CURRENT LITERATURE

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

General and Historical

Nagao, E. [The present conditions and the future measure at national leprosaria in Japan.] Jpn. J. Lepr. **64** (1995) 93–99.

In Japan, 90% of leprosy in-patients are housed in leprosaria. As of the end of 1994, the overall number of in-patients hospitalized in Japan in leprosaria was 5811, of whom 5767 in-patients were hospitalized in national leprosaria. In recent years, the number of Japanese patients newly affected by leprosy in Japan has decreased to within 10. Accordingly, the number of new admissions to leprosaria has decreased to as low as zero. The number of readmissions, on the other hand, is about 30 a year, and about 15 patients a year return to their social lives. It is estimated that the number of patients hospitalized in leprosaria will decrease to about 4000 by the year 2005, about 2000 by the year 2015, and further to about 700 by the year 2025, as a result of deaths due to patient ageing. At the end of 1994, patients who were leprosy bacillus-positive in the skin-smear test accounted for only 2% in the overall hospitalized patients, and the counterplan for leprosy is almost terminating in the leprosaria. However, about 40% of the patients hospitalized in leprosaria are in fact still on medication, and there still remain unresolved problems. Treatment at the leprosaria is thought to be insufficient to deal with the anxiety of recurrence (the reason for the medication), leprous neuralgia, iritis and glaucoma, leprous acute reaction, and intractable leprosy, and a joint counterplan on a nationwide scale is needed. In the future, it would be necessary to secure medical welfare for "the decrease and ageing of hospitalized patients" at leprosaria. [The 14 figures all have legends in English.]—Author's English Abstract

Naik, S. S., Revankar, C. R. and Ganapati, R. Women workers in leprosy. Indian J. Lepr. **67** (1995) 329–331.

Resulting from a questionnaire survey of leprosy administrators in selected regions in India in low endemic areas, hyperendemic areas, and major states, this short article draws attention to the very small proportion of leprosy workers who are women: 142 (2.3%) out of 6155. The proportion of women working on leprosy is higher for technicians (19%), medical officers (8.5%), and physiotherapy technicians (6.6%) than that for nonmedical supervisors and paramedical workers (1–2%); none of 40 health educators was a woman. Rural areas are particularly deprived of female workers. The authors comment that this paucity of women workers may be a handicap to the National Leprosy Eradication Programme in total population surveys, jeopardizing the proper clinical examination of women and the full cooperation of female leprosy patients for drug intake and disability prevention and care of deformities. As an immediate remedy, leprosy training of existing women workers in general health services is suggested and their utilization in leprosy work as well as their routine duties.—C. A. Brown (Trop. Dis. Bull.)

Vaz, M., Jacob, A. J. W. and Rajendran, A. An evaluation of the future role of non-governmental organizations currently engaged in leprosy control in India. S.E. Asian J. Trop. Med. Pub. Health **26** (1995) 297–300.

The mass implementation of short-term multidrug therapy in India has led to dramatic falls in the prevalence of leprosy.

This paper addresses the future role of non-governmental organizations currently involved in leprosy control. This evaluation is based on current trends in leprosy control, projected health needs in the future and the necessity to maximize health care outputs in the face of limited resources.—Authors' Abstract

Chemotherapy

Ahmadi, M., Khalaf, L. F., Smith, H. J. and Nicholls, P. J. A dapsone-induced blood dyscrasia in the mouse: evidence for the role of an active metabolite. J. Pharm. Pharmacol. **48** (1996) 228–232.

In the female mouse, dapsone (50–500 mg kg^{-1}, p.o.) caused a dose-related methemoglobinemia which peaked at 0.5–1 hr with recovery to baseline values occurring by 4 hr. Cimetidine (100 mg kg^{-1}, p.o.), a known inhibitor of several hepatic P450 isozymes administered 1 hr before dapsone, prevented the methemoglobinemia. *In vitro,* dapsone required activation by mouse hepatic microsomes to cause methemoglobin formation in mouse erythrocytes and cytotoxicity to human mononuclear leukocytes. In both instances, the toxic effects were markedly reduced by cimetidine. Daily dosing of mice with dapsone cell (50 mg kg^{-1}, p.o.) for 3 weeks induced a blood dyscrasia, characterized by a fall of platelet and white blood cell counts, which was inhibited by cimetidine (100 mg kg^{-1}, p.o. daily). It is concluded that an active metabolite of dapsone arising from a P450-dependent pathway is involved in the genesis not only of the methemoglobinemia but also the blood dyscrasia arising from repeated administration of the drug in this species.—Authors' Abstract

Ahmed, K. M. and El Tahir, M. S. The role of village leaders in the implementation of multidrug therapy for leprosy, Sudan—a pilot study in the Angasana Hills. Lepr. Rev. **67** (1996) 39–46.

The purpose of this study is to implement multidrug therapy (MDT) and to evaluate the possible role of village leaders in supervising MDT treatment in remote and inaccessible areas in Sudan where health facilities are poor.

Three villages from the Angasana Hills southeast of Sudan, where leprosy is endemic, have been chosen for this study. C

A health education course for village leaders in the area was conducted. Three medical assistants from a nearby village were identified to examine all leprosy suspects and to put the diagnosed cases on treatment. The village leaders were to supervise the treatment of the patients during the rainy season.

Out of 43 cases detected all paucibacillary (PB) cases detected (11 cases) completed their treatment and 28 out of 32 multibacillary (MB) cases were regularly on treatment.

It has been obvious that the village leaders were useful in supervising MDT in the Angasana area, a process which can be extended to other inaccessible areas in the Sudan.—Authors' Summary

Chio, L. C., Bolyard, L. A., Nasr, M. and Queener, S. F. Identification of a class of sulfonamides highly active against dihydropteroate synthase from *Toxoplasma gondii, Pneumocystis carinii,* and *Mycobacterium avium.* Antimicrob. Agents Chemother. **40** (1996) 727–733.

Sulfanilanilides with 3',5'-halogen substitutions had Ki values 6- to 57-fold lower than the Ki of sulfamethoxazole when tested against dihydropteroate synthase from *Toxoplasma gondii.* The compounds acted as competitive inhibitors. These compounds were also active against dihydropteroate synthase from *Pneumocystis carinii, Mycobacterium avium,* and *Escherichia coli* but were not significantly more active than sulfamethoxazole. The compounds were significantly more active in culture than were standard agents. Against *T. gondii* in culture, 50% inhibitory

concentrations were 7- to 30-fold lower than that of sulfadiazine; against *P. carinii* in culture, a concentration of 100 μM caused 33% to 95% inhibition of growth, compared with 9% inhibition with 100 μM sulfamethoxazole.—Authors' Abstract

Dhople, A. M. and Dimova, V. *In vitro* activity of a new rifamycin derivative against *Mycobacterium leprae.* Arzneimittelforschung **46** (1996) 210–212.

The antimicrobial effects of T-9 (3-(4-cinamyl-1-piperazinyl)iminomethyl rifamycin SV) alone and in combination with ofloxacin, against strains of *M. leprae* were evaluated, using an *in vitro* cell-free culture system. The minimum inhibitory concentrations (MICs) of T-9 against rifampin-sensitive and rifampin-resistant strains of *M. leprae* were 0.1 μg/ml and 0.4 μg/ml, respectively. Furthermore, in common with rifabutin, but not with rifamycin, T-9 demonstrated synergy with ofloxacin against both rifampin-sensitive and rifampin-resistant strains of *M. leprae.* The results suggest that T-9, in combination with ofloxacin as part of multidrug regimens, warrants further evaluation as treatment for patients with leprosy.—Authors' Abstract

Feenstra, P. Achieving multidrug therapy for all leprosy patients—ILEP Medical Bulletin. Lepr. Rev. **66** (1995) 169–176.

This is an important and salutary document which needs to be made available to all national health boards and services dealing with the treatment of leprosy. Fifteen recommendations are made to correct problems identified in a global survey of 228 ILEP projects, in Africa, Asia, Latin America and Europe, involving 239,574 patients registered for chemotherapy; this survey covered about 40% of all ILEP programs. Only 2 in every 3 patients are receiving multidrug therapy (MDT) in these programs. This situation is clearly very unsatisfactory and cause for concern. The recommendations are made in response to problems categorized as patient management or program management obstacles. The 8 recommendations for improved patient management emphasize the importance of beginning MDT at the earliest possible moment–at diagnosis, screening or immediately following register review. A flexible attitude to patient drug supply is called for where patients cannot be effectively supervised throughout treatment. Patient registers dealing with dapsone monotherapy need to be reviewed as soon as possible and patients either released from treatment or transferred to MDT. The WHO recommended treatment regimens need to be followed for both paucibacillary and multibacillary leprosy; even when skin smears are still positive no further chemotherapy is needed. Patient education and care are top priorities which are an essential part of limiting nerve damage and preventing the worsening of existing disabilities. Rigorous and standardized absentee retrieval procedures need to be uniformly established. Seven recommendations address program management obstacles. Inadequate management capacity, lack of standardized operational guidelines, inadequate recording and reporting (absentee control and follow up) all militate against the implementation of MDT programs. Insufficient facilities/trained staff, inadequate drug supply and lack of infrastructure and security all need to be addressed. General health care staff should be drawn into the leprosy control programs, and the good practice manuals and procedures established by ILEP/WHO should be followed. The writers of the recommendations remain confident that the goal of MDT for all leprosy patients by the year 2000 remains an achievable target.—M. Hooper (Trop. Dis. Bull.)

Fleming, C. J., Hunt, M. J., Salisbury, E. L. C., McCarthy, S. W. and Barnetson, R. S. Minocycline-induced hyperpigmentation in leprosy. Br. J. Dermatol. **134** (1996) 784–787.

A 36-year-old man was treated with dapsone, rifampin and clofazimine for borderline lepromatous leprosy. After 9 months, his leprosy plaques became progressively more red and after 23 months, the clofazimine was stopped and he was given minocycline instead. Six weeks later, he developed blue-black pigmentation in his leprosy lesions. The histology was consistent with minocycline-induced hyperpigmentation. This is the first report of minocycline-

induced pigmentation in leprosy. We suggest it is important to consider this side effect before the administration of minocycline in leprosy, particularly if it is prescribed in place of clofazimine.—Authors' Abstract

Ji, B. H., Perani, E. G., Petinom, C. and Grosset, J. H. Bactericidal activities of combinations of new drugs against *Mycobacterium leprae* in nude mice. Antimicrob. Agents Chemother. **40** (1996) 393–399.

The bactericidal activities of 12 regimens with various combinations of new drugs (clarithromycin [CLARI], minocycline [MINO], and ofloxacin [OFLO]) and the standard antileprosy drugs, especially rifampin (RMP), were compared in nude mice with established *Mycobacterium leprae* infection. The longest duration of treatment was 24 weeks for intermittent (once every 4 weeks) therapy and 8 weeks for daily therapy. Bactericidal effects were monitored by titrating the proportion of viable *M. leprae* isolates by subinoculating the organisms into the foot pads of immunocompetent and nude mice. The results indicate that RMP was more bactericidal than any combination of the new drugs. A single dose of CLARI-MINO, with or without OFLO, displayed bactericidal activity as great as that of 4 weeks of daily treatment with dapsone (DDS) plus clofazimine (CLO); thus, intermittent CLARI-MINO, with or without OFLO, may replace DDS and CLO of the standard multidrug regimen, and these will become regimens that can be administered monthly and under full supervision. Additional evidence that this may be the case is provided by the finding that intermittent RMP-CLARI-MINO or RMP-CLARI-MINO-OFLO administered for 12 or 24 weeks was as active as the standard multidrug regimen. While the intermittent treatment always displayed significantly greater bactericidal activity than the same number of doses of daily treatment, daily treatments with CLARI-MINO and CLARI-MINO-OFLO were more active than the drugs given as intermittent treatment for the same duration; therefore, unless these combinations are to be administered together with intermittent RMP, they should be given daily, especially for the treatment of RMP-resistant cases of infection. Finally, 12 weeks of daily treatment with DDS-CLO was more bactericidal than had been expected, suggesting that it may not be necessary to administer the standard multidrug regimen for multibacillary leprosy for as long as 24 months in order to minimize the risk of developing RMP resistance.—Authors' Abstract

Lau, Y. Y., Hanson, G. D. and Carel, B. J. Determination of rifampin in human plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatog. B **676** (1996) 147–152.

A simple, specific and sensitive high-performance liquid chromatographic (HPLC) method was developed for the determination of rifampin in human plasma. Rifampin and sulindac (internal standard) are extracted from human plasma using a C-2 Bond Elut extraction column. A 100-μl volume of 0.1 M HCl is added to the plasma before extraction to increase the retention of the compounds on the extraction column. Methanol (1 ml) is used to elute the compounds and 0.5 ml of 3 mg/ml ascorbic acid in water is added to the final eluate to reduce the oxidation of rifampin. Separation is achieved by reversed-phase chromatography on a Zorbax Rx C-8 column with a mobile phase composed of 0.05 M potassium dihydrogen phosphate-acetonitrile (55:45, v/v). Detection is by ultraviolet absorbance at 340 nm. The retention times of rifampin and internal standard are approximately 4.4 and 7.8 min., respectively. The assay is linear in concentration ranges of 50 to 35,000 ng/ml. The quantitation limit is 50 ng/ml. Both intra-day and inter-day accuracy and precision data showed good reproducibility.—Authors' Abstract

Mok, C. C. and Lau, C. S. Dapsone syndrome in cutaneous lupus erythematosus. J. Rheumatol. **23** (1996) 766–768.

Dapsone is increasingly used in the treatment of rheumatic diseases. It is indicated in many inflammatory skin conditions characterized by polymorphonuclear cell infiltration. Minor reactions to the drug are very common, but severe idiosyncratic reactions are rare considering the large number of pa-

tients taking this drug worldwide. We describe 2 patients with cutaneous lupus erythematosus who developed severe dapsone reaction after low dose therapy, with a fatal outcome in one. Physicians should be aware of the potentially lethal side effects of dapsone.—Authors' Abstract

Clinical Sciences

Abbot, N. C., Beck, J. S., Mostofi, S. and Weiss, F. Sympathetic vasomotor dysfunction in leprosy patients: comparison with electrophysiological measurement and qualitative sensation testing. Neurosci. Lett. **206** (1996) 57–60.

Testing of skin vasomotor reflexes (VRs) by laser Doppler flowmetry (LDF) is now a recognized method of measuring peripheral dysautonomia. To assess its specificity as an indicator of impairment to unmyelinated autonomic fibers, VR testing at the fingerpulp was compared with standard qualitative sensation (QST) and with sensory electrophysiological (SNVC) measurements in 39 Iranian leprosy patients. There was a significant relationship between VR and SNCV values (but not QST): these were jointly measurable in 38.5% of fingers, and jointly absent in 35.3% of fingers which also showed significantly reduced LDF perfusion and skin temperatures. However, in 10.3% of fingers, predominantly index and otherwise apparently healthy, VRs were absent but SNCV present, suggesting early subclinical autonomic impairment. In a further 16% of fingers, predominantly ulnar and with poor microcirculation, intact (though impaired) VRs could be recorded despite the absence of SNCV responses, suggesting sparing or regeneration of these fibers. This evidence suggests that where there is heterogeneity of nerve damage a combination of VR and electrophysiological testing can indicate the functional status of distinct fiber types.—Authors' Abstract

Abbot, N. C., Terencio, J., Ferrell, W. R., Torres, P., Wilson, M. G., Lockhart, J. C., Quintana, M. V., Gimeno, V., Beck, J. S. and Lopez Pla, J. [Red and near-infrared laser Doppler perfusion imaging of the hands of leprosy patients in Spain.] Rev. Leprol. (Fontilles) **20** (1995) 847–856. (in Spanish)

The new technique of laser Doppler perfusion imaging at red and near infrared wavelengths has been employed to image blood flow of the fingers in a group of leprosy patients and healthy volunteers at Sanatorio Fontilles. The technique allows flow to be measured in both dermal and subdermal structures and so could be useful to locate apparent microcirculatory impairment. At the ambient temperatures in Fontilles (23–26°C) very high rates of flow can be measured at the finger pulps which, because of their abundant arteriovenous anastamoses, have a thermoregulatory role. However, in many patients, including those without obvious clinical involvement of the hands, blood flow and skin temperature were significantly reduced, confirming previous findings on leprosy patients in India and Iran. The preliminary findings indicate that these reductions in flow are not localized to one anatomical territory but can affect all measured fingers in one subject, suggesting a more generalized impairment of thermoregulatory function. While this may involve a central mechanism, the results could also be explained by a continuing subchronic vasculitis of the fingers. Our previous findings of an association between loss of sensation and reduction in flow suggest that neural factors, such as a depletion of sensory neuropeptides, may also be involved.—Authors' English Summary

Barbosa, A. D., Silva, T. M. C., Santos, M. I. R., Garrido, M. D. F., Patel, B. N., Cavalcanti, M. and Riccio, M. C. O. Coexistence of an unusual form of scabies and lepromatous leprosy—a case report. Pathol. Res. Practice **192** (1996) 88–90.

A case of unusual crusted (Norwegian) scabies involving the entire skin of a 26-year-old Brazilian patient with lepromatous leprosy is reported. The more prominent histopathological findings were acanthosis, hyperkeratosis and crusting with many mites of *Sarcoptes scabiei.* In the dermis, numerous foamy histiocytes filled with abundant acid-fast bacilli were seen.—Authors' Abstract

El Harith, A., Chowdhury, S., Al Masum, A., SemiaoSantos, S., Das, P. K., Akhter, S., Vetter, J. C. M. and Haq, I. Reactivity of various leishmanial antigens in a direct agglutination test and their value in differentiating post-kala azar dermal leishmaniasis from leprosy and other skin conditions. J. Med. Microbiol. **44** (1996) 141–146.

A direct agglutination test (DAT) for the detection of post-kala azar dermal leishmaniasis (PKDL) was evaluated in conditions that simulate the disease clinically or immunologically. A reference strain of *Leishmania donovani* (LEM 1399) and antigen preparations from two *Leishmania* isolates from Bangladeshi patients with post-kala azar dermal leishmaniasis or visceral leishmaniasis were used. A titer of least 51,200 was obtained in tests of patients with PKDL with all three antigens; whereas a maximum titer of 1600 was recorded in patients with cutaneous leishmaniasis, mucocutaneous leishmaniasis or leprosy. Antigens from dermal isolates of *L. tropica* (LV 140) and *L. braziliensis* (LV 65) yielded titers of 1600–6400 in patients with PKDL. The lowest titer recorded in 70 patients tested with the homologous PKDL antigen was 409,600. In patients with leprosy, cutaneous leishmaniasis, syphilis, onchocerciasis, tuberculosis, blastomycosis or vitiligo, titers ranged from 100 to 1600. The DAT is better than current parasitological and histopathological methods for the diagnosis of PKDL in areas in which leprosy is co-endemic.—Authors' Abstract

Endoh, M., Ueki, A., Takahashi, K., Yamanaka, H., Izumi, S. and Tabira, T. Significantly increased frequency of the apolipoprotein E epsilon 4 allele in elderly non-demented leprosy patients. Neurosci. Lett. **207** (1996) 206–208.

Apolipoprotein E (apo E) genotypes in 350 leprosy patients were examined and compared with those of 870 age-matched controls. The allelic frequencies of the apo E gene did not differ between demented patients with leprosy and controls. However, the frequency of apo E epsilon 4 allele was significantly higher in non-demented leprosy patients than in controls ($p < 0.001$). of special interest is that the prevalence of E3/4 genotype in non-demented leprosy patients increased significantly with age, being 14.1%, 24.4%, and 28.3% in the 60s, 70s, and 80s, respectively ($p < 0.05$). These data suggest that apo E epsilon 4 is not a risk factor for senile dementia in elderly leprosy patients, and there exist factors to overcome the risk of apo E4 in leprosy patients.—Authors' Abstract

Gatti, C. F., Chá, D., Barquin, M. A. and Berben, V. [Wade's histoid leprosy.] Rev. Leprol. (Fontilles) **20** (1995) 857–861. (in Spanish)

We present a 65-year-old female patient coming from Corrientes (Argentina) who presented nodular tumors on face and neck with a 3-year evolution, which the diagnosis of histioid leprosy was confirmed by baciloscopy and microscopy.—Authors' English Summary

Kohli, M. M., Ganguly, N. K., Kaur, S. and Sharma, V. K. Urinary excretion of renal brush border membrane enzymes in leprosy patients—effect of multidrug therapy. Experientia **52** (1996) 127–130.

Renal function at the brush border membrane level has been studied using characteristic enzymes, such as alkaline phosphatase, leucine-aminopeptidase and gamma-glutamyl transpeptidase. Urinary enzyme studies were performed using leprosy patients, classified on the basis of bacteriological index (BI > 3; N = 20, BI < 3; N = 12, BI-ve; N = 10) and compared with control subjects (N = 10). The role of enzymuria in monitoring WHO-recommended multidrug therapy (MDT) has been evaluated in these patients. A significant increase in the enzyme activities ($p < 0.01$), as well as signif-

icant ($p < 0.01$) proteinurea in 24-hr urine samples of both the smear positive groups (Bf > 3, BI < 3) prior to therapy compared to control subjects, indicates proximal tubular functional impairment at brush border membrane level. In the smear-negative (BIve) group, no significant difference was observed in enzyme activities as compared with the control group. In a follow-up study (BI > 3; N = 13, BI < 3; N = 4) the activities of all the enzymes decreased significantly in all the groups when compared to a corresponding untreated group. The follow-up study was not carried out on the smear-negative group. The surprising finding was the differential behavior of γ-glutamyl transpeptidase, whose activity increased significantly ($p < -0.01$) even after therapy in BI > 3 group when compared with untreated patients. However, in a detailed work-up including hepatic and renal function tests, the serum biochemistry was found to be normal both before and after therapy. Urinary excretion of brush border enzymes seems to be related to bacterial load, and their potential in studying the effect of MDT remains unclear.—Authors' Abstract

Pal, S., Singh, S. B. and Bhattacharya, S. K. Immunological paramenters in leprosy patients with and without arthritis. J. Indian Med. Assoc. **93** (1995) 266–267.

Twelve leprosy patients (in Uttar Pradesh, India) with arthritis and 161 patients without arthritis were studied for immunological paramenters such as immunoglobulins (IgG, IgM, IgA), C-reactive proteins and rheumatoid factor. There was an increase in the levels of IgG, IgA value in leprosy patients with and without arthritis compared to 20 healthy controls. IgM level was decreased in both the groups compared to controls, but a significant decrease was observed ($p < 0.01$) in patients with arthritis. C-reactive protein was significantly positive in the leprosy with arthritis group ($p < 0.01$) and positive in 12 cases of the leprosy without arthritis group compared to the negative control group. Rheumatoid factor was present in leprosy with arthritis (16.6%) compared to both the control group and leprosy without arthritis group. This study concluded the presence of arthritis in leprosy patients as a definite entity which showed cahnges in immunological parameters.—Authors' Abstract

Qiu, W. [Oral diseases must be included into Leprosy Control Program.] China Lepr. J. **11** (1995) 90. (in Chinese)

For basic eradication of leprosy, making the prevalence < 0.01% and incidence < 0.5/100,000 by the year 2000, various projects of control and rehabilitation of leprosy were formulated and implemented by governments at different levels. Now, the number of leprosy patients decreased to 20,000 or less from some 500,000 in the early 1950s, and so the emphasis of leprosy control has been shifted to rehabilitation, but oral diseases of persons with and cured of leprosy all were neglected.

Based on a survey among 1155 patients and ex-patients in eight leprosaria of Guangdong in 1987, 976 persons have dental caries with 5108 decayed teeth, an average of 5.23 per person, of which only 28 decayed teeth have been filled, and so residual root rate is 70.7%, being about twice as much as in healthy people. Among these patients bad oral hygiene was seen in 81.5%, odontoliths in 87.4%, periodontal diseases in 76.8% and dentition defect in 69.5%. In addition, there was a need for dental filling in 50%, dental extraction in 72%, treatment of periodontal diseases in 78%, and inserting artificial teeth in 67%, but unfortunately no dentist was willing to do so because they fear leprosy patients. If all such severe oral diseases were not treated their health would be impaired.

"Health for all by 2000" ought to include oral health and oral conditions of leprosy patients, and it must be managed properly. For this, the following work should be done:

1. Health authorities and leprosy control institutions should provide the necessary resources for control of oral diseases in persons with and cured of leprosy.

2. To set up a leading group for the control of dental diseases in leprosy, formulating a relevant project and promoting its implementation.

3. To train leprosy workers in dentology, because the number of dentists is too few to complete the task.

4. To raise funds from various circles but most should be supplied by the government.

5. To popularize knowledge of oral hygiene in leprosy patients and ex-patients.—Author's English Abstract

Shimada, T., Nishimura, Y., Kimura, G., Eto, S. and Tomita, K. Vertebral osteomyelitis presenting with bilateral pleural effusions in a leprous patient. Diagn. Microbiol. Infect. Dis. **24** (1996) 101–103.

We present a highly rare case of vertebral osteomyelitis due to *Salmonella newport* that was associated with pleural effusion in a leprous woman. The salmonella infection was considered to be precipitated by her hemolytic anemia resulting from dapsone. The directs spread of infection from the vertebrae led to the pleurisy.—Authors' Abstract

Terencio de las Aguas, J. and Contreras Rubio, F. [Epidermoid carcinoma arising from neurotrophic ulceration in a leprosy patient.] Rev. Leprol. (Fontilles) **20** (1995) 865–873. (in Spanish)

The frequency of cancer and leprosy in our personal experience together with Cancer on chronic ulcers of leprosy patients is considered. A case of a 78-year-old female patient affected with lepromatous leprosy, inactive bacteriologically since 1958, with important neurological and bone problems is provided. Three years ago, she started to present an extensive neurotrophic ulcer on the right foot vegetating with a diagnosis of pseudoepitheliomatous hyperplasia and epidermoid carcinoma.—Authors' English Summary

Immuno-Pathology

Geitz, H., Handt, S. and Zwingenberger, K. Thalidomide selectively modulates the density of cell surface molecules involved in the adhesion cascade. Immunopharmacology **31** (1996) 213–221.

The mode of action of thalidomide (THD) in clinical cases of vasculitis is still not clear. Expression of adhesion molecules on endothelial cell lines was therefore assessed *in vitro*. THD is capable of changing the density of tumor necrosis factor alpha (TNF alpha) induced ICAM-1 (CD54), VCAM-1 (CD106) and E-selectin antigens on HUVEC. Furthermore, modulation of L-selectin (CD62L) by THD can be demonstrated on human leukocytes *in vitro*. The molecules investigated are involved in the neutrophil-endothelial cell interaction and participate in the adhesion cascade. Blunting of cytokine induced up-regulation of these adhesion molecules may account at least in part for anti-vasculitic effects of thalidomide.—Authors' Abstract

Goulart, I. M. B., Figueiredo, F., Coimbra, T. and Foss, N. T. Detection of transforming growth factor-beta 1 in dermal lesions of different forms of leprosy. Am. J. Pathol. **148** (1996) 911–917.

Immunohistochemical studies were performed to determine the presence and distribution of polypeptide transforming growth factor (TGF)-beta 1, a cytokine with macrophage-suppressing activity, in skin biopsies from 41 patients with different clinical forms of leprosy. We used an anti-TGF-beta 1 polyclonal antibody and the avidin-biotin-peroxidase (ABC complex) method. The results demonstrated that the lesions of the lepromatous and borderline lepromatous forms presented intense cytoplasm staining for TGF-beta 1 in the cells of the dermal infiltrate. A reaction of moderate intensity was observed in the cells of granulomas from borderline borderline cases; whereas no detectable immunoreaction was observed in granuloma cells from the tuberculoid and borderline tuberculoid forms. Considering that in the lepromatous leprosy form *Mycobacterium leprae* multiplies in the cytoplasm of macrophages and the lesions are diffuse and consist of poorly

differentiated young macrophages, we believe that these alterations may be explained at least in part by the presence of TGF-beta 1 in the dermal infiltrate. Production of the cytokine may be induced by the presence of the bacillus itself and of its constituents, causing a mechanism of parasite evasion. Similarly, the absence of TGF-beta 1 in tuberculoid leprosy, which progresses with a specific immune response to *M. leprae,* may explain the intense differentiation of macrophage cells with the formation of well-defined epithelioid granulomas capable of eliminating most of the bacilli.—Authors' Abstract

Gruber, R., Lederer, S., Bechtel, U., Lob, S., Riethmuller, G. and Feucht, H. E. Increased antibody titers against mycobacterial heat-shock protein 65 in patients with vasculitis and arteriosclerosis. Int. Arch. Allergy Immunol. **110** (1996) 95–98.

Heat-shock proteins (HSPs) are a group of highly conserved proteins that show extensive homology at the DNA and protein level among bacterial and mammalian species. Furthermore, bacterial HSPs induce specific cellular and humoral immune responses in mammals. Crossreacting antibodies may therefore be induced in chronic infections. Recently, it has been claimed that patients with arteriosclerosis (AS) of the carotid arteries have significantly elevated antibody titers to mycobacterial HSPs. In this study, we extended the spectrum of vascular diseases and analyzed sera from patients with systemic vasculitis and systemic lupus erythematosus (SLE) for the presence of anti-HSP antiboides. Anti-HSP antibodies, tested in an ELISA with recombinant mycobacterial HSP 65, were significantly elevated in patients with vasculitis ($N = 56$; $p < 0.01$) and AS ($N = 29$; $p < 0.0001$), but only marginally in patients with SLE ($N = 22$; $p > 0.05$ compared to healthy controls ($N = 90$). These findings further support the concept of infection-induced immune reactions playing a pathogenic role in the development of both AS and vasculitis.—Authors' Abstract

Kaufmann, S. H. E., Ladel, C. H. and Flesch, I. E. A. T cells and cytokines in intracellular bacterial infections: experiences with *Mycobacterium bovis* BCG. Autoimmune Dis. **195** (1995) 123–132.

Intracellular bacteria reside in mononuclear phagocytes, and protective immunity is dominated by T lymphocytes. *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) infection of mice represents an excellent model for studying immune mechanisms involved in defense against persistent intracellular bacteria that cause chronic disease. Gene disruption mutant mice include: A beta (–/–), which lack conventional CD4+ T-cell receptor alpha/beta (TCR alpha/beta)T lymphocytes; B-2 microglobulin (–/–), which lack conventional CD8+ TCR alpha/beta lymphocytes; TCR beta (–/–), which lack all TCR alpha/beta lymphocytes; TCR delta (–/–), which lack all TCR gamma/delta lymphocytes; and RAG-1 (–/–) mutants, which lack mature T and B lymphocytes. Studies of these mutants suggest that CD4+ TCR alpha/beta, CD8+ TCR alpha/beta and TCR gamma/ delta T lymphocytes all contribute to immunity against *M. bovis* BCG. Activation of antibacterial effector-functions in macrophages by T helper 1 (Th1) cell-derived gamma-interferon (IFN-gamma) is central to protection. In contrast, Th2 cells are only marginally involved. Activation of Th1 and Th2 cells is regulated by interleukin 10 (IL-10) and IL-12, which are induced early in infection with *M. bovis* BCG. Although IL-12 is stimulated by *M. bovis* BCG in immunocompetent mice, studies with IFN-gamma receptor-deficient and tumor necrosis factor alpha (TNF-alpha) receptor-deficient mutant mice suggest that *M. bovis* BCG-induced IL-12 secretion depends on IFN-gamma and TNF-alpha. Hence, IL-12 cannot be the first cytokine produced during *M. bovis* BCG infection.—Authors' Abstract

Kroger, H., Miesel, R., Dietrich, A., Ohde, M., Rajnavolgyi, E. and Ockenfels, H. Synergistic effects of thalidomide and poly(ADP-ribose) polymerase inhibition on type II collagen-induced arthritis in mice. Inflammation **20** (1996) 203–215.

The present study investigates synergistic effects of the TNF-alpha inhibitor thalidomide and the poly(ADP-ribose) polymerase

(PARP)-inhibitor nicotinic acid amide (NAA) in male DBA/1 hybrid mice suffering from type II collagen-induced arthritis. Parameters including the arthritis index, chemiluminescence and anti-collagen antibody titers were used for the assessment of disease activity. The disease courses demonstrated clearly an inhibitory effect of thalidomide on NAA inhibited established collagen arthritis in a dose-dependent manner. The combined application of thalidomide and NAA caused a powerful synergistic inhibition of arthritis. Furthermore, thalidomide and NAA were tested *ex vivo* for their inhibition of the NADPH oxidase-dependent generation of reactive oxygen species by activated neutrophils and monocytes in unseparated human blood. Our data show that type II collagen-induced arthritis can be suppressed by the simultaneous inhibition of TNF-alpha, PARP, and NADPH oxidase.—Author's Abstract

Lin, Y. G., Zhang, M., Hofman, F. M., Gong, J. H. and Barnes, P. F. Absence of a prominent Th2 cytokine response in human tuberculosis. Infect. Immun. **64** (1996) 1351–1356.

Depressed Th1 responses are a prominent feature of human tuberculosis, but an enhanced Th2 response has not been detected in peripheral blood T cells stimulated *in vitro* with *M. tuberculosis.* In disease due to *M. leprae,* Th2-cells predominate in tissue lesions of patients with extensive disease but are absent from peripheral blood. To determine if Th2 cells are present in tissue lesions of tuberculosis patients, we evaluated patterns of cytokine expression in lymph nodes from tuberculosis patients with or without human immunodeficiency virus infection and in controls without tuberculosis. Gamma interferon and interleukin-10 (IL-10) mRNA expression in tuberculosis patients with or without human immunodeficiency virus infection was high; whereas IL-4 expression in the same patients was low. Immunolabeling studies showed that macrophage production of IL-12 was increased in lymph nodes from tuberculosis patients, that gamma interferon was produced by T cells, and that IL-10 was produced by macrophages rather than Th2 cells. These results indicate that Th2 responses are not enhanced either systemically or at the site of disease in human tuberculosis.—Authors' Abstract

Mendez Samperio, P., Hernandez Garay, M., Badillo Flores, A. and Nunez Vazquez, A. Down-modulation of mycobacterial-induced IL-1 beta production in human mononuclear cells by IL-4. Clin. Exp. Immunol. **104** (1996) 374–379.

Tuberculosis is characterized by a cellular immune response mediated by various cytokines, including IL-I beta released by stimulated mononuclear cells. It is now well established that IL-I beta plays an important role in local and systemic inflammatory response in tuberculosis. Here we have demonstrated, for the first time, that addition of IL-4 to human mononuclear cells obtained from 11 healthy bacille Calmette-Guerin (BCG)-vaccinated donors reduced BCG-induced production of IL-1 beta 91.46 ± 2.2%. This inhibitory effect was found highly significant ($p < 0.001$) and was dose-dependent. The effect of IL-4 on the secretion of IL-1 beta was specific, since a complete reversion was obtained with a neutralizing MoAb to human IL-4. In addition, this inhibitory effect was not attributed to a cytotoxic effect, since trypan blue exclusion studies indicated no loss of cell viability in response to IL-4. Interestingly, the induction of IL-1 beta was regulated by IL-4, at least in part, by a direct mechanism mediated through the 130 extracellular domain of the IL-4 receptor, as demonstrated by incubation of the mononuclear cells with the neutralizing anti-IL-4 receptor MoAb. Finally, a significant down-regulation of IL-1 beta secretion was observed in hsp70-stimulated mononuclear cells cultured with IL-4. Further experimental work is needed to establish the relevance of IL-4 in human mycobacterial infection *in vivo.* However, an understanding of the mechanisms that control IL-1 beta secretion in human mycobacterial infections is essential to understand the pathogenesis of tuberculosis.—Authors' Abstract

Mustafa, A. S., Lundin, K. E. A., Meloen, R. H., Shinnick, T. M., Coulson, A. F. W. and Oftung, F. HLA-DR4-restricted

T-cell epitopes from the mycobacterial 60,000 MW heat-shock protein (hsp60) do not map to the sequence homology regions with the human hsp60. Immunology **87** (1996) 421–427.

The mycobacterial 60,000 MW heat-shock protein (hsp60) is a major antigen recognized by mycobacteria-reactive human CD4+ T cells with lymphokine profiles and effector functions consistent with protective immunity. In addition, the presence of a large number of T-cell epitopes presented by several HLA class II molecules makes this antigen relevant to subunit vaccine design. However, the results from animal models as well as human studies suggest that the mycobacterial hsp60 may induce T-cell-mediated autoimmune conditions. In humans, the expression of HLA-DR4 represents a risk factor for some autoimmune diseases. These observations suggest that the epitopes from the mycobacterial hsp60 presented to T cells in the context of HLA-DR4 could be relevant to autoimmunity. This is the first report on identification of HLA-DR4-restricted T-cell epitopes from the mycobacterial antigen hsp60. In total, five epitopes recognized in the context of HLA-DR4 by the *M. leprae* hsp60-reactive CD4+ T-cell clones from a subject immunized with *M. leprae* were defined by synthetic peptides. Two of the epitopes were *M. leprae*-specific (aa 343–355, aa 522–534); whereas three epitopes were common to *M. leprae* and *M. tuberculosis* (aa 331–345, aa 441–455, aa 501–515). However, all of these epitopes belong to the regions that are highly divergent between the mycobacterial hsp60 and the homologous human hsp60 sequence, suggesting that the T cells recognizing the mycobacterial hsp60 in the context of HLA-DR4 may not necessarily induce autoreactivity.—Authors' Abstract

Ruth, J. H., Bienkowski, M., Warmington, K. S., Lincoln, P. M., Kunkel, S. L. and Chensue, S. W. IL-1 receptor antagonist (IL-1ra) expression, function, and cytokine-mediated regulation during mycobacterial and schistosomal antigen-elicited granuloma formation. J. Immunol. **156** (1996) 2503–2509.

Granulomas (GR) mediated predominantly by Th1/type 1 (IFN-gamma) and Th2/type 2 (IL-4, IL-5, IL-10) cytokines were induced by i.v. injection of sensitized CBA/J mice with carbohydrate beads coated with *Mycobacterium tuberculosis* or *Schistosoma mansoni* egg Ags, respectively. GR macrophages (mϕ) from types 1 and 2 GR both produced IL-1ra, but the former showed accelerated IL-1ra-producing capacity, releasing two- to threefold greater amounts on day 4 than those of type 2 GR, as measured by sandwich ELISA. *In vivo* depletion of IL-1ra exacerbated GR size and augmented regional cytokine production in both types of responses. To determine the critical cytokines mediating IL-1ra expression, oil-elicited peritoneal mϕ were exposed to graded doses (0.1 to 10 ng/ml) of cytokines (IL-1 beta, IL-2, IL-4, IL-10, IL-12, IFN-gamma, and TNF-alpha) for 24 hr, then stimulated with opsonized zymosan. Of the cytokines tested, IFN-gamma and TNF-alpha were the best costimuli for IL-1ra production in the presence of zymosan; whereas IL-1 beta, IL-10, and IL-12 were not active. *In vivo* depletion of IL-4, IL-10, IL-12, IFN-gamma, or TNF-alpha with 5 mg of cytokine-specific neutralizing rabbit IgG revealed that IFN-gamma and TNF-alpha were required for maximal IL-1ra production by mϕ. Furthermore, the delayed IL-1ra production by type 2 GR mϕ could be related to later TNF-alpha production. Our findings indicate that IL-1ra is a common regulatory product of inflammatory mϕ and is particularly promoted by type 1 cytokines, IFN-gamma, and TNF-alpha.—Authors' Abstract

Shannon, E. J. and Sandoval, F. Thalidomide can be either agonistic or antagonistic to LPS evoked synthesis of TNF-alpha by mononuclear cells. Immunopharmacol. Immunotoxicol. **18** (1996) 59–72.

The effect of thalidomide on tumor necrosis factor alpha (TNF-alpha) produced *in vitro* by lipopolysaccharide (LPS)-stimulated human cells was investigated. In cultures of LPS-stimulated human mononuclear cells enriched for adherent cells and in cultures of LPS-stimulated human monocytes of the cell line-THP-1, thalidomide enhanced the synthesis of TNF-alpha.

When cultures of unfractionated peripheral blood mononuclear cells were stimulated with LPS, thalidomide decreased the synthesis of TNF-alpha. Depending on the type of cells stimulated with LPS *in vitro* thalidomide, at concentrations achieved *in vivo,* can either enhance or suppress the synthesis of TNF-alpha.—Authors' Abstract

Shiratsuchi, H., Hamilton, B., Toossi, Z. and Ellner, J. J. Evidence against a role for interleukin-10 in the regulation of growth of *Mycobacterium avium* in human monocytes. J. Infect. Dis. **173** (1996) 410–417.

Interleukin-10 (IL-10) inhibits intracellular *Mycobacterium avium* killing by cytokine-activated murine macrophages and may have a role in pathogenesis. Cytokine activities in supernatants of *M. avium*-infected human monocytes were maximal at 6–24 hr for tumor necrosis factor (TNF)-alpha and 24–48 hr for IL-10. TNF-alpha and IL-10 production increased with increasing *M. avium*-to-monocyte infection ratios (20:1 to 200:1). TNF-alpha production by monocytes infected with smooth, domed, and opaque organisms at 200:1 exceeded that of monocytes infected with smooth, flat, and transparent *M. avium* ($p < 0.01$). IL-10 induction demonstrated considerable strain-to-strain variability and did not correlate with intracellular *M. avium* growth. IL-10 significantly inhibited TNF-alpha, IL-1 beta, and IL-6 production by *M. avium*-infected monocytes. Coculturing monocytes with IL-10 after *M. avium* infection did not affect intracellular *M. avium* growth. Differential induction of TNF-alpha may be a factor in the intracellular growth of *M. avium* in human monocytes. IL-10, however, played no apparent role in pathogenicity in this model.—Authors' Abstract

Zerva, L., Cizman, B., Mehra, N. K., Alahari, S. K., Murali, R., Zmijewski, C. M., Kamoun, M. and Monos, D. S. Arginine at positions 13 or 70–71 in pocket 4 of HLA-DRB1 alleles is associated with susceptibility to tuberculoid leprosy. J. Exp. Med. **183** (1996) 829–836.

Evaluation of human histocompatibility leukocyte antigen (HLA) class II genes in 54 cases of tuberculoid leprosy (TL) and 44 controls has shown a positive association with HLA-DRB1 alleles that contain Arg(13) or Arg(70)–Arg(71). Among TL patients, 87% carry specific alleles of DRB1 Arg(13) or Arg(70)–Arg(71) as compared to 43% among controls ($p = 5 \times 10^{-6}$), conferring a relative risk of 8.8. Thus, susceptibility to TL involves three critical amino-acid positions of the beta chain, the side chains of which, when modeled on the DR1 crystal structure, line a pocket (pocket 4) accommodating the side chain of a bound peptide. This study suggests that disease susceptibility may be determined by the independent contribution of polymorphic residues participating in the formation of a functional arrangement (i.e., pocket) within the binding cleft of an HLA molecule.—Authors' Abstract

Microbiology

Basu, J., Mahapatra, S., Kundu, M., Mukhopadhyay, S., Nguyen Disteche, M., Dubois, P., Joris, B., VanBeeumen, J., Cole, S. T., Chakrabarti, P. and Ghuysen, J. M. Identification and overexpression in *Escherichia coli* of a *Mycobacterium leprae* gene, pon1, encoding a high-molecular-mass class A penicillin-binding protein, PBP1. J. Bacteriol. **178** (1996) 1707–1711.

Cosmid B577, a member of the collection of ordered clones corresponding to the genome of *Mycobacterium leprae,* contains a gene, provisionally called pon1, that encodes an 821-amino-acid-residue high-molecular-mass class A penicillin-binding protein, provisionally called PBP1. With similar amino acid sequences and modular designs, *M. leprae* PBP1 is related to *Escherichia coli* PBP1a and PBP1b, bienzymatic proteins with transglycosylase and transpeptidase activities. When produced in *E. coli,* His tag-labelled derivatives of *M. leprae* PBP1 adopt the correct membrane

topology, with the bulk of the polypeptide chain on the surface of the plasma membrane. They defy attempts at solubilization with all the detergents tested except cetyltrimethylammonium bromide. The solubilized PBP1 derivatives can be purified by affinity chromatography on Ni2+-nitrilotriacetic acid agarose. They have low affinities for the usual penicillins and cephalosporins.—Authors' Abstract

Berman, J. S., Blumenthal, R. L., Kornfeld, H., Cook, J. A., Cruikshank, W. W., Vermeulen, M. W., Chatterjee, D., Belisle, J. T. and Fenton, M. J. Chemotactic activity of mycobacterial lipoarabinomannans for human blood T lymphocytes *in vitro.* J. Immunol. **156** (1996) 3828–3835.

A crucial early event in tuberculosis is the ingestion of *Mycobacterium tuberculosis* (Mtb) by alveolar macrophages. Chemotactic factors released by infected macrophages are likely to initiate a granulomatous response, a key feature of host resistance to tuberculosis. To date, the role of mycobacterial products in regulating the granulomatous response has not been clearly defined. Here we report that the mycobacterial cell wall glycophospholipid lipoarabinomannan (LAM) could specifically induce human peripheral blood T-cell chemotaxis *in vitro.* Both terminally mannosylated LAM isolated from Mtb and LAM lacking the terminal mannosyl units isolated from an avirulent mycobacterium could induce T-cell migration in the absence of serum. In contrast, terminally mannosylated LAM isolated from *M. bovis* BCG failed to induce T-cell chemotaxis. These observations represent the first report that LAM is capable of directly inducing biologic responses in human T cells. Flow cytometry analysis revealed that CD4+, CD8+, and CD45RO+ lymphocytes were present in the migrating cell populations at ratios similar to those found in nonmigrating cells. The chemotactic response was found to require new protein synthesis, and could be blocked by inhibitors of protein tyrosine kinases at concentrations that did not affect random migration. Acyl groups at the reducing terminus of LAM appear to be required for the chemotactic activity of this mycobacterial glycolipid. Lastly, culture supernatants from human alveolar macrophages infected *in vitro* with a virulent strain of Mtb could induce T-cell migration. Much of the migratory activity present in these supernatants could be blocked using a mAb against LAM, suggesting that LAM is one of the chemotactic factors released by Mtb-infected alveolar macrophages.—Authors' Abstract

DeMaio, J., Zhang, Y., Ko, C., Young, D. B. and Bishai, W. R. A stationary-phase stress-response sigma factor from *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 2790–2794.

Alternative RNA polymerase sigma factors are a common means of coordinating gene regulation in bacteria. Using PCR amplification with degenerate primers, we identified and cloned a sigma factor gene, sigF, from *Mycobacterium tuberculosis.* The deduced protein encoded by sigF shows significant similarity to SigF sporulation sigma factors from *Streptomyces coelicolor* and *Bacillus subtilis* and to SigB, a stress-response sigma factor, from *B. subtilis.* Southern blot surveys with a sigF-specific probe identified cross-hybridizing bands in other slow-growing mycobacteria, *M. bovis* bacille Calmette-Guerin (ECG) and *M. avium,* but not in the rapid-growers *M. smegmatis* or *M. abscessus.* RNase protection assays revealed that *M. tuberculosis* sigF mRNA is not present during exponential-phase growth in *M. bovis* BCG cultures but is strongly induced during stationary phase, nitrogen depletion, and cold shock. Weak expression of *M. tuberculosis* sigF was also detected during late-exponential phase, oxidative stress, anaerobiasis, and alcohol shock. The specific expression of *M. tuberculosis* sigF during stress or stationary phase suggests that it may play a role in the ability of tubercle bacilli to adapt to host defenses and persist during human infection.—Authors' Abstract

Dhople, A. M. *In vitro* susceptibility of *Mycobacterium leprae* to oxygen-mediated damage. Microbios **85** (1996) 35–44.

In order to evaluate factors responsible for the failure of *Mycobacterium leprae* to multiply in cell-free cultures *in vitro* studies

were undertaken to determine the possible poisoning of the organism by hydroxide and superoxide radicals produced in the growth medium. The superoxide dismutase activity was very low, 10% of the levels found in armadillo cells, while measured activity of database and glutathione peroxidase was negligible. Susceptibility of *M. leprae* to hydrogen peroxide was enhanced by potassium iodide but not by lactoperoxidase. The addition of high amounts of catalase completely prevented hydrogen peroxide-mediated killing of *M. leprae.* Superoxide generated by the action of xanthine oxidase on xanthine was lethal to *M. leprae,* but superoxide dismutase added to the reaction mixture gave significant protection. Thus, superoxide radicals may be a major cause for the sudden termination of growth of *M. leprae* in primary cultures and also for failure of subcultures.—Author's Abstract

Dobner, P., Feldmann, K., Rifai, M., Loscher, T. and Rinder, H. Rapid identification of mycobacterial species by PCR amplification of hypervariable 16S rRNA gene promoter region. J. Clin. Microbiol. **34** (1996) 866–869.

A total of 0.3 to 0.4 kb of the promoter region of the 16S rRNA genes from *Mycobacterium tuberculosis, M. gordonae, M. xenopi,* and *M. leprae* was PCR amplified, cloned, and sequenced. The observed number of substitutions, insertions, and deletions exceeded those found in previously used targets sequences, including the entire 16S coding region. A simple and generally applicable restriction fragment length polymorphism method that can be used to distinguish between mycobacterial species is described.—Authors' Abstract

Dobos, K. M., Khoo, K. H., Swiderek, K. M., Brennan, P. J. and Belisle, J. T. Definition of the full extent of glycosylation of the 45-kilodalton glycoprotein of *Mycobacterium tuberculosis.* J. Bacteriol. **178** (1996) 2498–2506.

Chemical evidence for the true glycosylation of mycobacterial proteins was recently provided in the context of the 45-kDa MPT 32 secreted protein of *Mycobacterium tuberculosis* (K. Dobos, K. Swiderek, K.-H. Khoo, P. J. Brennan, and J. T. Belisle, Infect. Immun. 63:2846–2853, 1995), However, the full extent and nature of glycosylation as well as the location of glycosylated amino acids remained undefined. First, to examine the nature of the covalently attached sugars, the 45-kDa protein was obtained from cells metabolically labeled with D-[U-C-14]glucose and subjected to compositional analysis, which revealed mannose as the only covalently bound sugar. Digestion of the protein with the endoproteinase subtilisin and analysis of products by liquid chromatography-electrospray-mass spectrometry on the basis of fragments demonstrating neutral losses of hexose (m/z 162) or pentose (m/z 132) revealed five glycopeptides, S-7, S-18, S-22, S-29, and S-41, among a total of 50 peptides, all of which produced only m/z 162 fragmentation ion deletions. Fast atom bombardment-mass spectrometry, N-terminal amino acid sequencing, and cy mannosidase digestion demonstrated universal O glycosylation of Thr residues with a single alpha-D-Man, mannobiose, or mannotriose unit. Linkages within the mannobiose and mannotriose were all alpha 1-2, as proven by gas chromatography mass spectrometry of oligosaccharides released by beta-elimination. Total sequences of many of the glycosylated and nonglycosylated peptides combined with published information on the deduced amino acid sequence of the entire 45-kDa protein demonstrated that the sites of glycosylation were located in Pro-rich domains near the N terminus and C terminus of the polypeptide backbone. Specifically, the Thr residues at positions 10 and 18 were substituted with alpha-D-Manp(1→2)-alpha-D-Manp, the Thr residue at position 27 was substituted with a single alpha-n-Manp, and Thr-277 was substituted with either alpha-D-Manp, alpha-D-Manp(1→2)-alpha-D-Manp, or alpha-D-Manp(1→2)-alpha-D-Manp (1→2)-alpha-D-Manp. This report further corroborates the existence of true prokaryotic glycoproteins, defines the complete structure of a mycobacterial mannoprotein and the first complete structure of a mannosylated mycobacterial protein, and establishes the principles for the study of other mycobacterial glycoproteins.—Authors' Abstract

Escuyer, V., Haddad, N., Frehel, C. and Berche, P. Molecular characterization of a surface-exposed superoxide dismutase of *Mycobacterium avium.* Microb. Pathogen. **20** (1996) 41–55.

Mycobacterium avium is an intracellular pathogen capable of growing inside the phagosomal compartment of macrophages. In this work, we characterized the superoxide dismutase of *M. avium* as a putative candidate to resist the oxidative stress. The gene sodA encoding superoxide dismutase (SOD:EC1.15.1.1) from *M. avium* TMC724 was cloned and sequenced. It encodes a 23-kDa protein (207 amino acids) showing identity with the *M. leprae* SOD (91%) and the *M. tuberculosis* SOD (83%). This enzyme was functionally expressed in both *Escherichia coli* and *M. smegmatis,* and identified as a manganese (Mn) SOD on the basis of sequence comparison with other MnSODs from different organisms, and by activity inhibition studies. By indirect immunogold labeling of *M. avium* with a mAb directed against *M. leprae* SOD, the enzyme was found to be exposed at the cell surface of *M. avium.* It was also shown that SOD was released in supernates of *M. avium* TMV724 during exponential growth, suggesting a role of this enzyme during interactions with the environment. When SOD was expressed in the nonpathogenic *M. smegmatis,* it was also exposed at the surface of bacteria and released in supernates, but this was not sufficient to protect this recombinant mycobacterium from the killing mechanisms of macrophages.—Authors' Abstract

Fernandes, N. D. and Kolattukudy, P. E. Cloning, sequencing and characterization of a fatty acid synthase-encoding gene from *Mycobacterium tuberculosis* var. *bovis* BCG. Gene **170** (1996) 95–99.

Mycobacterial cell walls contain unique lipids such as mycolic acids, very long chain fatty acids and multimethyl-branched fatty acids. A multifunctional fatty acid synthase (Fas) with the unique capability of catalyzing both *de novo* synthesis and chain elongation of fatty acids has been purified and characterized from *Mycobacterium tuberculosis* var. *bovis* BCG (bacillus Calmette-Geurin) [Kikuchi et al., Arch. Biochem. Biophys. 295 (1992) 318-326]. To understand how the various domains that catalyze the reactions involved in both *de novo* synthesis and elongation are organized in the mycobacteria, a fas gene was cloned from a cosmid library of genomic DNA from *M. bovis* BCG. Sequencing of the cosmid clone revealed a contiguous sequence of 11,577 bp of mycobacterial genome containing an 8389-bp open reading frame that could code for a protein of 2797 amino acids (301 kDa). By comparing the Fas aa sequence with the sequences in the active site regions of known fas and polyketide synthase-encoding genes, the functional catalytic domains in Fas were identified. This analysis revealed that the domains are organized in the following order: acyltransferase, enoyl reductase, dehydratase, malonyl/palmitoyl transferase, acyl carrier protein, beta-keto reductase, beta-ketoacyl synthase. This domain organization is like a head-to-tail fusion of the two yeast fas gene subunits. The results obtained constitute the first report of the cloning, sequencing and structural elucidation of a fas from the mycobacteria.—Authors' Abstract

Fsihi, H., Vincent, V. and Cole, S. T. Homing events in the gyrA gene of some mycobacteria. Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 3410–3415.

The A subunit of DNA gyrase in *Mycobacterium leprae,* unlike its counterpart in *M. tuberculosis,* is produced by protein splicing as its gene, gyrA, harbors a 1260-bp in-frame insertion encoding an intein, a putative homing endonuclease. Analysis of the gyrA locus from different mycobacterial species revealed the presence of inteins in *M. flavescens, M. gordonae,* and *M. kansasii* but not in 10 other pathogenic or saprophytic mycobacteria. In all four cases where intein coding sequences were found, they were localized in the same position in gyrA, immediately downstream of the codon for the key active-site residue Tyr-130, The intein products were similar, but not identical, in sequence and the splice junctions displayed all the features found in other polypeptides known to be produced by protein splicing from a precursor protein. Paired motifs, found in homing en-

donucleases encoded by some group I RNA introns, and inteins showing endonuclease activity were present in the gyrA inteins as were other intein-specific signatures. Some strains of *M. flavescens, M. gordonae,* and *M. kansasii* were shown by PCR analysis to have inteinless gyrA-genes, in contrast to the situation in *M. leprae* where all the isolates possessed insertions in gyrA. Sequencing of the corresponding regions revealed that, although the GyrA protein sequence was conserved, the nucleotide sequences differed in gyrA genes with and without inteins, suggesting that the homing endonuclease displays sequence specificity.—Authors' Abstract

Gobin, J. and Horwitz, M. A. Exochelins of *Mycobacterium tuberculosis* remove iron from human iron-binding proteins and donate iron to mycobactins in the *M. tuberculosis* cell wall. J. Exp. Med. **183** (1996) 1527–1532.

To multiply and cause disease in the host, *Mycobacterium tuberculosis* must acquire iron from the extracellular environment at sites of replication. To do so, the bacterium releases high-affinity iron-binding siderophores called exochelins. In previous studies, we have described the purification and characterization of the exochelin family of molecules. These molecules share a common core structure with another type of high-affinity iron-binding molecule located in the cell wall of *M. tuberculosis*: the mycobactins. The water-soluble exochelins differ from each other and from the water-insoluble mycobactins in polarity, which is dependent primarily upon the length and modifications of an alkyd side chain. In this study, we have investigated the capacity of purified exochelins to remove iron from host high-affinity iron-binding molecules, and to transfer iron to mycobactins. Purified desferri-exochelins rapidly removed iron from human transferrin, whether it was 95% or 40% iron saturated, its approximate percent saturation in human serum, and from human lactoferrin. Desferri-exochelins also removed iron, but at a slower rate, from the iron storage protein ferritin. Purified ferri-exochelins, but not iron transferrin, transferred iron to desferri-mycobactins in the cell wall of live bacteria. To explore the possibility that the transfer of iron from exochelins to mycobactins was influenced by their polarity, we investigated the influence of polarity on the iron affinity of exochelins. Exochelins of different polarity exchanged iron equally with each other. This study supports the concept that exochelins acquire iron for *M. tuberculosis* by removing this element from host iron-binding proteins and transferring it to desferri-mycobactins in the cell wall of the bacterium. The finding that ferri-exochelins but not iron transferrin transfer iron to mycobactins in the cell wall underscores the importance of exochelins in iron acquisition. This study also shows that the variable alkyl side chain on the core structure of exochelins and mycobactins, the principal determinant of their polarity, has little or no influence on their iron affinity.—Authors' Abstract

Gonzalez-Merchand, J. A., Colston, M. J. and Cox, R. A. The rRNA operons of *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*: comparison of promoter elements and of neighbouring upstream genes. Microbiology **142** (1996) 667–674.

Mycobacterium smegmatis has two rRNA (rm) operons designated rrnA(f) and rrnB(f). Appropriate restriction fragments of genomic DNA containing sequences immediately upstream from the 165 rRNA genes were cloned. We now report the nucleotide sequence of 552 bp upstream from the 5'-end of the Box A(L) antitermination element of the leader region of the rrnA(f) operon. The 5'-end of this segment of DNA was found to comprise 113 codons of an ORF encoding a protein which is significantly similar to UDP-N-acetylglucosamine 1-carboxyvinyl-transferase (EC 2.5.1.7), which is important to cell wall synthesis. A homologous ORF is located immediately upstream from the single rrn (rrnA(f)) operons of *M. tuberculosis* and *M. leprae.* Primer-extension analysis of the RNA fraction of *M. smegmatis* revealed four products which were related to transcription start points; the rrnB(f) operon appears to have a single promoter whereas the rmA(f) operon has three (p1, P2 and P3). Analysis

of *M. tuberculosis* RNA revealed two products corresponding to transcripts directed by promoters homologous with pi and P3 of the rmA of *M. smegmatis.* Thus, the promoter and upstream regions of the rmA(f) operon of *M. smegmatis* and the rrnA operon of *M. tuberculosis* are homologous. The presence of P2 in *M. smegmatis* and its absence from *M. tuberculosis* is attributable to insertions/deletions of 97 bp.—Authors' Abstract

Hermans, J. and deBont, J. A. M. Techniques for genetic engineering in mycobacteria—alternative host strains, DNA-transfer systems and vectors. Antonie Van Leeuwenhoek **69** (1996) 243–256.

The study of mycobacterial genetics has experienced quick technical developments in the past 10 years, despite a relatively slow start caused by difficulties in accessing these recalcitrant species. The study of mycobacterial pathogenesis is important in the development of new ways of treating tuberculosis and leprosy now that the emergence of antibiotic-resistant strains has reduced the effectiveness of current therapies. The tuberculosis vaccine strain *M. bovis* BCG might be used as a vector for multivalent vaccination. Also, nonpathogenic mycobacterial strains have many possible biotechnological applications. After giving an historical overview of methods and techniques, we will discuss recent developments in the search for alternative host strains and DNA transfer systems. Special attention will be given to the development of vectors and techniques for stabilizing foreign DNA in mycobacteria.—Authors' Abstract

Matsumoto, S., Tamaki, M., Yukitake, H., Matsuo, T., Naito, M., Teraoka, H. and Yamada, T. A stable *Escherichia coli*-mycobacteria shuttle vector 'pSO246' in *Mycobacterium bovis* BCG. FEMS Microbiol. Lett. **135** (1996) 237–243.

The most widely used plasmid vector system in mycobacteria is based on pAL5000 from *Mycobacterium fortuitum.* The derivatives of the pAL5000-based shuttle vectors between *Escherichia coli* and mycobacteria, which we have utilized to secrete recombinant antigens, were generated. The stability of the vectors was assessed in *M. bovis* BCG (BCG). The plasmid vector pSO246 was stable in BCG for at least 50 generations.—Authors' Abstract

Nakamura, M. Inoculum sizes necessary for maintaining the activity of *Mycobacterium leprae* in cell-free liquid media. Jpn. J. Lepr. **64** (1995) 119–123.

Evidence was presented in a previous paper that the activity of cells of *Mycobacterium leprae* was maintained in phosphate buffer (pH 7) containing fetal calf serum (10%) with/without glycerine (2%) for about 4 weeks during incubation of cells at 30°C, when an inoculum having more than 3000 pg ATP was used. In the present paper, it is confirmed that the definite inoculum sizes are essential for obtaining the reproducible results described above, by using inocula containing more and less than 3000 pg ATP.—Author's Abstract

Philipp, W. J., Poulet, S., Eiglmeier, K., Pascopella, L., Balasubramanian, V., Heym, B., Bergh, S., Bloom, B. R., Jacobs, W. R. and Cole, S. T. An integrated map of the genome of the tubercle bacillus, *Mycobacterium tuberculosis* H37Rv, and comparison with *Mycobacterium leprae.* Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 3132–3137.

An integrated map of the genome of the tubercle bacillus, *Mycobacterium tuberculosis,* was constructed by using a twin-pronged approach. Pulsed-field gel electrophoretic analysis enabled cleavage sites for Asn I and Dra I to be positioned on the 4 4-Mb circular chromosome, while, in parallel, clones from two cosmid libraries were ordered into contigs by means of fingerprinting and hybridization mapping. The resultant contig map was readily correlated with the physical map of the genome via the landmarked restriction sites. Over 165 genes and markers were localized on the integrated map, thus enabling comparisons with the leprosy bacillus, *M. leprae,* to be undertaken. Mycobacterial genomes appear to have evolved as mosaic structures since extended segments with conserved gene or-

der and organization are interspersed with different flanking regions. Repetitive sequences and insertion elements are highly abundant in *M. tuberculosis,* but the distribution of IS6110 is apparently nonrandom.—Authors' Abstract

Salazar, L., Fsihi, H. deRossi, E., Riccardi, G., Rios, C., Cole, S. T. and Takiff, H. E. Organization of the origins of replication of the chromosomes of *Mycobacterium smegmatis, Mycobacterium leprae* and *Mycobacterium tuberculosis* and isolation of a functional origin from *M. smegmatis.* Mol. Microbiol. **20** (1996) 283–293.

The genus *Mycobacterium* is composed of species with widely differing growth rates ranging from approximately three hr in *Mycobacterium smegmatis* to two weeks in *M. leprae.* As DNA replication is coupled to cell duplication, it may be regulated by common mechanisms. The chromosomal regions surrounding the origins of DNA replication from *M. smegmatis, M. tuberculosis,* and *M. leprae* have been sequenced, and show very few differences. The gene order, rnpA-rpmH-dnaA-dnaN-recF-orf-gyrS-gyrA, is the same as in other gram-positive organisms. Although the general organization in *M. smegmatis* is very similar to that of *Streptomyces* spp., a closely related genus, *M. tuberculosis* and *M. leprae* differ as they lack an open reading frame, between dnaN and recF, which is similar to the gnd gene of *Escherichia coli.* Within the three mycobacterial species, there is extensive sequence conservation in the intergenic regions flanking dnaA, but more variation from the consensus DnaA box sequence was seen than in other bacteria. By means of subcloning experiments, the putative chromosomal origin of replication of *M. smegmatis,* containing the dnaA-dnaN region, was shown to promote autonomous replication in *M. smegmatis,* unlike the corresponding regions from *M. tuberculosis* or *M. leprae.*—Authors' Abstract

Tokue, Y., Sugano, K., Noda, T., Saito, D., Shimosato, Y., Ohkura, H., Kakizoe, T. and Sekiya, T. Identification of mycobacteria by nonradioisotopic single-strand conformation polymorphism analysis. Diagn. Microbiol. Infect. Dis. **23** (1995) 129–133.

Clinical isolates of mycobacteria were identified to species levels using nonradioisotopic single-strand conformation polymorphism (non-RI SSCP) analysis of 16S rRNA gene fragments amplified by polymerase chain reaction with primers common to all of mycobacterial species. The method is based on a hypervariable region within the 16S rRNA in mycobacteria, which is characterized by species-specific nucleotide sequences. A total of 92 mycobacterial strains (*Mycobacterium tuberculosis, M. avium, M. gordonae, M. intracellulare, M. kansasii, M. chelonae, M. nonchromogenicum, M. xenopi,* and unidentified strain) were studied. They were classified into nine types of pattern showing single-strand DNA bands having different mobilities. Each strain was shown in the species-specific mobility by non-RI SSCP analysis. The results of non-RI SSCP analysis were identical to those of standard biochemical methods and 16S rRNA sequencing.—Authors' Abstract

Epidemiology and Prevention

Ganapati, R., Revankar, C. R. and Kingsley, S. Management of leprosy on the basis of the epidemiology of disabilities. Lepr. Rev. **67** (1996) 13–17.

With the reduction on caseload due to the impact of multidrug therapy (MDT) in most parts of India, we believe that there is a need to understand the epidemiology of disabilities in leprosy which may not necessarily correlate with the distribution pattern of active disease. We present a methodology of data collection and verification taking the district as a unit to calculate the prevalence rate of disability as an exclusive entity in

the district population, unrelated to the problems posed by the communicable component of leprosy. This study indicated that the prevalence rate of Grade II disabilities in 14 hyperendemic districts was 0.82/1000; whereas it was 0.22/1000 in low endemic districts. Limb disability data collected from three hyperendemic districts in Andhra Pradesh, following task-oriented training, enabled the paramedical worker to offer services to 5753 disabled patients after assessing the disability caseload per worker.—Authors' Summary

González-Abreu, E., Pon, J. A., Hernández, P., Rodriguez, J., Mendoza, E., Hernández, M., Cuevas, E. and González, A. B. Serological reactivity to a synthetic analog of phenolic glycolipid I and early detection of leprosy in an area of low endemicity. Lepr. Rev. **67** (1996) 4–12.

A total of 23,863 individuals living in an area of low endemicity for leprosy were tested by enzyme-linked immunosorbent assay with a semisynthetic analog of the phenolic glycolipid I antigen of *Mycobacterium leprae.* The proportion found positive was 3.86% which was significantly higher than that in a sample of a population known to be free of leprosy. Clinical examinations as well as Mitsuda and skin-smear tests were organized for those defined as seropositive. The proportion of individuals with lepromin reactions of < 3 mm increased 18.9% per serological interval as antibodies rose though it was not statistically significant. As a result of the clinical and bacteriological examinations, 2 cases with clinical signs and heavy bacillary load were found; whereas acid-fast bacilli were demonstrated in 2 other individuals without clinical manifestations of leprosy. The usefulness of the system for control purposes is discussed.—Authors' Summary

Myint, T. and Htoon, M. T. Leprosy in Myanmar, epidemiological and operational changes, 1958–92. Lepr. Rev. **67** (1996) 18–27.

The registered caseload and prevalence of leprosy have declined in Myanmar from a peak of 86.2 per 10,000 population (95% CI 85.43–86.97) in 1973–77 to 26.82 (95% CI 18.46–35.18) in 1988–92. The new case detection rates have also declined from 7.41 per 10,000 (95% CI 6.3–8.52) in 1968–72 to 1.96 (95% CI 1.43–2.52) in 1988–92. The increase in the multibacillary proportion of new cases from 11.85% (95% CI 11.84–11.86) in 1968–72 to 40.54% (95% CI 37.2–43.88) in 1988–92 and the decline in proportion of new cases under 14 years of age from 26.81% (95% CI 26.8–26.82) in 1968–72 to 11.22% (95% CI 10.92–11.52), coupled with the finding of declining detection rates among school children and in mass village surveys could mean that the incidence of leprosy may be declining.—Authors' Summary

Myrvang, B. Mycobacterial infections in Norway. Scand. J. Infect. Dis. **98** Suppl. (1995) 12–14.

Tuberculosis was a major health problem in Norway in the first part of the century, but since the 1930s there has been a dramatic and steady decline in incidence. However, for various reasons, including tuberculosis in foreign-born residents, there has been no definite decrease in notified cases during the last decade. The emergence of drug-resistant strains of *M. tuberculosis* has up to now not been a problem of any significance. Leprosy reached its peak incidence in the 19th century. Nowadays the few imported cases seen, on average less than one a year, may represent a diagnostic challenge. Therapeutically, we have adopted a modification of the multidrug regimen introduced and recommended by WHO a decade ago. Available figures indicate that diseases due to other mycobacteria, so-called atypical mycobacteria, may be an increasing problem. A small part of the observed increase is due to infections with *M. avium-intracellulare* complex in AIDS patients.—Author's Abstract

van Beers, S. M., deWit, M. Y. L. and Klatser, P. R. The epidemiology of *Mycobacterium leprae*: recent insight. FEMS Microbiol. Lett. **136** (1996) 221–230.

Leprosy is still a health problem in many countries. Because the causative organism, *Mycobacterium leprae,* cannot be cultured

in vitro, it is virtually impossible to assess exposure, and the onset of infection and disease. As a consequence, the chain of infection, considered as the relationships between *M. leprae,* transmission and human host, is poorly understood. Here, we discuss a number of organism-, host- and environmental-related factors which may be incriminated in the dynamic process of the development of leprosy disease. The use of modern molecular and immunological tools has become a valuable addition to epidemiological research. Understanding of the epidemiology of leprosy is a prerequisite for effective control of the disease.—Authors' Abstract

Rehabilitation

Deepak, S. Leprosy and community-based rehabilitation. Lepr. Rev. **66** (1995) 273–276.

The editorialist addresses the nature of community-based rehabilitation, the needs of disabled leprosy patients in terms of community-based rehabilitation, and whether the community-based rehabilitation approach can be adapted for rehabilitation of leprosy patients.—Trop. Dis. Bull.

Kets, C. M., van Leerdam, M. E. van Brakel, W. H., Deville, W. and Bèrtelsmann, F. W. Reference values for touch sensibility thresholds in healthy Nepalese volunteers. Lepr. Rev. **67** (1996) 28–38.

One-hundred thirty-six apparently healthy volunteers between the ages of 16 and 67 were used to determine normative thresholds of tactile sensibility in the Nepali adult population.

Tactile sensibility thresholds on standardized sites on hands and feet were assessed for two sensory tests: Semmes-Weinstein monofilaments (SWM) and moving-point discrimination (M2PD). Results are reported as the proportion of subjects able to feel a given threshold. The effect of age, sex, side, occupation, smoking habit and alcohol consumption on the results was examined with quantile regression.

On the hand 200 mg seemed an appropriate threshold for "normal" touch sensibility measured with monofilaments. About 99% (95% confidence interval 97–100) of individuals could detect this filament at all sites. A similar proportion could discriminate two points 4 mm apart which were moved from proximal to distal on the volar pad of the distal phalanx of the index and little finger. For the sole of the foot the thresholds were 2 g and 8 mm. Variability of results was greatest at the heel.

Normal thresholds for tactile sensibility were higher than those published for the North American population. Monofilament thresholds suitable for screening were 200 mg (log number 3.61) and 2 g (log number 4.31) for hand and foot, respectively. For moving 2-point discrimination on the hand this threshold was 4 mm.—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Al Majed, S. A. Study of paradoxical response to chemotherapy in tuberculous pleural effusion. Respir. Med. **90** (1996) 211–214.

Background: Paradoxical worsening of disease in spite of effective chemotherapy for tuberculosis has been reported to occur in cases of intracranial tuberculoma, lymph node, and pulmonary tuberculosis. However, only rare case reports describe such paradoxical response in tuberculosis pleurisy.

Methods: Sixty-one patients with proven tuberculous pleural effusion were retrospectively screened in Riyadh, Saudi Arabia, in three major hospitals to look systematically at the incidence and features of paradoxical response.

Results: Paradoxical increase in the size of the effusion was detected in 10 of 61 patients. In six patients, the effusion became massive with worsening of dyspnoea, requiring the use of corticosteroids in five patients and therapeutic aspiration in all six. However, complete resolution occurred in all 10 patients within 1–3 months. Three out of the 10 patients developed residual pleural thickening.

Conclusion: An incidence of 16% (10/61) paradoxical worsening of tuberculous effusion following the start of antituberculous treatment has been documented. This resulted in respiratory distress necessitating therapeutic re-aspiration in 6 of 10 patients.—Author's Abstract

Ang, S. C. and Moscovic, E. A. Cross-reactive and species specific *Mycobacterium tuberculosis* antigens in the immunoprofile of Schaumann bodies: a major clue to the etiology of sarcoidosis. Histol. Histopathol. **11** (1996) 125–134.

Sarcoidosis, once thought to be a variant of tuberculosis, is currently listed as a disease of unknown etiology. The present study was initiated by unpublished observations that Schaumann bodies—the laminated inclusions often encountered in sarcoid granulomas—crossreacted with commercial polyclonal antibodies to *Mycobacterium bovis, M. duvalii* and *M. paratuberculosis.* Given the broad crossreactivity of many mycobacterial antigens, those findings lacked specificity but warranted in-depth probing of the immunoprofile of the bodies, particularly for specific mycobacterial antigens. Formalin-fixed tissue from eight patients with an established diagnosis of sarcoidosis was studied with panels of antibodies against both common cytoplasmic proteins and various mycobacterial antigens, using a labeled streptavidin-biotin-alkaline phosphatase technique. Our findings indicate that Schaumann bodies are indeed residual bodies of heterophagic mycobacterial derivation. They immunostained intensely for the lysosomal proteins muramidase and CD68, variably for some cytoskeletal proteins (tubulin, desmin, vimentin) and not at all for cytokeratin, muscle actin, alpha-l-antichymotrypsin and ferritin. Both crossreactive and species-specific antigenic determinants of *M. tuberculosis* complex were shown be present. Affinity absorption with killed intact bacilli H37Rv resulted in virtually equal loss of binding by all polyclonal antimycobacterial antibodies to crossreactive ligands in Schaumann bodies. In addition, the bodies were clearly labeled with the monoclonal antibodies TB68 and TB71, known to recognize species-specific epitopes of *M. tuberculosis* complex. Although obtained on a small number of cases, our findings uphold Schaumann's original postulate that the laminated calcific inclusions represent remnants of "transformed tubercle bacilli."—Authors' Abstract

Bermudez, L. E. and Goodman, J. *Mycobacterium tuberculosis* invades and replicates within type II alveolar cells. Infect. Immun. **64** (1996) 1400–1406.

Although *Mycobacterium tuberculosis* is assumed to infect primarily alveolar macrophages after being aspirated into the lung in aerosol form, it is plausible to hypothesize that *M. tuberculosis* can come in contact with alveolar epithelial cells upon arrival into the alveolar space. Therefore, as a first step toward investigation of the interaction between *M. tuberculosis* and alveolar epithelial cells, we examined the ability of *M. tuberculosis* to bind to and invade alveolar epithelial cells *in vitro.* The H37Rv and H37Ra strains of *M. tuberculosis* were cultured to mid-log phase and used in both adherence and invasion assays. The A549 human type II alveolar cell line was cultured to confluence in RPMI 1640 supplemented with 5% fetal bovine serum, L-glutamine, and nonessential amino acids. H37Rv was more efficient in entering A549 cells than H37Ra, *M. avium,* and *Escherichia coli* HB101, a nonpiliated strain (4.7% ± 1.0% of the initial inoculum in 2 hr compared with 3.1% ± 0.8%, 2.1% ± 0.9%, and 0.03% ± 0.0%, respectively). The invasion was more efficient at 37°c than at 30°c (4.7% ± 1.0% compared with 2.3% ± 0.8%). H37Rv and H37Ra were both capable of multiplying intracellularly at a similar ratio over 4 days. Binding was inhibited up to 55.7% by anti-CD51 antibody (anti-vitronectin receptor), up to 55% with anti-CD29 antibody (beta(1) integrin), and 79% take of *M. tuberculosis* H37Rv was micro-

tubule and microfilament dependent. It was inhibited by 61.4% in the presence of 10 μM colchicine and by 72.3% in the presence of 3 μM cytochalasin D, suggesting two separate pathways for uptake. Our results show that *M. tuberculosis* is capable of invading type II alveolar epithelial cells and raise the possibility that invasion of alveolar epithelial cells is associated with the pathogenesis of lung infection.—Authors' Abstract

Beyers, N., Gie, R. P., Zietsman, H. L., Kunneke, M., Hauman, J., Tatley, M. and Donald, P. R. The use of a geographical information system (GIS) to evaluate the distribution of tuberculosis in a high-incidence community. S. Afr. Med. J. **86** (1996) 40–44.

Objective. To determine the geographical distribution of tuberculosis in the two Western Cape suburbs with the highest reported incidence of tuberculosis.

Design. Descriptive illustrative study.

Setting. Two adjacent Western Cape suburbs covering 2.42 km^2 with a population of 34,294 and a reported tuberculosis incidence of > 1,000/100,000.

Subjects. All patients notified as having tuberculosis over a 10-year period (1985–1994).

Interventions. None

Outcome measure. The geographical distribution of the cases was determined using a geographical information system (GIS) and the National Population Census (1991).

Results. One-thousand-eight-hundred thirty-five of the 5,345 dwelling units (34.3%) housed at least 1 case of tuberculosis during the past decade and in 483 houses 3 or more cases occurred. These cases were distributed unevenly through the community, with the tuberculosis incidence per enumerator subdistrict (ESD) varying from 78 to 3,150/100,000 population.

Conclusion. In a small area with a high incidence of tuberculosis, the cases are spread unevenly through the community and there are certain houses where tuberculosis occurs repeatedly. This information should be used to direct health services to concentrate on certain high-risk areas.—Authors' Abstract

Botha, F. J. H., Sirgel, F. A., Parkin, D. P., van de Wal, B. W., Donald, P. R. and Mitchison, D. A. Early bactericidal activity of ethambutol, pyrazinamide and the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater) in patients with pulmonary tuberculosis. S. Afr. Med. J. **86** (1996) 155–158.

The early bactericidal activity (EBA) of ethambutol, pyrazinamide and the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater: Mer National) was evaluated in patients with pulmonary tuberculosis who were sputum-positive on microscopy for acid-fast bacilli.

Twenty-eight patients (mean age 33 years and weight 51 kg on average; range 40–59 kg) were studied. The fall in viable counts of *Mycobacterium tuberculosis* in sputum collections during the 2 days following the start of treatment was estimated from counts of colony-forming units (CFUs) of *M. tuberculosis* per ml of sputum cultured on selective 7H10 agar medium. The EBA for ethambutol determined in 9 patients was 0.245 ± 0.046, $\log_{10}$ CFU/ml sputum/day, that for pyrazinamide was 0.003 ± 0.014 $\log_{10}$ CFU/ml sputum/day and that for Rifater 0.558 " 0.054 log, CFU/ml sputum/day. The results obtained are similar to those reported in a previous study of the first 2 days of treatment, but in smaller numbers of patients, and confirm the moderate EBA of ethambutol while pyrazinamide is again shown to have very little EBA. Rifater has a marked EBA which may be due mainly to the action of isoniazid. This methodology may be valuable in the rapid evaluation of the bactericidal activity of new antituberculosis agents and the comparison of different dose sizes of agents of the same class.—Authors' Abstract

Briglia, M., Eggen, R. I. L., deVos, W. M. and vanElsas, J. D. Rapid and sensitive method for the detection of *Mycobacterium chlorophenolicum* PCP-1 in soil based on 16S rRNA gene-targeted PCR. Appl. Environ. Microbiol. **62** (1996) 1478–1480.

A method based on 16S rRNA gene-targeted PCR and oligonucleotide probing was developed for detecting *Mycobacterium chlorophenolicum* PCP-1 in soil. The

primers and probe were specific for PCP-1 in DNA extracts of three soils. The method allowed for PCP-1 detection in soil with a detection limit of 3×10^2 cells per g.—Authors' Abstract

Caugant, D. A., Sandven, P., Eng, J., Jeque, J. T. and Tonjum, T. Detection of rifampin resistance among isolates of *Mycobacterium tuberculosis* from Mozambique. Microb. Drug Resis. **1** (1995) 321–326.

Rifampin resistance in respiratory isolates of *Mycobacterium tuberculosis* from Mozambique was detected by screening for point mutations using polymerase chain reaction (PCR) and DNA sequence analysis. The target template was a 350-bp fragment of rpoB encoding the beta-subunit of the RNA polymerase. Of the 66 strains studied, 38 were rifampin resistant by susceptibility testing with the radiometric method, 3 were intermediately resistant, and 25 were susceptible to rifampin. In 39 of the 41 rifampin-resistant strains, base-substitutions in the rpoB fragment were detected, and a total of 13 distinct mutations affecting 6 amino acids were observed. One of these mutations (His → Thr in amino acid 526) was not previously described. The isolates were also investigated by restriction fragment length polymorphism (RFLP) analysis using the insertion element IS6110 as a hybridization probe. A total of 47 RFLP patterns were identified, with up to 9 isolates having the same RFLP pattern. Strains with the same RFLP pattern harbored different mutations in rpoB, suggesting that acquisition of rifampin resistance followed the spread of a rifampin-susceptible clone. The data showed that rifampin resistance can be detected with a high sensitivity by DNA sequence analysis of this fragment of rpoB. However, a few strains with rifampin resistance due to factors other than base substitutions in rpoB could be missed.—Authors' Abstract

Cooksey, R. C., Morlock, G. P., McQueen, A., Glickman, S. E. and Crawford, J. T. Characterization of streptomycin resistance mechanisms among *Mycobacterium tuberculosis* isolates from patients in New York City. Antimicrob. Agents Chemother. **40** (1996) 1186–1188.

From a collection of 367 isolates of *Mycobacterium tuberculosis* from patients in New York City in 1994, 45 isolates (12.3%) were resistant *in vitro* to 2 μg or more of streptomycin (SM) per ml. We further evaluated these isolates for levels of SM resistance and for mutations previously associated with resistance in the rpsL (S12 ribosomal protein) gene and the rrs (16S rRNA)-coding region. Twenty-four isolates, representing nine distinct patterns of susceptibility to antituberculosis drugs, were resistant to 500 μg of SM per ml and shared a common point mutation at nucleotide 128 in the rpsL gene. This mutation, which substitutes lysine for arginine in the S12 ribosomal binding protein, was not present in isolates with low-level SM resistance or in SM-susceptible control isolates. Among 20 isolates with low-level SM resistance, one possessed a substitution [C→G(865)] in the 912 loop of the rrs gene. No mutations in the 530 loop of the ws coding region were detected, suggesting the presence of an alternative SM resistance mechanism in 19 isolates. Single-strand conformation polymorphisms of mutants were readily detected by a nonradioactive gel screen.—Authors' Abstract

deCian, W., Sassella, D. and Wynne, B. A. Clinical experience with rifabutin in the treatment of mycobacterial infections. Scand. J. Infect. Dis. **98** Suppl. (1995) 22–26.

Effective new therapies are required to combat the increasing incidence of mycobacterial infections. Rifabutin has been investigated in studies conducted in various countries around the world, and in the treatment of tuberculosis rifabutin in combination regimen has been shown to be as effective as rifampicin. Rifabutin is active in approximately 30% of patients with tuberculosis resistant to standard therapies, including rifampin and/or isoniazid. Placebo-controlled studies of rifabutin in the treatment of *Mycobacterium avium-intracellulare* complex (MAC) infection in AIDS patients have provided evidence for the inclusion of rifabutin in multidrug regimens. Rifabutin as a single agent is the only drug

approved for the prophylaxis of MAC infection. Clinical experience indicates that rifabutin is well tolerated and that it does not reduce the tolerability of combination regimens.—Authors' Abstract

Dubnau, E., Soares, S., Huange, T. J. and Jacobs, W. R. Overproduction of mycobacterial ribosomal protein S13 induces catalase peroxidase activity and hypersensitivity to isoniazid in *Mycobacterium smegmatis.* Gene **170** (1996) 17–22.

A bacillus Calmette Guerin (BCG) DNA fragment was identified which conferred hypersensitivity to isoniazid (INH) upon *Mycobacterium smegmatis* (Ms) when present on a multicopy plasmid. The gene cluster present on this fragment contains the genes encoding ribosomal proteins L36 (rpmJ), S13 (rpsM), S11 (rpsK) and S4 (rpsD), as well as the gene encoding initiation factor-1 (infA), an open reading frame of unknown function (ORFX) and a putative promoter region. The rpsM gene, from either BCG or Ms, is necessary and sufficient to produce the INH-hypersensitive phenotype in Ms. but the gene cluster has no effect on INH sensitivity when introduced into BCG on a multicopy plasmid. The presence of rpsM on a multicopy plasmid also causes an increase in catalase/peroxidase (Kat/Prx) activity in Ms. The overproduction of S13 may induce a stress response, resulting in increased expression of katG (encoding Kat/Prx) in Ms, thereby causing hypersensitivity to INH.—Authors' Abstract

Falkinham, J. O. Epidemiology of infection by nontuberculous mycobacteria. Clin. Microbiol. Rev. **9** (1996) 177.

The past 10 years have seen enormous increases in the number of nontuberculous mycobacterial infections, principally in patients with AIDS. Today, there is heightened awareness of the importance of nontuberculous mycobacterial infections. In addition to the increase in the number of infections with known mycobacteria (e.g., *Mycobacterium avium*), new species (e.g., *M. genavense*) have been identified some by nonconventional methods. For the most part, nontuberculous mycobacteria are opportunistic pathogens whose normal habitat is natural (e.g., rivers, swamps, and soils) and human-influenced (e.g., drinking water) environments. Focus has been on identification of sources and routes of transmission. Sources have been identified for some (e.g., *M. avium* and *M. xenopi*) but not others (e.g., *M. haemophilum*). Physiological studies have led to descriptions of the ecology and epidemiology for some species. Some grow in natural or potable water. All strains tested have been resistant to standard methods of disinfection. Consequently, they are found in drinking water. Characteristics that enhance environmental survival may also promote survival in infected animals. Tools for genetic analysis of nontuberculous mycobacteria (e.g., PCR) have led to identification of antibiotic resistance genes and to development of molecular markers for epidemiological studies. Molecular markers have confirmed the wide heterogeneity of natural mycobacterial populations and have been used to show the genetic identity of environmental and patient isolates.—Author's Abstract

Garbe, T. R., Hibler, N. S. and Deretic, V. Response of *Mycobacterium tuberculosis* to reactive oxygen and nitrogen intermediates. Mol. Med. **2** (1996) 134–142.

Background: *Mycobacterium tuberculosis* is a significant human pathogen capable of replicating in mononuclear phagocytic cells. Exposure to reactive oxygen and nitrogen intermediates is likely to represent an important aspect of the life cycle of this organism. The response of *M. tuberculosis* to these agents may be of significance for its survival in the host.

Materials and Methods: Patterns of *de novo* proteins synthesized in *M. tuberculosis* H37Rv exposed to compounds that generate reactive oxygen and nitrogen intermediates were studied by metabolic labeling and two-dimensional electrophoresis.

Results: Menadione, a redox cycling compound which increases intracellular superoxide levels, caused enhanced synthesis of seven polypeptides, six of which appeared to be heat-shock proteins. Chemical release of nitric oxide induced eight polypeptides of which only one could be identified as a heat-shock protein. Nitric ox-

ide also exhibited a mild inhibitory action on general protein synthesis in the concentration range tested. Hydrogen peroxide did not cause differential gene expression and exerted a generalized inhibition in a dose-dependent manner. Cumene hydroperoxide caused mostly inhibition but induction of two heat-shock proteins was detectable.

Conclusions: The presented findings indicate major differences between *M. tuberculosis* and the paradigms of oxidative stress response in enteric bacteria, and are consistent with the multiple lesions found in oxyR of this organism. The effect of hydrogen peroxide, which in *Escherichia coli* induces eight polypeptides known to be controlled by the central regulator oxyR, appears to be absent in *M. tuberculosis.* Superoxide and nitric oxide responses, which in *E. coli* overlap and are controlled by the same regulatory system soxRS, represent discrete and independent phenomena in *M. tuberculosis.*—Authors' Abstract

Gong, J. H., Zhang, M., Modlin, R. L., Linsley, P. S., Iyer, D., Lin, Y. G. and Barnes, P. F. Interleukin-10 downregulates *Mycobacterium tuberculosis*-induced Th1 responses and CTLA-4 expression. Infect. Immun. **64** (1996) 913–918.

To characterize the mechanism by which interleukin 10 (IL-10) inhibits Th1 responses to intracellular pathogens, we evaluated the interaction between IL-10 and *Mycobacterium tuberculosis*-induced gamma interferon (IFN-gamma) production by peripheral blood mononuclear cells from persons across the spectrum of tuberculous infection. *M. tuberculosis*-induced IFN-gamma production was highest in healthy tuberculin reactors, intermediate in human immunodeficiency virus (HIV)-negative tuberculosis patients, and lowest in HIV-infected tuberculosis patients. Neutralizing antibodies to IL-10 increased IFN-gamma production in HIV-infected and HIV-negative tuberculosis patients by enhancing monocyte IL-12 production. Expression of the T-cell-costimulatory molecule CTLA-4 was depressed in *M. tuberculosis*-stimulated peripheral blood mononuclear cells from tuberculosis patients, and anti-IL-10 and IL-12 upregulated expression of CTLA-4. These findings provide evidence that intracellular pathogens can inhibit Th1 responses and downregulate expression of specific costimulatory molecules.—Authors' Abstract

Guleria, I., Teitelbaum, R., McAdam, R. A., Kalpana, G., Jacobs, W. R. and Bloom, B. R. Auxotrophic vaccines for tuberculosis. Nature Med. **2** (1996) 334–337.

Tuberculosis is responsible for the deaths of more people each year than any other single infectious disease, with greater than 7 million new cases and 2 million deaths annually. It remains the largest attributable cause of death in HIV-infected individuals, responsible for 32% of deaths of HIV-infected individuals in Africa. The only currently available vaccine for tuberculosis, bacille Calmette-Guerin (BCG), is the most widely used vaccine in the world, being administered to approximately 100 million children each year. Although untoward effects were not seen in several studies of HIV-seropositive children, the safety of live attenuated BCG vaccine in HIV-positive adults remains unknown and a matter of some concern. To obviate potential adverse affects of BCG vaccines in immunodeficient individuals, we have studied five auxotrophic strains of BCG produced by insertional mutagenesis for safety in administration to mice with severe combined immunodeficiency disease (SCID), and for protection in a susceptible strain of mice. The results indicate that viable BCG could no longer be detected in mice receiving the auxotrophs after 16–32 weeks, and that infected SCID mice survived for at least 230 days. In contrast, all SCID mice succumbed within 8 weeks to conventional BCG vaccine. When susceptible BALB/c mice were immunized with auxotrophs and subsequently challenged virulent *Mycobacterium tuberculosis,* several of auxotrophs produced comparable protection against intravenous and intratracheal challenge with *M. tuberculosis* relative to conventional BCG. These results suggest that auxotrophic strains of BCG represent a potentially safe and useful vaccine against tuberculosis for populations at risk for HIV.—Authors' Abstract

Hirsch, C. S., Hussain, R., Toossi, Z., Dawood, G., Shahid, F. and Ellner, J. J. Cross-modulation by transforming growth factor beta in human tuberculosis: suppression of antigen-driven blastogenesis and interferon gamma production. Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 3193–3198.

In tuberculosis, *Mycobacterium tuberculosis* (MTB)-stimulated T-cell responses are depressed transiently, whereas antibody levels are increased. Lymphoproliferative responses of peripheral blood mononuclear cells (PBMCs) from Pakistani tuberculosis (TB) patients to both mycobacterial and candidal antigens were suppressed by approximately 50% when compared to healthy purified protein derivative (PPD)-positive household contacts. Production of interferon gamma (IFN-gamma) in response to PPD also was depressed by 78%. Stimulation with PPD and the 30-kD alpha antigen of MTB (30-kDa antigen) induced greater secretion of transforming growth factor beta (TGF-beta), but not interleukin 10 (IL-10) or tumor necrosis factor alpha (TNF-alpha), by PBMCs from TB patients compared to healthy contacts. The degree of suppression correlated with the duration of treatment; patients treated for < 1 month had significantly lower T-cell blastogenesis and IFN-gamma production and higher levels of TGF-beta than did patients treated for > 1 month. Neutralizing antibody to TGF-beta normalized lymphocyte proliferation in response to PPD, partially restored blastogenesis to candidal antigen, and significantly increased PPD-stimulated production of IFN-gamma in TB patients but not in contacts. Neutralizing antibody to IL-10 augmented, but did not normalize, T-cell responses to both PPD and candida in TB patients and candidal antigen in contacts. TGF-beta, produced in response to MTB antigens, therefore plays a prominent role in down-regulating potentially protective host effector mechanisms and looms as an important mediator of immunosuppression in TB.—Authors' Abstract

Hsieh, P. C., Shenoy, B. C., Samols, D. and Phillips, N. F. B. Cloning, expressing, and characterization of polyphosphate glucokinase from *Mycobacterium tuberculosis*. J. Biol. Chem. **271** (1996) 4909–4915.

Polyphosphate glucokinase from *Mycobacterium tuberculosis* catalyzes the phosphorylation of glucose using polyphosphate or ATP as the phosphoryl donor. The *M. tuberculosis* H37Rv gene encoding this enzyme has been cloned, sequenced, and expressed in *Escherichia coli*. The gene contains an open reading frame for 265 amino acids with a calculated mass of 27,400 daltons. The recombinant polyphosphate glucokinase was purified 189-fold to homogeneity and shown to contain dual enzymatic activities, similar to the native enzyme from H37Ra strain. The high G+C content in the codon usage (64.5%) of the gene and the absence of an *E. coli*-like promoter consensus sequence are consistent with other mycobacterial genes. Two phosphate binding domains conserved in the eukaryotic hexokinase family were identified in the polyphosphate glucokinase sequence; however, "adenosine" and "glucose" binding motifs were not apparent. In addition, a putative polyphosphate binding region is also proposed for the polyphosphate glucokinase enzyme.—Authors' Abstract

Klausner, J. D., Makonkawkeyoon, S., Akaresewi, P., Nakata, K., Kasinrerk, W., Corral, L., Dewar, R. L., Lane, H. C., Freedman, V. H. and Kaplan, G. The effect of thalidomide on the pathogenesis of human immunodeficiency virus type 1 and *M. tuberculosis* infection. J. Acq. Immun. Defic. Synd. Hum. Retrovirol. **11** (1996) 247–257.

Tumor necrosis factor alpha (TNF-alpha), a cytokine produced during the host defense against infection, is associated with fevers, weakness, and progressive weight loss. Thalidomide inhibits the synthesis of TNF-alpha both *in vitro* and *in vivo* and may have clinical usefulness. We therefore initiated a pilot study of thalidomide treatment in patients with human immunodeficiency virus type 1 (HIV-1)-associated wasting with or without concomitant infection with tuberculosis. Thirty-nine patients were randomly allocated to treatment with either thalidomide or placebo in a double-blind manner for 21 days. Thirty-two patients completed the study. In patients with

concomitant HIV-1 and tuberculosis infections, thalidomide therapy was associated with a reduction in both plasma TNF-alpha levels and HIV-1 levels. No significant reduction in either TNF-alpha or HIV-1 levels was observed in patients with HIV-1 infection only. During the study period, patients receiving thalidomide treatment (N = 16) showed a significant weight gain (mean ± SEM: 6.5 ± 1.2%; $p < 0.02$) relative to placebo-treated patients (N = 16). Patients with simultaneous HIV-1 and tuberculosis infections experienced a higher mean weight gain during thalidomide treatment than the group of patients with HIV-1 infection only. The results of this pilot study suggest that thalidomide may have a clinical role in enhancing weight gain and possibly reducing TNF-alpha and HIV-1 levels in patients with HIV-1 and concomitant mycobacterial infections.—Authors' Abstract

Klemens, S. P., Sharpe, C. A. and Cynamon, M. H. Activity of pyrazinamide in a murine model against *Mycobacterium tuberculosis* isolates with various levels of *in vitro* susceptibility. Antimicrob. Agents Chemother. **40** (1996) 14–16.

The activity of pyrazinamide (PZA) against eight isolates of *Mycobacterium tuberculosis* in a murine infection model was evaluated. *M. tuberculosis* isolates with various degrees of *in vitro* susceptibility to PZA (MIC range, 32 to > 2,048 μg/ml) were used. Four-week-old female mice were infected intravenously with approximately 10^7 viable *M. tuberculosis* organisms. PZA at 150 mg/kg of body weight was started 1 day postinfection and given 5 days/week for 4 weeks. Infected but untreated mice were compared with PZA-treated mice. Mice were sacrificed at the completion of the treatment period, and viable cell counts were determined from homogenates of spleens and right lungs. PZA had activity in the murine test system against *M. tuberculosis* isolates for which the MICs were ≤ 256 μg/ml. However, there was an inconsistent correlation between the absolute MICs and the reductions in organ viable cell counts. Studies with drug-resistant *M. tuberculosis* isolates with an isogenic background would improve evaluation of drug efficacy in the murine test system. Further evaluation of antimycobacterial agents against monodrug-resistant isolates Will provide data that will be useful for development of algorithms for treatment of infection with drug-resistant organisms.—Authors' Abstract

Mahairas, G. G., Sabo, P. J., Hickey, M. J., Singh, D. C. and Stover, C. K. Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis.* J. Bacteriol. **178** (1996) 1274–1282.

The live attenuated bacillus Calmette-Guerin (BCG) vaccine for the prevention of disease associated with *Mycobacterium tuberculosis* was derived from the closely related virulent tubercle bacillus, *M. bovis.* Although the BCG vaccine has been one of the most widely used vaccines in the world for over 40 years, the genetic basis of BCG's attenuation has never been elucidated. We employed subtractive genomic hybridization to identify genetic differences between virulent *M. bovis* and *M. tuberculosis* and avirulent BCG. Three distinct genomic regions of difference (designated RD1 to RD3) were found to be deleted from BCG, and the precise junctions and DNA sequence of each deletion were determined. RD3, a 9.3-kb genomic segment present in virulent laboratory strains of *M. bovis* and *M. tuberculosis,* was absent from BCG and 84% of virulent clinical isolates. RD2, a 10.7-kb DNA segment containing a novel repetitive element and the previously identified mpt-64 gene, was conserved in all virulent laboratory and clinical tubercle bacilli tested and was deleted only from substrains derived from the original BCG Pasteur strain after 1925. Thus, the RD2 deletion occurred after the original derivation of BCG. RD1, a 9.5-kb DNA segment found to be deleted from all BCG substrains, was conserved in all virulent laboratory and clinical isolates of *M. bovis* and *M. tuberculosis* tested. The reintroduction of RD1 into BCG repressed the expression of at least 10 proteins and resulted in a protein expression profile almost identical to that of virulent *M. bovis* and *M. tuberculosis,* as determined by two-dimensional gel electrophoresis. These data indicate a role for RD1 in the regulation of multiple genetic loci, suggesting that the loss of viru-

lence by BCG is due to a regulatory mutation. These findings may be applicable to the rational design of a new attenuated tuberculosis vaccine and the development of new diagnostic tests to distinguish BCG vaccination from tuberculosis infection.—Authors' Abstract

Mattila, J. O., Vornanen, M., Vaara, J. and Katila, M. L. Mycobacteria in prurigo nodularis: the cause or a consequence? J. Am. Acad. Dermatol. **34** (1996) 224–228.

Background: Prurigo nodularis (PN) is a chronic skin disorder; its cause remains unknown.

Objective: we evaluated mycobacteria as a possible cause of PN.

Methods: Forty-three patients with PN were examined. Skin biopsy specimens were obtained for microbiologic and histopathologic studies. The patients were tested for intracutaneous reactivity to 12 mycobacterial antigens with the Mantoux technique.

Results: Six specimens (14%) grew mycobacteria in culture: *M. avium-intracellulare* (3), M. *malmoense* (1), and *Mycobacterium* sp. (2). Histopathologically, 12 samples (28%) were positive for acid-fast bacilli, and granulomatous changes were present in one sample. Patients whose cultures were positive for mycobacteria had significantly larger skin reactions to mycobacterial antigens. Two patients underwent 2 years of antituberculous chemotherapy; one had an excellent response and the other a partial response.

Conclusion: Detection of mycobacteria by culture or staining, combined with elevated skin reactivity to mycobacteria in a high proportion of patients with PN, suggests a mycobacterial cause.—Authors' Abstract

Mikusova, K., Mikus, M., Besra, G. S., Hancock, I. and Brennan, P. J. Biosynthesis of the linkage region of the mycobacterial cell wall. J. Biol. Chem. **271** (1996) 7820–7828.

The "core" structure of the cell wall of *Mycobacterium* and related genera is unique among prokaryotes, consisting of a covalently linked complex of mycolic acids, D-arabinan and D-galactan (mycolylarabinogalactan, mAG), which, in turn, is linked to peptidoglycan via a special linkage unit, -alpha-L-Rhap(1→3)-D-GlcNAc-P-. Little is known of the biosynthesis of this complex, although it is the site of action of several common antituberculosis drugs. Isolated cell membranes of *Mycobacterium smegmatis* catalyzed the incorporation of [C-14]GlcNAc from UDP-[C-14]GlcNAc into two glycolipids (1 and 2) and of [C-14]Rha from TDP-[C-14]Rha into glycolipid 2. These products were characterized as polyprenol-P-P-GlcNAc (glycolipid 1) and polyprenol-P-P-GlcNAc-Rha (glycolipid 2) based on sensitivity of synthesis to tunicamycin, chromatographic characterization of the products of mild acid hydrolysis, and mass spectral analysis of the glycosyl and polyprenyl units. Glycolipids 1 and 2 were shown to be precursors of the linkage unit in polymerized cell wall. The inclusion in the assays of UDP-[C-14]Galp and a preparation of cell walls allowed the incorporation of [C-14]Gal into two further glycolipids (3 and 4). Preliminary evidence indicates a precursor-product relationship among glycolipids 1, 2, 3, and 4. Thus, the first steps in the biosynthesis of the mycobacterial cell wall involve synthesis of the linkage disaccharide on a polyprenyl-P-P carrier followed by growth of the galactan unit. Assays are thus defined for the screening of new antituberculosis drugs active against cell wall synthesis.—Authors' Abstract

Munk, M. E., Mayer, P., Anding, P., Feldmann, K. and Kaufmann, S. H. E. Increased numbers of interleukin-12-producing cells in human tuberculosis. Infect. Immun. **64** (1996) 1078–1080.

Numbers of interleukin-12 (IL-12)-producing cells were quantitated in the peripheral blood of healthy donors and tuberculosis patients by the ELISPOT assay. We observed that (i) stimulation with mycobacteria increases numbers of IL-12 producers from healthy donors and (ii) tuberculosis patients have larger numbers of IL-12 producers than healthy donors. Our data emphasize the importance of IL-12 in immunity to tuberculosis.—Authors' Abstract

Nichols, C. W. *Mycobacterium avium* complex infection, rifabutin, and uveitis—is there a connection? Clin. Infect. Dis. **22** (1996) S43–S47.

Rifabutin, an antimycobacterial agents, has been recommended by the U.S. Public Health Service as prophylaxis against infection due to *Mycobacterium avium* complex (MAC) in patients with AIDS. When rifabutin is administered as prophylaxis, uveitis has been reported only rarely. However, uveitis has been reported in two studies in which rifabutin was administered at higher doses in combination with an azole, a macrolide, or both for treatment of disseminated MAC infection. The uveitis that has been reported has been predominantly anterior and mild-to-moderate in nature, although severe hypopyon uveitis has occasionally been reported. No etiologic infectious agent has been isolated from any of these patients, and treatment with topical steroids and cycloplegics usually leads to rapid resolution of the uveitis. It is necessary to discontinue prophylaxis or therapy with rifabutin only in cases of uveitis that are refractory to treatment or when the uveitis recurs. Immunologic factors, rather than direct drug toxicity, appear to be the most likely explanation for the occurrence of uveitis in patients receiving rifabutin; however, further study is required to elucidate the mechanisms involved.—Author's Abstract

Pollard, M. Thalidomide promotes metastasis of prostate adenocarcinoma cells (PA-III) in L-W rats. Cancer Lett. **101** (1996) 21–24.

Two contradictory actions have been ascribed to thalidomide relative to tumor metastasis: immunosuppression and antiangiogenesis. The latter effect was determined with basic fibroblast growth factor in a rabbit cornea micropocket assay system. The prostate adenocarcinoma (PA-III) transplanted tumor line in Lobund-Wistar (L-W) rats produces a tumor at the subcutaneous implant site from which tumor cells metastasize uniformly only through lymphatic channels through the heart to the lungs in which secondary tumors develop. L-W rats were implanted with PA-III cells and administered, by gavage, thalidomide (50 mg/kg body wt per day) in corn oil. Control rats with PA-III cells were administered corn oil. Autopsy examinations on day 30 revealed that the thalidomide-treated rats developed more metastatic tumor foci in the lungs than in the controls.—Author's Abstract

Raad, I., Hachem, R., Leeds, N., Sawaya, R., Salem, Z. and Atweh, S. Use of adjunctive treatment with interferon-gamma in an immunocompromised patient who had refractory multidrug-resistant tuberculosis of the brain. Clin. Infect. Dis. **22** (1996) 572–574.

We describe a patient with acute lymphocytic leukemia and multidrug-resistant tuberculosis of the brain and spinal cord. Despite treatment with six antituberculous drugs and a steroid medication for 11 months, there was no appreciable clinical or radiological improvement in the patient's condition. Within 5 months of initiating adjunctive therapy with IFN-gamma and granulocyte colony stimulating factors, substantial neurological and radiological improvement was noted. Therapy with IFN-gamma was continued for 12 months, resulting in complete resolution of the lesions in the brain and spinal cord.—Authors' Abstract

Ragno, S., Winrow, V. R., Mascagni, P., Lucietto, P., DiPierro, F., Morris, C. J. and Blake, D. R. A synthetic 10-kD heat-shock protein (hsp10) from *Mycobacterium tuberculosis* modulates adjuvant arthritis. Clin. Exp. Immunol. **103** (1996) 384–390.

The heat-shock protein, hsp10, is an abundant protein in *Mycobacterium tuberculosis* (Mtb), its nucleotide sequence encoding a protein of 99 amino acids with a molecular mass of 10.7 kD. This sequence is phylogenetically conserved, being represented by the GroES homologue of *Escherichia coli.* Hsp10 and GroES are members of the ciaperonin 10 family of molecular chaperones, and GroES is necessary for the optimal activity of GroEL, a member of the chaperonin 60 family and the *E. coli* homologue of mycobacterial hsp65. Since

hsp65 has been implicated in both experimental and human rheumatoid arthritis, we aimed to assess the immunomodulatory effects of its co-chaperonin, hsp10, in experimental arthritis. Our results show that an aqueous solution of a mycobacterial hsp10 delayed the onset and severity of adjuvant-induced arthritis in rodents when administered after disease induction but before joint involvement occurred. This biological activity was specific for the hsp10 of Mtb, since neither GroES nor the rat homologue was effective. Using synthetic hsp10 fragments, the activity was localized to the N-terminal region of the molecule. Assessment of circulating antibody levels to mycobacterial hsp10 and hsp65 indicated that all arthritic rats had increased titers to both hsp10 and hsp65: hsp10-treated rats showed further elevation of this humoral response not only to hsp10 but also to hsp65 when compared with the untreated arthritic control. This is the first report of the immunomodulatory activity of mycobacterial hsp10 experimental arthritis, and exhibits a potential role for this co-chaperonin in pathophysiological situations.—Authors' Abstract

Rajalingam, R., Mehra, N. K., Jain, R. C., Myneedu, V. P. and Pande, J. N. Polymerase chain reaction-based sequence-specific oligonucleotide hybridization analysis of HLA class II antigens in pulmonary tuberculosis: relevance to chemotherapy and disease severity. J. Infect. Dis. **173** (1996) 669–676.

HLA antigens were studied by serology and polymerase chain reaction-based sequence-specific oligonucleotide hybridization techniques in 153 patients with pulmonary tuberculosis (PTB) and 289 healthy controls. HLA-DR2 was present more frequently in PTB patients than in controls (51% vs. 36.3%; corrected P [Pc] = 0.029, relative risk [RR] = 1.8). The DR2 association was stronger in patients in the drug-failure group (N = 56; Pc = 0.000012, RR = 3.7) than in healthy controls and patients in the drug-responder group. No significant deviation was observed in HLA allelic frequencies in various patient groups, as determined by radiographs of lung lesions. Molecular subtyping of DR2 revealed that the bulk of the allele was DRBI*1501 and DRBI*1502 in patients and controls. There was no skewing of the frequency of these molecular subtypes of DR2 in patients, suggesting that the whole DR2 molecule or its closely linked gene(s) may be involved in governing patient susceptibility to PTB and, particularly, development of the severe drug-resistant form of the disease.—Authors' Abstract

Sanchez Carrillo, C., Cotarelo, M., Cercenado, E., Vicente, T., Blazquez, R. and Bouza, E. Comparative *in-vitro* activity of sparfloxacin and eight other antimicrobial agents against clinical isolates of non-tuberculous mycobacteria. J. Antimicrob. Chemother. **37** (1996) 151–154.

The *in-vitro* activity of sparfloxacin and eight other antimicrobial agents against 64 nontuberculous mycobacteria (non-MAI) (40 rapidly growing and 24 slowly growing) was compared with those of ciprofloxacin, ofloxacin, amikacin, tobramycin, cefoxitin, imipenem, clarithromycin and doxycycline. Sparfloxacin presented the same activity as ciprofloxacin, being highly active against rapidly growing mycobacteria and showing good *in-vitro* activity against slowly growing mycobacteria. Amikacin was very active against rapidly growing mycobacteria and clarithromycin presented good activity against all mycobacteria tested. These results suggest that sparfloxacin is a valid agent to be considered in the treatment of *Mycobacterium* spp. infections.—Authors' Abstract

Sreevatsa, S., Pan, X., Stockbauer, K. E., Williams, D. L., Kreiswirth, B. N. and Musser, J. M. Characterization of rpsL and rrs mutations in streptomycin-resistant *Mycobacterium tuberculosis* isolates from diverse geographic localities. Antimicrob. Agents Chemother. **40** (1996) 1024–1026.

Two genes (rpsL and rrs) with mutations associated with streptomycin resistance in *Mycobacterium tuberculosis* were characterized in 78 streptomycin-resistant and 61 streptomycin-susceptible isolates recovered

from patients living in the United States, South America, Europe, Africa, and Asia. Fifty-four percent of the 78 resistant organisms had missense mutations in codon 43 of rpsL, resulting in a K-43→R substitution. Mutations in codon 88 of rpsL were also identified in four Asian isolates.—Authors' Abstract

Vandercam, B., Gala, J., Vandeweghe, B., Degraux, J., Wauters, G., Larsson, L., Bourlond, A. and Portaels, F. *Mycobacterium simiae*-disseminated infection in a patient with acquired immunodeficiency syndrome. Infection **24** (1996) 49–51.

Mycobacterium simiae is commonly found in nature and its role as a pathogen has been controversial. A case of disseminated *M. simiae* infection with blood, pulmonary and cutaneous localization is reported here. The pathogenic role of *M. simiae* was clearly demonstrated as it was the only organism isolated from sputum, broncho-alveolar lavage fluid, as well as blood and skin tissue. Identification of *M. simiae* by conventional testing may be difficult. Analysis of conventional testing may be difficult. Analysis of fatty and mycolic acid patterns, as performed in this case, is necessary to confirm its identification.—Authors' Abstract

Walsh, G. P., Tan, E. V., dela Cruz, E. C., Abalos, R. M., Villahermosa, L. G., Young, L. J., Cellona, R. V., Nazareno, J. B. and Horowitz, M. A. The Philippine cynomolgus monkey (*Macaca fascicularis*) provides a new nonhuman primate model of tuberculosis that resembles human disease. Nature Med. **2** (1996) 430–436.

A nonhuman primate model of tuberculosis (TB) that closely resembles human disease is urgently needed. We have evaluated the Philippine cynomolgus monkey, *Macaca fascicularis,* as a model of TB. Cynomolgus monkeys challenged intratracheally with extremely high doses of *Mycobacterium tuberculosis* (10^5 or 10^4 CFU) developed an acute, rapidly progressive, highly fatal multilobar pneumonia. However, monkeys challenged with moderate or low doses of *M. tuberculosis* ($\leq 10^3$ CFU) developed a chronic, slowly progressive, localized form of pulmonary TB, akin to the disease in humans, that was frequently accompanied by such clinical syndromes as ocular tuberculosis, meningitis and tuberculous spondylitis. A significant proportion of monkeys challenged with 10^2 or 10^1 CFU contained the infection in a subclinical state. The Philippine cynomolgus monkey model is an excellent model of chronic TB and provides an opportunity to study subclinical and potentially latent disease in an animal model.—Authors' Abstract

Warren, R., Hauman, J., Beyers, N., Richardson, M., Schaaf, H. S., Donald, P. and van Helden, P. Unexpectedly high strain diversity of *Mycobacterium tuberculosis* in a high-incidence community. S. Afr. Med. J. **86** (1996) 45–49.

Objective. To characterize *Mycobacterium tuberculosis* strains present in a community experiencing an epidemic, in order to establish whether a high rate of transmission results in low strain diversity.

Design. Sputum specimens collected for 18 months; IS6110-based DNA fingerprinting.

Setting. The communities of Ravensmead and Uitsig, Cape Town, South Africa.

Participants. Three-hundred-thirty-four pulmonary tuberculosis patients attending the Local Authority Health Care Clinic.

Main outcome measure. DNA fingerprinting.

Results. A total of 334 *M. tuberculosis* isolates were characterized by IS6110-based DNA fingerprinting; 209 strains were identified, 199 having 5 or more insertions. Forty of these strains were present in 2 or more patients (clustering—126 patients in total), which indicates a recent transmission rate of 30%. The 163 unique strains suggest reactivation of latent infections. Computer analysis showed a high degree of strain diversity, and a common progenitor could only be linked to 33% of the strains. Clustering was shown in 50% of drug-resistant isolates.

Conclusions. The low rate of transmission (30%) and the high degree of strain diversity (209 strains) was unexpected and unexplained, given the high burden of dis-

ease in this community. The clustering of drug-resistant strains suggests that transmission, rather than lack of compliance, drives the spread of antibiotic resistance in this community. Preliminary indications are that BCG vaccination, while having little effect on the incidence of tuberculosis in this community, may have altered the strain dynamics.—Authors' Abstract