CURRENT LITERATURE

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

General and Historical

Andrade, V., Moreira, T. M. A., Tardin, R. T., de Castro, A. J. W. and de Sousa, A. C. M. [A leprosy elimination campaign combined with a vaccine against poliomyelitis, Rio de Janeiro, Brazil.] An. Bras. Dermatol. **73** (1998) 159–165. (in Portuguese)

A leprosy elimination campaign in the city of Rio de Janeiro (LEC-RIO) was coupled with an antipolio campaign—the second dose of oral Sabin vaccine. Following World Health Organization's recommendation the basis of this campaign was its divulging, in order to stimulate people who present signs and symptoms suggesting this sickness, especially contagious patients who live in areas not covered by the program, to show up spontaneously at the Health Services. LEC-RIO's main strategies were the divulgation about the disease on mass means of communication and community exam, increasing the 28 normal sanitary health service points to 70 for diagnosis and treatment scouts that day. We highlight the participation of several associations, represented by MORHAN, Brazil Scouts, residents' associations and service clubs (Rotary Club, Lions) in recruiting and training volunteers for pamphlet distribution and operation of the telephone information service, called Telehansen. Three thousand-eleven persons attended, 52 new leprosy cases were diagnosed, of which 11 (21.2%) were multibacillary cases; among 35 paucibacillary cases, 16 (30.8%) had the indeterminate clinical form and 6 cases were not classified. Besides that, 2300 cases of other dermatoses were detected and 603 cases did not present abnormalities on dermatological exam.—Authors' English Summary

Stitch-Groh, V. and Bretzel, G. [Leprosy—current aspects.] Hautarzt **48** (1997) 297–302.

The current aspects of epidemiology, transmission, bacteriology, clinical features, reactions, diagnosis, chemotherapy and treatment of leprosy are reviewed.—Authors' English Abstract

Chemotherapy

Gillis, T. P. and Krahenbuhl, J. L. Global elimination of leprosy. Rev. Med. Microbiol. **9** (1998) 39–48.

This review considers historical aspects of infectious diseases control before summarizing the epidemiology of leprosy and basic underlying mechanisms of clinical disease, with emphasis on areas which are poorly understood and which remain potential obstacles in leprosy elimination on a worldwide scale. Multidrug therapy (MDT) and the risk of relapse, early detection of leprosy, and prevention of disabilities and rehabilitation are discussed.—Authors' Abstract

Ji, B., Sow, S., Perani, E., Lienhardt, C., Diderot, V. and Grosset, J. Bactericidal activity of a single-dose combination of ofloxacin plus minocycline, with or without rifampin, against *Mycobacterium leprae* in mice and in lepromatous patients. Antimicrob. Agents Chemother. **42** (1998) 1115–1120.

To develop a fully supervisable, monthly administered regimen for treatment of leprosy, the bactericidal effect of a single-dose combination of ofloxacin (OFLO) and minocycline (MINO), with or without rifampin (RMP), against *Mycobacterium leprae* was studied in the mouse foot pad system and in previously untreated lepromatous leprosy patients. Bactericidal activity was measured by the proportional bactericidal method. In mouse experiments, the activity of a single dose of the combination of OFLO-MINO was dosage related; the higher dosage of the combination displayed bactericidal activity which was significantly inferior to that of a single dose of RMP, whereas the lower dosage did not exhibit a bactericidal effect. In the clinical trial, 20 patients with previously untreated lepromatous leprosy were treated with a single dose consisting of either 600 mg of RMP plus 400 mg of OFLO and 100 mg of MINO or 400 mg of OFLO plus 100 mg of MINO. The OFLO-MINO combination exhibited definite bactericidal activity in 7 of 10 patients but was less bactericidal than the RMP-OFLO-MINO combination. Both combinations were well tolerated. Because of these promising results, a test of the efficacy of multiple doses of ROM in a larger clinical trial appears justified.—Authors' Summary

Li, W., *et al.* [Bacterial change and relapse after MDT in 157 cases of MB leprosy.] China Lepr. J. **14** (1998) 6–10 (in Chinese)

In 1983 to 1993, 711 cases of MB leprosy have been registered and treated with WHO-MDT until their skin smears became negative in Yangzhou and Dongtai, Jiangsu. Among them 157 cases who had BI of ≥2.6 at the beginning of treatment and stopped MDT before December 1990 were followed up, of which 65 (41.4%) were followed up over 7 years. Their skin smears became negative within 2 to 6 years, with average time of 47.7 months after starting MDT, being within 4 years in 75.8% and within 5 years in 97.5%. For five years their BI decreased by 0.69 yearly on an average. They have been followed up for a mean of 6.02 years, amounting to 946 person-years. Two cases were found to have relapsed 3 and 4.5 years after stopping MDT, respectively, being a relapse rate of 1.3% or 2.1/1000 person-years. So, in this group of MB leprosy patients with higher bacterial load the relapse rate was no higher.—Authors' English Abstract

Li, H., *et al.* [FD MDT for patients with MB leprosy.] China Lepr. J. **14** (1998) 1–6. (in Chinese)

In 1983, the first author with her fellows had treated 80 cases of MB leprosy for 24 and 27 months with WHO-MDT and then follow up was done annually, showing that they were improving clinically, bacteriologically and histopathologically. In 1986 to 1994, the authors had treated 657 MB leprosy patients with fixed duration MDT for 24 months again in Liangshan and Panzhihua, Sichuan, and by the end of 1995 a total of 425 cases, including patients treated only with MDT in 190 and with other drugs or regimens in 235 previously, had been followed up for 5 years after stopping MDT. The results showed that among the patients only with MDT (1477 person-years) there was no relapse and among the cases treated with other methods (1172 person-years) two relapsed (0.17%). The skin smears became negative in 99.6% (226/227) within 5 years and only one LL case in the 5th and 6th year still had a BI of 0.33. Becoming bacterial negativity in B form was faster than that in L form. Among 35 cases with a BI of >4.0 before MDT only one relapsed, being 0.24% or 1/420 person-years and among 358 cases with a BI of >3.0 before MDT also one relapsed, being 0.08% or 1/1224 person-years. Six months after beginning of the treatment lepra reaction was decreasing and by the fourth year of follow up there was no reaction. Before MDT visible disability accounted for 22.7% (149/657), and during the treatment 12 cases had new or deteriorated disabilities, being 1.8%. There were 11 cases with hypersensivity to DDS and damage of the liver, but the antileprosy treatment was not greatly affected.—Authors' English Abstract

Mayorga Rodriguez, J. A., Vargas Salas, F., Morales Ortiz, R., Munoz Estrada,

V. F., Garcia Vargas, A. and Barba-Gomez, J. F. [Bacilloscopic study of patients with Hansen's disease, treated by the WHO scheme.] Dermatol. Rev. Mex. **41** (1997) 103–104. (in Spanish)

A total of 30 patients from Mexico, with multibacillary Hansen's disease (BL or LL), who had completed their controlled treatment according to the scheme of the World Health Organization (WHO) were studied. The bacilloscopy of each patient was examined in their bacteriological, morphological and stain indexes during the initiation, 1st and 2nd year of treatment. As a result of the treatment, 40% (12 patients) tested negative on their initial bacteriological index and the remainder of the patients remained positive. It is suggested that in patients with multibacillary Hansen's disease the time frame of the treatment implemented by the WHO should be prolonged to avoid future relapse.—Authors' English Abstract

Qu, T. [Efficacy of MDT in 59 cases of MB leprosy.] China Lepr. J. **14** (1998) 25–26. (in Chinese)

Since May 1986, 59 cases of MB leprosy have received WHO-MDT for 2 years, including 46 who had taken DDS or DDS plus RMP previously and 13 new patients with disease duration of 21.5 months, being 8 months as an average. At stop of MDT two cases were cured, and among all the patients the mean BI decreased from 2.13 to 0.43. The skin smears of 68 became negative 7 years after stopping MDT while one case (1.69%) who had taken DDS previously relapsed. The author thought that the monitoring should be continued for over 10 years.—Author's English Abstract

Zhu, R. [MDT plus ofloxacin in MB leprosy.] China Lepr. J. **14** (1998) 33–34. (in Chinese)

In 1993 to 1994, three cases of MB leprosy still had a BI of from 2.2 to 4.0 after completion of a 2-year MDT course. So, ofloxacin 400 mg was given daily while continuing to take MDT for 4 months and their BIs decreased by 0.6 to 1.5, showing quicker efficiency.—Author's English Abstract

Clinical Sciences

Batistella, G. G. G., Maakaroun, M. and Vilela de Castro, A. Extracapsular cataract extraction and intraocular lens implantation in leprosy patients: visual outcome and complications. Indian J. Lepr. **70** (1998) 5–10.

In Belo Horizonte, Brazil, 70 eyes of 53 leprosy patients had extracapsular cataract extraction and intraocular lens implantation done during a period of 4 years. The authors analyzed the outcome regarding restoration of vision and complications after this procedure. Visual acuity improved in 92.9% of the eyes and in 65.7% acuity had improved by four lines or more on the Snellen chart. The postoperative complications could not be associated only to leprosy infiltration; in any case, they were not too serious and could be controlled.—Authors' Abstract

Biedermann, T., Degitz, K., Schirren, C. G., Burgdorf, W., Plewig, G. and Bieber, T. Leprosy type 1 reaction as the first manifestation of borderline lepromatous leprosy in a young native German. Br. J. Dermatol. **137** (1997) 1006–1010.

While leprosy is usually a chronic disease, leprosy reactions may lead to acute problems. These reactions most often occurred after initiation of therapy but can also develop before treatment. Leprosy rarely presents with a reaction. We describe a German patient who presented in this unusual way in order to demonstrate the various tools used to confirm the diagnosis. A young German woman suddenly developed progressive functional loss of the left hand and within a few weeks an increasing number of erythematous macules and nodules

appeared. Histological examination of a skin biopsy revealed tuberculoid granulomas, some located around small nerves; acid-fast bacilli were detected microscopically and DNA from *Mycobacterium leprae* was identified by polymerase chain reaction in the biopsy and a nasal swab; serological tests were positive. The disease was classified as borderline lepromatous leprosy.

The acute neuritis followed by skin lesions represented a leprosy type 1 reaction in which the immune system reacts to bacilli previously unrecognized in nerve tissue, both in nerve and skin.—Authors' Abstract

Biswas, J. and Raizada, S. Immunotherapy of uveitis. Indian J. Lepr. **70** (1998) 11–25.

The role of retinal autoimmunity in the pathogenesis of retinochoroidal inflammation has now been established. The discovery of retinal autoantigens, the production of experimental autoimmune uveitis, the identification of uveitogenic peptide determinants in retinal autoantigens, and the pathogenesis and role of T-cell-mediated immune mechanism in EAU have contributed to enhance our understanding of the concept of retinal autoimmunity. Moreover, in recent years, the roles of retinal autoimmunity and the cell-mediated immune mechanism have been demonstrated in clinical conditions like sympathetic ophthalmia and Vogt-Koyanagi-Harada's disease. The role of various cytokines and cell adhesion molecules has also been studied. The clinical application of such a concept has resulted in the therapeutic usage of several immunomodulators like cyclosporine and FK-506 in autoimmune retinochoroidal inflammations.—Authors' Conclusion

Campos, W. R., Orefice, F., Sucena, M. A. and Rodrigues, C. A. F. Bilateral iridocyclitis caused by *Mycobacterium leprae* diagnosed through paracentesis. Indian J. Lepr. **70** (1998) 27–31.

The authors conducted an anterior chamber paracentesis in a patient with lepromatous leprosy showing bilateral iridocyclitis. The paracentesis was performed in the outpatient's department. The aqueous humor was studied by the Ziehl-Nielsen staining method and the result was the isolation of the *M. leprae* in the anterior chamber. This study shows that *M. leprae* can promote uveitis in leprosy patients. Therefore, it should be looked for in patients having this type of disease.—Authors' Abstract

Courtright, P. The epidemiology of ocular complications of leprosy. Indian J. Lepr. **70** (1998) 33–37.

The greatest limitation to our understanding of the epidemiology of the ocular complications of leprosy is the lack of incidence data. Data collection and analysis, currently underway in India and The Philippines will provide much of the information necessary to determine the incidence of specific eye findings as well as the factors responsible for eye pathology in patients on MDT. Data collection should not end with the completion of MDT; the risk of eye complications following MDT and during surveillance is also required.

We also require information on appropriate and effective interventions. Methods to ensure the early recognition of lagophthalmos and optimal timing of steroid treatment need to be defined. A clinical trial of different surgical procedures for the correction of lagophthalmos and ectropion would provide the information necessary for health workers, clinicians and patients to choose the procedure most likely to result in clinical and cosmetic success. Our continuing lack of understanding of chronic uveitis hinders our ability to identify and treat patients with this condition. We need better ways to detect early changes as well as pharmacological agents to prevent severe miosis. We need to better clarify the contribution of cataract (both age-related and complicated) to blindness and vision loss in leprosy patients.

There has been little work on the social or economic implications of eye disease in leprosy patients. Findings from Korea have demonstrated a threefold risk in age-adjusted mortality in blind leprosy patients compared to their nonblind peers. As life expectancy increases, leprosy patients will

face additional years with eye disease and vision loss. There is much we need to understand about the progression of eye disease if these patients are going to have an improved quality of life.—From the article

Daniel, E. Lagophthalmos in leprosy. Indian J. Lepr. **70** (1998) 39–47.

The occurrence of facial paralysis in leprosy is well known. Paralysis of the orbicularis oculi muscle is by far the most serious consequence of the loss of function of the facial nerve. Lagophthalmos is due to the failure of the orbicularis oculi muscle. At a minimum, 290,000 people worldwide are purported to have leprosy-related lagophthalmos. It occurs in about 20% of all leprosy patients, and it occurs in both paucibacillary (PB) and multibacillary (MB) leprosy. In a study of 14,257 cases of leprosy in China, lagophthalmos was found in 2114 patients (14.83%), and 1214 of these were unilateral while 900 were bilateral. It was found that most of the cases of unilateral lagophthalmos occurred in PB cases while bilateral lagophthalmos was common in MB patients. In most cases (90%) of bilateral lagophthalmos, the condition occurred on both sides within 2 months of each other. In MB patients, the onset of lagophthalmos was more gradual, it occurred later in the disease and was not as severe as that seen in PB patients.—From the article

Date, A., John, G. T., Thomas, P. P. and Jacob, C. K. Leprosy and renal transplantation. Lepr. Rev. **69** (1998) 40–45.

Nine cases of leprosy in patients treated at a large renal transplant center in South Asia are described. Three had leprosy diagnosed before transplantation and had either completed or were continuing chemotherapy at the time of transplantation. One showed exacerbation of undisclosed leprosy after transplantation. Five patients developed the disease for the first time 22 months to 12 years after transplantation. Immunosuppression did not adversely affect the treatment of leprosy in any of the patients although concurrent liver disease required cessation of rifampin in one patient.—Authors' Summary

ffytche, T. J. The prevalence of disabling ocular complications of leprosy: a global study. Indian J. Lepr. **70** (1998) 49–59.

A world-wide study on the ocular complications of leprosy has been carried out over the past 10 years. The data from 4772 patients, designed to give baseline information for a 5-year incidence study, have been analyzed. Blindness due to leprosy was seen in 3.2% of the sample and 7.1% had Grade 2 visual disability. The causes of visual impairment in the disease are discussed and it is emphasized that a high proportion of these are preventable, particularly through the early use of multidrug therapy. The active participation of ophthalmologists in the management of the disease is still required since many of the blinding complications respond well to surgery.—Author's Abstract

Hogeweg, M. Strategies for improvement of management of ocular complications in leprosy. Indian J. Lepr. **70** (1998) 61–70.

Responsibility for eye care of leprosy-affected persons should be shared between leprosy and eye care staff. Leprosy and PHC staff should be responsible for:

—treatment of reversal reactions in the face, and of recent lagophthalmos, with prednisolone

—conservative treatment of mild lagophthalmos

—referral of patients with severe lagophthalmos and/or exposure keratitis, unless there is sufficient expertise within the program

—recognition of the acute red eye and treatment of acute conjunctivitis

—referral of all other conditions of acute red eye, unless there is sufficient expertise within the program

—recognition of severe visual impairment and referral as needed

—recognition of the need for reading glasses in patients aged over 40 years, in rehabilitation services

—encouraging medical colleges, Control of Blindness Societies, and staff of general eye care facilities, to actively take part in

the treatment of eye complications in patients affected by leprosy

—encouraging charitable organizations to provide special eye care programs for patients affected by leprosy, in particular for those who are disabled and are living in leprosy settlements.

Eye care services (a visiting ophthalmologist or paramedical ophthalmic assistant to the specialized leprosy centers for consultation is an appropriate alternative and may sometimes be even more feasible) should take the responsibility for:

—eyelid surgery in patients with large lid gaps (>6 mm) or signs of exposure keratitis

—treatment and follow up of acute iritis, corneal ulcers, foreign bodies, and other causes of "the acute red eye" in cooperation with the leprosy service or PHC staff.

—the eye care services should offer "positive discrimination" in the treatment of cataract-blind leprosy patients, realizing the great difficulties that these patients have in avoiding injuries or taking care of injuries once they have occurred, especially in the case of limbs that have lost protective sensation.—From the article

Jiang, J. and Wei, X. Y. The current situation of lagophthalmos and keratopathy of leprosy in PR China. Indian J. Lepr. **70** (1998) 71–77.

It is estimated that there might be 45,000 cases with lagophthalmos and 70,000 cases with keratopathy among the 300,000 leprosy-affected persons still alive in China. Restoring or preserving eyesight in such a big leprosy population is a great challenge.—From the article

Job, C. K., Ebenezer, G. J., Thompson, K. and Daniel, E. Pathology of eye in leprosy. Indian J. Lepr. **70** (1998) 79–91.

The eye is involved in all forms of leprosy, more in lepromatous than in tuberculoid spectrum. With the advent of adequate antileprosy therapy beginning with the introduction of dapsone and enhanced by multidrug therapy, and with the effective implementation of leprosy eradication programs sponsored by the World Health Organization, all eye complications are significantly and unquestionably reduced. Surgical correction of deformities involving the eye has greatly helped in ameliorating the sufferings of many who had been already affected seriously. Considering the seriousness of eye complications, repeated and careful examination of the eye especially of those with lepromatous leprosy and those with nerve involvement affecting the eye cannot be overemphasized.

It has been said that *M. leprae* can survive in the iris and ciliary body long after skin lesions have become negative which may be due to insufficient therapy. The indolent nature of eye lesions may require life-long surveillance and therapeutic intervention. Therefore, training of frontline personnel and ophthalmologists in endemic countries to recognize and manage eye complications of leprosy is imperative. Let us look forward to the day when leprosy changes in the eye will only be considered pathologic curiosities.—Authors' Conclusion

Knuuttila, J. P., van Brakel, W. H. and Anderson, A. M. Ocular impairments in an impairment survey of leprosy-affected persons in Nepal. Indian J. Lepr. **70** (1998) 93–96.

An impairment survey was carried out in Nepal. The study subjects (N = 318) were a mixture of outpatients and patients admitted less than 1 month before the survey. Of the subjects, 101 were attending the hospital outpatients' clinic or were admitted and the rest were examined in the field. The patients studied included those on MDT and care-after-cure cases. Ocular impairments were found in 25% of these cases. The most common ocular impairment was poor vision followed by lagophthalmos and insensitive cornea.—Authors' Abstract

Orefice, F., Miranda, D. and Boratto, L. M. Presence of *M. leprae* in the conjunctiva, vitreous body and retina of a patient having lepromatous leprosy. Indian J. Lepr. **70** (1998) 97–102.

Histopathological study of the ocular globe of a lepromatous leprosy patient re-

vealed the presence of lepra bacilli in the conjunctiva, sclera, episclera, cornea, iris, ciliary body, vitreous body and retina.—Authors' Abstract

Passerotti, S., Salotti, R. A. and Vieth, H. Assessment and treatment of the dry eye in leprosy. Indian J. Lepr. **70** (1998) 103–108.

During the period of existence of the Ophthalmologic Prevention Center we have come across a large number of patients with corneal dehydration (dryness cornea, dry eye) due to various causes. We find that a majority of the patients had a big improvement in their symptoms and the signs of the dry eye with just a simple prevention technique.—Authors' Abstract

Rajan, M. A. Longitudinal follow-up of eyes in leprosy. Indian J. Lepr. **70** (1998) 109–114.

Early detection of leprosy with adequate management of the disease and reactions will prevent eye complications and ocular morbidity. Testing of corneal sensation in all cases of lagophthalmos and routine eye examination is mandatory as most of the eye complications are insidious in their onset and progress. Intraocular surgery and intraocular implants can be done safely. Early cataract surgery should be done to prevent the complications of hypermaturity which could lead to permanent blindness.—Author's Conclusion

Sundar Rao, P. S. S., Daniel, E., Kurian, N. and Gayathri, K. J. Epidemiological aspects of ocular morbidity in leprosy-affected persons. Indian J. Lepr. **70** (1998) 115–122.

Preliminary data on the incidence of ocular morbidity during multidrug therapy (MDT) in terms of existing morbidity at start of MDT (baseline) and the proportional contribution of various conditions to ocular complications while on MDT.

About half the patients had no further eye complications. Another 20% had only some degradation of corneal sensation. Thus, nearly 35% had some continuing ocular morbidity even while on MDT.

It is obvious that ocular morbidity is quite significant in leprosy-affected persons. These patients are still being followed up, to provide incidence rates by age and as well as relating to duration of the disease and treatment. More such studies would be required not only to assess the impact of the disease but also for the identification of risk factors. There is now also a great need to embark on case control as well as intervention studies to formulate strategies to prevent or at least delay ocular morbidity.—From the article

Thompson, K. Lid surgery to reduce discomfort produces an unexpected improvement in visual acuity—a case presentation. Indian J. Lepr. **70** (1998) 123–125.

Surgical correction of lagophthalmos and ectropion supplemented by ocular lubricants (and vitamin A if necessary) may significantly improve visual acuity even where corneal and conjunctival exposure damage is severe and long standing. It is necessary to distinguish as far as possible the relative extent of active, inflammatory corneal opacity and inactive, irreversible scarring, and in addition to permanent surgical measures (lateral tarsorrhaphy) to consider temporary central tarsorrhaphy if a significant inflammatory element is suspected.—Author's Conclusion

Thompson, K. Ocular morbidity in a sample of 150 treated leprosy patients. Indian J. Lepr. **70** (1998) 127–130.

A preliminary study carried out as part of a larger screening program among treated leprosy patients revealed that about 75% of the eyes examined had grade 1 or grade 2 ocular disability according to WHO grading. There needs to be a concerted effort at this present time to ensure that prevention of ocular morbidity is given its rightful priority by all leprosy health personnel, and that adequate resources are allocated to ocular aspects for training, prevention of dis-

ability activities, and for treatment of eye complications in leprosy.—Authors' Conclusion

Waddell, K. The challenge: organizing services to prevent blindness in leprosy patients. Indian J. Lepr. **70** (1998) 131–137.

Blindness from leprosy should be avoidable with well-organized services allowing prompt treatment of complications close to where the patients live. Unfortunately, this is not happening in many places. To allow full coverage of all in need, paramedical eye workers must be the foundation of these services. The main challenge is organization and training. In summary: firstly, eye care must be integrated into leprosy care programs and secondly, these programs must be integrated into the regional eye care/blindness prevention programs. The leprosy community is making progress with the first part (though there is still a long way to go), but it should now add its input to the second or else eye care for leprosy patients will not be sustainable.—Author's Conclusion

Wang, J., *et al.* [Analysis of 108 aged cases of leprosy.] China Lepr. J. **14** (1998) 22–23. (in Chinese)

In the period of 1950 to 1994, there were 108 leprosy patients with age of over 60 years at diagnosis, being 3.9% of all the patients in Dongtai, Jiangsu. In general, these aged patients all had shorter disease duration, more skin lesions and damaged nerves with less lepra reaction and more severe disability at diagnosis, but relapse was less after the cure among them.—Authors' English Abstract

Wu, Y., *et al.* [Histopathological feature of type 2 reaction in leprosy.] China Lepr. J. **14** (1998) 29–31. (in Chinese)

Histopathological observation of the skin lesions in 18 cases of leprosy with reversal reaction showed that there were epidermal edema, lymphectasia in the dermis and decrease of BI among all of them while edema inside and outside leprosy granuloma having loose structure, fibroblastic hyperplasia, lymphocytosis and granulomatous transformation can be seen in some cases. The authors consider the reversal reaction as a hypersensitivity indeed.—Authors' English Abstract

Xu, M. [Comprehensive control of plantar ulcers in leprosy.] China Lepr. J. **14** (1998) 26–27. (in Chinese)

Protective footwear was given to 80 leprosy patients under 60 years old, in whom plantar ulcers of 24 patients had healed within 24 months. Self-care will be successful for controlling plantar ulcer if a habit of doing it is formed, on the basis of a three-year observation. The author emphasized the importance of surgical intervention for healing the ulcers.—Author's English Abstract

Yang, J., Jian, D., Hu, L., Liu, J. and Wang, K. Blindness and low vision in leprosy patients in Sichuan Province, China. Indian J. Lepr. **70** (1998) 139–143.

The acuity of vision of 2145 leprosy patients was examined. Twenty-six patients had bilateral blindness and 80 had diminution of vision bilaterally, according to WHO's standard. The vision disability rate was 4.94%. In addition, 136 patients (6.34%) had blindness or low vision involving one eye. The causes of blindness and low vision were leukoma and corneal ulcer.—Authors' Abstract

Yao, J. and Qian, J. Present situation of eye care and research in leprosy in China. Indian J. Lepr. **70** (1998) 149–151.

Ocular complications in leprosy often develop insidiously and with few, if any, symptoms. They are frequently overlooked and neglected with the consequences of serious problems developing later that may cause loss of sight. Therefore, more and more leprosy-cured persons have eye problems now in China.

There are about 300,000 living leprosy-affected persons in China, and of them about 190,000 will be having eye problems. The number of persons with blindness due

to complications of leprosy are more than 40,000.—From the article

Yao, J. and Qian, J. Surgical treatment of secondary cataract in leprosy. Indian J. Lepr. **70** (1998) 145–148.

Eye problems of leprosy affect around two million people worldwide and cause blindness in approximately 250,000. Damage to the uveal tract and the complications thereof are the main causes of blindness in leprosy, and it was reported that 42.57% of vision loss in leprosy-affected persons was due to cataract secondary to uveitis. Surgical treatment may be considered for some of these problems in an effort to improve their vision. This paper retrospectively reviews the results of cataract surgery in 18 such cases.

Although it is more difficult to do artificial lens implantation for secondary cataract in leprosy, and more complications will occur, we believe good results can be obtained if cases are carefully selected and the operations are performed by experienced ophthalmologists.—From the article

Zhou, L., *et al.* [Effect of prednisone on silent neuritis in leprosy.] China Lepr. J. **14** (1998) 24.

From July 1990 to October 1993, 24 cases of silent neuritis from 296 leprosy patients, including 129 active cases and 147 cases who completed MDT, were found during regular follow up yearly. They were given prednisone according to a standard regimen for 6 months. The result showed that 60 nerves in 22 patients have recovered wholly or partly. Only one case had slight maldigestion as a side effect.—Authors' English Abstract

Immuno-Pathology

Altare, F., Durandy, A., Lammas, D., Emile, J. F., Lamhamedi, S., LeDeist, D., Drysdale, P., Jouanguy, E., Doffinger, R., *et al.* Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science **280** (1998) 1432–1435.

In humans, interferon gamma (IFN-γ) receptor deficiency leads to a predisposition to mycobacterial infections and impairs the formation of mature granulomas. Interleukin-12 (IL-12) receptor deficiency was found in otherwise healthy individuals with mycobacterial infections. Mature granulomas were seen, surrounded by T cells and centered with epithelioid and multinucleated giant cells, yet reduced IFN-γ concentrations were found to be secreted by activated natural killer and T cells. Thus, IL-12-dependent IFN-γ secretion in humans seems essential in the control of mycobacterial infections, despite the formation of mature granulomas due to IL-12-independent IFN-γ secretion.—Authors' Abstract

Batoni, G., Esin, S., Harris, R. A., Kallenius, G., Svenson, S. B., Andersson, R., Campa, M. and Wigzell, H. gamma delta+ and CD4+ alpha beta+ human T cell subset responses upon stimulation with various *Mycobacterium tuberculosis* soluble extracts. Clin. Exp. Immunol. **11** (1998) 52–62.

By using a flow cytometric technique which allows direct identification of proliferating cells within mixed cell populations, we have previously described that soluble extracts obtained from *Mycobacterium tuberculosis* or *M. avium* represent strong stimuli for human gamma delta+ T cells. In the present study, we demonstrate that the protocol used for the preparation of *M. tuberculosis* soluble extracts may have an impact on their gamma delta+ T-cell stimulatory capacity. In agreement with our previous data, soluble extracts prepared from bacteria killed at 85°C and directly disrupted by prolonged sonication (TBe), elicited a strong proliferation of gamma

delta+ T cells after 6–7 days of stimulation. In contrast, when soluble extracts were obtained from bacteria autoclaved (121°C, 25 min) and then washed by centrifugation, a predominant proportion of CD4+ alpha beta+ T cells was achieved in the responding population. The stimulatory activity for gamma delta+ T cells was recovered in the supernatant of the autoclaved bacteria, indicating that autoclaving of *M. tuberculosis* bacilli releases an antigen(s) into the supernatant which stimulates human gamma delta+ T cells. While protease digestion of TBe only partially reduced its stimulatory capacity on gamma delta+ T cells, the stimulatory component(s) released into the supernatant after autoclavation of bacilli was found to be sensitive to protease digestion. Interestingly, in contrast to the preponderant proportion of gamma delta+ T cells induced in the responding population by unfractionated TBe, when the extract was fractionated by fast performance liquid chromatography (FPLC), most of the fractions exhibited a strong stimulatory capacity on CD4+ alpha beta+ T cells only. The gamma delta+ T-cell stimulatory activity was confined to the low molecular weight range FPLC fractions. Such results may suggest a possible regulatory role of gamma delta+ T cells on CD4+ alpha beta+ T cells.—Authors' Abstract

Britton, W. J., Meadows, N., Rathjen, D. A., Roach, D. R. and Briscoe, H. A tumor necrosis factor mimetic peptide activates a murine macrophage cell line to inhibit mycobacterial growth in a nitric oxide-dependent fashion. Infect. Immun. **66** (1998) 2122–2127.

The control of mycobacterial infections depends on the cytokine-mediated activation of mononuclear phagocytes to inhibit the growth of intracellular mycobacteria. Optimal activation requires the presence of T-cell-derived gamma interferon (IFN-γ) and other signals, including tumor necrosis factor (TNF). Recently, an 11-mer peptide based on amino acids 70 to 80 of the human TNF sequence, TNF(70–80), was found to have TNF mimetic properties, which include the activation of human and mouse neutrophils to kill *Plasmodia* spp. Therefore, we investigated the capacity of TNF(70–80) to activate the murine macrophage cell line RAW264.7 infected with the vaccine strain *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). When RAW264.7 cells were pretreated with human TNF or TNF(70–80) in the presence of IFN-γ, there was a dose-dependent reduction in the replication of BCG as measured by the uptake of H-3-labeled uracil and a concomitant release of nitric oxide as measured by the nitrite in the culture supernatants. TNF- or TNF(70–80)-induced macrophage activation was dependent on IFN-γ and was inhibited by neutralizing monoclonal antibody to human TNF and by anti-TNF-γ antisera. Both nitrite release and BCG growth inhibition were abrogated by competitive inhibitors of L-arginine, which blocked the activation of inducible nitric oxide synthase. A soluble form of the Type 1 TNF receptor blocked the activation of BCG-infected macrophages by human TNF and TNF(70–80), demonstrating that the effect of TNF(70–80) is dependent on signaling through TNF receptor I. The mimetic effects of TNF(70–80) on macrophage activation *in vitro* suggest that treatment with TNF(70–80) may modulate mycobacterial infections *in vivo*.—Authors' Abstract

de Jong, R., Altare, F., Haagen, I. A., Elferink, D. G., de Boer, T., Vriesman, P. J. C. V., Kabel, P. J., Draaisma, J. M. T., van Dissel, J. T., Kroon, F. P., Casanova, J. L. and Ottenhoff, T. H. M. Severe mycobacterial and *Salmonella* infections in interleukin-12 receptor-deficient patients. Science **280** (1998) 1435–1438.

Interleukin-12 (IL-12) is a cytokine that promotes cell-mediated immunity to intracellular pathogens by inducing type 1 helper T cell (Th1) responses and interferon-gamma (INF-γ) production. IL-12 binds to high-affinity beta 1/beta 2 heterodimeric IL-12 receptor (IL-12R) complexes on T cells and natural killer cells. Three unrelated individuals with severe, idiopathic mycobacterial and *Salmonella* infections were found to lack IL-12R beta 1 chain expression. Their cells were deficient

in IL-12R signaling and INF-γ production, and their remaining T-cell responses were independent of endogenous IL-12. IL-12R beta 1 sequence analysis revealed genetic mutations that resulted in premature stop codons in the extracellular domain. The lack of IL-12R beta 1 expression results in a human immunodeficiency and shows the essential role of IL-12 in resistance to infections due to intracellular bacteria.—Authors' Abstract

Gao, J., *et al.* [TNF-α and SIL-2R in the sera of MB leprosy patients.] China Lepr. J. **14** (1998) 10–12. (in Chinese)

In Lanxi and Tongxiang, Zhejiang, SIL-2R and TNF-α in the sera of 10 MDT-treated cases and 18 persons cured of MB leprosy were tested, with 19 local health residents as controls. The results showed that the levels of SIL-2R and TNF-α were raised in the MB cases and significantly correlated ($r = 0.667$, $t = 2.536$, $p<0.025$), being higher than those of the cures and residents ($p<0.001$). SIL-2R in LL cases and TNF-α in relapsed MB were the highest, and those in the cures were significantly lower than in the residents ($p<0.001$). It was indicated that there are abnormalities in regulation of SIL-2R and TNF-α and inhibition of immune function among patients with MB leprosy.—Authors' English Abstract

Hess, J., Miko, D., Catic, A., Lehmensiek, V., Russell, D. G. and Kaufmann, S. H. E. *Mycobacterium bovis* bacille Calmette-Guerin strains secreting listeriolysin of *Listeria monocytogenes.* Proc. Natl. Acad. Sci. U.S.A. **95** (1998) 5299–5304.

Recombinant (r) *Mycobacterium bovis* strains were constructed that secrete biologically active listeriolysin (Hly) fusion protein of *Listeria monocytogenes.* The r-BCG strains pAT261:Hly or pMV306:Hly expressed plasmid multicopies or chromosomal single copies of the hly gene, respectively. Human and murine macrophage-like cell lines were infected with r-BCG pAT261:Hly and pMV306:Hly strains. Interestingly, intracellular persistence of both r-BCG strains was reduced in macrophages as compared with the parental BCG strain. By immunogold labeling Hly was detected in membrane structures and within the phagosomal space of macrophages. In addition, Hly was localized within cytoplasmic vacuoles outside the mycobacteria-containing phagosome of host cells infected with r-BCG pAT261:Hly or r-BCG pMV306:Hly. Hly fusions consistently colocalized with a lysosome-associated membrane glycoprotein, suggesting that membrane-attack conformation of Hly was not altered. Although r-BCG pAT261:Hly and r-BCG pMV306:Hly microorganisms apparently did not egress into the cytoplasmic compartment of host cells, they both improved major histocompatibility complex class I presentation of cophagocytosed soluble protein as compared with wild-type BCG microbes. These data suggest the Hly secretion endows BCG with an improved capacity to stimulate CD8 T cells. Because CD8 T cells play a major role in protection against tuberculosis such Hly secreting r-BCG constructs are antituberculosis vaccine candidates.—Authors' Abstract

Machado, P., Abrams, J., Santos, S., Brennan, P., Barral, A. and Barral-Netto, M. Production of host-protective (IFN gamma), host-impairing (IL-10, IL-13) and inflammatory (TNF alpha) cytokines by PBMC from leprosy patients stimulated with mycobacterial antigens. Eur. J. Dermatol. **9** (1998) 98–103.

The production of IFN-gamma, IL-10, IL-13 and TNF-alpha was determine using PBMC from 7 tuberculoid (TT) and 7 lepromatous leprosy (LL) patients, after stimulation with several mycobacterial antigens, in an attempt to characterize the cytokine responses to these antigens. The results showed that TT patients displayed higher IFN-gamma levels than LL patients with the mycobacterial antigens tested, but no differences in IL-10 production were observed between the two groups. MLSC antigen was associated with the lowest IFN-gamma production in TT and LL groups. Only BCG could be identified with stimulation of IFN-gamma production in some LL

patients. The mycobacterial antigens SP+, SP– and BCG were associated with higher TNF-alpha production in patients and controls, suggesting that these antigens could be involved in immunopathological effects. Our findings showed that the antigens tested were associated with a heterogeneous cytokine production in leprosy patients. Further studies are required to establish if an individual antigen can be identified as inducing a protective immune response in leprosy.—Authors' Abstract

Manca, F., Valle, M. T., Megiovanni, A., Pira, G. L., Fenoglio, D., Kunkl, A., Merlo, A., Terranova, P., Bottone, L., Balbi, B., Lantero, S. and Rossi, G. A. Requirement for different presenting cells and for different processing mechanisms by human CD4 T helper clones specific for *M. tuberculosis* antigens. Hum. Immunol. **59** (1998) 265–274.

Human T-helper cells specific for mycobacterial antigens have been extensively investigated. Differences have been detected according to antigen specificity and to fine epitope specificity. In this work we have analyzed two additional parameters that allow discrimination among antigen-specific T-helper cells: requirement for certain types of antigen-presenting cells (APC) and requirement for protease-sensitive antigen-processing pathways. We used T-cell clones from peripheral blood or from pleural exudates, and specific for different antigenic fractions of *M. tuberculosis.* APC were autologous peripheral blood mononuclear cells, adherent monocytes, adherent pleural monocytes, EBV transformed B lymphocytes and dendritic cells. Seven clones out of 12 were stimulated by all APC irrespective of their specificity, whereas other clones had more selective requirements. When protease inhibitors were used during antigen pulsing of APC, the production of certain epitopes, and thus T-cell activation, was impaired with six clones out of 16. These results demonstrate that the human T-helper repertoire specific for mycobacterial antigens is highly diverse also according to APC populations needed for presentation and to processing mechanisms required for production of the relevant T epitopes.—Authors' Abstract

Matsumoto, K., Yajima, M. and Asano, G. [Pathological findings of the liver in Hansen's disease.] Jpn. J. Lepr. **66** (1997) 97–102. (in Japanese)

Histopathological lesions in the liver of leprosy patients from Japan who had been treated long term were reviewed based on autopsy findings of 31 cases, including 24 cases with lepromatous leprosy (LL) and 7 cases with tuberculoid leprosy (TL). Except one case of LL in the progressive stage, most of the cases were diagnosed as retrogressive or quiescent stage. Morphologically, acid-fast bacilli and granuloma were seen in a few cases. No changes suggestive of drug- and virus-induced liver injury were recognized. However, sclerotic changes to the portal vein were encountered in tuberculoid leprosy.—Authors' English Abstract

Matsuo, E., Furuno, Y., Komatsu, A., Maekawa, S., Murata, K., Sidik, H., Kikuchi, T. and Sasaki, N. [Hansen's disease and nephropathy as its sequence.] Jpn. J. Lepr. **66** (1997) 106–108. (in Japanese)

Nephropathy as a consequence of leprosy before and after the introduction of chemotherapy was compared using a report from 1943 and autopsy reports from 1978 to 1981 from the Japanese National Hansen's disease hospital Zensein. The death rate from nephritis (including arteriolitis, glomerulonephritis, and interstitial nephritis) in 1943 was 21.2% and in 1979–1981, the rate of glomerulonephritis was 37.3% (although not necessarily listed as a cause of death). It was concluded that the rate of nephritis did not seem to have decreased after the introduction of chemotherapy.—Authors' English Abstract

Sato, K., Akaki, T. and Tomioka, H. Differential potentiation of antimycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal

macrophages activated by interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha). Clin. Exp. Immunol. **112** (1998) 63–68.

The anti-mycobacterial activities of IFN-gamma and TNF-alpha-treated murine peritoneal macrophages were determined. Resident macrophages pretreated with IFN-gamma or TNF-alpha for 2 days were infected with test organisms and subsequently cultured for up to 7 days. First, the early-phase growth of *Mycobacterium tuberculosis* (days 0–3) was strongly suppressed in IFN-gamma-treated macrophages, and progressive bacterial elimination was subsequently observed. Although TNF-alpha treatment of macrophages did not affect the early-phase growth of organisms, bacterial killing was observed in the later phase of cultivation. Second, although IFN-gamma-treated macrophages killed *M. avium* during the first 3 days of culture, regrowth of the intracellular organisms was subsequently observed. TNF-alpha treatment of macrophages did not influence the mode of intracellular growth of *M. avium.* Third, IFN-gamma but not TNF-alpha enhanced production of reactive nitrogen intermediates (RNI) by macrophages infected with *M. tuberculosis* or *M. avium,* whereas both cytokines increased macrophage release of reactive oxygen intermediates (ROI). The present findings therefore show that IFN-gamma and TNF-alpha potentiated the anti-mycobacterial activity of murine peritoneal macrophages in different fashions. They also suggest that RNI played more important roles than did ROI in the expression of macrophage anti-mycobacterial, particularly anti-*M. avium,* activity.—Authors' Abstract

Schoel, B. and Kaufmann, S. H. E. Influence of mycobacterial virulence and culture condition on gamma delta T cell activation. Microb. Pathogen. **24** (1998) 197–201.

Activation of human gamma delta T cells by culture supernatants of virulent and avirulent mycobacteria was examined. The stimulatory potential of mycobacteria was influenced by the type of culture media and independent from their virulence. Activation of gamma delta T cells by phagocytes infected with viable virulent *Mycobacterium tuberculosis* H37Rv and avirulent *M. bovis* BCG was comparable. We conclude that gamma delta T cell stimulation occurs in response to infection with mycobacteria independent from their virulence.—Authors' Abstract

Scollard, D. M., Gillis, T. P. and Williams, D. L. Polymerase chain reaction assay for the detection and identification of *Mycobacterium leprae* in patients in the United States. Am. J. Clin. Pathol. **109** (1998) 642–646.

The differentiation of leprosy from other cutaneous granulomatous diseases is routinely based on characteristic histopathologic features and the demonstration of *Mycobacterium leprae* by acid-fast staining. Increased ascertainment of other mycobacterial infections in the skin has made this task more difficult, but the distinction remains fundamental for the selection of appropriate treatment. Experience with formalin-fixed, paraffin-embedded tissues, frozen tissues, and tissue lysates referred for detection of *M. leprae* DNA by a polymerase chain reaction (PCR) assay during the past 4 years was reviewed. This assay was done by using primers and probes previously developed in our laboratory to amplify a 360-base-pair fragment of the gene for an 18-kD protein of *M. leprae.* Among biopsy samples obtained from 37 patients, PCR results were positive for 10 of 20 samples diagnosed as leprosy by histopathologic criteria and in 0 of 17 not diagnosed as leprosy. The specificity of the assay was 100% in this clinical referral material; sensitivity ranged from 50% to 83%. The PCR assay also identified *M. leprae* in one third of samples in which acid-fast organisms were seen and the histopathologic features were consistent with but not definitive of leprosy. In a nonendemic population, the sensitivity and specificity of PCR assay recommend its use primarily to identify *M. leprae* when acid-fast organisms are discernible but atypical clinical or histopathologic features obscure

the diagnosis. The assay is not highly informative when acid-fast bacilli are not detectable by light microscopy.—Authors' Abstract

Seitzer, U., Scheel Toellner, D., Mattern, T., Haas, H., Flad, H. D. and Gerdes, J. Staining pattern of seven monoclonal anti-CD26 antibodies in leprosy: implications for the use of CD26 as a surrogate marker of a human Th1-like reaction. Virchow Arch. **432** (1998) 343–347.

In a previous study using the monoclonal anti-CD26 antibody MIB-DS2/7 in leprosy and other granulomatous diseases, it was shown that CD26 may be a candidate for use as an operational marker of a human Th1-like reaction. In this follow-up study, we compared seven different monoclonal anti-CD26 antibodies with respect to their staining pattern in lepromatous and tuberculoid leprosy tissues. Three distinct staining patterns became apparent in this anti-CD26 antibody panel: staining of T lymphocytes and of connective tissue; staining of T lymphocytes, connective tissue and macrophages; and almost no staining of T lymphocytes but staining of connective tissue and macrophages. The two antibodies assigned to the first staining pattern, including MIB-DS2/7, were found to be most suitable for the operational discrimination between Th1-like and Th2-like reactions in leprosy. The antibodies assigned to staining patterns 2 and 3 did not allow this discrimination. Although all seven monoclonal antibodies investigated were specific for CD26, only two were found to be useful in identifying a Th1-like immune reaction in human tissue.—Authors' Abstract

Siew, L. K., Beech, J. T., Thompson, S. J. and Elson, C. J. Effect of T-helper cytokine environment on specificity of T-cell responses to mycobacterial 65,000 MW heat-killed shock protein. Immunology **93** (1998) 493–497.

The purpose of this work was to determine if the fine specificity of T cells differed between mice immunized with an antigen in a T-helper 1 (Th1) cytokine-dominated environment as compared with a T-helper 2 (Th2) cytokine-dominated environment. It was found that splenic T cells protein (hsp 65) and interleukin-12 (IL-12) produced less interleukin-4 (IL-4) and more interferon-gamma (IFN-γ) in response to stimulation with hsp 65 *in vitro* than did T cells from mice immunized with hsp 65 alone. The T-cell proliferative response to hsp 65 did not differ between the two groups of mice, although the responses were higher than those of T cells from non-immunized mice. Strikingly, T cells from mice given hsp 65 and IL-12 gave significantly higher responses to six peptides (corresponding to the sequence of hsp 65) to which T cells from mice immunized with hsp 65 alone did not respond. It is considered that different epitopes are presented to T cells (possibly owing to changes in antigen processing) if the environment is shifted, by IL-12, from Th2, toward Th1 cytokines.—Authors' Abstract

Suneetha, L. M., Satish, P. R., Korula, R. J., Suneetha, S. K., Job, C. K. and Balasubramanian, A. S. *Mycobacterium leprae* binds to a 25-kDa phosphorylated glycoprotein of human peripheral nerve. Neurochem. Res. **23** (1998) 907–911.

Mycobacterium leprae, the causative agent of leprosy, specifically invades and destroys the peripheral nerve, which results in the main clinical manifestation of the disease. Little is known about the bacteria-nerve protein interaction. We show in the present work that *M. leprae* binds to a 25-kDa glycoprotein from human peripheral nerve. This protein is phosphorylatable and it binds to lectins which have alpha-mannose specificity.

This *M. leprae*-protein interaction could be of importance in the pathogenesis of leprosy.—Authors' Abstract

Young, S. P., Epstein, E. and Potter, V. Determinant capture by MHC class II DR3 during processing of *Mycobacterium leprae* 65 kDa heat shock protein by human B cells. Hum. Immunol. **59** (1998) 259–264.

Using T-cell immunoblotting we have characterized the immunogenic fragments derived from the *Mycobacterium leprae* 65-kDa heat shock protein that become associated with MHC class II DR3 during processing by a human B-cell line. After 5 hr incubation with antigen, a peptide of approximately 12-kDa (similar to 110 amino acids) was the only major fragment found associated with the class II MHC. The association of this oligopeptide was abolished if an excess of a synthetic peptide representing the minimal epitope was included in the culture or when cells were incubated at 4°C. This suggests that the generation of this moiety is dependent on cell metabolism and that its binding to MHC is specific. This large fragment may represent an intermediate in the processing pathway, directly demonstrating the role of MHC in determinant capture during antigen degradation.—Authors' Abstract

Microbiology

Bashyam, M. D. and Tyagi, A. K. Identification and analysis of "extended-10" promoters from mycobacteria. J. Bacteriol. **180** (1998) 2568–2573.

Earlier studies from our laboratory on randomly isolated transcriptional signals of mycobacteria had revealed that the –10 region of mycobacterial promoters and the corresponding binding domain in the major sigma factor are highly similar to their *Escherichia coli* counterparts. In contrast, the sequences in –35 regions of mycobacterial promoters and the corresponding binding domain in the major sigma factor are vastly different from their *E. coli* counterparts (M. D. Bashyam, D. Kaushal, S. K. Dasgupta, and A. E. Tyagi, J. Bacteriol. 178:4847–1853, 1996). We have now analyzed the role of the TGN motif present immediately upstream of the –10 region of mycobacterial promoters. Sequence analysis and site-specific mutagenesis of a *Mycobacterium tuberculosis* promoter and a *M. smegmatis* promoter reveal that the TGN motif is an important determinant of transcriptional strength in mycobacteria. We show that mutation in the TGN motif can drastically reduce the transcriptional strength of a mycobacterial promoter. The influence of the TGN motif on transcriptional strength is also modulated by the sequences in the –35 region. Comparative assessment of these extended –10 promoters in mycobacteria and *E. coli* suggests that functioning of the TGN motif in promoters of these two species is similar.—Authors' Abstract

Basu, D., Narayankumar, D. V., van Beeumen, J. and Basu, J. Characterization of a beta-lactamase from *Mycobacterium smegmatis* SN2. Biochem. Mol. Biol. Int. **43** (1997) 557–562.

Beta-lactamases have been reported to be largely responsible for beta-lactam resistance in *Mycobacteria.* We report the characterization of a cell-associated beta-lactamase from *Mycobacterium smegmatis.* The enzyme hydrolyzed the "beta-lactamase-stable" oximinocephalosporins. Nitrocefin was the best substrate. 6-Beta-iodopenicillanate, clavulanate and sulbactam were effective inhibitors; whereas the K_i value aztreonam was high. From its substrate and inhibitor, the enzyme appeared to be a cephalosporinase of group 2e.—Authors' Abstract

Brosch, R., Gordon, S. V., Billault, A., Garnier, T., Eiglmeier, K., Soravito, C., Barrell, B. G. and Cole, S. T. Use of a *Mycobacterium tuberculosis* H37Rv bacterial artificial chromosome library for genome mapping, sequencing, and comparative genomics. Infect. Immun. **66** (1998) 2221–2229.

The bacterial artificial chromosome (BAC) cloning system is capable of stably propagating large, complex DNA inserts in *Escherichia coli.* As part of the *Mycobacterium tuberculosis* H37Rv genome sequencing project, a BAC library was constructed in the pBeloBAC11 vector and used for genome mapping, confirmation of sequence

assembly, and sequencing. The library contains about 5000 BAC clones, with inserts ranging in size from 25 to 104 kb, representing theoretically a 70-fold coverage of the *M. tuberculosis* genome (4.4 Mb). A total of 840 sequences from the T7 and SP6 termini of 420 BACs were determined and compared to those of a partial genomic database. These sequences showed excellent correlation between the estimated sizes and positions of the BAC clones and the sizes and positions of previously sequenced cosmids and the resulting contigs. Many BAC clones represent linking clones between sequenced cosmids, allowing full coverage of the H37Rv chromosome, and they are now being shotgun sequenced in the framework of the H37Rv sequencing project. Also, no chimeric, deleted, or rearranged BAC clones were detected, which was of major importance for the correct mapping and assembly of the H37Rv sequence. The minimal overlapping set contains 68 unique BAC clones and spans the whole H37Rv chromosome with the exception of a single gap of ~150 kb. As a postgenomic application, the canonical BAC set was used in a comparative study to reveal chromosomal polymorphisms between *M. tuberculosis, M. bovis,* and *M. bovis* BCG Pasteur, and a novel 12.7-kb segment present in *M. tuberculosis* but absent from *M. bovis* and *M. bovis* BCG was characterized. This region contains a set of genes whose products show low similarity to proteins involved in polysaccharide biosynthesis. The H37Rv BAC library therefore provides us with a powerful tool both for the generation and confirmation of sequence data as well as for comparative genomics and other postgenomic applications. It represents a major resource for present and future *M. tuberculosis* research projects.—Authors' Abstract

Cilliers, F. J., Warren, R. M., Hauman, J. H., Wiid, I. J. F. and van Helden, P. D. Oligonucleotide $(GTG)_5$ as an epidemiological tool in the study of nontuberculous mycobacteria. J. Clin. Microbiol. **35** (1997) 1545–1549.

Analysis of restriction fragment length polymorphisms in the genome of *Mycobacterium tuberculosis* (DNA fingerprinting) has proved to be a useful epidemiological tool in the study of tuberculosis within populations or communities. However, to date no similar method has been developed to study the epidemiology of nontuberculous mycobacteria (NTM). In this communication, it is reported that a simple oligonucleotide repeat, $(GTG)_5$, can be used to genotype accurately all species and strains of NTM tested. It is suggested that this technology is an easily applied and accurate tool which can be used for the study of the epidemiology of NTM.—Authors' Abstract

Fitzmaurice, A. M. and Kolattukudy, P. E. An acyl-CoA synthase (acoas) gene adjacent to the mycocerosic acid synthase (mas) locus is necessary for mycocerosyl lipid synthesis in *Mycobacterium tuberculosis* var. *bovis* BCG. J. Biol. Chem. **273** (1998) 8033–8039.

An open reading frame, ORF3, first identified adjacent to the mycocerosic acid synthase gene in *Mycobacterium bovis* BCG encodes a protein with acyl-CoA synthase (ACoAS) activity. Genes homologous to acoas are found adjacent to other multifunctional polyketide synthase genes in the mycobacterial genome. To test whether these gene products are necessary to esterify the fatty acids generated by the adjacent polyketide synthase gene products, the acoas gene was disrupted in *M. bovis* BCG using a suicide vector containing the acoas gene with an internal deletion and the hygromycin-resistant gene as selection marker. Allelic exchange at the acoas locus was confirmed by Southern hybridization and polymerase chain reaction amplification of both flanking regions expected from homologous recombination. Immunoblot analysis indicated that the 65-kDa ACoAS protein product was absent in the mutant. Chromatographic analysis of lipids derived from [1-C-14]propionate showed that the mutant did not produce mycocerosyl lipids, although it produced normal levels of mycocerosic acid synthase. These results suggest that ACoAS is involved in the synthesis of mycocerosyl lipids of the mycobacterial cell wall.—Authors' Abstract

Klatser, P. R., Kuijper, S., van Ingen, C. W. and Kolk, A. H. J. Stabilized, freeze-dried PCR mix for detection of mycobacteria. J. Clin. Microbiol. **36** (1998) 1798–1800.

We report here the development of a freeze-drying procedure allowing stabilization at ambient temperature of preoptimized, premixed, and predispensed PCR mixes aimed at the detection of mycobacteria in clinical materials. The freeze-dried mixes retained activity at 4°C and at 20°C for 1 year and for 3 months at 37°C, as judged by their performance with 50 fg and 500 fg of purified *Mycobacterium bovis* BCG target DNA.—Authors' Abstract

Kurabachew, M., Wondimu, A. and Ryon, J. J. Reverse transcription-PCR detection of *Mycobacterium leprae* in clinical specimens. J. Clin. Microbiol. **36** (1998) 1352–1356.

A reverse transcription (RT)-PCR assay targeting the 16S rRNA of *Mycobacterium leprae* was developed to detect the organism in clinical specimens. A 171-bp fragment was amplified when *M. leprae* RNA was used as a template but not when a panel of RNAs from 28 potentially cross-reacting mycobacterial species, seven genera related to *Mycobacterium*, and three organisms normally found among skin or nose flora were tested. As few as 10 organisms isolated from infected tissue could be detected, confirming the sensitivity of the assay. When the test was applied to clinical specimens, *M. leprae* was detected in 82% of skin biopsy specimens obtained from untreated leprosy patients, while skin-biopsy specimens from healthy volunteers and patients with other dermatological disorders were negative. The sensitivity of the RT-PCR was higher than that of slit-skin smear staining for acid-fast bacilli or acid-fast staining of fixed biopsy specimens since 53% of acid-fast bacillus-negative biopsy specimens were RT-PCR positive. Because 16S rRNA is rapidly degraded upon cell death, the assay may detect only viable organisms and may prove to be useful in assessing the efficacy of chemotherapy.—Authors' Abstract

Lee, R. E., Brennan, P. J. and Besra, G. S. Synthesis of beta-D-arabinofuranosyl-1-monophosphoryl polyprenols: examination of their function as mycobacterial arabinosyl transferase donors. Bioorg. Med. Chem. Lett. **8** (1998) 951–954.

A convenient synthetic strategy has been developed to produce libraries of beta-D-arabinofuranosyl-monophosphorylpolyprenol. Those containing C-50 and C-55 polyprenols were the most active as donors for the cell-free synthesis of the arabinans of mycobacterial cell walls.—Authors' Abstract

Mahadevan, U. and Padmanaban, G. Cloning and expression of an acyl-CoA dehydrogenase from *Mycobacterium tuberculosis*. Biochem. Biophys. Res. Comm. **244** (1998) 893–897.

A gene from *Mycobacterium tuberculosis* coding for acyl-CoA dehydrogenase was cloned, overexpressed and characterized on the basis of enzyme activity with various chain length substrates. The results show that the protein is a medium chain acyl-CoA dehydrogenase (MCADH). The mycobacterium protein expressed appears to be unique since, by comparison, the active site glutamic acid of the protein does not lie in the same position as other well characterized MCADH, but in a position present in long chain and isovaleryl acyl-CoA dehydrogenases (LCADH and IVDH).—Authors' Abstract

Mdluli, K., Swanson, J., Fischer, E., Lee, R. E. and Barry, C. E., III. Mechanisms involved in the intrinsic isoniazid resistance of *Mycobacterium avium*. Mol. Microbiol. **27** (1998) 1223–1233.

Isoniazid (INH), which acts by inhibiting mycolic acid biosynthesis, is very potent against the tuberculous mycobacteria. It is about 100-fold less effective against *Mycobacterium avium*. This difference has often been attributed to a decreased permeability of the cell wall. We measured the rate of conversion of radiolabelled INH to 4-pyridylmethanol by whole cells and cell-free extracts and estimated the permeability

barrier imposed by the cell wall to INH influx in *M. tuberculosis* and *M. avium*. There was no significant difference in the relative permeability to INH between these two species. However, the total conversion rate in *M. tuberculosis* was found to be four times greater. Examination of *in vitro*-generated mutants revealed that the major resistance mechanism for both species is loss of the catalase-peroxidase KatG. Analysis of lipid and protein biosynthetic profiles demonstrated that the molecular target of activated INH was identical for both species. *M. avium*, however, formed colonies at INH concentrations inhibitory for mycolic acid biosynthesis. These mycolate-deficient *M. avium* exhibited altered colony morphologies, modified cell wall ultrastructure and were 10-fold more sensitive to treatment with hydrophobic antibiotics, such as rifampin. These findings may significantly impact the design of new therapeutic regimens for the treatment of infections with atypical mycobacteria.—Authors' Summary

Pelicic, V., Reyrat, J.-M. and Gicquel, B. Genetic advances for studying *Mycobacterium tuberculosis* pathogenicity. Mol. Microbiology **28** (1998) 413–420.

Tuberculosis remains the greatest cause of death worldwide because of a single pathogen. Despite its importance, the genetic basis of the pathogenicity of *Mycobacterium tuberculosis* remains poorly understood, mainly because the most productive investigative approach, molecular genetic analysis, has been severely hampered by a lack of efficient tools. However, significant advances, including the development of methods for inactivating genes and studying their expression with reporter genes, have been recently made. This progress may lead to opportunities for developing new vaccines and antituberculous drugs. The aim of this review is to examine the present state of the art in mycobacterial molecular genetics and pinpoint some expected or promising areas for future research.—Authors' Summary

Philipp, W. J., Schwartz, D. C., Telenti, A. and Cole, S. T. Mycobacterial genome structure—minireview. Electrophoresis **19** (1998) 573–576.

Genome maps have been constructed for the mycobacterial pathogens *Mycobacterium leprae* and *M. tuberculosis*, as well as for the attenuated vaccine strain *M. bovis* BCG Pasteur. While the chromosomes of *M. tuberculosis* and *M. bovis* BCG Pasteur show extensive conservation at the gross level, comparison with *M. leprae* revealed a high degree of diversification, with a mosaic-like pattern apparent. The ordered libraries of *M. tuberculosis* and *M. leprae* produced during the course of these studies played a central role in the genome sequencing projects of these two bacilli, showing the utility of this approach for systematic sequencing of bacterial genomes.—Authors' Abstract

Williams, D. L., Spring, L., Collins, L., Miller, L. P., Heifets, L. B., Gangadharam, P. R. J. and Gillis, T. P. Contribution of *rpoB* mutations to development of rifamycin cross-resistance in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **42** (1998) 1853–1857.

The contributions of 23 insertion, deletion, or missense mutations within an 81-bp fragment of *rpoB*, the gene encoding the β-subunit of the DNA-dependent RNA polymerase of *Mycobacterium tuberculosis*, to the development of resistance to rifamycins (rifampin, rifabutin, rifapentine, and KRM-1648) in 29 rifampin-resistant clinical isolates were defined. Specific mutant *rpoB* alleles led to the development of cross-resistance to all rifamycins tested, while a subset of mutations were associated with resistance to rifampin and rifapentine but not to KRM-1648 or rifabutin. To further study the impact of specific *rpoB* mutant alleles on the development of rifamycin resistance, mutations were incorporated into the *rpoB* gene of *M. tuberculosis* H37Rv, contained on a mycobacterial shuttle plasmid, by *in vitro* mutagenesis. Recombinant *M. tuberculosis* clones containing plasmids with specific mutations in either codon 531 or 526 of *rpoB* exhibited high-level resistance to all rifamycins tested, whereas clones containing a plasmid

with a mutation in codon 516 exhibited high-level resistance to rifampin and rifapentine but were susceptible to both rifabutin and KRM-1648. These results provided additional proof of the association of specific *rpoB* mutations with the development of rifamycin resistance and corroborate previous reports of the usefulness of *rpoB* genotyping for predicting rifamycin-resistant phenotypes.—Authors' Abstract

Wren, B. W., Stabler, R. A., Das, S. S., Butcher, P. D., Mangan, J. A., Clarke, J. D., Casali, N., Parish, T. and Stoker, N. G. Characterization of a haemolysin from *Mycobacterium tuberculosis* with homology to a virulence factor of *Serpulina hyodysenteriae*. Microbiology **144** (1998) 1205–1211.

Scrutiny of sequence data from the *Mycobacterium leprae* genome sequencing project identified the presence of a gene encoding a 268-amino-acid polypeptide which is highly similar to a pore-forming hemolysin/cytotoxin virulence determinant, TlyA, from the swine pathogen *Serpulina hyodysenteriae*. Using degenerate oligonucleotide primers based on the TlyA sequences, the *Mycobacterium tuberculosis* homolog was amplified and this product was used to obtain the clone and sequence a 2.5 kb fragment containing the whole *M. tuberculosis tlyA* gene. *tlyA* encodes a 267-amino-acid protein with a predicted molecular mass of 28 kDa. *tlyA* homologs were identified by PCR in *M. leprae, M. avium* and *M. bovis* BCG, but appeared absent in *M. smegmatis, M. vaccae, M. kansasii, M. chelonae* and *M. phlei*. The *M. tuberculosis* gene appeared to be the first gene in an operon containing at least two other genes. Introduction of the *M. tuberculosis tlyA* gene into *M. smegmatis* using a mycobacterial shuttle expression plasmid converted non-hemolytic cells into those exhibiting significant hemolytic activity. Similarly, inducible hemolytic activity was observed in sonicated bacteria when *tlyA* was expressed as a His_6-tagged fusion protein in *Escherichia coli*. *tlyA* mRNA was detected in both *M. tuberculosis* and *M. bovis* BCG using RT-PCR, confirming that this gene is expressed in organisms cultured *in vitro*.—Authors' Abstract

Experimental Infections

Gormus, B. J., Baskin, G. V., Xu, K., Bohm, R. P., Mack, P. A., Ratterree, M. S., Cho, S.-N., Meyers, W. M. and Walsh, G. P. Protective immunization of monkeys with BCG or BCG plus heat-killed *Mycobacterium leprae*: clinical results. Lepr. Rev. **69** (1998) 6–23.

Rhesus and sooty mangabey monkeys (RM and SMM) were vaccinated and boosted with BCG or BCG + low dose (LD) or high dose (HD) heat-killed *Mycobacterium leprae* (HKML). One group was not vaccinated. Except for a group of controls, all monkeys were challenged with live *M. leprae*. All animals were studied longitudinally to determine antileprosy protective efficacy. BCG reduced the numbers of RM with histopathologically diagnosed leprosy by 70% and slowed and ameliorated the appearance of symptoms. BCG + LDHKML reduced the number of RM with leprosy by 89% and BCG + HDHKML by 78%. BCG did not protect SMM from developing leprosy, but disease progress was slowed; disease in SMM was exacerbated by the addition of HKML to the vaccine. RM, as a species, are prone to paucibacillary (PB) forms of leprosy, whereas SMM are prone to multibacillary (MB) forms. Thus, BCG vaccination offers significant protection from clinical disease and slows/ameliorates the rate of progression/degree of disease at the PB end and appears to at least ameliorate symptoms at the MB end of the leprosy spectrum. BCG +

HKML protects at the PB end and exacerbates disease progress at the MB end of the leprosy spectrum.—Authors' Abstract

Gormus, B. J., Murphey-Corb, M., Martin, L. N., Baskin, G. B., Mack, P. A., Xu, K., Ratterree, M. S., Gerone, P. J., Scollard, D. M. and Gillis, T. P. Impaired responses to *Mycobacterium leprae* antigens in rhesus monkeys experimentally inoculated with simian immunodeficiency virus and *M. leprae*. Lepr. Rev. **69** (1998) 24–39.

Seven of eight rhesus monkeys (RM) coinfected with simian immunodeficiency virus (SIV) and *Mycobacterium leprae* harbored acid-fast bacilli (AFB) at sites of dermal inoculation and/or at disseminated sites at times of humane sacrifice (up to 270 days post-*M. leprae* inoculation) due to SIV-induced debilitation or, in one long-term survivor's case, to date over 3 years post-*M. leprae* inoculation. Detectable AFB were cleared in biopsies of inoculation sites of RM inoculated with *M. leprae* alone after 63 days postinoculation; these sites have, so far, remained AFB-negative, thereafter.

Compared to animals infected with *M. leprae* alone, RM coinfected with SIV plus *M. leprae* showed: 1) completely suppressed serum antibody responses to *M. leprae*-specific PGL-I antigen, but strong anti-SIV Gp120 antibody responses; 2) impaired sensitization of blood mononuclear cells (MNC) to *in vitro* recognition of *M. leprae*-specific antigens in blastogenic stimulation assays; 3) impaired *in vitro* responses of blood MNC to nonspecific (ConA) blastogenic stimuli; and 4) early post-*M. leprae* inoculation, there was a significant incremental diminution of percentages of blood CD4+CD29+T-cells in addition to the existing SIV-induced diminished percentages of CD4+CD29+T-cells.

The results indicate that humoral and cellular immune responses to *M. leprae* antigens are compromised in *M. leprae*-inoculated RM previously infected with SIV. These results provide an immunologic basis for the demonstration of enhanced *M. leprae* persistence or leprosy susceptibility in SIV-*M. leprae* coinfected RM.—Authors' Abstract

Wang, H., *et al.* [Effect of minocycline made in China on vitality of *M. leprae* in nude mice.] China Lepr. J. **14** (1998) 16–18. (in Chinese)

The activities of 0.02% and 0.04% China-made minocycline against *Mycobacterium leprae* in nude mice were evaluated by the kinetic method, and compared with those of 0.02% imported minocycline and 0.02% rifampin. The nude mice were treated for 105 days. The results revealed that the activity of China-made minocycline against *M. leprae* is similar to that of imported minocycline, and the larger the dosage of the drug the stronger the activity of the drug. During the early period of stopping treatment, 0.04% minocycline showed the best antileprosy effect with some bactericidal activity. However the activity of rifampin against *M. leprae* was more persistent and stable than that of minocycline. —Authors' English Abstract

Epidemiology and Prevention

Mo, S. [Monitoring for ten years after basic eradication of leprosy.] China Lepr. J. **14** (1998) 31–32. (in Chinese)

In Binyang county, in the south of Guangxi Province, with a population of 922,073, there were 316 leprosy patients to be registered accumulatively up to 1986, of which only four were active cases by the end of that year. Hereafter, ten-year serious surveillance showed that the prevalence and incidence did not go up.—Author's English Abstract

van den Broek, J., O'Donoghue, J., Ishengoma, A., Masao, H. and Mbega, M. Evaluation of a sustained 7-year health education campaign on leprosy in Rufiji District, Tanzania. Lepr. Rev. **69** (1998) 57–74.

To assess the impact of a 7-year intensive health education campaign about leprosy delivered by workers of the Kindwitwi Leprosy Trust to schoolchildren and general public in Rufiji District. Knowledge, attitude and beliefs toward leprosy were measured in Rufiji and compared to neighboring Kisarawe District as control. Lessons learned from this analysis may be useful for the planning and evaluating of health education campaigns.

Interviews were made of school children, general public, community leaders, traditional healers and medical staff in both districts.

A stratified randomized sampling scheme was used, with stratification for urban and rural settings. A representative sample of school children, general public, community leaders, traditional healers and medical staff in Rufiji District and in the control area of Kisarawe District were interviewed. The interviews were partly structured and partly open. The results of the interviews were analyzed in the context of epidemiological leprosy data from 1985 until 1995, and demographic data of both districts. Data entry and statistical analysis were done using FileMaker Pro, Stata and Excel computer packages.

We did not observe positive effects of the health education campaign on the indicators regarding early diagnosis of leprosy with less disability. Leprosy case detection was declining in both districts.

We found that the campaign had a favorable impact on the knowledge and the attitude of school children in Rufiji District. We could demonstrate a relationship between increased knowledge of leprosy and a positive, less stigmatizing attitude. Knowledge of leprosy was better in Rufiji as compared to Kisarawe, but only among school children. We found indications that low level of education, rural residence, older age, female gender and Moslem religion were associated with stigmatizing attitudes and beliefs toward leprosy. Knowledge about leprosy reactions among medical staff interviewed was not optimal.

The exact outcome of the sustained campaign in Rufiji District was difficult to assess because no comparison could be made with the situation prior to the campaign. However, the health education campaign was associated with increased knowledge and diminished tendency to stigmatize leprosy among school children. Health education campaigns have to be sustained and have to cover a broad sector of the society in order to induce behavioral changes in the community. The focus of health education should be rural communities and schools, and pay special attention to women, religious leaders and traditional healers. Awareness of diagnosis and treatment of leprosy reactions among medical staff should be improved.—Authors' Summary

Wen, Y., *et al.* [On correlation between health education and early case-finding in leprosy.] China Lepr. J. **14** (1998) 13–16. (in Chinese)

The results of a systematic health education were analyzed with stratified analysis and it was indicated that health education has raised the leprosy knowledge level in the local doctors and population, and made the patients see doctors earlier so as to get early diagnosis and treatment.—Authors' English Abstract

Wittenhorst, B., Vree, M. L., Ten Ham, P. B. G. and Velema, J. P. The National Leprosy Control Programme of Zimbabwe; a data analysis, 1983–1992. Lepr. Rev. **69** (1998) 46–56.

Prevalence and detection rates of leprosy in Zimbabwe as well as patient characteristics were reported by the National Leprosy Control Programme over the 10-year period 1983–1992. The control program made a new start in 1983 when multidrug therapy was introduced. Prevalence per 10,000 population declined steeply from 3.78 in 1983 to 0.52 in 1987. Prevalence continued to decline to 0.22 in 1992 and was highest in the northeastern provinces. After an initial increase, the detection rate per 10,000

had declined from 0.19 in 1985 to 0.08 in 1992. The proportion of refugees among new cases had gradually increased since 1988 and amounted to one third in 1991 and 1992.

An analysis of records of 802 cases who were newly detected from 1983 to 1992 showed that 51% were of the multibacillary (MB) type, 33% had visible disabilities at detection, 5% were under 15 years of age while the average delay time was 2.6 years. Patients with disabilities reported a longer delay time, were more often men and had more often the MB type of leprosy.

The data suggest that transmission of leprosy is low but that cases are not diagnosed early enough to prevent transmission altogether.—Authors' Summary

Rehabilitation

Shen, J., *et al.* [Efficacy of self-care for six years among 337 persons disabled by leprosy.] China Lepr. J. **14** (1998) 19–21. (in Chinese)

Three-hundred-thirty-seven leprosy patients have been taught to do self-care, and for 6 years exposure keratoconjunctivitis decreased from 66 eyes to 15 eyes, palmar rhagades and wounds lessened from 128 to 28 sites and from 4 to 0, and sole rhagades reduced from 93 to 3 sites and ulcers in the feet from 67 to 28, respectively. The authors pointed out that for long-term self-care, regular supervision and guidance and supply of needed materials are essential.—Authors' English Abstract

Wang, J., *et al.* [Surgical correction of clawhand in leprosy.] China Lepr. J. **14** (1998) 27–29. (in Chinese)

Surgical methods used and their effect on claw hand in 30 leprosy patients are reported. With dynamic method in 21 cases, the short-term effective rate was 90.5% and long-term rate was 85.7%. With static method in nine cases, the effective rates were 100% and 66.6%, respectively. The authors think that for better long-term effect on claw hand in leprosy the best should be dynamic operation.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entitities

Amara, R. R., Shanti, S. and Satchidanandam, V. Characterization of novel immunodominant antigens of *Mycobacterium tuberculosis.* Microbiology **144** (1998) 1197–1203.

Seven novel antigens of *Mycobacterium tuberculosis,* which had previously been identified based on reactivity to sera from patients with tuberculosis, were characterized. Nucleotide sequence analysis of the genes encoding these seven antigens identified one of them as the FtsH and a second as the aminoimidazole ribotide synthase of *M. tuberculosis.* Antisera raised to the recombinant forms of each of these seven antigens were used to study the distribution of these proteins within mycobacterial species as well as to determine their subcellular localization and hydrophobicity. Four of the seven antigens were conserved only among pathogenic strains of mycobacteria. Of the seven proteins studied, FtsH and a second protein of unknown identity were localized in membranes. Two were cytosolic, while two others, which had a high proline content, were tightly associated with the cell wall. One protein was secreted. This secreted protein could be identified by serum from a majority of tuberculosis patients but

not BCG-vaccinated individuals, suggesting its potential use in the immunodiagnosis of tuberculosis.—Authors' Abstract

Bonecini Almeida, M. G., Chitale, S., Boutsikakis, I., Geng, J. Y., Doo, H., He, S. H. and Ho, J. L. Induction of *in vitro* human macrophage anti-*Mycobacterium tuberculosis* activity: requirement for IFN-gamma and primed lymphocytes. J. Immunol. **160** (1998) 4490–4499.

Mycobacterium tuberculosis (Mtb) is the world's leading infectious cause of mortality. Despite the overwhelming data supporting the critical role of cellular immunity, little is known of the early microbial and immune cell interactions and whether human macrophages can be activated to express anti-Mtb activity. We report the reconstitution of an *in vitro* system whereby human macrophages express anti-Mtb activity only in coculture with PBL and with interferon gamma (IFN-γ). Omission of IFN-γ in the co-cultures or of Mtb lysate/ IFN-γ-primed lymphocytes was associated with high growth of Mtb, high IL-10 and IL-12 p40, nearly undetectable IL-12 p70 levels, and the highest percentages of CD4 and CD8 T cells. In contrast, IFN-γ treatment of co-cultures containing Mtb lysate/ IFN-γ-primed PBL reduced the bacilli count by ~2.5 log, decreased the production of IL-10 by 5.7-fold, increased IL-12 p70 by ~50-fold, and reduced the percentages of CD4 and CD8 cells. Activation of anti-Mtb activity was time and dose dependent. At 2000 U/ml of IFN-γ, bactericidal activity was achieved (10-fold reduction from initial inoculum). Anti-Mtb activity against several strains of *M. tuberculosis* (H37Ra and H37Rv, and C, a clinical isolate) was observed and was associated with expression of inducible nitric oxide synthase. These data suggest that induction of human macrophage anti-Mtb activity required dual signaling from PBL and IFN-γ. Thus, the development of an *in vitro* human system may greatly facilitate studies to delineate immune cells, cytokines, and effector functions/genes critical in controlling Mtb. Defining the mechanisms may also provide novel treatment strategies for tuberculosis. —Authors' Abstract

Brzychy, M., Zwolska, Z., Andrzejczyk, Z. and Rudnicka, W. Cellular reaction to *Mycobacterium avium* complex (MAC) clinical isolates differing in hemolytic activity and virulence for C57BL/6 mice. Microbiol. Immunol. **42** (1998) 357–363.

In this study we showed that *Mycobacterium avium* complex (MAC) clinical isolates differed by the expression of hemolytic activity. Two hemolytic MAC strains were less susceptible to the mycobactericidal effect of murine macrophages than two unhemolytic MAC isolates. *In vivo,* hemolytic MAC bacilli survived in the spleens of infected mice for a longer time than unhemolytic MAC strains. This suggested a role of hemolysins in the virulence of MAC strains. There was no difference in the cytotoxicity of T cells from mice immunized with *M. bovis* BCG toward macrophages infected *in vitro* with MAC strains expressing or not expressing hemolytic activity.—Authors' Abstract

Calder, K. M. and Horwitz, M. A. Identification of iron-regulated proteins of *Mycobacterium tuberculosis* and cloning of tandem genes encoding a low iron-induced protein and a metal transporting ATPase with similarities to two-component metal transport systems. Microb. Pathogen. **24** (1998) 133–143.

Iron plays a central role in the pathogenesis of *Mycobacterium tuberculosis,* the principal causative agent of tuberculosis. To learn more about iron acquisition by this bacterium, its iron regulated proteins (IRPs) were investigated. Seven IRPs were identified—three increased by high iron concentrations, and four by low iron concentrations. The smallest protein induced by low iron, Irp10, is tightly iron regulated as it is virtually absent in bacteria cultured in the presence of high iron concentrations. The gene (irpA) encoding this protein and an adjacent open reading frame, mtaA, were cloned and sequenced. The protein encoded by mtaA (Mta72) has striking homology to metal transporting P-type ATPases. This study suggests that IRp10 and Mta72 function as a two-component metal transport

system in *M. tuberculosis.*—Authors' Abstract

Chambers, H. F., Kocagoz, T., Sipit, T., Turner, J. and Hopewell, P. C. Activity of amoxicillin/clavulanate in patients with tuberculosis. Clin. Infect. Dis. **26** (1998) 874–877.

Some beta-lactam antibiotics are active *in vitro* against *Mycobacterium tuberculosis.* There are anecdotal reports of successful treatment of tuberculosis caused by multiple-drug-resistant strains of *M. tuberculosis* with regimens that included amoxicillin/clavulanate. Reduction of *M. tuberculosis* in the sputum of patients with pulmonary tuberculosis during administration of amoxicillin/clavulanate was measured by a quantitative culture method to determine the activity *in vivo.* Patients were randomized to receive isoniazid, ofloxacin, or amoxicillin/clavulanate for 7 days. Isoniazid was the most effective agent, reducing *M. tuberculosis* after 2 days at a mean rate (±standard deviation) of 0.60 ± 0.30 $\log^{10}$ cfu/ml per day, compared with 0.32 ± 0.05 and 0.34 ± 0.03 for ofloxacin and amoxicillin/clavulanate, respectively. The early bactericidal activity of amoxicillin/clavulanate was comparable to that reported for antituberculous agents other than isoniazid. Further studies of beta-lactam antibiotics with *in vitro* activity against *M. tuberculosis* are warranted to define their role in treatment of tuberculosis. —Authors' Abstract

Denis, O., Tanghe, A., Palfliet, K., Jurion, F., van den Berg, T. P., Vanonckelen, A., Ooms, J., Saman, E., Ulmer, J. B., Content, J. and Huygen, K. Vaccination with plasmid DNA encoding mycobacterial antigen 85A stimulates a CD4+ and CD8+ T-cell epitopic repertoire broader than that stimulated by *Mycobacterium tuberculosis* H37Rv infection. Infect. Immun. **66** (1998) 1527–1533.

Vaccination of mice with plasmid DNA carrying the gene for the major secreted mycobacterial antigen 85A (Ag85A) from *Mycobacterium tuberculosis* is a powerful technique for generating robust specific Th1 helper T-cell responses, CD8+-mediated cytotoxicity, and protection against *M. tuberculosis* challenge. We have now analyzed in more detail the antigen-specific immune CD4+- and CD8+-T-cell responses induced in BALB/c mice vaccinated with Ag85A DNA and have compared these responses to those generated by intravenous infection with *M. tuberculosis.* T-cell-epitope mapping, as measured by interleukin-2 and gamma interferon secretion from splenic T cells restimulated *in vitro* with synthetic 20-mer peptides spanning the complete mature sequence of Ag85A, demonstrated that DNA vaccination stimulated a stronger and broader T-cell response than did *M. tuberculosis* infection. Moreover, elevated cytotoxic T lymphocyte (CTL) activity against Ag85A-transfected and peptide-pulsed P815 target cells could be generated exclusively by vaccination with plasmid DNA, not following *M. tuberculosis* infection. By using DNA vaccination, three Ag85A CTL epitopes with predicted major histocompatibility complex class I binding motifs were defined. One of them was previously reported as a dominant, promiscuously recognized T-cell epitope in healthy humans with primary infections. These data strengthen the potential of DNA vaccination with respect to inducing antituberculous immunity in humans.—Authors' Abstract

Doenhoff, M. J. A role for granulomatous inflammation in the transmission of infectious disease: schistosomiasis and tuberculosis. Parasitology **115** Suppl. (1997) S113–S135.

The relationship between cell-mediated granulomatous inflammation and transmission of disease in schistosomiasis and tuberculosis has been explored. In two experiments involving *Schistosoma mansoni*-infected normal and T cell-deprived mice, and infected deprived mice that had been variously reconstituted with immune or normal lymphocytes or immune serum, there was a significant positive numerical correlation between mean liver granuloma diameters and fecal egg counts in individual

animals. Lymphocytes from donors with recently patent infections were more active than cells from chronically infected or uninfected donors in reconstituting egg excretion rates in deprived recipients, and mesenteric lymph node (MLN) cells were more active than spleen cells. Modulation of granulomatous activity with increasing chronicity of infection in the donors, resulting in a decrease in granuloma size around freshly produced tissue-bound eggs, was paralleled by a waning of the capacity of transferred lymph node cells to reconstitute egg excretion in the recipients. Serum taken from chronically infected donor mice over the same period and transferred to infected deprived recipients became more active in enhancing egg excretion in the recipients as the cell-mediated activity declined. A recent study in Kenya has found that *S. mansoni*-infected patients with concurrent human immunodeficiency virus (HIV) infection excrete fewer eggs than patients exposed to the same levels of schistosome infection, but who are not HIV-infected, thus indicating that schistosome egg excretion in humans is also immune-dependent. Attention is drawn to an apparently parallel situation in human tuberculosis, another pathogen which induces a cell-mediated granulomatous immune response. Several studies have shown that patients with tuberculosis who are also HIV-seropositive tend to have fewer tubercle bacilli detectable in their saliva than those with tuberculosis, but who are HIV-negative. This discrepancy, associated with differences in lung pathology in HIV-positive patients, suggests that in tuberculosis immune cell-mediated granulomatous inflammation causes the destruction of host tissue in a manner which facilitates onward transmission of the bacterial pathogen.—Author's Abstract

El Hay, M. J. and Andersen, P. Immunological requirements for a subunit vaccine against tuberculosis. Immunol. Cell Biol. **75** (1997) 595–603.

Some of the background issues associated with the development of a subunit vaccine for tuberculosis as well as the requirements for a subunit vaccine and progress toward this goal are reviewed.—Authors' Abstract

Erb, K. J., Kirman, J., Woodfield, L., Wilson, T., Collins, D. M., Watson, J. D. and LeGros, G. Identification of potential CD8+ T-cell epitopes of the 19 kDa and AhpC proteins from *Mycobacterium tuberculosis.* No evidence for CD8+ T-cell priming against the identified peptides after DNA-vaccination of mice. Vaccine **16** (1998) 692–697.

Mycobacterium tuberculosis is one of the major killers among infectious agents. It is of great importance to develop an efficient vaccine against *M. tuberculosis* since the only available vaccine *M. bovis*-BCG has a low efficacy. Furthermore, the emergence of multidrug-resistant *M. tuberculosis* strains makes it difficult to cure the disease, CD8+ T cells have been implied to play an important role in protective immunity against *M. tuberculosis.* A good vaccination strategy for the induction of cytotoxic CD8+ T-cell responses is naked DNA-injection of eukaryotic expression vectors. The use of DNA-injection in an attempt to induce cytotoxic CD8+ T-cell responses against epitopes of the 19-kDa or AhpC proteins from *M. tuberculosis* in mice was studied. MHC class I binding assays, of peptides derived from these proteins, demonstrated the presence of potential CD8+ T-cell epitopes. However, CD8+ T-cell responses against the peptides after DNA-injection were not detected. Furthermore, no difference in the kinetics of bacterial clearance was observed in vaccinated versus unvaccinated animals, even though 19-kDa and AhpC-specific antibodies were readily detected in the serum of vaccinated animals. Taken together these results suggest that the 19-kDa and AhpC genes are not good candidates for DNA vaccines against *M. tuberculosis.*—Authors' Abstract

Flynn, J. L., Scanga, C. A., Tanaka, K. E. and Chan, J. Effects of aminoguanidine on latent murine tuberculosis. J. Immunol. **160** (1998) 1796–1803.

A unique feature of *Mycobacterium tuberculosis* is its ability to establish latent infection in the human host, which can reactivate to cause disease years later. In the present study, the mechanisms involved in the control of latent tuberculous infection were examined using two murine experimental tuberculosis models. Analysis of the model involving infection of mice with a relatively low inoculum of the virulent Erdman strain of *M. tuberculosis* indicated that *in vivo* inhibition of reactive nitrogen intermediate (RNI) production by the nitric oxide synthase inhibitor aminoguanidine resulted in reactivation. This reactivation was evidenced by hepatosplenomegaly, a robust tissue granulomatous reaction, and increased bacillary load. IFN-gamma, TNF-alpha, and inducible nitric oxide synthase were all expressed throughout the latent phase of infection. Reactivation of latent tuberculous infection by aminoguanidine treatment was confirmed using a second murine tuberculosis model based on treatment with antimycobacterial drugs. Results obtained using this drug-based model also suggested the existence of an RNI-Índependent antimycobacterial mechanism(s) operative in the latent phase of infection. Together, these data suggest that both RNI-dependent and -independent mechanisms contribute to the prevention of tuberculous reactivation.—Authors' Abstract

Frothingham, R. and Meeker-O'Connell, W. A. Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. Microbiology **144** (1998) 1189–1196.

Genetic loci containing variable numbers of tandem repeats (VNTR loci) form the basis for human gene mapping and identification, forensic analysis and paternity testing. The variability of bacterial tandem repeats has not been systematically studied. Eleven tandem repeat loci in the *M. tuberculosis* genome were analyzed. Five major polymorphic tandem repeat (MPTR) loci contained 15-bp repeats with substantial sequence variation in adjacent copies. Six exact tandem repeat (ETR) loci contained large DNA repeats with identical sequences in adjacent repeats. These 11 loci were amplified in 48 strains to determine the number of tandem repeats at each locus. The strains analyzed included 25 wild-type strains of *M. tuberculosis, M. bovis, M. africanum* and *M. microti* and 23 substrains of the attenuated *M. bovis* BCG vaccine. One of the five MPTR loci and all six ETR loci had length polymorphisms corresponding to insertions or deletions of tandem repeats. Most ETR loci were located in intergenic regions where copy number may influence expression of downstream genes. Each ETR locus had multiple alleles in the panel. Combined analysis identified 22 distinct allele profiles in 25 wild-type strains of the *M. tuberculosis* complex and five allele profiles in 23 *M. bovis* BCG substrains. Allele profiles were reproducible and stable, as demonstrated by analyses of multiple isolates of particular reference strains obtained from different laboratories. VNTR typing may be generally useful for strain differentiation and evolutionary studies in bacteria.—Authors' Abstract

Goletti, D., Weissman, D., Jackson, R. W., Collins, F., Kinter, A. and Fauci, A. S. The *in vitro* induction of human immunodeficiency virus (HIV) replication in purified protein derivative-positive HIV-infected persons by recall antigen response to *Mycobacterium tuberculosis* is the result of a balance of the effects of endogenous interleukin-2 and proinflammatory and antiinflammatory cytokines. J. Infect. Dis. **177** (1998) 1332–1338.

Coinfection with *Mycobacterium tuberculosis* and human immunodeficiency virus (HIV) is a serious problem, particularly in developing countries. Recently, *M. tuberculosis* and purified protein derivative (PPD) were demonstrated to induce HIV replication in CD8 T cell-depleted peripheral blood mononuclear cells from HIV-positive, PPD-positive persons but not in cells from PPD-negative persons. The role of endogenous and exogenous cytokines in modulating *M. tuberculosis*-induced HIV replication was evaluated. *M. tuberculosis*-induced HIV replication decreased following simultaneous inhibition of endogenous interleukin (IL)-2, IL-1-beta, and tumor necrosis

factor-alpha by the addition of soluble receptors and receptor antagonists or following exogenous IL-10 and transforming growth factor (TGF)-beta. In contrast, neutralization of endogenous IL-10 and TGF-beta augmented *M. tuberculosis*-induced HIV replication by increasing cellular activation. Thus, the balance between IL-2 and proinflammatory and antiinflammatory cytokines plays a major role in *M. tuberculosis*-induced replication of HIV.—Authors' Abstract

Johnson, C. M., Cooper, A. M., Frank, A. A. and Orme, I. M. Adequate expression of protective immunity in the absence of granuloma formation in *Mycobacterium tuberculosis*-infected mice with a disruption in the intracellular adhesion molecule 1 gene. Infect. Immun. **66** (1998) 1666–1670.

It remains unknown whether the expression of cell-mediated protective immunity and the capacity to mount a delayed-type hypersensivity (DTH) reaction in tuberculosis infection represent two manifestations of a basic response or are dissociable events. In this study, we present data in favor of the latter hypothesis, by showing that tuberculosis infection in the lungs of mice possessing only a truncated form of intracellular adhesion molecule 1 due to gene disruption was still adequately controlled by the expression of protective immunity in the absence of any sustained influx of macrophages and the lack of formation of appreciable granulomas. These animals also had no detectable DTH response to mycobacterial proteins in the foot pad assay, indicating that the accumulation of blood-borne macrophages at sites of mycobacterial infection or antigen deposition is not essential to control of the infection. These data support the hypothesis that the DTH component of the cellular response is not protective but contributes by walling off the sites of infection to prevent dissemination and reactivation disease.—Authors' Abstract

Juffermans, N. P., Verbon, A., van Deventer, S. J. H., van Deutekom, H., Speelman, P. and van der Poll, T. Tumor necrosis factor and interleukin-1 inhibitors as markers of disease activity of tuberculosis. Am. J. Respir. Crit. Care Med. **157** (1998) 1328–1331.

Serum concentrations of tumor necrosis factor-alpha (TNF), interleukin (IL)-1 beta, and their circulating inhibitors soluble TNF receptor type I (sTNFRI), type II (sTNFRII), IL-1 receptor antagonist (IL-1ra), and soluble IL-1 receptor type II (sIL-1RII) were measured for 123 patients with tuberculosis (TB) in various stages of disease, in persons who had been in close contact with patients with contagious pulmonary TB, and in healthy controls. Levels of sTNFRI, sTNFRII, and IL-1ra, but not of sIL-1RII, were elevated in patients with active TB compared with contacts and controls and declined during treatment. The concentrations of these mediators did not differ between patients with pulmonary and extrapulmonary TB. The levels of sTNFRI and IL-1ra were higher in patients with fever and anorexia. Neither TNF nor IL-1 beta was detectable. We conclude that serum concentrations of sTNFRs I and II and IL-1ra may serve as markers of disease activity of TB. Sequential measurements of these cytokine inhibitors may be useful in the monitoring of antituberculous therapy.—Authors' Abstract

Kirk, S. M., Schell, R. F., Moore, A. V., Callister, S. M. and Mazurek, G. H. Flow cytometric testing of susceptibilities of *Mycobacterium tuberculosis* isolates to ethambutol, isoniazid, and rifampin in 24 hours. J. Clin. Microbiol. **36** (1998) 1568–1573.

Susceptibility testing of *Mycobacterium tuberculosis* is seriously limited by the time required to obtain results.

We show that susceptibility testing of clinical isolates of *M. tuberculosis* can be accomplished rapidly with acceptable accuracy by using flow cytometry. The susceptibilities of 35 clinical isolates of *M. tuberculosis* to various concentrations of isoniazid, rifampin, and ethambutol were tested by the agar proportion method and by flow cytometry. Agreement between the results from

the two methods was 95%, 92%, and 83% for isoniazid, ethambutol, and rifampin, respectively. Only 11 discrepancies were detected among 155 total tests. The results of cytometric susceptibility tests were available within 24 hr of inoculation of drug-containing medium, while the proportion method required 3 weeks to complete. The flow cytometric method is also simple to perform.—Authors' Abstract

Lightbody, K. A., Girvin, R. M., Pollock, D. A., Mackie, D. P., Neill, S. D. and Pollock, J. M. Recognition of a common mycobacterial T-cell epitope in MPB59 of *Mycobacterium bovis.* Immunology **93** (1998) 314–322.

Bovine tuberculosis, which persists as a residual level of infection in many European countries, has implications not only for the economy of farming communities but also for human health. The aim of this study was to identify a common mycobacterial antigen which was recognized in bovine tuberculosis and to characterize the response to this antigen at the epitope level. A T-cell clone, phenotype CD4+, raised from an animal experimentally infected with *Mycobacterium bovis* was shown to proliferate in response to a panel of sonicates derived from different mycobacterial species indicating recognition of an antigen with broad specificity. This antigen was subsequently shown to be MPB59. Recognition of MPB59 at the epitope level was determined in experimental and field cases of bovine tuberculosis using a panel of synthetic peptides (20-mers with 10-residue overlaps) incorporating the signal sequence and mature protein. The results showed that *in vitro* interferon-gamma was predominantly produced in response to adjacent peptides numbers 10 and 11, suggesting that the dominant epitope was contained in the overlap, correlating to residues 101–110 (YYQSGLSVIM). This epitope was recognized by 54% of tuberculous cattle of mixed breeds, which suggests that it may be genetically permissive in terms of major histocompatibility complex presentation. Sequence analysis confirmed that there were only minor differences in the amino acid composition within this region for various mycobacterial species, which could explain the common T-cell recognition described in this study. Common recognition of this epitope indicates that it would have limited potential for use as diagnostic reagent *per se* but may have potential for inclusion in a subunit vaccine.—Authors' Abstract

Miesel, L., Weisbrod, T. R., Marcinkeviciene, J. A., Bittman, R. and Jacobs, W. R. NADH dehydrogenase defects confer isoniazid resistance and conditional lethality in *Mycobacterium smegmatis.* J. Bacteriol. **180** (1998) 2459–2467.

Isoniazid (INH) is a highly effective drug used in the treatment and prophylaxis of *Mycobacterium tuberculosis* infections. Resistance to INH in clinical isolates has been correlated with mutations in the inhA, katG, and ahpC genes. In this report, we describe a new mechanism for INH resistance in *M. smegmatis.* Mutations that reduce NADH dehydrogenase activity (Ndh; type II) cause multiple phenotypes, including (i) coresistance to INH and a related drug, ethionamide; (ii) thermosensitive lethality; and (iii) auxotrophy. These phenotypes are corrected by expression of one of two enzymes: NADH dehydrogenase and the NADH-dependent malate dehydrogenase of the *M. tuberculosis* complex. The genetic data presented here indicate that defects in NADH oxidation cause all of the mutant traits and that an increase in the NADH/NADH+ ratio confers INH resistance.—Authors' Abstract

Mshana, R. N., Tadesee, G., Abate, G. and Miorner, H. Use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide for rapid detection of rifampin-resistant *Mycobacterium tuberculosis.* J. Clin. Microbiol. **36** (1998) 1214–1219.

We describe a test which uses the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to detect resistance to a bacte-

ricidal drug, rifampin, in *in vitro*-cultured *Mycobacterium tuberculosis*. The assay shows a linear relationship between the number of viable bacteria and the ability to reduce MTT. Dead mycobacteria were unable to reduce MTT. Rifampin-sensitive *M. bovis* (BCG) and *M. tuberculosis* exposed to rifampin showed a rifampin concentration-dependent inhibition of the ability to reduce MTT, while the resistant strains were unaffected. The inhibition of MTT reduction after treatment with rifampin paralleled the reduction in the number of CFU. By using mixing experiments in which the population percentages of rifampin-sensitive and -resistant strains were varied, the assay could detect the presence of rifampin resistance in the mixture when at least 1% of the bacterial population was composed of drug-resistant strains. The assay is cheap, can be usually read, and requires less than 3 days to obtain susceptibility results. The total time required to obtain results, from the time sputum is received in the laboratory, is, in most cases, less than 4 to 5 weeks, which is the time required for primary culture of the bacteria. The MTT assay could, in combination with a test to detect resistance to isoniazid, be a cheap and rapid screening method for multidrug-resistant *M. tuberculosis* that is affordable even by low-income countries where tuberculosis is a major public health problem.—Authors' Abstract

Oliver, S. J., Cheng, T. P., Banquerigo, M. L. and Brahn, E. The effect of thalidomide and two analogs on collagen induced arthritis. J. Rheumatol. **25** (1998) 964–969.

Objective. Thalidomide has been described as an inhibitor of both angiogenesis (which may account for its teratogenic effects on limb bud formation) and tumor necrosis factor-alpha (TNF-α) production. We evaluated its therapeutic potential in collagen-induced arthritis (CIA), a rat model of rheumatoid arthritis (RA).

Methods. Rats were administered orally 200 mg/kg/day thalidomide (N = 10) or either of two analogs, EM-12 (N = 9) or supidimide (N = 9). An additional group was given thalidomide (N = 10) at 200 mg/kg twice daily, and a control group (N = 13) was given vehicle only. At completion of the protocols, serum levels of TNF-α and vascular endothelial growth factor (VEGF) were measured.

Results. Suppression of inflammatory synovitis by clinical and radiographic criteria was significantly lower in all experimental protocols except the lower dose thalidomide group. The EM-12 analog was the most efficacious, and twice daily thalidomide was better than once daily. The incidence of arthritis onset was comparable among all groups. Strong cell-mediated and humoral responses to type II collagen, measured by a radiometric delayed-type hypersensitivity assay and anti-type II collagen IgG ELISA, respectively, were similar in the experimental and control groups. TNF-α and VEGF levels were increased in all rats immunized with collagen compared to naive controls.

Conclusion. Thalidomide and its analogs can suppress the clinical severity of rat CIA, but the mechanism of action is not a result of TNF-alpha or VEGF downregulation.—Authors' Abstract

Piatek, A. S., Tyagi, S., Pol, A. C., Telenti, A., Miller, L. P., Kramer, F. R. and Alland, D. Molecular beacon sequence analysis for detecting drug resistance in *Mycobacterium tuberculosis*. Nature Biotechnol. **16** (1998) 359–363.

We developed a new approach to DNA sequence analysis that uses fluorogenic reporter molecules—molecular beacons—and demonstrated their ability to discriminate alleles in real-time PCR assays of genomic DNA. A set of overlapping molecular beacons was used to analyze an 81-bp region of the *Mycobacterium tuberculosis* rpoB gene for mutations that confer resistance to the antibiotic rifampin. In a blinded study of 52 rifampin-resistant and 23 rifampin-susceptible clinical isolates, this method correctly detected mutations in all of the resistant strains and in none of the susceptible strains. The assay was carried out entirely in sealed PCR tubes and was simple to perform and interpret. This approach can be

used to analyze any DNA sequence of moderate length with single base pair accuracy.—Authors' Abstract

Ruff, P., Chasen, M. R., Long, J. E. H. and van Rensburg, C. E. J. A phase II study of oral clofazimine in unresectable and metastatic hepatocellular carcinoma. Ann. Oncol. **9** (1998) 217–219.

Background. Hepatocellular carcinoma is highly refractory to most chemotherapeutic agents. Clofazimine, a riminophenazine compound used to treat leprosy since 1962, inhibits various cancer cell lines, including hepatocellular carcinoma cell lines, via phospholipase A(2) dependent processes. Clofazimine also inhibits p170-glycoprotein, the mdr1 gene product.

Patients and methods. Thirty patients (26 males and four females) with unresectable (25) or metastatic (5) hepatocellular carcinoma received oral clofazimine 600 mg daily for 2 weeks, followed by 400 mg daily until progression or death.

Results. There were three responses (10%)—one of a soft tissue metastasis, and two of local disease—with 13 patients' disease stabilizing for up to 20 months. The overall median survival was 13 weeks. Adverse events included hyperpigmentation, eczematous skin rashes and palpitations.

Conclusion. Although only three patients had an objective response (10%), the 13 patients with stable disease for up to 20 months, and an overall median survival of 13 weeks, suggest that clofazimine, or other riminophenazine compounds may prove to be of value in hepatocellular carcinoma.—Authors' Abstract

Thompson, S. J., Francis, J. N., Siew, L. K., Webb, G. R., Jenner, P. J., Colston, M. J. and Elson, C. J. An immunodominant epitope from mycobacterial 65-kDa heat shock protein protects against pristane-induced arthritis. J. Immunol. **160** (1998) 4628–4634.

Previous studies showed that mice with pristane-induced arthritis (PIA) and those protected from the disease by preimmunization with mycobacterial 65-kDa heat shock protein (hsp65) possess raised immune responses to hsp65.

Additionally, T cells from hsp65-protected mice, but not from pristane-injected or normal mice, produced the Th2-associated cytokines IL-4, IL-5, and IL-10 in response to stimulation with hsp65. Here we demonstrate that the specificity of the immune response to hsp65 and related heat shock proteins (hsps) differs between protected and PIA mice. T cells from hsp65-protected mice respond to the bacterial hsps tested but not to the mammalian homolog, hsp58. Similarly, they exhibit high serum titers of anti-hsp65 antibodies, yet they have virtually undetectable levels of anti-hsp58 IgG. By contrast, both cellular and humoral immune responses are detectable to bacterial and mammalian hsps in mice with PIA. An immunodominant T-cell epitope has been identified in hsp65-protected mice corresponding to amino acids 261–271 from hsp65. Immunization of mice, either before or after the induction of arthritis, with this bacterial peptide, but not its mammalian homolog, protects mice from the development of PIA, and protection is associated with the production of Th2-type cytokines. Other experiments revealed that T cells primed with bacterial 261–271 or the mammalian homolog do not crossreact at the proliferative or cytokine level. These results demonstrate that an hsp65 peptide-specific Th2 response confers protection from PIA but do not support the idea that protection is mediated by a crossreaction with self hsp58 in the joints.—Authors' Abstract

Uh, S. T., Ki, S. Y., Lim, G. I., Moon, S. H., Jeong, S. W., Kim, H. T., Kim, Y. H. and Park, C. S. The T cell receptor subsets of lymphocytes in bronchoalveolar lavage in patients with active pulmonary tuberculosis. Respir. Med. **92** (1998) 408–414.

Study objective. To determine whether or not the levels of gamma/delta lymphocytes increase in bronchoalveolar lavage (BAL) fluid from patients with pulmonary tuberculosis.

Design. Prospective data collection relating to cells in BAL fluid and peripheral blood mononuclear cells (PBMC) from patients with pulmonary tuberculosis and control subjects.

Setting. A university hospital, from March 1990 to December 1993.

Patients. Thirteen patients with pulmonary tuberculosis who were diagnosed by culture of *Mycobacterium tuberculosis* from their sputum of BAL fluid and/or clinical response were enrolled in the study. Fifteen healthy volunteers participated as control subjects.

Measurements and results. The differential cell counts in BAL fluid were made by Diff-Quik stain. The percentages of T-cell receptor (TCR) (gamma/delta and alpha/beta)-positive lymphocytes and interleukin 2 (IL-2) receptor-positive CD3 lymphocytes in BAL fluid and peripheral blood were measured by dual scan with flow-cytometry. The percentage and absolute number of lymphocytes and the percentages of CD3+, IL2R+ lymphocytes in BAL fluid significantly increased in patients with tuberculosis when compared with those of control subjects. The percentages and numbers of gamma/delta and alpha/beta TCR-positive lymphocytes in BAL fluid and PBMC from patients with tuberculosis are indistinguishable from those of control subjects.

Conclusions. Gamma/delta lymphocytes do not appear to have as much meaning in patients as they do in animal studies.—Authors' Abstract

Wang, C. C. and Rook, G. A. W. Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed *Mycobacterium vaccae*. Immunology **93** (1998) 307–313.

Allergic disorders are mediated by T lymphocytes secreting T-helper 2 (Th2) cytokines, interleukin-4 (IL-4) and interleukin-5 (IL-5), resulting in high levels of serum immunoglobulin E (IgE) and recruitment of eosinophils. One of the treatment strategies is to downregulate the Th2 component by inducing a T-helper 1 (Th1) response to the relevant allergen, because Th1 and Th2 cytokines are thought to be mutually antagonistic. In this study, we examined the effects of *Mycobacterium vaccae,* a potent inducer of Th1 immunity, on allergic responses in a murine model. A single injection of *M. vaccae* into ovalbumin (OVA)-preimmunized BALB/c mice suppressed serum IgE over a wide dose range (10^7, 10^8 or 10^9 *M. vaccae*). Further experiments, using 10^7 *M. vaccae* injected twice, showed that this treatment inhibited not only serum IgE, but also the potential for ovalbumin-induced IL-5 production by spleen cells. This nonspecific ability of a mycobacterium to decrease Th2 activity, even when not presented together with the allergen, is in agreement with recent epidemiological studies on the impact of bacillus Calmette-Guerin (BEG) vaccination, and of other potent Th1 stimuli, on the incidence of atopy. The suppression of serum IgE and allergen-specific IL-5 synthesis by *M. vaccae* suggest that this organism is likely to have clinical application in the immunotherapy of allergy.—Authors' Abstract