constitute an operon. Considering that multiple isoforms of PstS are present in mycobacteria the possible role of pstS1 genes for pathogenesis is discussed.—Authors' Abstract

Tseng, S., Pak, G., Washenik, K., Pomeranz, M. K. and Shupack, J. L. Rediscovering thalidomide: a review of its mechanism of action, side effects, and potential uses. J. Am. Acad. Dermatol. **35** (1996) 969–979.

Thalidomide, a hypnosedative drug introduced in the 1950s, has been used in a variety of dermatologic conditions during the past few decades. Although originally withdrawn from the world market on discovery of its teratogenic effect, it has since been selectively reintroduced for use in various disorders thought to have an autoimmune or inflammatory basis. A review of the literature focused on clinical uses of thalidomide in the treatment of dermatologic diseases was performed. Diseases for which thalidomide has been found effective include erythema nodosum leprosum, prurigo nodularis, actinic prurigo, discoid lupus erythematosus, aphthous stomatitis, Behcet's syndrome, and graft-versus-host disease. Side effects such as teratogenicity and peripheral neuropathy remain its limiting factor. Thalidomide is a useful addition to the therapeutic armamentarium for treatment-resistant dermatoses as long as proper vigilance for adverse effects is maintained.—Authors' Abstract

Wards, B. J. and Collins, D. M. Electroporation at elevated temperatures substantially improves transformation effi-

ciency of slow-growing mycobacteria. FEMS Microbiol. Lett. **145** (1996) 101–105.

The effects of electroporation temperature, biochemical pretreatment of cells and stage of culture on electroporation efficiency for slow-growing mycobacteria were investigated. The efficiency of transformation into *Mycobacterium tuberculosis, M. bovis* and *M. intracellulare* increased markedly with temperature. In contrast, the efficiency of transformation into *M. smegmatis,* a fast-growing species, was higher at 0°C and decreased with temperature. While stage of culture had little effect, a further increase in efficiency of 2–4-fold was obtained following glycine or ethionamide pretreatment. Electroporation at 37°C has been chosen as a standard condition for slow-growing species as it usually resulted in a transformation efficiency several orders of magnitude higher than that obtained at 0°C.—Authors' Abstract

Yuan, Y. and Barry, C. R. A common mechanism for the biosynthesis of methoxy and cyclopropyl mycolic acids in *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 12828–12833.

Mycobacterium tuberculosis produces three classes of mycolic acids that differ primarily in the presence and nature of oxygen-containing substituents in the distal portion of the meromycolate branch. The methoxymycolate series has a methoxy group adjacent to a methyl branch, in addition to a cyclopropane in the proximal position. Using the gene for the enzyme that introduces the distal cyclopropane (Cma1) as a probe, we have cloned and sequenced a cluster of genes coding for four highly homologous methyl transferases (mmal-4). When introduced into *M. smegmatis,* this gene cluster conferred the ability to synthesize methoxymycolates. By determining the structure of the mycolic acids produced following expression of each of these genes individually and in combination, we have elucidated the biosynthetic steps responsible for the production of the major series of methoxymycolates. The mma4 gene product (MMAS-4) catalyzes an unusual S-adenosyl-L-methionine-dependent transformation of the distal cis-olefin into a secondary alcohol with an adjacent methyl branch. MMAS-3 O-methylates this secondary alcohol to form the corresponding methyl ether, and MMAS-2 introduces a cis-cyclopropane in the proximal position of the methoxy series. The similarity of these reactions and the enzymes that catalyze them suggest that some of the structural diversity of mycolic acids results from different chemical fates of a common cationic intermediate, which in turn results from methyl group addition to an olefinic mycolate precursor.—Authors' Abstract