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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

Frankel, E. H. and Teng, B. P. A case of tuberculoid leprosy in Rhode Island. Rhode Island Med. J. **73** (1990) 623–625.

With the influx of people entering the United States from India, Southeast Asia and Central Africa, it is likely that future cases of leprosy will appear in Rhode Island.—Authors' Abstract

Go-Estrada, K. L. Pattern of leprosy: STUH experience. Santo Tomas J. Med. **38** (1989) 179–184.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that principally affects the skin, peripheral nerves and mucus membranes of the upper respiratory tract. Data on the epidemiological status of the disease at the Santo Tomas University Hospital Out Patient Department were collected from January 1981 to December 1988. A total of 344 diagnosed cases of Hansen's disease were included in this study. The disease was found commonly among adults with the predominance of males. It occurred frequently in the age group of 20–29 years and in most cases, the age of onset was also within this period. Most lesions were noted initially in the extremities, particularly the extensors, and a large percentage of these patients sought consult within 1 year of onset of symptoms. Noninfectious cases belonging to the tuberculoid and borderline tuberculoid groups were reported more frequently than the infectious groups of midborderline, borderline lepromatous and lepromatous leprosy. Hansen's disease, therefore, presents a wide spectrum of clinical manifestations. Present concepts regarding the disease in The Philippines range from physical factors to traditional folklore involving supernatural influences. These beliefs should be countered by accurate and effective health education to significantly decrease the physical and social disability brought about by the disease to the society.—Author's Abstract

Pattyn, S. R. Therapeutic regimens in leprosy. Verh. K. Acad. Geneeskd. Belg. **52** (1990) 301–310.

Insight into the bacteriology, general pathology, and pharmacology of the treatment of leprosy has increased most significantly during the last 30 years, through numerous studies, starting with the demonstration by Shepard of the multiplication of *Mycobacterium leprae* in the mouse foot pad. Our studies showed that the treatment of the disease can be considerably shortened. The introduction of more drugs highly bactericidal for *M. leprae,* provided toxicity and antagonism do not constitute a problem, can only improve the results in terms of length of treatment and absence of relapses. Only when the public realizes that leprosy can indeed be treated "like any other disease" will the fear for it decrease, will patients show up in earlier stages of the disease, will transmission and endemicity decline, and will a situation be reached comparable to that of tuberculosis in the western world. In the meantime there is an increasing need for better understanding, prevention, and treatment of the complications of the disease: reversal reactions and erythema nodosum leprosum. In endemic regions there will remain for some time to come a great need for rehabilitation activities.—Author's Summary

Chemotherapy

Blok, L. M., Bloos, L. J. and Van Den Berg, G. A retrospective study on seven years of multiple drug treatment for paucibacillary and multibacillary leprosy, in Bayara General Hospital, Nigeria. Lepr. Rev. **62** (1991) 193–200.

In Bauchi State, Nigeria, a retrospective study was carried out among 973 patients on multidrug therapy (MDT), multibacillary (MB) and paucibacillary (PB), and 118 patients on a dapsone-clofazimine therapy. These patients were registered between January 1983 and September 1989. Clinical results and the problem of defaulting were investigated. The most important conclusions drawn are: although relapses occur, MDT-PB can be a valuable treatment; health education, shorter duration of treatment and permission to come less often lower the default rate, but in spite of this, the distance between home and clinic remains a problem.—Authors' Summary

Cartel, J.-L., Boutin, J.-P., Spiegel, A., Plichart, R. and Roux, J.-F. Longitudinal study on relapses of leprosy in Polynesian multibacillary patients on dapsone monotherapy between 1946 and 1970. Lepr. Rev. **62** (1991) 186–192.

Between 1946 and 1970, 295 new leprosy patients were detected in French Polynesia, of whom 145 were multibacillary. Of these 145 put on dapsone monotherapy, 131 reached bacteriological negativity in a period of time ranging from 2 to 12 years (average 4.72 years) and were followed up for a period of time ranging from 19 to 43 years (median follow-up period after bacteriological negativity: 18 years). Among the 131 patients, 36 relapses were detected, the first one 4 years after bacteriological negativity and the last one 26 years after. The crude relapse rate was 27.5%, the risk of relapse was 1.39 per 100 patient years, and the cumulative relapse probability, calculated using the lifetable method, reached 0.38 ± 11 by year 31 of the study. From these findings one may assume that, at least in French Polynesia, one-third to one-half of multibacillary patients put on dapsone monotherapy would relapse if still present 36 years after bacteriological negativity. Such results re-emphasize the need for leprosy patients to be treated with multidrug therapy as recommended by WHO.—Authors' Summary

Dhople, A. M., Strong, L. C., Meindl, W., Schoenenberger, H. and Gardner G. D. In vitro and in vivo effects of N-methyl-3,5-dichlorobenzylamine hydrochloride on *Mycobacterium leprae.* Arzen. Forschung. **41** (1910) 253–256.

The antimicrobial effects of a new benzylamine, ME-93 (N-methyl-3,5-dichlorobenzylamine hydrochloride), alone and in combination with dapsone and rifampin, have been evaluated *in vitro* in cell-free culture system and *in vivo* in mouse foot pad system. Even at 50 μg/ml, ME-93 did not completely inhibit the *in vitro* growth of *M. leprae,* and the effects were bacteriostatic. However, there was a synergism when ME-93 was combined with rifampin, and the effects were bactericidal. Similar findings were also obtained in the mouse foot pad system. Thus, there is a new drug that needs further attention in the chemotherapy of leprosy.—Authors' Summary

Ekambaram, V. and Rao, M. K. Relapse rate in paucibacillary leprosy patients after multidrug therapy in North Arcot District. Indian J. Lepr. **63** (1991) 34–42.

Surveillance data from 14,227 paucibacillary (PB) patients who had been released from treatment 1 year earlier, after completing multidrug therapy (PB regimen) for 6 to 12 months, were analyzed to assess relapse rates and the influence of three variables: number of lesions, nerve involvement, and duration of treatment. The overall relapse rate at 1 year of surveillance was acceptably low at 0.34%. Relapse rates were about four times higher when there were many (4–9) lesions, or when nerve was involved (0.80% vs 0.20%). Extending the duration of treatment beyond 6 months did not reduce the relapse rates significantly in the high-risk groups. Detection of PB cases early, before these risk factors become op-

erative, and treating them with MDT would appear to be the best strategy to minimize relapse rates.—Authors' Abstract

Gelber, R. H., Sui, P., Tsang, M., Alley, P. and Murray, L. P. Effect of low-level and intermittent minocycline therapy on the growth of *Mycobacterium leprae* in mice. Antimicrob. Agents Chemother. **35** (1991) 992–994.

We evaluated the minimal concentrations of minocycline in the diet and in serum required to inhibit the growth of seven *Mycobacterium leprae* isolates in mice. Minocycline concentrations of 0.01% and 0.04% in the diet, which resulted in levels in serum of ≤ 0.17 and 0.51 μg/ml, respectively, were consistently and completely inhibitory. Even 0.004% dietary minocycline (levels in serum, ≤ 0.08 μg/ml) partially inhibited five of these strains, while 0.001% minocycline was consistently inactive. For five of these isolates, minocycline at a concentration of 0.04% in the diet given 3 days (Monday, Wednesday, Friday) and 1 day weekly completely inhibited the growth of *M. leprae,* and minocycline given even 1 day monthly was partially inhibitory for three of these five *M. leprae* isolates.—Authors' Abstract

Kesava Reddy, P. and Cherian, A. Relapse in leprosy after multidrug therapy and its differential diagnosis with reversal reaction. Indian J. Lepr. **63** (1991) 61–69.

Relapse may be caused either by persisters or through reinfection in a patient released from treatment after MDT. Differentiating relapse from reversal reaction is not always easy, on histological and clinical grounds. A therapeutic trial with steroids for 2–4 weeks can be used to differentiate relapse from reversal reaction occuring in the skin. However, if a patient develops nerve function deficit after release from treatment, it is best to initiate antileprosy treatment along with a long course of steroids.—Authors' Abstract

Pattyn, S. R., Groenen, G., Janssens, L., Kuykens, L., Mputu, L. B. and the Collaborative Study Group for the Treatment of Leprosy in Zaire. A controlled therapeutic trial in paucibacillary leprosy comparing a single dose of rifampicin with a single dose of rifampicin followed by one year of daily dapsone. Lepr. Rev. **62** (1991) 179–185.

The cure rates of two treatment regimens in PB leprosy were compared in a prospective randomized trial: treatment U consisting of a single dose of rifampin 40 mg/K bodyweight, and treatment A of rifampin 1500 mg in a single dose, followed by 1 year of daily dapsone 100 mg. In patients with a bacterial index (BI) = 0, the cure rates evaluated on the basis of histopathology of skin biopsies, were identical for the two regimens but in patients with a BI = 1, cure and relapse rates were unacceptable. For this reason and particularly the need to separate patients on the basis of the BI in skin biopsies, the single dose regimen does not appear to be suited for wide-scale application.—Authors' Summary

Tsutsumi, S. and Gidoh, M. Studies on the development of novel antileprous chemotherapeutics using nude mice with special reference to a new quinolone carboxylic acid, AT-4140. Jpn. J. Lepr. **58** (1989) 250–258.

In order to develop a novel drug for antileprosy chemotherapy, the inhibitory effects of three synthesized compounds, a supplied antituberculous one and three quinolone carboxylic acids, were examined on the growth of leprosy bacilli inoculated into the foot pads of nude mice. Among them, a new quinolone carboxylic acid, AT-4140 whose chemical structure was 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(cis-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, strongly inhibited the growth of leprosy bacilli at doses of 15 and 30 mg/kg; whereas, the effect of ofloxacin used as a positive control was limited at the same doses.—Authors' Summary

Wang, H.-Y., *et al.* [Effects of ofloxacin and minocycline on experimental leprosy.] China Lepr. J. **7** (1991) 17–19. (in Chinese)

A study on experimental chemotherapy of leprosy in nude mice (NIH/nu/nu) in-

fected by *Mycobacterium leprae* is reported. In those who have taken 0.05% ofloxacin and 0.01% minocycline in the diet for 83 days, beginning 90 days after inoculation, the antileprosy actions were observed and compared with that of 0.01% rifampin. The results reveal that the activity against *M. leprae* of 0.05% ofloxacin and 0.01% minocycline were similar to that of 0.01% rifampin. However, in the early period after the stopping of treatment, the antimicrobial activity of 0.01% minocycline was better than that of the others and it is worth further study. With 75 mg or 150 mg of ofloxacin per kg body weight, the normal mice (CFW) infected with *M. leprae* were also tested along with the experiments in the nude mice. The drug was administered by esophageal cannula 5 days weekly. The results showed that 75 mg and 150 mg of ofloxacin have bacteriostatic and highly bactericidal effects, respectively.—Authors' English Abstract

Clinical Sciences

Annichino-Bizzacchi, J. M. and Machado, T. F. G. S. Leprosy and acquired factor VIII inhibitor: a case report. Lepr. Rev. **62** (1991) 155–157.

In this report we describe a case of factor VIII inhibitor appearing in a man with leprosy, with comments on the clinical presentation of the disease, laboratory findings and outcome of the patient.—Authors' Summary

Byrd, S. R. and Gelber, R. H. Effect of dapsone on haemoglobin concentration in patients with leprosy. Lepr. Rev. **62** (1991) 171–178.

Hemolysis and frank anemia from dapsone therapy of leprosy has been long recognized. However, the frequency and severity of this side-effect have not been well documented. We report herein a retrospective analysis of the effect of daily dapsone (generally 100 mg/day) on the hemoglobin concentration of 100 leprosy patients undergoing initial chemotherapy. The average hemoglobin was found to fall significantly by almost 2 g/dl, from 14.25 ± 1.27 g/dl to a nadir of 12.31 ± 1.61 ($p < 0.001$). Eighty-three percent of patients had a fall of hemoglobin concentration of 1 g/dl or more, while in 16% of patients the hemoglobin fell ≥ 3 g/dl. Increasing age was found associated with an increased magnitude of dapsone-related hemolysis ($p \leq 0.004$). Decreasing the daily dose of dapsone was associated with an increased hemoglobin concentration ($p < 0.001\%$). We have concluded that dapsone commonly results in not only hemolysis but a significant decrease in hemoglobin concentration. This may have serious clinical implications, especially in endemic areas, where, owing to nutrition, malaria, and intestinal parasitism, the hemoglobin concentration is already compromised.—Authors' Summary

Foster, R., Sanchez, A., Foulkes, J. and Cameron, L. J. Profile of blood elements in leprosy patients. Indian J. Lepr. **63** (1991) 12–33.

Blood levels of 40 elements in 14 leprosy patients and 5 control subjects living near Mukinge Hospital in the North Western Province of Zambia were determined by spectrophotometry. In patients, compared to controls, serum levels of titanium, silicon, potassium and platinum were significantly higher; red cell levels of phosphorus were lower but those of antimony, bismuth, nickel, titanium, yttrium, silicon and platinum were higher; and whole blood levels of phosphorus, selenium, antimony and silver were lower. There were also significant differences in levels of certain elements when histologically active and inactive patients were compared and between the polar forms of leprosy. The data are consistent with a hypothesis of metabolic and nutritional involvement in the etiology of leprosy.—Authors' Abstract

Hogeweg, M., Kiran, K. U. and Suneetha, S. The significance of facial patches and Type I reaction for the development of facial nerve damage in leprosy. A retro-

spective study among 1226 paucibacillary leprosy patients. Lepr. Rev. **62** (1991) 143–149.

Charts of 1226 paucibacillary leprosy patients, registered between 1982 and 1987 were reviewed for recent facial nerve damage, facial patches, and the presence of type 1 reaction. Twenty-six (2.1%) patients with recent lagophthalmos were identified. In a great majority (85%) patients with recent lagophthalmos showed significant patches over the malar region or around the eye, at the same side as the nerve damage together with clinical signs of type 1 reaction. This combination of significant patches in certain locations and type 1 reaction seems to be a precondition for facial nerve damage. The clinical implication is that a small group of patients may be identified, who are at risk of facial nerve damage. By examining these patients more carefully, it will be possible to detect nerve damage early and to prevent permanent damage of the facial nerve by timely treatment with an appropriate steroid regimen.—Authors' Summary

Jain, V. K., Archana, Seth, S., Chaudhary, S. D. and Lal, H. Gamma-Glutamyl transpeptidase in leprosy. Indian J. Lepr. **63** (1991) 93–96.

Activity of the enzyme gamma-glutamyl transpeptidase (GGTP) was measured in the sera of 20 patients each of paucibacillary and multibacillary leprosy and 20 healthy controls. None of the subjects had any systemic or hepatic disease and none had taken any hepatotoxic or antileprotic drugs in the past 3 months. Mean values in the paucibacillary group (38.62 ± 1.99 U/L) and in the multibacillary group (59.04 ± 3.13 U/L) were significantly higher compared to that in controls (32.04 ± 0.66 U/L). The mean value in the multibacillary group was also significantly higher compared to that in the paucibacillary group.—Authors' Abstract

Jha, P. K., Talwar, S., Suresh, M. S. and Panvelkar, V. Localized borderline lepromatous leprosy. Lepr. Rev. **62** (1991) 212–216.

A 48-year-old soldier presented with three small leprosy lesions localized over the flexor area of the forearm. There was no nerve thickening and clinically the lesions looked like borderline tuberculoid leprosy. However, these lesions demonstrated a bacterial index (BI) of 4+ while no acid-fast bacilli could be demonstrated from any other site of the body. A lepromin test was negative. Histologically, evidence of borderline lepromatous leprosy was conspicuous. The case was diagnosed as localized borderline lepromatous leprosy and treated with multidrug therapy. After 1 year of treatment, the lesions regressed, a lepromin test was positive (5 mm), and the BI from the lesions fell to 1+.—Authors' Summary

Karaçorlu, M. A., Cakiner, T., Sürel, Z., Ersoy, N., Saylan, T. and Sütlas, M. The protective effects of methyl cellulose and conoid shields for lagophthalmos and corneal hypaesthesia in leprosy. Lepr. Rev. **62** (1991) 201–205.

Lagophthalmos and corneal hypesthesia are among the most frequently encountered lesions in leprosy, and they can easily give rise to blindness. Many measures (such as eye drops, protective conoid shields, muscle exercises, surgical treatment, etc.) have been used to protect the eyes under such circumstances, and this paper examines the protective role of methyl cellulose and conoid shields in 41 patients. All of them had lagophthalmos (5 mm or more) and corneal hypesthesia. They were divided into three groups. Group one had 15 leprosy control patients (27 eyes) who did not use methyl cellulose or eye shields. Group two had 16 leprosy patients (28 eyes), and they used methyl cellulose and eye shields when they felt discomfort in their eyes. Group three had 10 leprosy patients (17 eyes), and they used methyl cellulose and eye shields regularly. Statistically significant improvement was seen in group three. Further studies on larger groups of patients including the effects of different concentrations of methyl cellulose, on Schirmer test and tear break up time, may be of value.—Authors' Summary

Kiran, K. U., Hogeweg, M. and Suneetha, S. Treatment of facial nerve damage with lagophthalmos, using a semistandardized

steroid regimen. Lepr. Rev. **62** (1991) 150–154.

Twenty-seven patients with borderline leprosy and facial nerve damage of ≤ 6 months duration (36 eyes) were treated with a semistandardized regimen of steroids (the average starting dose was 25–30 mg, duration 5–6 months) on an outpatient basis. Red and raised reactive patches were usually present in the upper malar area or around the eye(s) in patients with recent lagophthalmos. The lid gap was measured in millimeters during gentle and strong closure. After completion of the steroid course 75% of the eyes had complete closure or only a slight gap of ≤ 2 mm on gentle closure. Steroids were found to be beneficial and safe, in the dosage that we prescribed.—Authors' Summary

Ramesh, V., Saxena, U., Misra, R. S. and Mukherjee, A. Post-kala-azar dermal leishmaniasis: a case report strikingly resembling lepromatous leprosy. Lepr. Rev. **62** (1991) 217–221.

An adult man with post-kala-azar dermal leishmaniasis who had lesions distributed in a manner strikingly similar to lepromatous leprosy is described. He was mistakenly treated with multidrug therapy as recommended by the WHO Expert Committee on leprosy. All investigations including slit-skin smears, histopathology, culture for *Leishmania donovani* and an indirect fluorescent antibody test to confirm post-kala-azar dermal leishmaniasis proved futile. The diagnosis was ultimately based on the previous history of kala-azar, the absence of other disorders which were ruled out by relevant laboratory tests and the good therapeutic response to sodium antimony gluconate. The epidemiological significance of this case and the salient points to distinguish this condition from leprosy are discussed.—Authors' Summary

Roche, P. W., Britton, W. J., Neupane, K. P., Failbus, S. S., Cho, S.-N. and Theuvenet, W. J. The response to chemotherapy of serum *Mycobacterium leprae*-specific antigen in multibacillary leprosy patients. Am. J. Trop. Med. Hyg. **44** (1991) 702–708.

We have examined the *Mycobacterium leprae* phenolic glycolipid-I (PGL-I) antigen levels in the sera of 45 multibacillary leprosy patients commencing chemotherapy. The PGL-I antigen levels correlated with the bacterial and morphological indices, but not with the serum IgM anti-PGL-I antibody levels. Antigen levels were significantly higher in patients with diffuse skin infiltration, but did not vary significantly with other parameters reflecting the duration and extent of untreated disease. The PGL-I antigen levels in 27 patients examined serially decreased consistently over the first year of multidrug therapy.—Authors' Abstract

Satyawan, I., Chin-A-Lien, R. A. M., Vuzevski, V. D. and Naafs, B. Granuloma disciformis chronica et progressiva (Miescher) mimicking tuberculoid leprosy. Int. J. Dermatol. **30** (1991) 445–447.

A 47-year-old caucasian man presented with a more than 15-years history of a skin lesion below his left knee. In 1958 and 1960, he was detached to the Dutch West Indies as a marine. In 1977, he developed a partially anesthetic hypopigmented skin lesion below his left knee. In 1978, the diagnosis of tuberculoid leprosy was made based on the clinical aspect, the loss of sensation, the histopathology, and the positive lepromin test. Treatment was started with dapsone 100 mg daily and later rifampin 600 mg was added. It was noted that clinically the lesion did not improve and the histopathological picture remained the same. Rifampin was discontinued because of side effects. In 1982, therefore, clofazimine 100 mg daily was added and clinically the lesion started to improve. In 1984, the histopathology showed only some fibrosis but no infiltrates. The treatment was discontinued. In 1988, the skin lesion reactivated.

On examination a single well-demarcated hypo- and de-pigmented lesion was seen below the left knee. The periphery of the lesion showed a slight induration which was shiny and erythematous with some telangiectases. The center of the lesion was depigmented, with atrophy. There was anesthesia to light touch, heat, and cold within the lesion. The peripheral nerves were not enlarged.

Histopathological examination of a biopsy taken from the periphery of the lesion showed a normal epidermis with underneath, both in the papular and reticular dermis, a granulomatous infiltrate consisting of lympho- and histiocytes together with Langhans' giant cells. Although the nerves that were present within the granuloma showed some degeneration, there were no lymphocytes present within the nerve bundles. Collagen staining (periodic acid-Schiff stain) did not show much necrobiosis of the collagen, but the elastin staining (van Gieson stain) showed remarkable decrease or complete absence of elastin material in the granulomatous infiltrate. No bacilli were demonstrated using the Wade-Fite stain.

The previous diagnosis of tuberculoid leprosy was rejected and the new diagnoses of granulomatous form of necrobiosis lipoidica (NL) and granuloma disciformis chronica et progressiva (Miescher) (GDCP) were considered. Based on the previous response to clofazimine 100 mg daily, treatment with this drug was restarted and the lesion was observed to resolve.—From the article

Saxena, U., Ramesh, V., Misra, R. S. and Mukherjee, A. Persistent reaction in paucibacillary leprosy: case reports. Lepr. Rev. **62** (1991) 206–211.

Three patients of histopathologically confirmed borderline-tuberculoid leprosy showing no acid-fast bacilli and with lesions confined to the face, 2 on the cheek and 1 on the forehead, were given multidrug therapy as recommended by the WHO for paucibacillary cases. Within 3 months the lesions showed signs of upgrading (or reversal) reaction which was substantiated by histopathology. In 1 patient the facial nerve was affected leading to facial palsy. The lymphocyte transformation test did not show a significant rise. All 3 patients were given oral prednisolone for periods varying between 5 and 7 months, but the response was poor except in 1 patient in whom the facial palsy responded favorably. Injections of sodium antimony gluconate tried in 1 patient after stoppage of steroids did not control the reaction. After 18 months of regular follow-up during therapy, the cutaneous reaction in the patient with facial nerve involvement subsided leaving significant atrophy. However, in the other 2 patients the skin lesion persisted with clinical and histopathological evidence of upgrading reaction. The reasons for the unnatural persistence of reaction in these patients is not clear.—Authors' Summary

Sen, R., Yadav, S. S., Singh, U., Sehgal, P. and Dixit, V. B. Patterns of erythropoeisis and anaemia in leprosy. Lepr. Rev. **62** (1991) 158–170.

A total of 128 leprosy patients were investigated for the morphological type of anemia, the underlying disturbances in iron metabolism and patterns of erythropoiesis and other cytomorphological changes in the bone marrow. The anemia was a mild to moderate degree in paucibacillary (PB) leprosy, while in multibacillary (MB) leprosy it was of a severe degree. Iron deficiency was observed in only a few patients. Impaired iron utilization as observed in anemia of a chronic disorder was a common finding in MB leprosy (41.7%) and more so in new cases (50%). Megaloblastic erythropoiesis was also more frequent in MB leprosy (45.2%) as compared to PB leprosy (16%), accounting for the severe degree of anemia in the former type. In 17.2% of the total patients (MB, 21.4%; PB, 9%) both megaloblastic erythropoiesis and features of impaired iron utilization were observed in bone marrow. Disturbances in iron metabolism and erythropoiesis were also observed but to a lesser degree in patients receiving specific antileprosy treatment. Irrespective of the type of disease and duration of treatment, increasing frequency of acid-fast bacilli (AFB) positivity and granulomas was observed in the bone marrow with an increasing severity of anemia.—Authors' Summary

Sharma, Y., Pahwa, V. K. and Dash, B. M. An unusual presentation of lepromatous leprosy: a case report. Med. J. Armed Forces India **46** (1990) 221–222.

A 23-year-old Indian man presented with several painful and tender keloid plaques over pressure points of knee, elbow and shoulder joints. There were no clinical signs

suggestive of leprosy, and unaffected skin appeared normal. Slit-skin smears from the lesions contained enormous numbers of "lepra bacilli," but a smear from unaffected skin, and others from nasal mucosa, were negative. Biopsy of a lesion showed foam cells and epithelioid cells in dermis. Staining by Fite-Faraco method "showed macrophages containing acid-fast bacilli morphologically resembling *Mycobacterium leprae.*" A diagnosis of an extremely rare variant of histoid leprosy was made.—W. H. Jopling (*Trop. Dis. Bull.*)

Tounkara, A., Fofana, Y., Diabate, N. and Sangared, D. [Anti-HIV seroconversion among patients with leprosy in Mali.] (Letter) Rev. Fr. Transfu. Hemobiol. **33** (1990) 447–450. (in French)

We measured the prevalence of serological positivity toward HIV among three populations in Mali: one group of 105 individuals with tuberculoid leprosy, a group of 105 lepromatous leprosy patients, and a group of 160 blood donors. Screening was done by ELISA and Western blot during the period between October 1988 and April 1989. No significant differences could be demonstrated in the seroprevalence of HIV infections among these three populations.—Translated from Authors' Résumé.

Immuno-Pathology

Accolla, R. S., Auffray, C., Singer, D. S. and Guardiola, J. The molecular biology of MHC genes. Immunol. Today **12** (1991) 97–99.

Antigenic peptides become associated with major histocompatibility complex (MHC) class I and class II surface antigens, are then presented to T cells and thereby elicit an antigen-specific cellular or humoral immune response. MHC molecules are genetically heterogeneous and polymorphic; their structure is therefore relevant to modulation of the immune system. The selective pressure resulting from this modulation is, in turn, the main driving force for the evolution of the complex genetic system. The density of MHC molecules on the cell surface is another parameter that influences immune responsiveness. The study of the evolution and regulation of MHC genes is, therefore, of great interest. These and other themes were discussed at the Third [International Institute of Genetics and Biophysics] Workshop which was recently held in Capri.—Authors' Abstract

Band, H., Panchamoorthy, G., McIean, J., Morita, C. T., Ishikawa, S., Modlin, R. and Brenner, M. B. Recognition of mycobacterial antigens by $\gamma\delta$ T cells. Res. Immunol. **141** (1990) 645–651.

In summary, $\gamma\delta$ T-cell responses elicited by mycobacterial antigens offer a system to examine novel features of T-cell receptor $\gamma\delta$ recognition of antigens and superantigens, and thus should help us move closer to understanding the physiological role(s) of this unique subset of T cells.—From the article

Castells Rodellas, A., Terencio de las Aguas, J., Luelmo Aguilar, J., Roca Miralles, M., Gonzalez Castro, U. and Rodriguez Cano, L. [Immunology of leprosy, 1987–1990.] Rev. Leprol. Fontilles **18** (1991) 19–44 (131 refs.). (in Spanish)

The modern concepts of the immunology of leprosy are reviewed. There are differing immunologic responses on the cellular and human levels in the different clinical forms of the disease and in the two types of lepra reactions. Humoral immune mechanisms are, in general, normal in leprosy although there may be deficits regarding their control. In cellular immune responses there are specific deficiencies which are sometimes total with no granuloma formation in response to *Mycobacterium leprae.* In these cases, the intracellular parasite is well tolerated. The different antigens of *M. leprae* are reviewed. Attempts are made to explain the basis of the immunodeficiency in leprosy, emphasizing the future role of genetic engineering.—RCH

Chatterjee, D., Bozic, C. M., McNeil, M. and Brennan, P. J. Structural features of

the arabinan component of the lipoarabinomannan of *Mycobacterium tuberculosis*. J. Biol. Chem. **266** (1991) 9652–9660.

The recent availability of pure lipoarabinomannan (LAM) from *Mycobacterium* spp. has resulted in its implication in host-parasite interaction, which events may be mediated by the presence of a phosphatidylinositol unit at the reducing end of LAM. Herein we address the structure of the antigenic, nonreducing end of the molecule. Through the process of ^{13}C NMR analysis of the whole molecule and gas chromatography/mass spectrometry of alditol acetates derived from the differential per-*O*-alkylated lipopolysaccharide, the majority of the arabinosyl residues were recognized as furanosides. Second, through analysis of per-*O*-alkylated oligoarabinosyl arabinitol fragments of partially hydrolyzed LAM, it was established that the internal segments of the arabinan component consists of branched 3,5-linked α-D-arabinofuranosyl (Ara*f*) units with stretches of linear 5-linked α-D-Ara*f* residues attached at both branch positions; whereas the nonreducing terminal segments of LAM consist of either of the two arrangements, β-D-Ara*f*-(1 → 2)-α-D-Ara*f*-(1 → 5)-α-D-Ara*f* → or [β-D-Ara*f*-(1 → 2)-α-D-Ara*f*-(1 →]$_2$ → (3 and 5)-α-D-Ara*f* →. Since this latter arrangement also characterizes the terminal segments of the peptidoglycan-bound arabinogalactan of *Mycobacterium* spp., we propose that mycobacteria elaborate unique terminal arabinan motifs in two distinct settings. In the case of the bound arabinogalactan, these motifs provide the nucleus for the esterified mycolic acids, entities which dominate the physicochemical features of mycobacteria and their peculiar pathogenesis. In the case of LAM, these motifs, non-mycolylated, are the dominant B-cell antigens responsible for the majority of the copious antibody response evident in most mycobacterial infections.—Authors' Abstract

Chaturvedi, V., Sinha, S., Girdhar, B. K., Katoch, K., Bhatia, A. S. and Sengupta, U. Association of mycobacterial-specific and *Mycobacterium leprae* specific antibody levels with clinical activity in tuberculoid leprosy: a comparative study of three serological enzyme-immunoassays. Lepr. Rev. **62** (1991) 122–133.

The ELISAs for polyclonal antibodies against *Mycobacterium leprae* (ML-ELISA) and specific antibodies against epitopes on 35-kDa protein (SACT-ELISA) and phenolic glycolipid-I (PG-ELISA) of *M. leprae* were evaluated comparatively in a group of 88 tuberculoid leprosy patients. The overall seropositivity rate with a battery of three tests (68%) was not significantly higher than that obtained with ML-ELISA alone (55%) for IgG class of antibodies. Seropositivities for SACT-ELISA and PG-ELISA were, respectively, 38% and 26%. ML-ELISA for IgM class of antibodies was least sensitive, showing only 8% positivity. A significant correlation was noted between individual values of the three assays, but the positive proportions overlapped maximally in the case of ML-ELISA (IgG) and SACT-ELISA. Further, positivity for the latter two assays, particularly SACT-ELISA, showed significant associations with the extent of "active" (largely untreated) infection. Immunoblotting revealed that the main antibody response was directed toward *M. leprae* antigens in the molecular weight range of 20–40 kDa and the densitometry results of this zone correlated significantly with corresponding SACT-ELISA and ML-ELISA (IgG) values.—Authors' Summary

Cohen, I. R. and Young, D. B. Autoimmunity, microbial immunity and the immunological homunculus. Immunol. Today **12** (1991) 105–110.

Clonal deletion and anergy are believed by many immunologists to be the fundamental mechanisms responsible for self tolerance. Nevertheless, as Irun Cohen and Douglas Young point out, such notions of nonreactivity cannot explain certain key features of immune behavior: the immunological dominance of microbial antigens that mimic self, the uniformity of autoimmune diseases and the prevalence of natural autoimmunity among the healthy. The theory of the immunological homunculus is presented here as a unifying principle.—Authors' Abstract

de Vries, R. R. P. Genetic control of immunopathology induced by *Mycobacterium leprae.* Am. J. Trop. Med. Hyg. **44** Suppl. (1991) 12–16.

The pathogenesis of leprosy is almost totally attributable to the immune response of the host toward *Mycobacterium leprae,* a virtually nontoxic intracellular parasite. At one end of the leprosy spectrum are tuberculoid leprosy patients, who develop immunity but also delayed-type hypersensitivity toward *M. leprae*; at the other end are lepromatous leprosy patients, who lack helper-T-cell activity and therefore do not develop immunity, but who can nevertheless produce antibodies that may cause immunopathology due to immune complexes. A range of immunopathology is seen between these poles.—Author's Abstract

Fine, P. and Dockrell, H. Leprosy vaccines. Vaccine **9** (1991) 291–293.

Leprosy is the clinical manifestation of chronic infection with *Mycobacterium leprae,* an intracellular parasite with a predilection for skin and nerves. Disabilities and mutilations associated with this disease, which are attributable primarily to nerve involvement, have made leprosy among the most feared and stigmatizing of all diseases. It is still widespread in the warmer regions of the globe, including southern Europe, southern U.S.A. and most of the developing countries. Though widespread, the distribution of the disease in endemic regions is sparse (a prevalence rate of 1 per 1000 is high) and predominantly rural, for reasons which are not understood, but which add to the difficulty of providing effective disease control.—Authors' Abstract

Hogervorst, E. J. M., Boog, C. J. P., Wagenaar, J. P. A., Wauben, M. H. M., Van der Zee, R. and Van Eden, W. T cell reactivity to an epitope of the mycobacterial 65-kDa heat-shock protein (hsp 65) corresponds with arthritis susceptibility in rats and is regulated by hsp 65-specific cellular responses. Eur. J. Immunol. **21** (1991) 1289–1296.

Adjuvant arthritis (AA) can be induced in genetically susceptible rats by immunization with heat-killed mycobacteria suspended in mineral oil. From our analysis of arthritogenic T-cell clone A2b, obtained from an arthritic Lewis rat and specific for the 180–188 epitope of mycobacterial 65-kDa heat-shock protein (hsp 65), the possible origin of AA was explained by the existence of a molecular mimicry of the 180–188 epitope with a cartilage-associated self antigen. We now have shown that Lewis rats respond to the 180–188 epitope after *Mycobacterium tuberculosis* immunization and that arthritis-resistant Fisher and (Lewis × Fisher) F_1 rats, although major histocompatibility complex class II identical with Lewis, do not respond to this epitope. However, in rare cases of arthritis in Fisher rats, responses to the epitope were seen. We obtained no evidence for a defect at the level of antigen processing and presentation or for suppression in Fisher rats. Thus, nonresponsiveness in Fisher rats was likely due to a difference at the level of the T-cell repertoire.

Previously, we have reported that pretreatment with hsp 65 in experimental arthritis, and not only in AA, caused resistance to arthritis induction. We now present evidence that immunization with hsp 65 or *in vitro* stimulation with hsp 65 may lead to inhibition of responses specific for epitope 180–188. Thus, the hsp 65-induced resistance to arthritis is probably caused by the induction of regulatory control specifically targeted at the 180–188 epitope. Especially in rats that tend to focus their responses on the critical 180–188 sequence, such as Lewis, regulation seems to develop following immunization with hsp 65. Since recent evidence suggests that hsp 65 and also the 180–188 epitope have a role in human arthritic conditions, the present findings are expected to contribute to further experimentation directed at exploiting hsp 65 or its epitopes for the development of new therapeutical approaches in humans.—Authors' Abstract

Kaplan, G. and Cohn, Z. A. Leprosy and cell-mediated immunity. Curr. Opinion Immunol. **3** (1991) 91–96.

The intradermal injection of the purified protein derivative of tuberculin into lepromatous leprosy patients leads to a local cell-mediated immune response and to the

extensive destruction of *Mycobacterium leprae*. This local response also occurs after intradermal injection of recombinant human interleukin-2; when administered over an 8-day period interleukin-2 evokes a systemic cell-mediated immune response and a reduction in the bacillary burden.—Authors' Abstract

Kaufmann, S. H. E. Interleukins, mycobacteria, and listeriae. Diagn. Microbiol. Infect. Dis. **13** (1990) 429–433.

From the data discussed, the following conclusions and speculations can be drawn. Both interleukin-mediated helper activities and cytolytic mechanisms participate in the immune response against mycobacteria, listeriae, and many other intracellular pathogens, and both events occur in granulomatous lesions. The concerted action of different interleukins leads to granuloma formation and maintains containment of bacteria within the lesion. Although interleukin-activated mononuclear phagocytes gain the capacity to inhibit their bacterial predators, this activity is often not sufficient for complete elimination. It may therefore become necessary for the bacteria to be released from their protective environment, and that step requires cytolysis. Under the influence of different interleukins, new T cells, blood monocytes, and granulocytes arrive at the lesion. Freshly immigrant blood monocytes and/or granulocytes with high antibacterial potential can take up bacteria released through target cell lysis and, upon appropriate activation by T-cell-derived interleukins, achieve microbial elimination more effectively.—From the article

Maestre Mesa, J. L. and Gonzalez Segredo, A. [Trial of a skin test with the soluble antigen of *Mycobacterium leprae* in a group at risk.] Rev. Leprol. Fontilles **18** (1991) 55–62. (in Spanish)

Skin tests with *Mycobacterium leprae* soluble antigen (MlSA) and purified protein derivative (PPD) were performed on 192 household contacts of multibacillary leprosy patients in order to assess the utility of the method for the identification of individuals presumably infected with *M. leprae*. They were all living in the municipality of Guantánamo which is the area with the highest prevalence rate in Cuba. The mean reaction size to MlSA was 5.03 mm. Control groups were composed of lepromatous patients in whom the mean reaction size to the same antigen was 0 mm, tuberculoid patients who showed a mean of 18.62 mm, pulmonary tuberculosis patients with a mean of 2.75 mm, and healthy individuals whose mean reading was 1.72 mm. The criterion for positivity was 6 mm for both MlSA and PPD reactions. Skin tests with the MlSA were found useful for epidemiological studies since among the positive household contacts the reaction size to this antigen was significantly higher ($p < 0.01$) than that elicited by PPD.—Authors' English Summary

Pereira, J. H., Palande, D. D. and Gschmeissner, S. E. Mycobacteria in nerve trunks of long-term treated leprosy patients. Lepr. Rev. **62** (1991) 134–142.

Mycobacteria were present in 4 out of 8 mixed peripheral nerve trunks from patients (3 BT and 1 BL) treated with dapsone (DDS) and/or MDT for periods ranging from 21 months to 8 years. Most of the bacilli appeared to be "whole." Nerve destruction with areas of granulomatous infiltration appeared more active than expected. Possible reasons for a continued presence of bacilli in treated nerves and its implications in "relapse" are discussed.—Authors' Summary

Roche, P. W., Britton, W. J., Failbus, S. S., Theuvenet, W. J., Lavender, M. and Adiga, R. B. Serological responses in primary neuritic leprosy. Trans. R. Soc. Trop. Med. Hyg. **85** (1991) 299–302.

The serological responses to two *Mycobacterium leprae* specific epitopes and one common mycobacterial antigen were examined in 46 untreated patients with primary neuritic (PN) leprosy. *M. leprae*-specific antibodies to the terminal disaccharide of phenolic glycolipid and/or the ML-04 defined epitope on the 35-kDa protein were detected in 41% of PN patients and 47% responded to one of the three antigens. This serological response mirrored that observed in paucibacillary leprosy patients. There was a significant increase in the level of antibody

response when more nerve trunks were involved. Changes in antibody levels in seropositive PN patients may prove useful in monitoring the response to chemotherapy.—Authors' Abstract

Salgame, P., Modlin, R. and Bloom, B. R. On the mechanism of human T cell suppression. Int. Immunol. **1** (1989) 121–129.

Previous evidence from several laboratories suggests that CD8+ T-suppressor (Ts) cells may be important regulatory elements governing specific unresponsiveness of lepromatous leprosy patients to *Mycobacterium leprae.* To analyze the mechanism of suppression, CD8+ Ts clones were established from lesions and peripheral blood of lepromatous patients and tested for ability to suppress antigen-responsive CD4+ T-helper (Th) clones or PBL. Suppression required induction by specific *M. leprae* antigen, but was effected in an antigen-nonspecific fashion. The Ts clones failed to exhibit cytotoxicity of four antigen-exposed MHC-matched target cells: a) an ori$^-$-SV40 transformed macrophage line; b) EBV transformed B cell lines; c) primary macrophages; and d) *M. leprae* responsive CD4+ cells. The possibility that Ts clones induce functional inactivation of CD4+ clones *in vitro* was investigated. *M. leprae*-responsive CD4+ clones were preincubated with Ts CD8+ clones, APC, and antigen for 16 hr after which the CD8+ cells were removed. The CD4+ clones with *M. leprae* and APC remained unresponsive to restimulation with APC and antigen for at least 10 days, although they responded to IL-2. Addition of IL-2 to the pre- or post-incubation cultures neither prevented the induction of unresponsiveness, nor reversed it. Earlier models of tolerance have suggested that receptor occupancy in the absence of second signals induces tolerance in B and T cells. Under conditions in which antigen responses of Th clones were HLA-DR-restricted, the Ts clones were able to suppress the response of DR mismatched Th clones. Thus, the effect of the Ts cells, like mechanisms requiring antigen presentation without a second signal, appears to be induction of clonal anergy in Th cells, perhaps by a novel mechanism.—Authors' Abstract

Scollard, D. M. Inside the skin: the local immune and inflammatory milieu in leprosy. Am. J. Trop. Med. Hyg. **44** Suppl. (1991) 17–23.

Skin lesions of leprosy have become a rich source of new information about the mechanisms involved in the uniquely broad spectrum of human responsiveness to *Mycobacterium leprae.* Recent technological advances in immunology and molecular biology have been applied to the study of skin lesions using three approaches: immunohistologic studies of skin biopsies from leprosy lesions, correlated assessment of cell subsets and soluble immunologic mediators, and studies of the effects of the inoculation of exogenous lymphokines into the lesions. Results from these studies suggest that an immunologic equilibrium may exist among long-established lesions across the spectrum so that, although T-helper and -suppressor cells are present in different proportions, immunologic activity is at a low, similar level in all types of lesions. Exogenous lymphokines can alter this equilibrium and temporarily change the histologic picture. Spontaneous immunologic changes occurring in acute leprosy reactions may also lead to changes in T-cell subsets and quantities of lymphokines.—Author's Abstract

Watson, J. D., Backstrom, B. T. and Doherty, T. M. New generation vaccines: does antibody play a directional role in antigen-processing? Am. J. Trop. Med. Hyg. **44** Suppl. (1991) 28–33.

Analyses of recombinant proteins isolated from genomic libraries of pathogenic organisms represent the beginning of identifying immunologically reactive epitopes. The induction of cell-mediated and humoral immune responses to any pathogen begins with the uptake and processing of antigen by antigen-presenting cells and the display of specific epitopes to the immune system of the host. Little emphasis is placed on the molecular mechanisms underlying transport of foreign proteins into antigen-presenting cells and factors that influence degradation to the peptides which represent the epitopes that associate with newly synthesized class II molecules of the major histocompatibility complex. These cellular

processes are crucial to the design of any new generation vaccine. We describe our analysis of the 18-kDa protein antigen of *Mycobacterium leprae* and consider a possible role for antibody in antigen processing. In both macrophage/dendritic cells and B lymphocytes, we suggest that antibody plays a directional role in antigen uptake, subcellular compartmentalization, and antigen degradation to yield peptides. These steps will all have an impact on the construction of new generation vaccines.—Authors' Abstract

Zhang, L., English, D. and Andersen, B. R. Activation of human neutrophils by *Mycobacterium tuberculosis*-derived sulfolipid-1. J. Immunol. **146** (1991) 2730–2736.

The principal sulfatide of a group of acidic lipids from virulent *Mycobacterium tuberculosis,* sulfolipid-1 (SL-1), stimulates neutrophil superoxide (O_2^-) generation and, at lower concentrations, primes neutrophil response to several other metabolic agonists including FMLP, and PMA. These responses to SL-1 were examined in relation to diacylglycerol (DAG) generation. Ca^{2+} availability and activation of guanine nucleotide binding proteins to clarify the signal transduction pathways involved. Pertussis toxin inhibited the ability of SL-1 to both stimulate neutrophils directly and to prime neutrophils for subsequent responses induced by PMA, suggesting a role for one or more guanine nucleotide-regulating proteins in both responses. SL-1 induced a rise in neutrophil DAG levels. DAG generation was inhibited by pretreatment of cells with pertussis toxin. Depletion of extracellular Ca^{2+} ablated O_2^- release induced by stimulatory levels of SL-1 but did not inhibit the priming effect induced by substimulatory concentrations of the lipid. Investigation of the activation of the neutrophil NADPH oxidase in a cell-free system revealed that the SL-1 priming effect was associated with translocation of the soluble cytosolic factors required for activation of the enzyme. Cytosolic factor translocation was not observed in pertussis toxin pretreated cells. Our results provide evidence for the role of a guanine nucleotide binding protein in both priming and direct activation of neutrophils by SL-1. This G protein regulates both SL-1-induced DAG generation and cytosolic cofactor translocation involved in neutrophil activation and priming. The multiplicity of effects of SL-1 on signal transduction pathways leading to phagocyte activation and priming may exert a profound influence on the pathogenicity of *M. tuberculosis.*—Authors' Abstract

Microbiology

Chakrabarty, A. N., Pal, N. K. and Dastidar, S. G. Mouse footpad pathogenicity of leprosy derived nocardioform bacteria cultivated *in vitro.* Indian J. Lepr. **63** (1991) 43–60.

In vitro cultures of the neocardioform bacteria from leprosy-infected tissues consisted of granules and bacilli. Inoculation of these granules into mouse foot pads (MFP) produced a mild, localized, inflammation for 4–6 weeks. The granules evoked typical granulomatous response in the subcutaneous tissue and showed gradual disintegration. Infiltration of muscles, connective tissue and epithelial cells by bacillary/mycelial masses was seen very frequently, and that of nerve bundles occasionally. Plenty of mycelial tufts emanated from many "macrophage globi." By 6–8 months, the granules disintegrated nearly completely releasing a large number of acid-fast bacilli (AFB), single layered rings of AFB, small globi and some residual mycelia. These AFB, harvested from the MFP, were similar to or indistinguishable from the bacillary preparations from the *in vitro* cultures and from the leprosy bacillus obtained directly from

humans or as passaged into the MFP, on the basis of many criteria studied, including the 36k gene positivity.—Authors' Abstract

Charvin, M., Rastogi, N. and Lévy-Frébault, V. V. An easy and rapid method for isolation of entire mycobacterial genome for application in pulsed-field gel electrophoresis. Curr. Microbiol. **22** (1991) 327–331.

A DNA extraction suitable for mycobacterial lysis in gentle conditions compatible with genome analysis by pulsed-field gel electrophoresis is presented. Effects of preliminary treatments with SDS, Triton X-100, and hexane on mycobacterial outer layer were observed by electron microscopy. The most efficient procedure, performed on cells from liquid or solid medium, consisted of treatment by Triton X-100, agarose embedding of the cells, and further treatment with α-amylase followed by lysozyme and SDS-proteinase K.—Authors' Abstract

Dhople, A. M. and Lamoureux, L. C. Factors influencing the *in vitro* growth of *Mycobacterium leprae*: effect of sulfhydryl compounds. Microbiol. Immunol. **35** (1991) 209–213.

In an attempt to determine the factors that influence the *in vitro* growth of *Mycobacterium leprae* in DH medium, the effects of sulfhydryl compounds were studied. Growth of *M. leprae* was monitored using two biochemical indicators. Only the sulfhydryl compounds, in reduced form, containing carboxyl group could support the growth of *M. leprae*. Higher cell yields were obtained when these sulfhydryl compounds were supplemented with dithiothreitol, presumably to keep the monothiols in reduced state during long incubation periods. Ascorbic acid could not replace dithiothreitol for this purpose. It is suggested that these carboxylated sulfhydryl compounds play a role in the metabolic activity of *M. leprae* along with maintaining low redox potential of the medium.—Authors' Abstract

Franzblau, S. G. In vitro activities of aminoglycosides, lincosamides, and rifamycins against *Mycobacterium leprae*. Antimicrob. Agents Chemother. **35** (1991) 1232–1234.

The *in vitro* activities of a variety of aminoglycosides, lincosamides, and rifamycins against *Mycobacterium leprae* were evaluated with the BACTEC 460 system. At 20 μg/ml, gentamicin, kanamycin, tobramycin, streptomycin, and amikacin were inactive. Lincomycin was active at 20 μg/ml, and clindamycin was active at 0.31 μg/ml. Rifamycin SV, rifabutin, and rifampin were active at 3.1, 3.1 to 12.5, and 200 ng/ml, respectively. The *in vitro* assay correlates well with the *in vivo* response of *M. leprae* to antimicrobial agents, with the exception of the aminoglycosides.—Author's Abstract

Liesack, W., Sela, S., Bercovier, H., Pitulle, C. and Stackebrandt, E. Complete nucleotide sequence of the *Mycobacterium leprae* 23 S and 5 S rRNA genes plus flanking regions and their potential in designing diagnostic oligonucleotide probes. FEBS **281** (1991) 114–118.

The complete nucleotide sequences of the *Mycobacterium leprae* 23 S and 5 S rRNA genes and their flanking regions are presented. As compared to other eubacterial homologous molecules the 23 S rRNA exhibits two insertions. A 16-nucleotide-long insertion is almost unique to members of the genus *Mycobacterium,* while the second represents an extended version of helix 54. The potential of both insertions to serve as target for diagnostic oligonucleotide probes was proven by comparative sequence analysis of 23 S rRNA of several *Mycobacterium* species and by dot blot hybridization. In addition, a 19-mer oligonucleotide probe is described, which can be considered genus *Mycobacterium*-specific.—Authors' Abstract

Sela, S. and Clark-Curtiss, J. E. Cloning and characterization of the *Mycobacterium leprae* putative ribosomal RNA promoter in *Escherichia coli*. Gene **98** (1991) 123–127.

The putative promoter region of the 16S ribosomal RNA-encoding gene (*r*RNA) of *Mycobacterium leprae* was cloned and characterized in *Escherichia coli*. A 932-bp *Hae*III restriction fragment, containing the

5′ end of the 16S *r*RNA gene and flanking upstream region, was cloned in front of a promoterless reporter gene in the shuttle vector, pMH109, to generate the plasmid, pYA1101. This clone exhibits promoter activity both in gram⁻ (*E. coli*) and gram⁺ (*Bacillus subtilis*) bacteria. Sequence analysis and primer extension experiments with mRNA derived from the *M. leprae* clone were used to determine the structure and the location of the promoter, as well as the transcription start point in *E. coli*. The promoter region contains sequences that resemble the −35 and −10 consensus sequences found in many bacteria. A region located 34 bp distal to the promoter is a putative *r*RNA processing signal, based on sequence homology with processing signals involved in the maturation of the *r*RNA precursor in *B. subtilis* and several *Mycoplasma* species.—Authors' Summary

Tsukamura, M. Growth physiology of mycobacteria in modified Dubos liquid medium. Microbiol. Immunol. **34** (1990) 995–1003.

Although mycobacteria grow in Dubos liquid medium showing an arithmetic linear growth, the initial few days of growth were found to correspond to an "induction" period. In this period, rapid increase of the amount of growth occurred; whereas increase of the number of colony-forming units (cfu) remained at a low level. This finding shows that the rapid increase of the amount of growth is accompanied by rapid death of multiplied bacteria. In a successive period, which was considered to correspond to the logarithmic growth phase, a 1:1 correspondence existed between the amount of growth and the number of cfu. The induction period is not considered to be a lag phase, in which the bacteria grow slowly, but a period of unbalanced relationship between the growth and the viability. Even when we inoculated different sizes of bacteria, the amounts of growth became similar in both inoculations after several days of incubation. However, the number of cfu remained always smaller in the use of small inocula than in the use of large inocula. In the use of small inocula, much more rapid increase of the amount of growth occurred. However, this rapid increase gave rise to rapid death of bacteria.—Author's Abstract

Wheeler, P. R., Bulmer, K. and Ratledge, C. Fatty acid oxidation and the β-oxidation complex in *Mycobacterium leprae* and two axenically cultivable mycobacteria that are pathogens. J. Gen. Microbiol. **137** (1991) 885–893.

Intact, nongrowing *Mycobacterium leprae, M. avium* and *M. microti* oxidized a wide range of 1-^{14}C-labeled fatty acids (C_8 to C_{24}) to $^{14}CO_2$. Laurate (C_{12}) was oxidized most rapidly, and its oxidation by *M. leprae* was inhibited by the antileprosy agents dapsone, clofazamine and rifampin. Key enzymes of β-oxidation were detected in extracts from all three mycobacteria. All these activities (both in intact mycobacteria and the enzymes) were stimulated in *M. avium* grown in Dubos medium plus palmitate, but activities in *M. microti* or *M. avium* grown either in Dubos medium with added liposomes or triolein or *in vivo* were similar to those detected in the same strain grown in Dubos medium alone. *M. avium* could be grown in medium in which 95% of its fatty acyl elongase activity is acetyl-CoA dependent. In this medium growing *M. avium* organisms oxidized [1-^{14}C]palmitate to $^{14}CO_2$ but simultaneously elongated palmitate to C_{24} acids and even longer. Acetyl-CoA-dependent elongase activity is similar but clearly not identical to reversed β-oxidation, but the exact point(s) of difference have not yet been identified.—Authors' Abstract

Experimental Infections

Dhople, A. M. Changes in hydrolytic enzyme activities in armadillos infected with *Mycobacterium leprae*. Microbios **66** (1991) 55–64.

The activities of hydrolytic enzymes in various organs of armadillos infected with *Mycobacterium leprae* were compared with those in normal armadillos. Except for as-

partate aminopeptidase and esterase, the levels of the other enzymes in liver, spleen and inguinal lymph nodes were significantly higher in armadillos infected with *M. leprae* compared with those in noninfected ones. These enzyme levels were at a maximum when the animals were sacrificed 22 to 30 months post-inoculation, a period when the bacterial load in the animals had also reached a maximum. Animals infected with *M. leprae* but not showing any signs of disseminated infection behaved similar to those in the noninfected group. The observed changes in enzymatic activities were not due to bacterial enzymes and so can be related to tissue damage caused by *M. leprae.*—Author's Abstract

Kohli, M., Sharma, V. K., Vaishnavi, C., Ganguly, N. K., Kaur, S. and Chuch, K. S. Renal brushborder membrane vesicle study of marker enzymes and uptake of nutrients in *Mycobacterium leprae* infected mice. Jpn. J. Exp. Med. **60** (1990) 285–290.

The renal brush-border membrane vesicles (BBMV) were used to elucidate the early biochemical functional status during the course of experimental *Mycobacterium leprae* infection in mice. The activities of the characteristic brush-border enzymes viz: alkaline phosphatase, leucine amino peptidase and γ-glutamyl transpeptidase were found to be significantly decreased ($p < 0.001$) at 3 and 6 months after infection. The transport of nutrients viz: D-glucose, L-alanine, L-lysine and L-aspartate across BBMV showed a similar pattern. The activity of brush-border enzymes and transport of nutrients across the membrane returned to normal at 9 months post-infection, suggesting regeneration of the brush-border membrane.—Authors' Summary

Meyers, W. M., Gormus, B. J., Walsh, G. P., Baskin, G. B. and Hubbard, G. B. Naturally acquired and experimental leprosy in nonhuman primates. Am. J. Trop. Med. Hyg. **44** Suppl. (1991) 24–27.

Naturally acquired leprosy has been observed in chimpanzees and sooty mangabey monkeys. Experimental multibacillary leprosy was established in 24 of 36 mangabey monkeys, 7 of 34 rhesus monkeys, and 15 of 19 African green monkeys following intravenous and intradermal inoculation of *Mycobacterium leprae.* The experimental disease strongly resembles leprosy in humans clinically, histopathologically, and immunologically. Thus, in addition to nine-banded armadillos in Louisiana and Texas [U.S.A.], chimpanzees and sooty mangabeys in Africa, in the wild or in captivity, may serve as a zoonotic source of *M. leprae.* Investigators using chimpanzees and monkeys should be alerted to the possibility of naturally acquired leprosy.—Authors' Abstract

Mistry, Y., Antia, N. H. and Mukherjee, R. Radiolabeling of *Mycobacterium leprae* lipids with schwannoma cells, a potential drug screening system. Antimicrob. Agents Chemother. **35** (1991) 1444–1447.

This study describes a novel method which could be developed into a test system of evaluating the efficacy of antileprosy drugs. The method estimates incorporation of [^{14}C]acetate into lipids of *Mycobacterium leprae* maintained within the 33B Schwannoma cell line. Schwannoma cell-resident *M. leprae* cells incorporated significant levels of radiolabel within their lipids during 12 days of incubation *in vitro.* This incorporation was markedly reduced by 5 μg of rifampin per ml (decrease, 81.62%); this decrease was observed within 24 hr of addition of the drug. Dapsone also reduced the radiolabel incorporation into the lipids, but to a lesser extent (decrease, 27.58%). This system was also able to differentiate between rifampin-sensitive and -resistant strains of mycobacteria. It is suggested that since the effect of bacteriostatic (dapsone) and bactericidal (rifampin) drugs could be detected by using this technique, it may prove useful in screening novel drugs acting against *M. leprae.*—Authors' Abstract

Epidemiology and Prevention

Baodebiligc, *et al.* [General situation of leprosy in Neimenggu Autonomous Region.] China Lepr. J. **7** (1991) 10–12. (in Chinese)

By 1980 in Neimenggu Autonomous Region with a population of 21,220,000 there have accumulated 71 cases of leprosy, including 56 L type, 14 T and 1 B group, but since 1981 no leprosy patient was found among local inhabitants. The authors review the history of the region and find no record of leprosy prevalence in the past 100 years and more, which could be related to natural conditions and life style of the residents. There were no special institutions for leprosy control because the leprosy patients have been few, but all the patients who have been found were sent to leprosaria in other provinces for treatment.—Authors' English Abstract

Krishna Murthy, P., Subramanian, M., Reddy, B. N., Rao, P. S. and Neelan, P. N. A computerized information system for evaluation of NLEP through monthly progress reports. Indian J. Lepr. **63** (1991) 70–77.

A computerized system for monitoring district-wise operational performance and epidemiological progress using existing regular and special monthly reports of the National Leprosy Eradication Programme (NLEP) is presented. The same system, with some minor modifications, could be used for program assessment at the Leprosy Control Unit level also. The advantage of the system is the speed with which it can generate output in the form of comparative tables and graphs for different regions for use by program managers for making overall assessments in time and for sending feedback reports to workers at various levels, for self-assessment, and for taking timely corrective action. The system presented provides immediate and easy access to the stored and/or processed information (indicators etc.,) at any time. The system has been pilot-tested using monthly reports from 18 districts of Tamil Nadu.—Authors' Abstract

Pattyn, S. R., Bourland, J. and Kakeze, C. Evolution of the leprosy endemicity in Burundi during the years 1981–88. Ann. Soc. Belg. Med. Trop. **71** (1991) 57–61.

Between 1981 and 1988 the detection rate of leprosy in Burundi increased from 2.4 per 100,000 during 1981–1984 to 3.09 per 100,000 during 1985–1988. The proportion of multibacillary disease remained constant at 30%. The detection rate in children decreased significantly from 21% to 9% for paucibacillary disease (PB) but remained constant for multibacillary disease (MB). The proportion of patients with disabilities doubled in both PB and MB patients. It seems thus that the detection rate is high but that cases are diagnosed fairly late. Health education of the public and staff in the health centers should stress the importance of better and earlier detection.—Authors' Summary

Pattyn, S. R. and Grillone, S. Leprosy in the Comores 1981–88. Ann. Soc. Belg. Med. Trop. **71** (1991) 51–55.

The evolution of the leprosy endemic in the République Fédérale Islamique des Comores between 1981 and 1988 is described. Leprosy on Grande Comore seems to be extinct. On the island of Anjouan the yearly detection rate is 0.38 per 1000 with a high multibacillary rate (34%). Leprosy is highly endemic in children; during the last 6 years, 30% of multibacillary and 44% of paucibacillary cases are detected in the < 15 years age group. Detection seems to be early as illustrated by the high proportion of paucibacillary patients with a small number of skin lesions and a low proportion of patients with severe infirmity. In the detection process both patient's and doctor's delays are short. Most diagnoses are suspected by relatives or others who had the disease in the past and who referred the suspects directly to the specialized service.—Authors' Summary

Raveendranathan, O., Nair, B. K. H. and Sarojini, P. A. Epidemiological significance of indeterminate leprosy—a hos-

pital based study. Indian J. Lepr. **63** (1991) 5–11.

An epidemiological analysis of 100 cases of indeterminate leprosy attending the Department of Dermatology and Venereology of Medical College Hospital, Trivandrum, (India), is presented. It was found that indeterminate leprosy formed 13.23% of all cases of leprosy and 1.3% of all outpatients attending this department. Only 27% of patients with indeterminate leprosy were below 15 years of age. There was a predominance of males, especially over 20 years of age. There was no history of contact with leprosy in any of the patients with indeterminate leprosy. All patients with indeterminate leprosy came for hypopigmented patches, suspecting leprosy. The majority had the disease for more than 6 months. A single lesion on the outer aspect of extremity was the most common presentation. The lepromin test was positive in only 2% of patients with indeterminate leprosy, while it was positive in 80% of control subjects. Three cases of dapsone resistance were suspected in this series. The epidemiological significance of the findings is discussed.—Authors' Abstract

Shu, H.-W., *et al.* [A study of subclinical infection with *M. leprae*. I. Paired analysis of contacts with leprosy.] China Lepr. J. **7** (1991) 12–16. (in Chinese)

Determination of subclinical infection with *Mycobacterium leprae* in 452 household contacts of leprosy patients (HC) and random residents (RR) around the patients' homes paired with the contacts, using PGL-I ELISA, showed the results as follows: a) the positivity in the HC is 26.11% higher than 20.79% in the RR ($p < 0.01$), suggesting that long-term and intimate contact with leprosy patients could enhance the probability of infection; b) the positive rate is 22.10% in males and 28.78% in females among the HC while the rate is 16.57% in males and 23.62% in females among the RR, manifesting higher levels in females than in males among both the HC and RR; c) the positive rates in the group aged 15–25 among both the HC and RR are 29.13% and 27.56%, respectively, but in the group aged 10–14 24.49% in the HC is higher than 17.35% in the RR ($p < 0.05$); d) the positive rates in the HC with LL and BL patients are 34.84% and 28.40%, respectively, being higher than in the HC with PB ($p < 0.01$), but there is no such phenomenon in the RR; e) either in the HC or in the RR the positive rates all are higher in those contacting with and living around active cases of leprosy, being 27.75% and 23.79%, respectively, but among the contacts with cured cases, the rate in the HC is 24.44%, being higher than 17.77% in the RR. The authors believe that the load and viability of *M. leprae* in the contacted patients plus the frequency and intimate degree of the contact are the main factors which could cause subclinical infection with *M. leprae*.—Authors' English Abstract

Rehabilitation

Zhang, G.-C., *et al.* [Epidemiological investigation of disability in leprosy. (I) Factors relevant to disability.] China Lepr. J. **7** (1991) 3–10. (in Chinese)

Examination of 14,257 leprosy patients showed that the rate of disability and deformity is 56.97% (81.15% in MB and 53.04% in PB). The authors find that the disability rate is higher in relapsed cases, and the longer the duration of the disease, the higher the rate. In those who have a disease duration of over 40 years the disability rate was as high as 76.93%, but most of the disabilities occurred less than 2 years after the leprosy had been diagnosed. The factors which could influence the disability and deformity, such as lepra reaction, level of education, occupation, and so on, are analyzed and suggestions relevant to reducing and preventing the disabilities are raised.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Al-Kassimi, F. A., Abdullah, A. K., Al-Orainey, I. O., Benar, A. B., Al-Hajjaj, M. S., Al-Majed, S. and Al-Wazzan, A. The significance of positive Mantoux reactions in BCG-vaccinated children. Tubercle **72** (1991) 101–104.

As the interpretation of tuberculin skin tests is controversial in subjects who have received BCG vaccine, we administered Mantoux tests to 2588 randomly selected Saudi children aged 5–13, 1945 of whom had been vaccinated with BCG at birth and 643 were unvaccinated. Only 7.8% of the BCG-vaccinated children were Mantoux positive (≥ 10 mm induration) at the age of 5 years, which was not significantly different from the unvaccinated children. The tuberculin sensitivity rose more steeply with age in the BCG-vaccinated than the unvaccinated children so that the difference between both groups became statistically significant in those aged 12 and 13 (20% versus 3.9%, 15.5% versus 4.1%, respectively). These findings support the previously expressed theoretical postulates that BCG-vaccinated subjects display an increased ability to respond immunologically to encounters with environmental mycobacteria. In communities with low prevalence of environmental mycobacteria, this would result in a slow but persistent rise of skin reactivity to tuberculin which, if given time, will become greater than that of unvaccinated subjects.—Authors' Summary

Al-Orainey, I. O. M. Effect of initial isoniazid resistance on response to chemotherapy of tuberculosis: a review of clinical trials. Ann. Saudi Med. **11** (1991) 3–8.

This study evaluated 17 clinical trials [in Africa, Asia, and Europe] of short-course chemotherapy of tuberculosis and assessed the influence of initial resistance to isoniazid on the response to therapy. High failure rates were observed for the nonrifampin-containing regimens. When rifampin was included, there was a very good response among patients suffering from sensitive and resistant strains. Regimens that included both rifampin and pyrazinamide yielded the best results, with low failure rates observed for sensitive and resistant patients. The addition of streptomycin and ethambutol to these regimens did not improve the response in patients with isoniazid-resistant bacilli. Prolonged therapy generally yielded a better response; however, in rifampin-containing regimens, the use of pyrazinamide for more than 2 months did not influence the outcome of therapy. In patients with isoniazid-resistant strains, 4- and 6-month regimens containing rifampin and pyrazinamide both yielded low failure rates. Initial isoniazid resistance had very little impact on the response to such regimens when therapy was carried out for 6 months.—Author's Abstract

Ambrosio, R. E., Harris, Y. and Huchzermeyer, H. F. A. K. A DNA probe for the detection of *Mycobacterium paratuberculosis*. Vet. Microbiol. **26** (1991) 87–93.

A genomic library of DNA extracted from *Mycobacterium paratuberculosis* was constructed in the expression vector λgt11. The library was screened by plaque hybridization with labeled *M. paratuberculosis* genomic DNA as probe. Strongly hybridizing plaques were isolated and their DNA extracted and characterized for *M. paratuberculosis* specificity by hybridization to DNA from other Mycobacteriaceae. A clone was obtained which was specific for *M. paratuberculosis*. DNA from this clone could detect 7 ng *M. paratuberculosis* DNA.—Authors' Abstract

Andersen, P., Askgaard, D., Ljungqvist, L., Bennedsen, J. and Heron, I. Proteins released from *Mycobacterium tuberculosis* during growth. Infect. Immun. **59** (1991) 1905–1910.

Proteins secreted from *Mycobacterium tuberculosis* during growth are believed to be important for protective immunity against tuberculosis. We have investigated the growth of *M. tuberculosis* in an enriched liquid medium. The release of isocitrate dehydrogenase from the bacilli served as a marker of autolysis and was observed dur-

ing the late logarithmic growth phase. The release of proteins during the culture period was investigated by enzyme-linked immunosorbent assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Three major groups of proteins, which differed markedly with respect to profile of release and location in intact bacilli, were defined. A short-term filtrate devoid of autolytic products was defined and found to be composed of 33 major components. Five proteins were identified by monoclonal antibodies. Pronounced superoxide dismutase activity was detected in the filtrate. The enzyme was purified and identified as a dominating component of short-term filtrate.—Authors' Abstract

Annamma, M., Radhakrishnan, V. V. and Shobha, S. Diagnosis of tuberculous meningitis by enzyme-linked immunosorbent assay to detect mycobacterial antigen and antibody in cerebrospinal fluid. Med. Microbiol. Immunol. **179** (1991) 281–288.

Enzyme-linked immunosorbent assay (ELISA) was used to detect mycobacterial antigen and antimycobacterial antibody in cerebrospinal fluid (CSF) specimens of 50 patients with tuberculous meningitis (TBM) and 50 patients with nontuberculous neurological diseases (control group). The assay gave no false-negative results in 10 culture-positive patients with TBM. Detection of mycobacterial antigen in CSF is more sensitive and specific for the diagnosis of TBM than detection of antibody. ELISA should be considered as one of the alternative methods in the laboratory diagnosis of TBM, particularly in culture-negative patients with TBM.—Authors' Abstract

Beck, J. S. Skin changes in the tuberculin test. Tubercle **72** (1991) 81–87.

This review describes the recent advances in knowledge of the nature and range of physiological changes that occur in the skin at the site of a positive tuberculin reaction. The infiltration of T cells and monocyte/macrophages shows a marked compartmentalization, suggesting that the functions of particular cell types depend on their localization. The extent of cutaneous edema (detectable as induration) is not closely related to other features of the reaction or to systemic indicators of cell-mediated immunity. The intensity of hyperemia is maximal at the center of the reaction and is correlated in most cases with the density of cellular infiltration in the dermis suggesting a functional coordination. Despite this correlation between cell numbers and velocity of blood flow, the reaction normally shows hypoxia, hypercapnia and local acidosis, but this metabolic modification may not be a wholly disadvantageous effect since these conditions appear to facilitate the growth and metabolism of activated lymphocytes and macrophages. In very strong reactions, there is central relative slowing of the circulation and this may lead to necrosis in extreme cases.

There are however a minority of cases where cell infiltration occurs but induration is not palpable: this situation has been named pseudoanergy, and its pathogenesis has not yet been established. The occurrence of pseudoanergy must throw some doubt on the conventional criteria for positivity in the reading of tuberculin skin tests (induration > 5 mm) and this may have relevance to future strategies for assessment of new vaccines.

The human tuberculin reaction should prove a valuable model for coordinating knowledge of the cellular and molecular mechanisms induced by mycobacterial immunity with the pathogenesis of tissue reactions in clinical tuberculosis. This should lead to the rational development of therapy for limiting inflammatory and scarring damage in antibiotic treated mycobacterial disease.—Author's Summary

Blanchard, D. K., Michelini-Norris, M. B., Pearson, C. A., Freitag, C. S. and Djeu, J. Y. *Mycobacterium avium-intracellulare* induced interleukin-6 from human monocytes and large granular lymphocytes. Blood **77** (1991) 2218–2224.

Mycobacterium avium-intracellulare (MAI) is an opportunistic pathogen commonly found in acquired immunodeficiency syndrome patients, whose immune systems

are severely compromised. However, normal responses to this bacterium are apparently sufficient to prevent disseminated infection because disease is rarely found unless an immunocompromised state is present. Because interleukin-6 (IL-6) is an inflammatory cytokine with a multitude of activities, we investigated the potential of MAI to induce IL-6 from normal human leukocytes. Peripheral blood mononuclear cells were fractionated into monocytes (Mo), large granular lymphocytes (LGL), and T cells and stimulated with bacteria. Culture supernatants were collected and assayed for IL-6 activity by bioassay. Mo and LGL, but not T cells, were found to release IL-6 within 12 hr of stimulation, with optimal production occurring by 2 days of culture. Production of IL-6 from human leukocyte subsets was confirmed by Northern blot analysis and by neutralization of biologic function of the culture supernatants with specific antisera. Taken together, these results indicate that production of IL-6 is a key response of Mo and LGL to MAI. The role of IL-6 in MAI infection, therefore, needs to be further investigated.—Authors' Abstract

Bobrovik, S. A. and Lyashchenko, K. P. [Determination of antigen-specific immune complexes by the method of the enzyme immunoassay.] Zh. Mikrobiol. Epidemiol. Immunol. **1** (1991) 67–70. (in Russian)

The methodological approach permitting the detection of immune complexes containing specific antibodies to a definite antigen in the enzyme-linked immunosorbent assay (ELISA) is described. The basic conditions of the assay were optimized. Immune complexes were precipitated from blood serum with 3.5% polyethylene glycol 6000 for 4 hr. The precipitate thus obtained was dissolved and incubated in polystyrene plates with immobilized antigen at 37°C for a long time (at least 6 hr) in a humid chamber. The amount of bound antibodies, determined by ELISA techniques, was conjectured from the level of antigen-specific immune complexes. The proposed approach can be used in the immunodiagnosis of infectious diseases.—Authors' English Abstract

Brahmajothi, V., Pitchappan, R. M., Kakkanaiah, V. N., Sashidhar, M., Rajaram, K., Ramu, S., Palanimurugan, K., Paramasivan, C. N. and Prabhakar, R. Association of pulmonary tuberculosis and HLA in South India. Tubercle **72** (1991) 123–132.

In 204 patients with smear-positive pulmonary tuberculosis HLA-A10, B8 and DR2 were more frequently found than in 404 control subjects ($p = 0.01$); the greatest attributable risk (0.29) was associated with HLA-DR2. The radiographic extent of disease was also associated with HLA-DR2 ($p = 0.0001$). In 152 patients with smear-negative pulmonary tuberculosis, the frequencies of HLA-A10 and B8, but not DR2, were greater in the control subjects ($p = 0.001$ and 0.01, respectively). HLA-DR2 may be involved in the pathogenesis of advanced pulmonary tuberculosis. Study of endogamous, genetically disparate populations (caste) revealed other HLA associations (A3, B12 and DR4) unique to them, suggesting that genes linked with the HLA complex might also be significant in the pathogenesis of tuberculosis.—Authors' Summary

Bubochkin, B. P. and Ivanova, E. S. [Specific tuberculosis prophylaxis by BCG vaccine in young subjects.] Probl. Tuberk. **3** (1991) 16–19. (in Russian)

The efficacy of antituberculous BCG revaccination studied in workers, students and young subjects of the penitentiary labor establishments (PLE) was assessed by the level of tuberculin sensitivity, the presence of a postvaccination skin sign, tuberculosis morbidity and severity of a tuberculosis process. The highest efficacy was achieved in workers and students as a result of systematic revaccination. Tuberculosis morbidity in them was 26.2 and 34.5 per 100,000, respectively. In the PLE contingent, it was several times higher because they received revaccination as early as their school years. The postvaccination skin signs were found in 91.8% of workers, 76.4% students, and 60.5% young subjects of PLE. In students and workers reactions with an infiltrate size of 5–11 mm were predominant and in the PLE contingent those with an

infiltrate of 12 mm and more. Subjects having a skin sign had a more benign tuberculosis course. Bacillary excretion was found in 48.9% and in those without a skin sign in 72% of cases ($p < 0.05$).—Authors' English Abstract

Buckner, C. B., Leithiser, R. E., Walker, C. W. and Allison, J. W. The changing epidemiology of tuberculosis and other mycobacterial infections in the United States: implications for the radiologist. Am. J. Roentgenol. **156** (1991) 255–264.

Diseases consequent to infection with mycobacterial organisms, such as *Mycobacterium tuberculosis* and other mycobacterial species, remain a significant health problem in the United States. Over the past decade several new factors have amplified this problem, the most significant of which is the ongoing epidemic of infection with the human immunodeficiency virus. This review discusses the changing epidemiology of mycobacterial disease and emphasizes the significance of these changes to the radiologist.—Authors' Abstract

Cave, M. D., Eisenach, K. D., McDermott, P. F., Bates, J. H. and Crawford, J. T. IS6110: conservation of sequence in the *Mycobacterium tuberculosis* complex and its utilization in DNA fingerprinting. Molec. Cell. Probes **5** (1991) 73–80.

Multiple copies of an insertion sequence, IS6110, were shown to be present in the genome of members of the *Mycobacterium tuberculosis* complex (*M. tuberculosis* and *M. bovis*). Ten to 12 copies are present in various strains of *M. tuberculosis,* while strains of *M. bovis* contain only one to three copies. IS6110 was not detected in the DNA of other species of mycobacteria. Restriction endonuclease analysis indicated that the sequence of IS6110 is conserved across strain and species lines. Hybridization to the insertion sequence can be used to detect restriction fragment length polymorphism reflecting divergence in the sequence of regions flanking the various copies of IS6110. These differences were used to fingerprint various strains of the *M. tuberculosis* complex.—Authors' Abstract

Denis, M. *In vivo* modulation of atypical mycobacterial infection: adjuvant therapy increases resistance to *Mycobacterium avium* by enhancing macrophage effector functions. Cell. Immunol. **134** (1991) 42–53.

Susceptible BALB/c mice were infected iv with a strain of *Mycobacterium avium* and infused with different biological response modifiers (BRM) in a gel delivery system so as to modify the progression of the infection in a beneficial fashion. Infusion of IL-2 or IL-4 in hydrophobic gels led to no significant enhancement of resistance. Infusion of muramyl dypeptide in hypromellose led to a significant enhancement of resistance against the *M. avium,* as seen by a significant reduction of colony-forming units (CFU) in the spleens of infected mice. Similarly, infusion of interleukin-1β in hypromellose in infected mice led to a significant reduction in CFU counts in the organs of mice. The mechanism(s) responsible for this enhanced resistance was studied further. It was found that infected mice developed profound immunosuppression, as judged by mitogenic and antigenic stimulation. Mice infused with MDP/hypromellose developed a similar immunosuppression, suggesting that this adjuvant immunotherapy did not act by stimulating a T-cell response or by abrogating a putative suppressive phenomenon. Macrophages from mice infused with MDP alone were no more bacteriostatic for a virulent *M. avium* than control cells. However, macrophages from infected mice infused with MDP/hypromellose were more bacteriostatic for *M. avium* than cells from mice infected with *M. avium* and infused with the hydrophobic gel only. Overall, these results suggest that adjuvant immunotherapy is beneficial in *M. avium* infections.—Author's Abstract

Devadoss, P. O., Klegerman, M. E. and Groves, M. J. A scanning electron microscope study of mycobacterial developmental stages in commercial BCG vaccines. Curr. Microbiol. **22** (1991) 247–252.

Scanning electron microscopy (SEM) studies were performed on freshly prepared

and freeze-dried Tice®-substrain *Mycobacterium bovis* BCG vaccine as well as Tice BCG grown on Middlebrook 7H10 agar. Intact colonies of the Tice and Glaxo BCG substrains growing on agar were also examined. The presence of developmental stages of the mycobacterial life cycle previously reported in the literature was confirmed in actively growing BCG and in commercial vaccine preparations. The pleomorphic forms consisted of various size coccal and bacillary cells. Propagation appeared to occur by fission of both forms to produce aggregate bodies and by a coccal-bacillary cycle. Filterable (30–200 nm) granular cocci and coccal microcolonies were also observed in commercially prepared BCG vaccines. The implications of pleomorphism on the biologic activities of various BCG vaccines are discussed.—Authors' Abstract

Diver, J. M., Schollaardt, T., Rabin, H. R., Thorson, C. and Bryan, L. E. Persistence mechanisms in *Pseudomonas aeruginosa* from cystic fibrosis patients undergoing ciprofloxacin therapy. Antimicrob. Agents Chemother. **35** (1991) 1538–1546.

The mechanisms of persistence to ciprofloxacin in nine sets of *Pseudomonas aeruginosa* strains isolated during ciprofloxacin therapy of chronic lung infections in cystic fibrosis patients were studied. Low-to-moderate levels of ciprofloxacin resistance developed in each case. Each set of pretherapy ciprofloxacin-susceptible, during-therapy ciprofloxacin-resistant, and post-therapy ciprofloxacin-susceptible isolates were shown to be genotypically related by using a radiolabeled epidemiological gene probe. All ciprofloxacin-resistant isolates were found to have altered susceptibilities to both nalidixic acid and various chemically unrelated antibiotics. Analysis of possible resistance mechanisms showed that the strains had altered outer membrane protein or lipopolysaccharide profiles. Complementation of possible DNA gyrase mutations with a plasmid-borne, wild-type *Escherichia coli gyrA* gene indicated that altered DNA gyrase was at least partly responsible for ciprofloxacin resistance in all strains tested. Attempts to generate ciprofloxacin-susceptible revertants *in vitro* showed that in some strains reversion was rapid in the absence of ciprofloxacin, while in other strains it was not possible to generate revertants. These data indicate that persistence of *Pseudomonas aeruginosa* to ciprofloxacin involves changes in DNA gyrase and is associated with pleiotropic changes in outer membrane proteins and lipopolysaccharide.—Authors' Abstract

Forster, D., Behrens, R. H., Campbell, H. and Byass, P. Evaluation of a computerized field data collection system for health surveys. Bull. WHO **69** (1991) 107–111.

A customized field data collection system (FDCS) has been developed for a hand-held computer to collect and check questionnaire data. The data quality, preparation time, and user acceptability of the system were evaluated during a malaria morbidity survey in Bakau, the Gambia. Eight field workers collected data with either the FDCS or on paper questionnaire forms in alternate weeks over a 6-week period. Significantly fewer item errors occurred with the FDCS, and by the end of the survey period interview times were significantly less with the FDCS than with the paper and pencil questionnaire.

Advanced appropriate technology may have a useful role in providing accurate and rapid information, particularly in overcoming bottlenecks in data processing, and in obviating the need for costly expertise and equipment. In developing countries this could help to improve the quality of data on health care.—Authors' Abstract

Frehel, C., de Chastellier, C., Offredo, C. and Berche, P. Intramacrophage growth of *Mycobacterium avium* during infection of mice. Infect. Immun. **59** (1991) 2207–2214.

Growth of the virulent *Mycobacterium avium* strain TMC 724 in host tissues during persistent infection of mice was studied. Following intravenous infection of C57BL/6 mice, the kinetics of bacterial growth was biphasic in the spleen and liver, with a significant reduction of the multiplication rate after day 21 to 28 of infection. An electron-

microscopic study of the liver and spleen of infected mice showed that the bacteria were strictly intracellular. They were observed within inflammatory macrophages populating granulomas disseminated in host tissues. The bacteria were confined to the phagosome compartment, and they were encapsulated. Phagosome-lysosome fusions were encountered, but the bacteria showed no visible signs of degradation and continued to multiply. These results are the first *in vivo* evidence that virulent *M. avium* multiplies exclusively intracellularly and that encapsulated bacteria resist the microbicidal mechanisms of macrophages inside the phagosomal compartment.—Authors' Abstract

Gangadharam, P. R. J., Ashtekar, D. R., Farhi, D. C. and Wise, D. L. Sustained release of isoniazid *in vivo* from a single implant of a biodegradable polymer. Tubercle **72** (1991) 115–122.

In order to solve the problem of poor patient compliance, attempts were made to prolong the bioavailability of antimycobacterial drugs after a single administration. A single implant of polylactic-co-glycolic acid (PLGA) co-polymer containing isoniazid ensured its sustained release up to 6 weeks. The levels are comparable with those obtained from daily doses. Homogenates of liver and lungs from animals killed at 6 weeks after a single implant showed high antimycobacterial activity against *Mycobacterium tuberculosis.* Sera from the implant and daily dose group animals showed no significant difference in renal, hepatic and hematological parameters. The implant caused no local or systemic toxicity.—Authors' Summary

Hellyer, T. J., Brown, I. N., Dale, J. W. and Easmon, C. S. F. Plasmid analysis of *Mycobacterium avium-intracellulare* (MAI) isolated in the United Kingdom from patients with and without AIDS. J. Med. Microbiol. **34** (1991) 225–231.

One-hundred-forty-seven isolates (128 strains) of *Mycobacterium avium-intracellulare* (MAI) were screened by agarose gel electrophoresis for the presence of plasmids. Plasmids were characterized according to size and by Southern hybridization analysis of intact and restriction endonuclease-digested DNA. Two cloned MAI plasmids, pLR7 and pLR20, were used as probes. There was no significant difference in the rate of plasmid carriage in MAI strains isolated from patients with the acquired immuno-deficiency syndrome (AIDS) and from non-AIDS patients in the UK, but a higher rate of plasmid carriage was observed in a panel of American strains from AIDS patients. Plasmids were grouped into two broad categories: small (mostly 14–30 kb) and large (> 150 kb). Southern blot analysis identified two distinct groups of small plasmids, the majority of which showed homology with pLR7. Plasmids from this group were significantly more common in strains of serotypes 4 and 8 which are particularly associated with AIDS.—Authors' Summary

Hubbard, R. D., Flory, C. M. and Collins, F. M. Memory T cell-mediated resistance to *Mycobacterium tuberculosis* infection in innately susceptible and resistant mice. Infect. Immun. **59** (1991) 2012–2016.

The memory T-cell immune response to *Mycobacterium tuberculosis* infection was examined in strains of mice which vary in their natural susceptibility to *M. bovis* BCG infection. Naturally susceptible (NS) C57BL/6 and naturally resistant (NR) B6D2 F_1 hybrid mice were infected with a sublethal dose of *M. tuberculosis* and then given antibiotic therapy beginning 2 weeks postinfection. T cells from both strains of mice transferred significant levels of resistance to syngeneic mice challenged aerogenically with *M. tuberculosis.* This memory response was not substantially reduced by depletion of either L3T4+ or Lyt2+ T cells from the donor mice but was ablated by depletion of both T-cell subsets. Cyclophosphamide pretreatment of C57BL/6 memory T-cell donors also ablated the resistance transferred to recipient mice. In contrast, B6D2 memory T cells were not affected by cyclophosphamide treatment, suggesting that differences may exist in the metabolic state of the memory T cells in the two donor strains, despite the fact that they both develop similar levels of acquired resistance to a sub-

sequent tuberculous challenge.—Authors' Abstract

Kanaujia, G. V., Katoch, V. M., Shivannavar, C. T., Sharma, V. D. and Patil, M. A. Rapid characterization of *Mycobacterium fortuitum-chelonei* complex by restriction fragment length polymorphism or ribosomal RNA genes. FEMS Microbiol. Lett. **77** (1991) 205–208.

Using labeled, γ-^{32}P rRNA of mycobacteria as a probe restriction fragment length polymorphism (RFLP) of rRNA genes of strains belonging to the *Mycobacterium fortuitum-chelonei* complex was analyzed. Each DNA sample was cleaved with *Eco*RI restriction endonuclease, the fragments were separated by agarose gel electrophoresis and transferred to nitrocellulose membrane. Fragments of DNA containing rRNA genes were identified by hybridization with γ-^{32}P-labeled rRNA. Patterns were found to be species specific and both the species were distinguishable from each other. Results indicate that this approach can be used for rapid genomic characterization of the *M. fortuitum-chelonei* complex.—Authors' Summary

Kiroshka, V. S., Shinkareva, T. I., Litvinov, V. I., Chernousova, L. N., Ginda, S. S. and Gilburd, B. S. [Characterization of the spectrum of antibody response to *Mycobacterium tuberculosis* determinants by the method of immunoblotting.] Zh. Mikrobiol. Epidemiol. Immunobiol. **1** (1991) 61–63. (in Russian)

The spectrum of antibody response to *Mycobacterium tuberculosis* antigenic determinants H37Rv and *M. bovis* antigenic determinants BCG was studied in serum samples from 33 healthy donors and 31 patients with infiltrative pulmonary tuberculosis by the method of immunoblotting. The study revealed that most frequently tuberculosis patients showed response to Ag-H37Rv with molecular weights of 52, 39, 35, 21, 31, 68 kDa (44.4–22.2%) and Ag-BCG with molecular weights of 60, 58, 50, 25, 54, 70 kDa (33.3–22.2%). By month 9 of effective chemotherapy binding predominantly with Ag-H37Rv determinants of 31, 62, 35, 75, 56, 28, 19, 5, 13 kDa (75–37.5%) and Ag-BCG determinants of 13, 34, 38, 44, 19, 36, 45, 52, 58, 60, 70 kDa (37.5–25%) were registered. Some differences in the spectra of antibody response to Ag-H37Rv and Ag-BCG determinants were noted.—Authors' English Abstract

Klyshev, T. L., Muminov, T. A., Sharmanoy, A. T. and Park, A. V. [The role of precipitating antibodies in the serum of tuberculosis patients.] Probl. Tuberk. **1** (1991) 59–60. (in Russian)

The content of serum α_1-proteinase inhibitors (α_1-PI), α_2-macroglobulins, transferrins, albumin, immunoglobulins A, M and G and C_3- and C_4-complement factors was investigated in a group of patients (N = 35) with pulmonary tuberculosis who showed precipitating antituberculous circulating antibodies having the properties of autoantibodies. A direct relationship between the individual circulating antibodies crossreacting with the tissues and the rate of an extrapulmonary localization of tuberculous process in pulmonary tuberculosis patients, on the one hand, and their inverse relationship to the level of blood α_1-PI, on the other, were revealed. It is recommended to define crossreacting antibodies and α_1-PI concentration to predict the risk of extrapulmonary foci of a tuberculous inflammation.—Authors' English Abstract

Lazraq, R., Houssaini-Iraqui, M., Clavel-Sérès, S. and David, H. L. Cloning and expression of the origin of replication of mycobacteriophage D29 in *Mycobacterium smegmatis*. FEMS Microbiol. Lett. **80** (1991) 117–120.

Libraries of mycobacteriophage D29 genes were obtained by transforming *Escherichia coli* with constructs derived from pUC19. The genomic libraries were used to transform *Mycobacterium smegmatis* MC^{2}155, and one of the vectors designated pRM64 was found to stably replicate in the mycobacterial recipients. The pRM64 vector contained a 2.65-kb fragment that was used as a probe and was then located on the physical map of D29. Vectors containing this fragment replicated stably in *M. smegmatis* for at least 144 generations in medium without the selective agent used (kana-

mycin); vectors not containing the fragment did not replicate in the recipient *M. smegmatis*. This is the first report showing that the cloning of the OriR of a mycobacteriophage allowed the stable replication of shuttle vectors for mycobacterial genetics.—Authors' Summary

Lim, S. D., Todd, J., Lopez, J., Ford, E. and Janda, J. M. Genotypic identification of pathogenic *Mycobacterium* species by using a nonradioactive oligonucleotide probe. J. Clin. Microbiol. **29** (1991) 1276–1278.

Commercial DNA hybridization assays utilizing alkaline phosphatase-labeled oligonucleotide probes for the identification of *Mycobacterium tuberculosis* complex and *M. avium* complex (MAC) were evaluated with 261 isolates of mycobacteria. On the basis of biochemical criteria, the test for MAC was 98% specific and more sensitive (95 of 99, 95%) than Gen-Probe (88 of 99, 89% sensitivity); the major difference in sensitivity noted between the two systems was related to the hybridization of seven MAC strains to the SNAP X probe. The *M. tuberculosis* complex probe correctly identified all 62 isolates of *M. tuberculosis* and all 11 isolates of *M. bovis*, for a sensitivity of 100%. There were two discrepant reactions with mycobacteria other than *M. tuberculosis* complex isolates.—Authors' Abstract

Lyashchenko, K. P., Bobrovnik, S. A. and Komissarenko, S. V. [Development of delayed hypersensitivity to mycobacterial antigens.] Zh. Mikrobiol. Epidemiol. Immunobiol. **4** (1991) 66–68. (in Russian)

In experiments on guinea pigs and BALB/c mice delayed hypersensitivity to mycobacterial antigens was induced by the sensitization of the animals with live BCG or killed *Mycobacterium bovis* or *M. avium* in incomplete Freund's adjuvant. In the study of the dynamics of the development of skin reactivity to tuberculin some advantages of the sensitization of guinea pigs with live mycobacteria were revealed; while after the revaccination of the animals no development of secondary cell-mediated immune response was observed. The immunization of guinea pigs with atypical mycobacteria prior to their sensitization with BCG was found to lead to the development of higher skin reactivity to allergen prepared from atypical mycobacteria than skin reactivity to tuberculin.—Authors' English Abstract

Mayrink, W., Michalick, M. S. M., Melo, M. N., Williams, P., Nascimento, E., Magalhaes, P. A., da Costa, C. A., de Oliveira Lima, A. and Dias, M. [Treatment of American cutaneous leishmaniasis by means of vaccine.] An. Bras. Dermatol. **66** (1991) 55–59. (in Portuguese)

Sixty-two patients with cutaneous leishmaniasis were treated by immunotherapy, using a vaccine composed of killed promastigotes. The therapeutic scheme proved to be an adequate alternative method for the treatment of the disease. The cure rate was 75.8% and no side effects were recorded. The treatment was effective for both cutaneous and mucocutaneous lesions.—Authors' English Summary

Mayurnath, S., Vallishayee, R. S., Radhamani, M. P. and Prabhakar, R. Prevalence study of tuberculous infection over fifteen years, in a rural population in Chingleput district (south India). Indian J. Med. Res. [A] **93** (1991) 74–80.

As in the earlier BCG trial against tuberculosis conducted in Chingleput District in South India (in 1969), the entire study population was tuberculin tested (Survey I), a study was undertaken subsequently to see whether in this population there was any change in the tuberculosis situation in terms of prevalence of infection in children. For this purpose, in two of the panchayat unions, in a random sample of panchayats, tuberculin testing was repeated twice at an interval of 10 yr (Survey II) and 15 yr (Survey III) after the initial testing in children aged 1–9 yr. High coverages were obtained for tuberculin testing and reading. Data from 8703 and 9709 children at Surveys I and II, respectively, was used for comparing the prevalence of infection over a period of 10 yr and from 4808, 4965 and 4889 children at Surveys I, II and III, respectively, for comparing the prevalence of infection over a period of 15 yr. The results showed that

although the prevalence of infection varied in the two panchayat unions, within each panchayat union it did not differ significantly at the three surveys. The overall prevalence of infection at the three surveys was 9.0%, 10.2% and 9.1%, respectively. The average annual risk of tuberculosis infection was estimated to be 1.7%, 1.9% and 1.7% at the three surveys, respectively. Thus, the results clearly showed that, over a period of 15 yr, there was no change in the tuberculosis situation, in terms of prevalence of infection, in the study population.—Authors' Abstract

Medda, S., Das, N., Bachhawat, B. K., Mahato, S. B. and Basu, M. K. Targeting of plant glycoside-bearing liposomes to specific cellular and subcellular sites. Biotechnol. Appl. Biochem. **12** (1990) 537–543.

The possibility of using liposomes as an effective drug delivery system has been studied by incorporation of two plant glycosides of varying terminal sugar residues onto the surface of liposomes and examination of their distribution in different tissues. The two glycosides, corchorusin D and asiaticoside having glucose and rhamnose, respectively, at the terminal ends were selected for the purpose. The hepatic uptake of liposomes made from egg lecithin, cholesterol and dicetyl phosphate and either of the two glycosides was compared. The hepatic uptake of asiaticoside bearing liposomes was reduced; whereas that of corchorusin D-bearing liposomes was enhanced and was specific for glucose. Liver perfusion followed by cell separation showed that the uptake is mostly into the nonparenchymal cells of liver. The distribution of corchorusin D-bearing liposomes was maximal in the lysosomal fraction of the nonparenchymal cells. Ways of using corchorusin D-bearing liposomes as delivery systems for drugs or enzymes to lysosomes have been sought.—Authors' Abstract

Momotani, E., Yoshino, T., Ishikawa, Y. and Nakajima, Y. Immunohistochemical study of bovine lymph nodes with antibodies against S100 protein subunits: comparison between lymph nodes of healthy and *Mycobacterium paratuberculosis*-infected cattle. Res. Immunol. **141** (1990) 771–782.

Using immunohistochemistry, the differential distribution of the α subunit (S100α) and β subunit (S100β) of S100 protein was studied in mesenteric lymph nodes from normal or *Mycobacterium paratuberculosis*-infected cattle. In epithelioid cell granulomas, S100α-positive epithelioid cells and some giant cells were scattered among S100α-negative cells, which were predominant. The S100β-positive and -negative cells contained acid-fast bacilli. The presence of S100β-positive cells was not demonstrated in the granulomas. In normal component cells in the lymph nodes, follicular dendritic cells in the germinal centers and endothelium of lymphatic sinus and lymph vessels were positive for S100α. S100β was positive only in the endothelial cells of blood vessels. Results shown in the present paper are discussed in light of results obtained in other work on human tissues using the same sources of antibodies.—Authors' Summary

Orrell, J. M., Brett, S. J., Ivanyi, J., Coghill, G., Grant, A. and Beck, J. S. Measurement of the tissue distribution of immunoperoxidase staining with polyclonal anti-BCG serum in lung granulomata of mice infected with *Mycobacterium tuberculosis*. J. Pathol. **164** (1991) 41–45.

Mice inoculated with *Mycobacterium tuberculosis,* strain H37Rv were used as a model of human tuberculosis. The microanatomical location of immunoperoxidase staining with a polyclonal anti-BCG serum was within macrophages and appeared granular rather than delineating whole bacilli. Immunoperoxidase staining appears to demonstrate degraded mycobacterial antigens from disrupted organisms and so reflects prior turnover of bacilli. On Ziehl-Neelsen staining, intact or almost intact bacilli are seen and so the extent of this form of staining reflects the current bacillary load. Both methods have limited sensitivity, but with larger mycobacterial loads the area of immunoperoxidase stain measured on a semi-automated image analyzer correlated with the numbers of bacilli observed. The

immunoperoxidase method will be useful in the evaluation of residual antigen in studying the pathogenesis of experimental murine tuberculosis. In human mycobacterial granulomata, this immunohistochemical technique should provide an alternative method of estimating the extent of bacillary load: this approach may also provide evidence of mycobacterial infection from residual antigen deposits in the tissue when whole bacilli have been successfully cleared.—Authors' Summary

Perrone, C., Gikas, A., Truffot-Pernot, C., Grosset, J., Vilde, J.-L. and Pocidalo, J.-J. Activities of sparfloxacin, azithromycin, temafloxacin, and rifapentine compared with that of clarithromycin against multiplication of *Mycobacterium avium* complex within human macrophages. Antimicrob. Agents Chemother. **35** (1991) 1356–1359.

The activities of sparfloxacin, azithromycin, temafloxacin, and rifapentine against two virulent strains of the *Mycobacterium avium* complex isolated from patients with AIDS were evaluated in a model of intracellular infection and were compared with that of clarithromycin. Human monocyte-derived macrophages were infected with the *M. avium* complex at day 6 of culture. The intracellular CFU was counted 60 min after inoculation. The intracellular and supernatant CFU was counted on days 4 and 7 after inoculation. The concentrations used, which were equal to peak levels in serum, were 10 μg of rifapentine per ml (MICs for the two strains, 4 and 16 μg/ml), 4 μg of clarithromycin per ml (MICs, 8 and 4 μg/ml), 1 μg of azithromycin per ml (MICs, 32 and 16 μg/ml), 4 μg of temafloxacin per ml (MICs, 2 and 16 μg/ml), and 1 μg of sparfloxacin per ml (MICs, 0.5 and 2 μg/ml). Compared with controls on day 7 after inoculation, clarithromycin ($p < 0.001$), sparfloxacin ($p < 0.001$), and azithromycin ($p < 0.001$ for the first strain, $p < 0.02$ for the second) slowed intracellular replication. Rifapentine ($p < 0.001$) and temafloxacin ($p < 0.001$) slowed intracellular replication of the first strain but not of the second strain. Azithromycin plus sparfloxacin was as effective as sparfloxacin alone. In this macrophage model, sparfloxacin or clarithromycin (difference not significant) exhibited a better efficacy than rifapentine, azithromycin, or temafloxacin against intracellular *M. avium* complex infection.—Authors' Abstract

Pitchappan, R. M., Brahmajothi, V., Rajaram, K., Subramanyam, P. T., Balakrishnan, K., and Muthuveeralakshmi, R. Spectrum of immune reactivity to mycobacterial (BCG) antigens in healthy hospital contacts in South India. Tubercle **72** (1991) 133–139.

In an effort to study the immunological responses to antigens of tubercle bacilli, 49 tuberculin-positive and 41 tuberculin-negative hospital contacts aged 20–29 years (staff nurses and students working in Government Rajaji Hospital, Madurai, South India) were studied for serum antibodies (IgG, IgM and IgA classes) to BCG by ELISA and the diameter of induration to PPD by Mantoux procedures. The two immunological parameters were correlated in regression analysis. The results have revealed higher anti-BCG serum antibody levels in hospital contacts than in noncontacts, significantly higher antibodies in tuberculin-negative hospital contacts than in tuberculin-positive hospital contacts, an inverse correlation of tuberculin reactivity and antibodies and a bimodal decline (regression) of antibodies against the increase in skin test induration. This study has thus suggested the existence of an immunological spectrum in hospital contacts from South India; persons at one pole of the spectrum were tuberculin negative and possessed significantly elevated antibody levels, and those at the other pole of the spectrum were tuberculin positive and possessed low antibody levels. Thus, the spectrum of immune reactivity may be due to an inherent susceptibility/resistance of an individual to *Mycobacterium tuberculosis*.—Authors' Summary

Res, P. C. M., Orsini, D. L. M., Van Laar, J. M., Janson, A. A. M., Abou-Zeid, C. and de Vries, R. R. P. Diversity in antigen recognition by *Mycobacterium tuberculosis*-reactive T cell clones from the synovial fluid of rheumatoid arthritis patients. Eur. J. Immunol. **21** (1991) 1297–1302.

In a previous study we have shown that synovial fluid mononuclear cells from many rheumatoid arthritis (RA) patients exhibit an enhanced response to *Mycobacterium tuberculosis* antigens as compared to peripheral blood mononuclear cells. The 65-kDa heat-shock protein of *M. tuberculosis* was shown not to play an important role in this response, therefore other mycobacterial proteins must be involved. In this study we have investigated the possibility that synovial fluid T cells from RA patients predominantly recognize a limited number of *M. tuberculosis* antigens, as a result of a lesion-specific activation of only those *M. tuberculosis*-reactive T cells that have cross-reacted with joint-related autoantigens. From the synovial fluid of four RA patients *M. tuberculosis*-reactive T-cell clones were isolated and analyzed for their phenotype, HLA-DR restriction and proliferation to immunoblot fractions containing sodium dodecyl sulfate-polyacrylamide gel-separated *M. tuberculosis* proteins of known molecular weight range. The overall *M. tuberculosis* immunoblot recognition pattern of the clones was strikingly heterogeneous. Within a panel of 15 clones, 12 different antigenic specificities could be distinguished. In other words, we did not observe a dominant recognition of a few *M. tuberculosis* antigens by synovial fluid T cells. This argues against the hypothesis that the elevated synovial T-cell reactivity against *M. tuberculosis* is a reflection of an *in vivo* expansion of a limited number of different types of *M. tuberculosis*-reactive T cells as a result of a crossreaction with putative joint autoantigens.—Authors' Abstract

Seledtsova, G. V. and Koslov, V. A. [Immunoregulatory properties of monocytes/macrophages in patients with pulmonary tuberculosis.] Probl. Tuberk. **5** (1991) 54–56. (in Russian)

Antigen-presenting ability of monocytes/macrophages (Mc/Mph) was studied in 26 patients with pulmonary tuberculosis and in 21 healthy donors. The above investigation has demonstrated that the ability of the Mc/Mph patients to present mycobacterial antigens was not only intact, but also appeared to be even higher than the reference values of Mc/Mph antigen presentation in healthy donors (0.7 and 0.28, respectively). The ability of Mc/Mph to secrete interleukin-1 (IL-1) both spontaneously and as a response to muramil dipeptide, a synthetic macrophage activator, was studied in 24 tuberculosis patients and 15 healthy donors. During the experiment it was revealed that the affected and healthy cells binding to a plastic surface do not differ in their spontaneous production of IL-1 (0.8 and 0.79, respectively). At the same time in response to muramil dipeptide stimulation, the Mc/Mph patients produced lesser IL-1 than those of the healthy donors stimulated under the similar conditions (3.2 ± 1.1 and 10.6 ± 3.6, respectively).—Authors' English Abstract

Shafer, R. W. and Jones, W. D. Relapse of tuberculosis in a patient with the acquired immunodeficiency syndrome despite 12 months of antituberculous therapy and continuation of isoniazid. Tubercle **72** (1991) 149–151.

A 33-year-old man with AIDS and pleuro-pulmonary tuberculosis was treated with a combination of antituberculous medications for 12 months and with continuation of isoniazid. A total of 2 months after completing combination therapy the patient developed fever, malaise, and anorexia. Mycobacterial blood cultures grew *Mycobacterium tuberculosis* and the patient improved with the re-administration of rifampin and pyrazinamide. Phage typing of the patient's isolates of *M. tuberculosis* confirmed that he had experienced a relapse and not a re-infection. The patient had received 5 months of his treatment while hospitalized. We believe he was compliant with therapy outside the hospital because he attended all of his clinic appointments. Follow-up studies of HIV-infected patients with tuberculosis are therefore needed.—Authors' Summary

Sirawaraporn, W., Sirawaraporn, R., Chanpongsri, A., Jacobs, W. R., Jr. and Santi, D. V. Purification and characterization of dihydrofolate reductase from wild-type and trimethoprim-resistant *Mycobacterium smegmatis.* Exper. Parasitol. **72** (1991) 184–190.

Dihydrofolate reductase (DHFR) from extracts of *Mycobacterium smegmatis* strain mc²6 and trimethoprim-resistant mutant mc²26 was purified to homogeneity. In crude extracts, the specific activity of the enzyme from the trimethoprim resistant strain was comparable to that from the sensitive strain. The DHFR from both sources was purified using affinity chromatography on MTX-Sepharose followed by Mono Q FPLC. The enzyme has an apparent molecular mass of 23 kDa from gel filtration on Sephadex G-100 and from SDS-PAGE. Amino terminal sequence analysis showed homology with DHFRs from a subset of other gram-positive organisms. The purified enzyme from the trimethoprim-sensitive organism exhibited K_m values for H_2folate and NADPH of 0.68 ± 0.2 μM and 21 ± 4 μM, respectively. The K_m values for H_2folate and NADPH for the enzyme from the drug-resistant organism were 1.8 ± 0.4 μM and 5.3 ± 1.5 μM, respectively. A k_{cat} of 4.5 sec^{-1} was determined for the DHFR from both sources. The enzyme from both sources was competitively inhibited by pyrimethamine and trimethoprim. The K_i value of trimethoprim, for the enzyme from the drug-resistant organism was about sixfold higher than for the enzyme from drug-sensitive strain. Our data suggest that mutation of DHFR contributes to trimethoprim resistance in the mc²26 strain of *M. smegmatis.*—Authors' Abstract

Söderström, K., Halapi, E., Nilsson, E., Grönberg, A., Van Embden, J., Klareskog, L. and Kiessling, R. Synovial cells responding to a 65-kDa mycobacterial heat shock protein have a high proportion of a TcRγδ subtype uncommon in peripheral blood. Scand. J. Immunol. **32** (1990) 503–515.

We have analyzed the ability of T cells from synovial fluid mononuclear cells (SFMC) and from peripheral blood mononuclear cells (PBMC) of inflammatory arthritic diseases to proliferate in response to mycobacterial antigens (65-kDa heat-shock protein [hsp] of BCG, whole BCG) and to rat collagen type II. The SFMC demonstrated a significantly greater ability to respond to 65-kDa hsp of BCG, and to whole BCG, compared with PBMC from the same patients. With collagen type II, only a small proportion of the patients showed a proliferative response, although with this antigen also SFMC responded better than PBMC. There was no difference between SFMC and PBMC in the response to control antigen (tetanus toxoid), phytohemagglutinin (PHA), or interleukin 2 (IL-2). A high proportion of cells in SFMC-derived short-term T-cell lines were of TcRγδ type, often exceeding the number of TcRγβ type. There was a significantly higher proportion of TcRγδ cells in the SFMC lines compared with the PBMC lines, and a large part of the TcRγδ cells in the SFMC cultures was CD8+. The SFMC lines had a high proportion of δ-TCS-1+ cells (Vδ1) among their TcRγδ cells, always exceeding the percentages of TiγA+ (Vγ9) and BB3+ (Vδ2). In the PBMC lines, the distribution of TcRγδ subtypes was markedly different, with a TiγA+/BB3+ population in the majority. These data argue for a different subpopulation distribution of TcRγδ cells in synovial fluid compared with peripheral blood of patients with inflammatory arthritic diseases.—Authors' Abstract

Van Lieshout, L., De Jonge, N., Bassily, S., Mansour, M. M. and Deelder, A. M. Assessment of cure in schistosomiasis patients after chemotherapy with praziquantel by quantitation of circulating anodic antigen (CAA) in urine. Am. J. Trop. Med. Hyg. **44** (1991) 323–328.

The kinetics of circulating anodic antigen (CAA) levels in urine were studied in Egyptian male patients infected with *Schistosoma mansoni* or with both *S. mansoni* and *S. haematobium,* before treatment, and at 1, 3, and 6 weeks after chemotherapy. A quantitative enzyme-linked immunosorbent assay (ELISA) demonstrated CAA in 82% of the serum and 89% of the urine samples from these 28 patients. To evaluate the possibility of circadian variability in urine CAA levels, samples were examined in 15 patients at four intervals during a 24-hr period. No significant differences in CAA titers were observed. Seventeen patients were subsequently treated with praziquantel and followed for 6 weeks. CAA titers in serum and urine decreased significantly 1 week after therapy. Thereafter, the profile of CAA

titer in urine continued to show a parallel but delayed decline compared to that in serum. While all serum CAA titers became negative 3–6 weeks after treatment, urine titers were negative in 47% at 3 weeks and 69% at 6 weeks. The remaining positive patients had low titers. A significant quantitative correlation in CAA titer was found between serum and urine before and after treatment. Seventeen Egyptian control subjects with no active schistosome infection were negative for CAA in both serum and urine. Our results confirm that the CAA urine assay could be used as a sensitive and noninvasive method to diagnose the disease, and indicate that the assay can be used to monitor efficacy of schistosome chemotherapy.—Authors' Abstract

Veeneman, G. H., Van Leeuwen, S. H., Zuurmond, H. and Van Boom, J. H. Synthesis of carbohydrate-antigenic structures of *Mycobacterium tuberculosis* using an iodonium ion promoted glycosidation approach. J. Carbohydr. Chem. **9** (1990) 783–796.

Analogs of the phenol-phthiocerol glycoside of *Mycobacterium tuberculosis* were synthesized starting from properly protected rhamnose and fucose ethyl thioglycosides. A recently developed iodonium ion promoted glycosidation procedure proved to be very efficient for the preparation of 3-aminopropyl 3-*O*-(α-L-rhamnopyranosyl)-2-*O*-methyl-α-L-rhamnopyranoside, 3-aminopropyl 3-*O*-[3-*O*-(2,3,4-tri-*O*-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranosyl]-2-*O*-methyl-α-L-rhamnopyranoside and 3-aminopropyl 3-*O*-(2,3,4-tri-*O*-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranoside.—Authors' Abstract

von Reyn, C. F., Hennigan, S., Niemczyk, S. and Jacobs, N. J. Effect of delays in processing on the survival of *Mycobacterium avium-M. intracellulare* in the isolator blood culture system. J. Clin. Microbiol. **29** (1991) 1211–1214.

Concentrations of *Mycobacterium avium-M. intracellulare* ranging from 10^{-1} to 10^{3} CFU/ml were added to blood, placed in Isolator tubes, and held at room temperature for intervals ranging from 4 hr to 56 days before being processed (centrifugation and culture on Middlebrook 7H10 agar). At all concentrations tested, *M. avium-M. intracellulare* was recovered after hold times ranging from 4 hr to 7 days; the number of final CFU actually increased progressively for hold times of 8 hr or more. Hold times of up to 7 days did not increase the time from processing to the first appearance of visible colonies. At an inoculum of 10^{2} CFU/ml, *M. avium-M. intracellulare* was recovered from Isolator tubes processed 56 days after inoculation. Two Isolator blood cultures were drawn from a patient with AIDS; *M. avium-M. intracellulare* was recovered from the sample processed immediately and from the sample processed after a hold time of 7 days. Since *M. avium-M. intracellulare* survives for prolonged periods in Isolator tubes, blood cultures may be collected in outpatient settings or in hospitals without mycobacterial culture facilities and shipped to reference laboratories for processing without loss of viability.—Authors' Abstract