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CURRENT LITERATURE

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

General and Historical

Bouree, P., Joseph, P. F., Fils-Aime, F., Morell-Gil, R. E. and Joseph, P. F. Leprosy in Haiti. Sante **12** (2002) 281–282.

Leprosy has yet a great impact on public health in Haiti. A study was carried out in Haiti from 1977 to 1999. On 2160 registered cases, mostly are paucibacillaries: 412 cases are under 15 year old patients and 1306 more than 15 years old. Multibacillaries cases are 85 in under 15 year old patients and 357 in more than 15 year old. By the improvement of sanitary conditions, detecting cases and multidrug therapy, leprosy had decreased during these last years, with an incidence from 0.1 to 0.2%. However, with the increasing poverty and political disorders, the control of leprosy remains difficult in Haiti.—Authors' Abtract

Kalk, A., and König, J. NGO and state: co-operation between a leprosy relief association and other institutions in South America. Lepr. Rev. **73** (2002) 160–166.

In a structured questionnaire format, the German Leprosy Relief Association (GLRA) interviewed its representatives in two Federal states of Brazil and four other Latin American countries about the distribution, between itself, the state and other institutions of a) responsibility for funding and b) implementation of activities, in relation to leprosy control. Wherever the political commitment was given, GLRA's role could be reduced to the highly effective support of the government structure in well-defined areas, most particularly in staff training, health education and eventually in program supervision. This public-private partnership under the umbrella of the host government sustains a small, but important specialized leprosy component while routine services are well integrated into the general health system.—Authors' Abstract

Kasturiaratchi, N. D., Settinayake, S., and Grewal, P. Processes and challenges: how the Sri Lankan health system managed the integration of Leprosy Services. Lepr. Rev. **73** (2002) 177–185.

At the end of 1999, the Ministry of Health in Sri Lanka took the bold decision to integrate its Leprosy Services within the country's general health system. The integration was completed in February 2001 and is already starting to bear fruit, but implementing the necessary changes has been a challenging task. Many new procedures had to be established, logistics improved, attitudes changed and health workers trained. A broad bridge between curative and preventative health services needed to be built. Integration efforts were supported by an advertising campaign to inform people that leprosy, like any other illness, can be treated at all health facilities. Contrary to the expectation that quality of service would drop following integration, more cases are now detected and an extensive network of government doctors is able to diagnose, treat and manage leprosy patients more efficiently. Prevalence has increased by 36% and the new case load by 41%. A few areas still need more attention, such as integrating MDT supplies within existing systems and improving the flow of information, but nonetheless the ownership of leprosy is shifting rapidly to local health services.—Authors' Abstract

Namadi, A., Visschedijk, J. and Samson, K. The leprosy elimination campaign in Jigawa, Nigeria: an opportunity for integration. Lepr. Rev. **73** (2002) 138–146.

Integration of leprosy control into the general health services is regarded as an important condition for increasing the accessibility and sustainability of leprosy services. However, it is often difficult to embark on such an integration process. In Jigawa State in Northern Nigeria, the leprosy elimination campaign was used as the initiator and catalyst for the integration process. In this article, this challenging process is described and analyzed. Available information is used to identify the constraints that emerged and to assess the consequences of integration for important aspects of leprosy control, such as case detection and case-holding and the accessibility and quality of the provided services. Some lessons from this experience are drawn that can be helpful for integration in other States or countries.—Authors' Abstract

Porter, J. D. H., Ogden, J. A., Ranganadha Rao, P. V., Prabhakar Rao, V., Rajesh, D., Buskade, R. A. and Soutar, D. Lessons in integration—operations research in an Indian leprosy NGO. Lepr. Rev. **73** (2002) 147–159.

Since the Alma Ata Declaration in 1978, health systems supporting the treatment and control of infectious diseases like leprosy and tuberculosis have been encouraged to 'integrate' into the primary health care structure within countries. Now, more than 20 years later, countries are still grappling with the concept of integration and looking for ways to achieve it. This study reports findings from a leprosy/Tuberculosis/AIDS awareness pilot project conducted by LEPRA India, a leprosy non-governmental organization (NGO), between 1996 and 2000 in Koraput district, Orissa. The project addressed the issue of integration on two levels. On the one hand LEPRA used the context of the project to explore ways in which to integrate TB services into their existing leprosy control structure. On the other hand, lessons from the pilot study were intended to help the organization find ways of linking with the government health care structure. Following a 'qualitative approach,' this operations research project assessed the perceptions of communities and providers about leprosy and tuberculosis services. Providers across the spectrum of this plural healthcare system were asked to provide comment on developing stronger networks with each other, with NGOs and with government, while patients and communities were asked to describe the resources available to them and the constraints they face in accessing health care in general, and for leprosy and TB in particular. LEPRA staff from top management to the outreach workers were also approached for their views. Patients and communities noted that physical access to treatment was a major constraint, while the existence of local providers and family support structures facilitated health and health care. Providers expressed a willingness to collaborate (with LEPRA and the government), but lacked training, adequate staff support and the appropriate equipment/technical resources. Also lacking were adequate information campaigns to inform the public about these diseases and their treatment. This information has provided LEPRA with an understanding of how they might best fill gaps in the existing system and therefore assist in the process of integrating services in their own organization and through the primary health care structure. To achieve this aim, LEPRA will increasingly become involved in developing relationships and partnerships with government in the delivery of training and services and in infrastructure development.—Authors' Abstract

Prabhakara Rao, V., Bhuskade, R. A., Sathi Raju, M., Ranganadha Rao, V., and Desikan, K. V. Initial experiences of implementation of functional integration (FI) in LEPRA India projects in Orissa. Lepr. Rev. **73** (2002) 167–176.

In 2000, the Government of the State of Orissa (population 37 million) in India decided to introduce functional integration for the control of leprosy, in place of the long-established vertical program, the general health services and the primary health care system. This paper describes the initial (9 months) experience of implementing this strategy in two projects run by LEPRA India. One of these, in the district of Koraput, was established in 1991 and covers a population of 1.5 million people.

The other, in Kalahandi district, started in 1997 and covers a population of 600,000. Both projects operate under difficult conditions with regard to terrain, the use of numerous tribal languages, illiteracy, water shortage, poor roads and communications. The preparatory phase included intensive health education of the public on leprosy, using a wide range of educational media and techniques. At the same time, LEPRA India supported the Government in the training and orientation of trainers, medical officers, primary health care staff and female health workers at village level. In all, over 2000 were trained. This paper describes all aspects of the implementation of functional integration in these two areas. In the 9-month period, 4207 suspect cases were referred to medical officers by health workers, but only 256 (6%) were confirmed as having leprosy. There were 169 confirmed self-reporting cases. Despite the clearly understood intention to involve primary health staff in case detection, 67% of all cases were in fact detected by LEPRA India, possibly due to overlapping attendance at clinics by vertical and general staff. There is obviously a need for further training of the general staff since only 6% of cases referred by them were confirmed as having leprosy. Steps must also be taken to ensure that the emphasis on case detection, confirmation and treatment shifts from the vertical to the general health staff. The supply of anti-leprosy drugs and steroids to primary health centers needs improvement. Appropriate teaching and learning material is urgently needed for both field staff and medical officers.—Authors' Abstract

Saunderson, P. R. and Ross, W. F. Training for integration. Lepr. Rev. **73** (2002) 130–137.

Training is often suggested as the solution to the inadequacies of the health care system, and there is little doubt that without it, service quality would suffer and new techniques and technologies would be difficult to introduce; clearly it is an important component in any drive to achieve quality of care. However, in this era of cost-effectiveness and cost cutting, which is part of the reason for integration, it is surprising that training is often not well planned and is rarely evaluated in a rational manner. This paper relies on recent discussions within ILEP about training and the use of training materials for leprosy in the present environment—one in which most programs are being integrated into the general health services. The development of a National Training Plan for Leprosy is proposed, with clear objectives, in order to best utilize the resources available.—Authors' Abstract

Smith, C. M. and Smith, W. C. S. Leprosy at the cutting edge: 2000 to 2005, and beyond. Trop. Doct. **32** (2002) 46–48.

This paper describes current strategies in the control and management of leprosy, and emphasizes the importance of early detection (especially of new cases) and treatment of leprosy, establishment of community-based rehabilitation and ongoing research (development of new diagnostic techniques, drugs and vaccines) in the elimination of this disease.—Trop. Dis. Bull.

Chemotherapy

Flageul, B., Wagner, L. and Cottenot, F. Immuno-allergic adverse effects of Rifampicin. Acta Leprol. **12** (2002) 71–78.

We report 2 new cases of immuno-allergic side-effects of rifampin (RMP), occurring in leprosy patients treated by multidrug therapy. These cases illustrate the various features of this type of complication. In one case, the patient exhibited a few days after restarting of RMP (600 mg daily), a typical flu syndrome associated to a thrombopenia. Previously, the patient had received RMP (300 mg, 3/5 days) that had to be stopped after 11 months for "general malaise" that in fact corresponded to a flu syndrome. The second patient developed a flu syndrome associated with a diffuse eczematous eruption one year after the onset of daily RMP (600 mg). Anti-RMP antibodies were detected only in the first case. The pathogenic mechanisms and the

clinico-biologic features are discussed.—Authors' Abstract

Rimoli, L. F. and de Godoy, M. F. Quantification of the oxidative stress in the blood of people with Hansen's disease undertaking or not a specific treatment. Hansen. Int. **26** (2001) 93–98.

In this study we evaluated the quantification of the oxidative stress in the blood of people with Hansen's disease undertaking or not a specific treatment. It was collected blood samples of 62 people carriers of Hansen and 13 healthy people. From these 62 patients, 35 were of multibacillary form and were being treated with clofazimine, dapsone and rifampin; 16 of the paucibacillary form treated with dapsone and rifampin; 11 of the patients were taking no treatment, being 5 of the multibacilar form and 6 of the paucibacilar form. The blood samples collected were taken for laboratory analyses where were made the amount of methemoglobin and count of Heinz's bodies. The obtained results were submitted to statistical analyses according to the Odds Ratio and Confidence Interval 95%. We concluded that the oxidative stress was caused by the therapy administered, not by the Hansen's disease itself, and the group that was taking clofazimine showed worse results, that is, higher oxidative stress.—Authors' Abstract

Clinical Sciences

Amador, M. do P. S. C., de Barros, V. R. S., Albuquerque, P. J. de B. S., Buna, M. I. F. and Campos, J. M. Childhood leprosy in the Curionópolis district—southeastern Pará state—a case report. Hansen. Int. **26** (2001) 121–125.

Childhood leprosy, especially advanced cases, show the extent of the problem and reflects the intensity of exposure to *Mycobacterium leprae* in a determined area. The authors report a case of borderline leprosy in a child three-years-old. The contact lepromatous leprosy (the father) with primary disease suspected. The child with a weight to age did not show any BCG scar at the diagnostic contact. The histology revealed: "Inflammatory infiltrated diffused." The special stain (Faraco-Fite) revealed a few acid-fast bacilli.—Authors' Abstract

Kalaiselvi, K., Rajaguru, P., Palanivel, M., Usharani, M. V. and Ramu, G. Chromosomal aberration, micronucleus and Comet assays on peripheral blood lymphocytes of leprosy patients undergoing multidrug therapy. Mutagenesis **17** (2002) 309–312.

To evaluate the genetic damage in leprosy patients, we carried out the alkaline Comet assay and chromosomal aberration (CA) and micronucleus (MN) tests in peripheral blood lymphocytes of 50 leprosy patients receiving multidrug treatment (MDT) and 50 healthy individuals. The Comet assay showed statistically higher mean values for length to width ratios of DNA mass ($p < 0.01$) and for mean frequencies of tailed cells ($p < 0.001$) in cells of leprosy patients than in those of controls. Similarly, the mean frequencies of micronucleated cells (per 1000 cytochalasin B-induced binucleated cells) were significantly greater ($p < 0.001$) in leprosy patients (19.92 ± 2.564) than in controls (1.6 ± 0.231). A statistically significant 10-fold increase in the frequency of CAs (11.16 ± 0.411) was observed in leprosy patients compared with controls (1.28 ± 0.242). In multiple regression analyses, when patients and controls were considered together, disease factor alone significantly influenced the genotoxicity markers. In the control group, age and alcohol consumption significantly influenced MN and length to width ratios and CA frequency, respectively. However, in MDT-treated leprosy patients none of the other confounding factors (sex, age, smoking and alcohol drinking) significantly affected the extent of genetic damage.—Authors' Abstract

Misra, V., Misra, S. P., Hatwal, D., Dwivedi, M., Singh, K. G. and Bajaj, A. K. *Helicobacter pylori* and associated histopathological changes in gastric biopsies of patients with leprosy. Indian J. Pathol. Microbiol. **44** (2001) 271–275.

Two antral biopsies each from 104 patients of leprosy and 100 controls were studied to find out the prevalence of *H. pylori* and associated histopathological changes. Sections were stained with hematoxylin and eosin, AB/PAS (pH 2.5) and Loeffler's methylene blue stains. Infection by *H. pylori,* inflammation and atrophy were found to be significantly more in leprosy patients as compared to controls ($p < 0.01$, < 0.005 and < 0.02 respectively). On comparing the histopathological changes in various subgroups of leprosy, *H. pylori,* inflammation and activity showed a statistically decreasing trend from tuberculoid to lepromatous subgroups ($p < 0.05$, < 0.001, < 0.01 respectively). Atrophy showed a significant increasing trend from tuberculoid to lepromatous group (< 0.001), it is concluded that despite a low prevalence of *H. pylori* and associated gastritis in patients with lepromatous leprosy, gastric epithelial damage is more marked due to altered immune response—Authors' Abstract

Sethuraman, G., Jeevan, D., Srinivas, C. R. and Ramu, G. Bullous erythema nodosum leprosum (bullous type 2 reaction). Int. J. Dermtol. **41** (2002) 362–364.

A 35-year-old man suffering from lepromatous leprosy with recurrent erythema nodosum leprosum (ENL) (bullous type 2 reaction) was admitted for acute exacerbation of the reaction. He had received regular WHO multibacillary multidrug therapy (WHO-MB-MDT) and corticosteroids for 11 months. The dose of corticosteroids could not be tapered below 20 mg/day due to sudden, frequent, and severe relapses of ENL. In hospital, he suddenly developed hypotension for which he was moved to the intensive care unit and managed. On the third day, he developed multiple, flaccid and tense bullae, some of which were hemorrhagic, over the face, trunk, and proximal extremities. Some of the ENL lesions showed a central bluish hue. Investigations revealed leukocytosis, thrombocytopenia, and hypokalemia. Blood culture yielded coagulase-negative *Staphylococcus aureus.* Slit skin smear and smear from the blister fluid showed numerous, fragmented, acid-fast bacilli (AFB) in clusters and many neutrophils. Skin biopsy revealed a subepidermal bulla that consisted of neutrophils and macrophage granulomas in the dermis together with perivascular neutrophilic infiltration; however, there were no features suggestive of vasculitis. Fite's stain demonstrated fragmented AFB. Both direct and indirect immunofluorescence studies did not show features of autoimmune bullous disorders; however, moderately strong discontinuous fibrinogen deposits were seen along the basement membrane and around the blood vessels. There were no deposits of immunoglobulin G (IgG) and C3. He was treated with antimicrobials, potassium chloride, and hydrocortisone, 100 mg intravenously, twice daily, which was subsequently switched to oral prednisolone. He responded well and was discharged. Upon lowering the dose of corticosteroids, he again developed severe ENL with fewer bullous lesions.—Authors' Abstract

Siddiqui, M. R., Moreira, A. L., Negesse, Y., Taye, G. A., Hansekom, W. A., Haslett, P. A., Britton, S. and Kaplan, G. Local nerve damage in leprosy does not lead to an impaired cellular immune response or decreased wound healing in the skin. J. Infect. Dis. **186** (2002) 260–265.

This study investigated whether peripheral nerve damage in patients with leprosy impairs local cellular immune responses, thereby reducing wound healing and leading to chronic skin ulceration. Anesthetic and contralateral sensitive skin sites in 42 patients with leprosy were compared for delayed-type hypersensitivity responses to purified protein derivative (PPD) of tuberculin. Leukocyte recruitment, epidermal activation, keratinocyte proliferation, and rates of wound healing after skin biopsy were compared. No significant differences in PPD-induced induration, epidermal activation and thickening or numbers of total T

cells, CD8(+) T cells, CD1a(+) Langerhans cells, and proliferating Ki67(+) keratinocytes were observed between anesthetic and sensitive skin sites. Similarly, rates of wound healing over 5 days after skin biopsy did not differ significantly. Thus, local leprosy-associated anesthesia does not appear to contribute to local immune compromise or impaired wound healing. Rather, chronic cutaneous ulceration in leprosy most likely results from repeated trauma associated with loss of sensation.—Authors' Abstract

Singh, N. Arora, V. K., Jain, A., Bhattacharya, S. N. and Bhatia, A. Cytology of testicular changes in leprosy. Acta Cytol. **46** (2002) 659–663.

OBJECTIVE: To study the changes in testicular aspirates and semen of patients with leprosy. STUDY DESIGN: A prospective study of 56 patients in the reproductive-age group, with no record of treatment for leprosy. Both Ridley-Jopling and WHO classification systems were used. Skin and/or nerve biopsies were performed for documentation of the diagnosis. Semen analysis and fine needle aspirates of the testes were performed. Smears from the testicular aspirates were stained with May-Grunwald-Giemsa and Ziehl-Neelsen stain. RESULTS: Five patients were unable to produce an ejaculate. Abnormal semen analysis and/or testicular aspirates were seen in 24 (42.8%) patients. Eleven had oligospermia and eight azoospermia. Abnormalities in testicular aspirates ranged from hypospermatogenesis (4) through maturation arrest (1) and atrophy (11). Two patients had hydrocoele, and two had associated microfilariae. Three patients with multibacillary leprosy had type 2 reaction. *Mycobacterium leprae* were demonstrable in testicular aspirates from all patients with multibacillary and in three with paucibacillary leprosy. CONCLUSION: Abnormal semen analysis and/or testicular aspirates occur in a very high percentage of patients with leprosy. While this is expected for multibacillary disease, the high incidence in the paucibacillary form was surprising. With the rapid elimination of leprosy, fertility-related disability might emerge as a major problem in these people.—Authors' Abstract

Ura, S., Opromolla, D. V. A., dos Santos Godoy, D. A. and Fleury, R. N. Type 1 reaction in lepromatous patients ten years after cure. Hansen. Int. **26** (2001) 117–121.

A case of lepromatous leprosy patient treated with rifampin and dapsone for six months, and then treated with dapsone only for 14 years, is presented. The patient remained without lesions and with negative bacilloscopy for 10 years, and after this period he started again to show lesions, now with borderline aspect and appearance of bacilli. The authors suggest that the patient has always been a borderline whose disease has become more severe to the point that he presented a lepromatous aspect. When bacilli started to show up again, the patient's cellular immunity destroyed them so that borderline lesions appeared, as it must have happened in the initial phase of the disease. They also discuss the possible causes that led bacilli, probably persistent, to multiply again.—Authors' Abstract

Immuno-Pathology

Fiallo, P., Clapasson, A., Favre, A. and Pesce, C. Overexpression of vascular endothelial growth factor and its endothelial cell receptor KDR in type 1 leprosy reaction. Am. J. Trop. Med. Hyg. **66** (2002) 180–185.

The sites of expression of vascular endothelial growth factor (VEGF) and of KDR, its endothelial cell receptor, were investigated in leprosy reaction Type 1, or reversal reaction (RR), by immunohistochemistry and *in situ* hybridization. In

comparison with nonreactional leprosy, overexpression of both VEGF and KDR was seen in granuloma cells, especially epithelioid and foreign body-type giant cells, the epithelium and the vascular endothelium of RR specimens. In granuloma cells, hybridization for VEGF was stronger than immunostaining, a finding that may reflect the rapid turnover of VEGF in an immunologically dynamic situation such as RR. In the epidermis, double immunohistochemistry revealed VEGF overexpression in CD1a-positive dendritic cells. The VEGF may not only be relevant for hyperpermeability and mononuclear cell differentiation (the key morphologic features in the acute, clinically evident phase of RR), but it could also be implicated in RR onset, when dendritic cells are activated in response to antigen stimulation.—Authors' Abstract

Goulart, I. M., Penna, G. O. and Cunha, G. Immunopathology of leprosy: the complexity of the mechanisms of host immune response to *Mycobacterium leprae*. Rev. Soc. Bras. Med. Trop. **35** (2002) 363–375. (In Portuguese)

Leprosy, whose etiologic agent *Mycobacterium leprae,* is an illness of ample clinical and immunopathological spectrum. Its clinical manifestations are correlated with distinct immunologic forms, varying from a vigorous immune response mediated by cells to *M. leprae* with Th1 standard in the tuberculoid polar region, to an absence of specific cellular response to antigens of *M. leprae* in the lepromatous polar region, with predominance of Th2 response and exacerbation of humoral response. It is probable that different polymorphic genes determine susceptibility to *M. leprae.* Additional studies are necessary to clarify the complex interactions between cytokines and the role of the phenotypic diversity of cells network that contribute to the host defense. The comprehension of such mechanisms will provide new insights for the identification of agonists and/or antagonists for pro- or anti-inflammatory effects, and also will indicate possible situations for its appropriate use in immunologic and/or immunotherapeutic interventions.—Authors' Abstract

Hashimoto, K., Maeda, Y., Kimura, H., Suzuki, K., Masuda, A., Matsuoka, M. and Makino, M. *Mycobacterium leprae* infection in monocyte-derived dendritic cells and its influence on antigen-presenting function. Infect. Immun. **70** (2002) 5167–5176.

Host defense against *Mycobacterium leprae* infection is chiefly mediated by gamma interferon (IFN-gamma)-secreting cytotoxic T cells. Since which antigen-presenting cell populations act to stimulate these T cells is not fully understood, we addressed the role of monocyte-derived dendritic cells (DCs). The DCs phagocytosed *M. leprae* and expressed bacterially derived antigens (Ags), such as phenolic glycolipid 1 (PGL-1), in the cytoplasm, as well as on the cell surface. The expression of HLA-ABC and -DR Ags on DCs was down-regulated by *M. leprae* infection, and that of CD86 was up-regulated, but not as fully as by *Mycobacterium bovis* BCG infection. Induction of CD83 expression required a large number of *M. leprae* cells. When a multiplicity of infection of >40 was used, the DCs induced a significant proliferative and IFN-gamma-producing response in autologous T cells. However, these responses were significantly lower than those induced by BCG- or *Mycobacterium avium*-infected DCs. A CD40-mediated signaling in *M. leprae*-infected DCs up-regulated the expression of HLA Ags, CD86, and CD83 but did not enhance T-cell-stimulating ability. Therefore, *M. leprae*-infected DCs are less efficient at inducing T-cell responses. However, when the surface PGL-1 on *M. leprae*-infected DCs was masked by a monoclonal antibody, the DCs induced enhanced responses in both CD4(+)- and CD8(+)-T-cell subsets. *M. leprae* is a unique pathogen which remains resistant to DC-mediated T-cell immunity, at least in the early stages of infection.—Authors' Abstract

Jaswal, T. S., Jain, V. K., Jain, V., Singh, M., Kishore, K. and Singh, S. Evaluation of leprosy lesions by skin smear cytology in comparison to histopathology. Indian J. Path. Microbiol. **44** (2001) 277–281.

Cytological evaluation of leprosy skin lesion was done to evaluate cytohistological

comparison with nonreactional leprosy, overexpression of both VEGF and KDR was seen in granuloma cells, especially epithelioid and foreign body-type giant cells, the epithelium and the vascular endothelium of RR specimens. In granuloma cells, hybridization for VEGF was stronger than immunostaining, a finding that may reflect the rapid turnover of VEGF in an immunologically dynamic situation such as RR. In the epidermis, double immunohistochemistry revealed VEGF overexpression in CD1a-positive dendritic cells. The VEGF may not only be relevant for hyperpermeability and mononuclear cell differentiation (the key morphologic features in the acute, clinically evident phase of RR), but it could also be implicated in RR onset, when dendritic cells are activated in response to antigen stimulation.—Authors' Abstract

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Hashimoto, K., Maeda, Y., Kimura, H., Suzuki, K., Masuda, A., Matsuoka, M. and Makino, M. *Mycobacterium leprae* infection in monocyte-derived dendritic cells and its influence on antigen-presenting function. Infect. Immun. **70** (2002) 5167–5176.

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Jaswal, T. S., Jain, V. K., Jain, V., Singh, M., Kishore, K. and Singh, S. Evaluation of leprosy lesions by skin smear cytology in comparison to histopathology. Indian J. Path. Microbiol. **44** (2001) 277–281.

Cytological evaluation of leprosy skin lesion was done to evaluate cytohistological

correlation. Twenty-five clinically suspected patients of leprosy were evaluated by performing fine needle aspiration (FNA) in nodular lesions and slit skin smear technique in flat lesions to classify across R-J scale. May-Grunwald-Giemsa (MGG) and Ziehl-Neelsen stain were employed on slit skin smears and fine needle aspiration material. Histopathological assessment of slides from same lesion was done. The overall diagnostic accuracy of fine needle aspiration was 76.1% and that of slit skin smear 50%. However, on adequate material diagnostic accuracy of slit skin smear was high, 100% as compared to 81.8% of fine needle aspiration smears. In cases of polar leprosy cytological findings paralleled histopathological diagnosis. Within the constraints of cytological interpretation the cases in borderline unstable spectrum of leprosy can be classified broadly.—Authors' Abstract

Parkash, O. and Girdhar, B. K. A comparative and combinatorial study of two serological assays for detection of *Mycobacterium leprae* infection. Acta Leprol. **12** (2002) 63–69.

The performances of *Mycobacterium leprae*-specific serological assays namely: phenolic glycolipid-1-based enzyme-linked immunosorbent assay (PGL-ELISA) and a monoclonal antibody-based inhibition test (MAIT) were studied for their efficiency to detect *M. leprae* infection. As usual, both the tests were more sensitive to detect lepromatous leprosy patients than tuberculoid type of leprosy patients. Considering the efficiency to detect leprosy patients, the MAIT was slightly more sensitive and specific than PGL-ELISA. When the results of both assays are considered together, a better sensitivity (over the sensitivity of individual assay) was obtained while maintaining good enough specificity. These findings point out that a combinatorial approach for detection of *M. leprae* infection would be a better strategy to detect *M. leprae* infection. Hence, it may act as a better tool for measurement of bacterial load in the patients.—Authors' Abstract

Microbiology

Chemlal, K., Huys, G., Laval, F., Vincent, V., Savage, C., Guiterrez, C., Laneelle, M. A., Swings, J., Meyers, W. M., Daffe, M. and Portaels, F. Characterization of an unusual mycobacterium: a possible missing link between *Mycobacterium marinum* and *Mycobacterium ulcerans.* J. Clin. Microbiol. **40** (2002) 2370–2380.

In an attempt to characterize an unusual mycobacterial isolate from a 44-year-old patient living in France, we applied phenotypic characterizations and various previously described molecular methods for the taxonomic classification of mycobacteria. The results of the investigations were compared to those obtained in a previous study with a set of temporally and geographically diverse *Mycobacterium ulcerans* (n = 29) and *Mycobacterium marinum* (n = 29) isolates (K. Chemical, G. Huys, P.-A. Fonteyne, V. Vincent, A. G. Lopez, L. Rigouts, J. Swings, W. M. Meyers, and F. Portaels. J. Clin. Microbiol. **39** (2001) 3272–3278). The isolate, designated ITM 00-1026 (IPP 2000-372), is closely related to *M. marinum* according to its phenotypic properties, lipid pattern, and partial 16S rRNA sequence. Moreover, fingerprinting by amplified fragment length polymorphism (AFLP) analysis unequivocally classified this strain as a member of the species *M. marinum,* although it lacked two species-specific AFLP marker bands. However, PCR and restriction fragment length polymorphism analysis based on *M. ulcerans*-specific insertion sequence IS2404 showed the presence of this element in a low copy number in isolate ITM 00–1026. In conclusion, the designation of this isolate as a transitional species further supports the recent claim by Stinear *et al.* (T. Stinear, G. Jenkin, P. D. Johnson, and J. K. Davies. J. Bacteriol. **182**

(2000) 6322–6330) that *M. ulcerans* represents a relatively recent phylogenetic derivative of *M. marinum* resulting from the systematic acquisition of foreign DNA fragments.—Authors' Abstract

Cole, S. T. Comparative mycobacterial genomics as a tool for drug target and antigen discovery. Eur. Respir. J. Suppl. **36** (2002) 78s–86s.

Genomics and the associated downstream technologies are generating vast data sets that provide new opportunities for understanding and combating both infectious and genetic diseases in humans. The genomic approach has been applied to tuberculosis, a major cause of transmissible morbidity and mortality, with notable success. Complete genome sequences are now available for three members of the *Mycobacterium tuberculosis* complex and the related intracellular pathogen *M. leprae.* Many of the predictions generated in silico by genomics have been validated through functional analysis, including studies of the transcriptome and proteome, and led to the identification of essential genes. Knowledge of the latter defines potential targets for new and existing drugs and their specificity can be assessed by comparative genomics with the host or other pathogens. Genomics is also furthering tuberculosis vaccine development by pinpointing potentially antigenic proteins as well as providing better diagnostic tools to detect infection.—Author's Abstract

Maeda, Y., Makino, K., Crick, D. C., Mahapatra, S., Srisungnam, S., Takii, T., Kashiwabara, Y. and Brennan, P. J. Novel 33-kilodalton lipoprotein from *Mycobacterium leprae.* Infect. Immun. **70** (2002) 4106–4111.

A novel *Mycobacterium leprae* lipoprotein LpK (accession no. ML0603) was identified from the genomic database. The 1116-bp open reading frame encodes a 371-amino-acid precursor protein with an N-terminal signal sequence and a consensus motif for lipid conjugation. Expression of the protein, LpK, in *Escherichia coli* revealed a 33-kDa protein, and metabolic labeling experiments and globomycin treatment proved that the protein was lipidated. Fractionation of *M. leprae* demonstrated that this lipoprotein was a membrane protein of *M. leprae.* The purified lipoprotein was found to induce production of interleukin-12 in human peripheral blood monocytes. The studies imply that *M. leprae* LpK is involved in protective immunity against leprosy and may be a candidate for vaccine design.—Authors' Abstract

Mougous, J. D., Green, R. E., A. S., Mo., Williams, S. J., Brenner, S. E. and Bertozzi, C. R. Sulfotransferases and sulfatases in mycobacteria. Chem. Biol. **9** (2002) 767–776.

Analysis of the genomes of *M. tuberculosis, M. leprae, M. smegmatis,* and *M. avium* has revealed a large family of genes homologous to known sulfotransferases. Despite reports detailing a suite of sulfated glycolipids in many mycobacteria, a corresponding family of sulfotransferase genes remains uncharacterized. Here, a sequence-based analysis of newly discovered mycobacterial sulfotransferase genes, named stf1–stf10, is presented. Interestingly, two sulfotransferase genes are highly similar to mammalian sulfotransferases, increasing the list of mycobacterial eukaryotic-like protein families. The sulfotransferases join an equally complex family of mycobacterial sulfatases: a large family of sulfatase genes has been found in all of the mycobacterial genomes examined. As sulfated molecules are common mediators of cell-cell interactions, the sulfotransferases and sulfatases may be involved in regulating host-pathogen interactions.—Authors' Abstract

Reeve, I., Hummel, D., Nelson, N. and Voss, J. Overexpression, purification, and site-directed spin labeling of the Nramp metal transporter from *Mycobacterium leprae.* Proc. Natl. Acad. Sci. **99** (2002) 8608–8613.

It has long been recognized that the pathogenicity of a broad range of intracellu-

lar parasites depends on the availability of transition metal ions, especially iron. Nramp1 (natural resistance-associated macrophage protein 1), a proton-coupled divalent metal ion transporter, has been identified as a controlling factor in the resistance or susceptibility to infection with a diverse range of intracellular pathogens such as *Toxoplasma, Salmonella, Mycobacterium,* and *Leishmania.* The role of divalent metal ion transport is even more compelling given the existence of Nramp homologs in several intracellular parasites, such as mycobacteria. We have confirmed the functional homology of the Nramp homolog from *Mycobacterium leprae* by using a yeast complementation assay for divalent cation uptake. To facilitate a concerted biochemical and structural analysis of this important class of transporters, the *M. leprae* Nramp was expressed in *Escherichia coli.* Dual affinity tags were engineerhe N and C termini to allow for isolation of full-length protein at >95% purity. Site-directed spin labeling of Cys-299 reveals a flexible hinge-like domain. A weak dipolar interaction is detected between the nitroxide and paramagnetic transition ions, indicating this position is approximately 19 A from the nearest high affinity binding site.—Authors' Abstract

Visca, P., Fabozzi, G., Petrucca, A., Ciaccio, C., Coletta, M., De Sanctis, G., Bolognesi, M., Milani, M. and Ascenzi, P. The truncated hemoglobin from *Mycobacterium leprae.* Biochem. Biophys. Res. Commun. **294** (2002) 1064–1070.

Truncated hemoglobins (trHb's) form a family of low molecular weight O_2 binding hemoproteins distributed in eubacteria, protozoa, and plants. TrHb's branch in a distinct clade within the hemoglobin (Hb) superfamily. A unique globin gene has recently been identified from the complete genome sequence of *Mycobacterium leprae* that is predicted to encode a trHb (*M. leprae* trHbO). Sequence comparison and modelling considerations indicate that monomeric *M. leprae* trHbO has structural features typical of trHb's, such as 20–40 fewer residues than conventional globin chains, Gly-based sequence consensu motifs, likely assembling into a 2-on-2 alpha-helical sandwich fold, and hydrophobic residues recognized to build up the protein matrix ligand diffusion tunnel. The ferrous heme iron atom of deoxygenated *M. leprae* trHbO appears to be hexacoordinated, like in *Arabidopsis thaliana* trHbO-3 (*A. thaliana* trHbO-3). Accordingly, the value of the second-order rate constant for *M. leprae* trHbO carbonylation (7.3×10^3 M^{-1} s^{-1}) is similar to that observed for *A. thaliana* trHbO-3 (1.4×10^4 M^{-1} s^{-1}) and turns out to be lower than that reported for carbon monoxide binding to pentacoordinated *Mycobacterium tuberculosis* trHbN (6.7×10^6 M^{-1} s^{-1}). The lower reactivity of *M. leprae* trHbO as compared to *M. tuberculosis* trHbN might be related to the higher susceptibility of the leprosy bacillus to toxic nitrogen and oxygen species produced by phagocytic cells.—Authors' Abstract

Epidemiology and Prevention

Chakrabarty, A. N. and Dastidar, S. G. Is soil an alternative source of leprosy infection? Acta Leprol. **12** (2002) 79–84.

Leprosy is believed to be transmitted only through human contacts. However, many anomalous observations have gradually accumulated which have weakened such beliefs. These are: only 1/3rd cases of leprosy give a definite history of being transmitted from other known cases; lifelong spouses, in whom only one has leprosy, seldom lead to leprosy to others; while MDT applied intensively in most leprosy endemic countries, could successfully reduce incidence of leprosy, however, simultaneously new cases arise unabated. Besides, a close look at animal leprosies also suggested a mode of transmission other than human-type contact. Thus, a search for alternative hypothesis led to the findings that leprosy bacillus (LB) could be a soil chemoautotroph and could facultatively live both in the human body and the soil

which could serve as an alternative source of infection. Evaluation of accumulated evidences points to this possibility.—Authors' Abstract

Hoal, E. G. Human genetic susceptibility to tuberculosis and other mycobacterial diseases. IUBMB Life **53** (2002) 225–229.

The existence of a genetic component in mycobacterial disease susceptibility is no longer in doubt and the investigations now being conducted aim to determine which genes are involved, to what extent, and in which disease phenotype they are relevant. In certain rare instances of susceptibility to poorly pathogenic mycobacteria, the genetic component is clear. The approaches employed to elucidate common disease susceptibility include linkage studies, particularly genome-wide linkage analysis of both tuberculosis and leprosy, and association studies. A number of candidate genes have shown association with tuberculosis, and in many cases, on replication of the study, association has been confirmed in a disparate population, indicating the wider importance of the gene in the disease process. In other instances, associations appear to be particular to a population or a subtype of disease.—Author's Abstract

Kocak, M., Balci, M., Pence, B. and Kundakci, N. Associations between human leukocyte antigens and leprosy in the Turkish population. Clin. Exp. Dermatol. **27** (2002) 235–239.

Leprosy is a chronic infection caused by an intracellular microorganism. Genetic predisposition to both disease susceptibility and to host immunological response has been postulated for many years. The aim of this study was to determine whether there is HLA-linked susceptibility to leprosy and its different types. HLA-class I (A, B, C) and II (DR, DQ) antigen frequencies in 80 patients with leprosy (35 borderline lepromatous, 25 lepromatous, 15 borderline tuberculoid, five tuberculoid) were compared with those in 120 healthy individuals. HLA-class I antigens A9, A10, A32, B5, B21, Bw4, Bw6, Cw1, Cw2 and HLA-class II antigens DR9, DR10, DRw52, DQ1, DQ3 were found to be significantly more frequent in patients with leprosy, whereas HLA-class I antigens A3, B44, B49 and HLA-class II antigen DQ5 were so in controls. However, there was no significant difference in HLA-class I and II antigen frequencies between subtypes of leprosy. HLA-A null antigen was found to have weak expression in patients with leprosy. In conclusion, factors other than HLA-class I and class II antigens may have a more critical role in the pathophysiology of leprosy infection in man.—Authors' Abstract

Santos, L. P. and de Oliveira Rabay, F. Epidemiological situation of leprosy control in Taubaté-SP municipality in 1999. Hansen. Int. **26** (2001) 112–116.

Brazil ranks second in the absolute number of leprosy cases in the world and first in the Americas. In 1999, a conference was held by the World Health Organization in Abdijan, Ivory Coast and there Brazil had set a goal to reduce the prevalence to below 1:10,000 inhabitants by the year 2005. Leprosy epidemiological data, collected by the Ministry of Health, were retrospectively studied to assess the epidemiological and operational situation of leprosy control of the patients seen in Taubaté municipality, São Paulo, in 1999. A prevalence of 3.24/10,000 inhabitants and a high incidence (1.27/10,000 inhabitants) were found for the year. The majority of patients (83.5%) had multibacillary leprosy and most received multiple drugs treatment (80% of the patients). It was also seen that 15.7% of the patients were not evaluated for disabilities during the year. While in 20.8% of the patients who were released from treatment and were evaluated for disabilities, a disabilities of grade II and III, was found. It is proposed to focus on assistance in basic health care units, because these are closer to the community and have health professionals for early diagnosis. To achieve in this way the so hoped for control of the disease.—Authors' Abstract

Wibawa, T., Soebono, H. and Matsuo, M. Association of a missense mutation of the laminin alpha2 gene with tuberculoid type of leprosy in Indonesian patients. Trop. Med. Int. Health **7** (2002) 631–636.

Leprosy, an infection caused by *Mycobacterium leprae,* has a specific tropism for the myelinating Schwann cells of peripheral nerves. Recently, the G domain of laminin alpha2 has been shown to be a mediator for *M. leprae* to bind to alpha-dystroglycan in Schwann cells. In order to analyze association of leprosy with the mediator, three genetic polymorphisms encoding the G domain of the laminin alpha2 chain were analyzed by direct sequencing in 53 leprosy patients and 58 healthy contact individuals from Indonesia. There was no significant difference in the incidence of the polymorphisms between patients and non-patients. Remarkably, it was found that a missense mutation (T7809C) substituting valine with alanine (V2587A) was found to be more frequent in the tuberculoid type than in the lepromatous type leprosy. It is supposed that this missense mutation is one of the determinant factors in the early onset of peripheral nerve damage in Indonesian tuberculoid leprosy patients.—Authors' Abstract

Rehabilitation

de Magalhães, H. M. and Duerksen, F. Transfer of the Peroneus Longus in footdroop deformity: results in leprosy. Hansen. Int. **26** (2001) 99–104.

This paper evaluates the surgical technique for the deep fibularis (peroneous) longus muscle transfer, described by Duerksen (1977), for dorsiflexion of the foot in cases of foot drop deformity in Hansen's disease patients. The patients were submitted to a pre and postsurgical 12-week physiotherapy and clinical evaluation program with satisfactory results, which gave the authors support to recommend this technique.—Authors' Abstract

Elui, V. M. C., de Oliveira, M. H. P. and dos Santos, C. B. The helpful use of splints in the rehabilitation of flexible claw hand in leprosy. Hansen. Int. **26** (2001) 105–111.

The objective of this study was the comparison of two kinds of dynamic splints, fabricated in leather and in thermoplastic material used to correct the flexible claw hand in leprosy affected individuals, regarding; the claw correction through active extension of proximal interphalangeal joint (PIP) goniometry. The subject population was formed by 30 leprosy patients, both sexes, which presented with flexible ulnar or ulnar-median claw hand, with ages between 20 and 81 years, submitted to the use of leather or thermoplastic splints. The two splints were compared. The results were statistically treated, showing that both splints improved the claw pattern, the thermoplastic splint had 85.5% claw correction, and the leather splint had 53%. It was concluded, that the splints, are helpful in correcting the claw hand deformities and self-esteem.—Authors' Abstract

Other Mycobacterial Diseases and Related Entities

Alexander, K. A., Pleydell, E., Williams, M. C., Lane, E. P., Nyange, J. F. C. and Michel, A. L. *Mycobacterium tuberculosis:* an emerging disease of free-ranging wildlife. Emerg. Infect. Dis. **8** (2002) 598–601.

Expansion of ecotourism-based industries, changes in land-use practices, and escalating competition for resources have increased contact between free-ranging wildlife and humans. Although human presence in wildlife areas may provide an im-

portant economic benefit through ecotourism, exposure to human pathogens may represent a health risk for wildlife. This report is the first to document introduction of a primary human pathogen into free-ranging wildlife. We describe outbreaks of *Mycobacterium tuberculosis,* a human pathogen, in free-ranging banded mongooses (*Mungos mungo*) in Botswana and suricates (*Suricata suricatta*) in South Africa. Wildlife managers and scientists must address the potential threat that humans pose to the health of free-ranging wildlife.—Authors' Abstract

Amofah, G., Bonsu, F., Tetteh, C., Okrah, J., Asiedu, K. and Addy, J. Buruli ulcer in Ghana: results of a national case search. Emerg. Infect. Dis. **8** (2002) 167–170.

From June to July 1999, a national search for cases of Buruli ulcer in Ghana identified 5619 patients, with 6332 clinical lesions at various stages. The overall crude national prevalence rate of active lesions was 20.7 per 100,000, but the rate was 150.8 per 100,000 in the most disease-endemic district. The case search demonstrated widespread disease and gross underreporting compared with the routine reporting system. The epidemiological information gathered will contribute to the design of control programs for Buruli ulcer.—Trop. Dis. Bull.

Asselineau, C., Asselineau, J., Laneelle, G. and Laneelle, M. The biosynthesis of mycolic acids by mycobacteria: current and alternative hypotheses. Prog. Lipid Res. **41** (2002) 501.

Experimental observations, accumulated during several decades, have allowed an overall scheme for the biosynthesis of the mycolic acids, which are very long chain fatty acids of Mycobacteria to be proposed. But, in almost every step, several hypotheses are compatible with the experimental results, leading to variations of the overall scheme. The aim of this review is to point to some additional possibilities. It is generally assumed that the classical elongation process of fatty acid synthesis produces two long chains, the condensation of which leads to the direct precursors of mycolic acids. But three condensations of four fatty acids, usually synthesized by Mycobacteria, is another hypothesis that could be considered. In the first hypothesis, some methyl or methylene substituents or oxygenated functions are added to the double bonds of an unsaturated precursor, whereas in the second hypothesis, the methylations could help in the building of very long aliphatic chains, and determine the location of double bonds or ramifications. The hypothetical coexistence of two pathways for mycolate biosynthesis is discussed.—Authors' Abstract

Baughman, R. P., Judson, M. A., Teirstein, A. S., Moller, D. R. and Lower, E. E. Thalidomide for chronic sarcoidosis. Chest **122** (2002) 227–232.

STUDY OBJECTIVES: Thalidomide therapy has been shown to modify granulomatous diseases, such as tuberculosis and leprosy. *Lupus pernio* is a skin manifestation of sarcoidosis that does not remit spontaneously, and was used as a marker of efficacy of thalidomide for sarcoidosis. DESIGN: An open-label, dose-escalation trial of thalidomide. SETTING: Patients were seen at one of four specialized sarcoidosis clinics in the United States. PATIENTS: Fifteen patients with *lupus pernio* and other manifestations of sarcoidosis unresponsive to prior therapy were enrolled. INTERVENTIONS: Skin lesions were assessed with visual examination by the treating physician, and photographic evaluation by a blinded panel of physicians reviewing photographs of the lesions before and after therapy. MEASUREMENTS AND RESULTS: Fourteen patients completed 4 months of therapy. All patients experienced some improvement in their skin lesions subjectively, and 10 of 12 evaluable patients showed improvement using photograph scoring. Five patients were better after 1 month (treated with 50 mg/d of thalidomide), seven more patients improved after 2 months (treated with 100 mg/d of thalidomide in the second month), and two patients required an additional month of 200 mg of thalidomide to achieve a response. Patients reported increased

somnolence (n = 9), numbness (n = 7), dizziness (n = 2), constipation (n = 6), rash (n = 1), and increasing shortness of breath (n = 1). One patient discontinued therapy because of new-onset dyspnea, due to probably unrelated new-onset congestive heart failure. CONCLUSION: Thalidomide was an effective form of treatment for chronic cutaneous sarcoidosis. The drug was well-tolerated and may be a useful alternative to systemic corticosteroids.—Authors' Abstract

Bowcock, S. J., Rassam, S. M., Ward, S. M., Turner, J. T. and Laffan, M. Thromboembolism in patients on thalidomide for myeloma. Hematol. **7** (2002) 51–53.

Seven cases of thromboembolism were found among 23 patients treated with thalidomide for myeloma over a total of 141.5 patient treatment months. Five thromboembolic events were venous (two severe) and two arterial. A historical control of 18 similar patients not given thalidomide had one thromboembolism over 289 months. We found no underlying thrombophilic tendency in the affected patients. We suggest that the thalidomide may predispose to thromboembolism at even lower doses than previously reported (mean dose 150 mg). The two most severe thromboses occurred on 100 mg thalidomide alone, not associated with chemotherapy or glucocorticoids. We raise the possibility that arterial thromboembolism may also occur in association with thalidomide. Some patients continued thalidomide after the event, together with warfarin, with no further thromboembolism.—Authors' Abstract

Chackerian, A. A., Alt, J. M., Perera, T. V., Dascher, C. C. and Behar, S. M. Dissemination of *Mycobacterium tuberculosis* is influenced by host factors and precedes the intitiation of T-cell immunity. Infect. Immun. **70** (2002) 4501–4509.

We report that dissemination of *Mycobacterium tuberculosis* in the mouse is under host control and precedes the initiation of T-cell immunity. Nine to eleven days after aerosol inoculation, *M. tuberculosis* disseminates to the pulmonary lymph nodes (LN), where *M. tuberculosis*-specific T cells are detected 2 to 3 days thereafter. This indicates that the initial spread of bacteria occurs via lymphatic drainage and that the acquired T-cell immune response is generated in the draining LN. Dissemination to peripheral sites, such as the spleen and the liver, occurs 11 to 14 days postinfection and is followed by the appearance of *M. tuberculosis*-specific T cells in the lung and the spleen. In all cases studied, dissemination to the LN or the spleen preceded activation of *M. tuberculosis*-specific T cells in that organ. Interestingly, bacteria disseminate earlier from the lungs of resistant C57BL/6 mice than from the lungs of susceptible C3H mice, and consequently, C57BL/6 mice generate an immune response to *M. tuberculosis* sooner than C3H mice generate an immune response. Thus, instead of spreading infection, early dissemination of *M. tuberculosis* may aid in the initiation of an appropriate and timely immune response. We hypothesize that this early initiation of immunity following inoculation with *M. tuberculosis* may contribute to the superior resistance of C57BL/6 mice.—Authors' Abstract

Chang, C. T., Wang, L. Y., Liao, C. Y. and Huang, S. P. Identification of nontuberculosis mycobacteria existing in tap water by PCR-restriction fragment length polymorphism. Appl. Environ. Microbiol. **68** (2002) 3159–3161.

This paper presents the finding of the possible cause of the high false-positive rate in acid-fast staining in histological examinations. Using acid-fast staining, culture, and PCR, acid-fast bacilli were detected in 83.7% of 49 hospital tap water samples and nontuberculous mycobacteria (NTM) were detected in 20.4%% of the same 49 samples. The 10 NTM isolates were also identified to the species level using PCR-restriction fragment length polymorphism. Our findings indicate that NTM in hospital tap water are the possible cause of false positives in acid-fast staining and of nosocomial infection in immunocompromised patients.—Authors' Abstract

Duong, D. J., Moxley, R. T. 3rd, Kellman, R. M., Pincus, S. H. and Gaspari, A. A. Thalidomide therapy for cicatricial pemphigoid. J. Am. Acad. Dermatol. **47** (2002) S193–S195.

Cicatricial pemphigoid is a chronic, presumed autoimmune, blistering disease of the mucous membranes and occasionally of the skin. The characteristic feature is scarring at the sites of healing. We report the use of thalidomide as a therapeutic agent to control previously resistant disease.—Authors' Abstract

Ginsberg, A. M. What's new in tuberculosis vaccines? Bull. World Health Organ. **80** (2002) 483–488.

Over the past 10 years, tuberculosis (TB) vaccine development has resurged as an active area of investigation. The renewed interest has been stimulated by the recognition that, although BCG is delivered to approximately 90% of all neonates globally through the Expanded Programme on Immunization, *Mycobacterium tuberculosis* continues to cause over 8 million new cases of TB and over 2 million deaths annually. Over one hundred TB vaccine candidates have been developed, using different approaches to inducing protective immunity. Candidate vaccines are typically screened in small animal models of primary TB disease for their ability to protect against a virulent strain of *M. tuberculosis.* The most promising are now beginning to enter human safety trials, marking real progress in this field for the first time in 80 years.—Author's Abstract

Hou, J. Y., Graham, J. E. and Clark-Curtiss, J. E. *Mycobacterium avium* genes expressed during growth in human macrophages detected by selective capture of transcribed sequences (SCOTS). Infect. Immun. **70** (2002) 3714–3726.

Selective capture of transcribed sequences (SCOTS) has been employed to identify 54 cDNA molecules that represent 46 genes that are expressed by *Mycobacteriumm avium* during growth in human macrophages. Some cDNA molecules correspond to genes that are apparently expressed 48 hr after infection of macrophages, while others correspond to genes expressed 110 hr after infection, and still others correspond to genes expressed throughout the course of infection in our model system. Genes expressed by *M. avium* during growth in macrophages include genes encoding enzymes of several biosynthetic pathways (pyrimidines, mycobactin, and polyketides); genes that encode enzymes involved in intermediary metabolism, energy metabolism (tricarboxylic acid cycle, glyoxalate shunt), and nitrogen metabolism; and genes that encode regulatory proteins. A number of genes of unknown function were also identified, including genes that code for proteins similar to members of the PPE family of proteins of *Mycobacterium tuberculosis* and proteins similar to those encoded by the *M. tuberculosis* mice genes, which have been previously associated with mycobacterial virulence. The SCOTS technique, followed by enrichment for cDNA molecules that are up-regulated or are uniquely expressed by *M. avium* during growth in human macrophages (compared to growth in laboratory broth culture), allows recovery and identification of a greater diversity of cDNA molecules than does subtractive hybridization between cDNA mixtures from macrophage-grown and broth-grown *M. avium.* Data are presented demonstrating the reproducibility of recovery of a subset of cDNA molecules from cDNA mixtures purified by SCOTS on several different occasions. These results further demonstrate the beneficial utility of the SCOTS technique for identifying genes whose products are needed for successful survival and growth by an organism in a specific environment.—Authors' Abstract

Kisich, K. O., Higgins, M., Diamond, G. and Heifets, L. Tumor necrosis factor alpha stimulates killing of *Mycobacterium tuberculosis* by human neutrophils. Infect. Immun. **70** (2002) 4591–4599.

The ability of human neutrophils to aid in defense against pulmonary infection with *Mycobacterium tuberculosis* is controversial. In this study, we have shown that neu-

trophils respond to and phagocytose *M. tuberculosis* in human lesions. Neutrophils from healthy individuals were able to kill significant fractions of an inoculum of *M. tuberculosis* within 1 hr of phagocytosis, and this ability was enhanced by tumor necrosis factor alpha but not by gamma interferon. The mycobactericidal mechanism was nonoxidative, as inhibitors of reactive oxygen or reactive nitrogen intermediates did not interfere with killing. However, the mycobactericidal mechanism was associated with increased exposure of intracellular *M. tuberculosis* to neutrophil defensins. *In vitro,* human neutrophil peptides 1 to 3 were not able to kill the bacilli even at much higher levels. These studies support the concept that human neutrophils are directly involved in defense against infection with *M. tuberculosis.*—Authors' Abstract

Kremer, L., Dover, L. G., Carrere, S., Nampoothiri, K. M., Lesjean, S., Brown, A. K., Breenan, P. J., Minnikin, D. E., Locht, C. and Besra, G. S. Mycolic acid biosynthesis and enzymic characterization of the beta-ketoacyl-ACP synthase A-condensing enzyme from *Mycobacterium tuberculosis.* Biochem. J. **364** (2002) 423–430.

Mycolic acids consist of long-chain alpha-alkyl-beta-hydroxy fatty acids that are produced by successive rounds of elongation catalyzed by a type II fatty acid synthase (FAS-II). A key feature in the elongation process is the condensation of a two-carbon unit from malonyl-acyl-carrier protein (ACP) to a growing acyl-ACP chain catalyzed by a beta-ketoacyl-ACP synthase (Kas). In the present study, we provide evidence that kasA from *Mycobacterium tuberculosis* encodes an enzyme that elongates *in vivo* the meromycolate chain, in both *Mycobacterium smegmatis* and *Mycobacterium chelonae.* We demonstrate that KasA belongs to the FAS-II system, which utilizes primarily palmitoyl-ACP rather than short-chain acyl-ACP primers. Furthermore, in an *in vitro* condensing assay using purified recombinant KasA, palmitoyl-AcpM and malonyl-AcpM, KasA was found to express Kas activity. Also, mutated KasA proteins, with mutation of Cys(171), His(311), Lys(340) and His(345) to Ala abrogated the condensation activity of KasA *in vitro* completely. Finally, purified KasA was highly sensitive to cerulenin, a well-known inhibitor of Kas, which may lead to the development of novel anti-mycobacterial drugs targeting KasA.—Authors' Abstract

Mitsiades, N., Mitsiades, C. S., Poulaki, V., Chauhan, D., Richardson, P. G., Hideshima, T., Munshi, N. C., Treon, S. P. and Anderson, K. C. Apoptotic signaling induced by immunomodulatory thalidomide analogs in human multiple myeloma cells: therapeutic implications. Blood **99** (2002) 4525–4530.

Thalidomide (Thal) achieves responses even in the setting of refractory multiple myeloma (MM). Although increased angiogenesis in MM bone marrow and the antiangiogenic effect of Thal formed the empiric basis for its use in MM, we have shown that Thal and its immunomodulatory analogs (IMiDs) directly induce apoptosis or growth arrest of MM cells, alter adhesion of MM cells to bone marrow stromal cells, inhibit the production of cytokines (interleukin-6 and vascular endothelial growth factor) in bone marrow, and stimulate natural killer cell anti-MM immunity. In the present study, we demonstrate that the IMiDs trigger activation of caspase-8, enhance MM cell sensitivity to Fas-induced apoptosis, and down-regulate nuclear factor (NF)-kappaB activity as well as expression of cellular inhibitor of apoptosis protein-2 and FLICE inhibitory protein. IMiDs also block the stimulatory effect of insulin-like growth factor-1 on NF-kappaB activity and potentiate the activity of TNF-related apoptosis-inducing ligand (TRAIL/Apo2L), dexamethasone, and proteasome inhibitor (PS-341) therapy. These studies both delineate the mechanism of action of IMiDs against MM cells *in vitro* and form the basis for clinical trials of these agents, alone and coupled with conventional and other novel therapies, to improve outcome in MM.—Authors' Abstract

Richardson, P., Hideshima, T. and Anderson, K. Thalidomide in multiple

myeloma. Biomed. Pharmacother. **56** (2002) 115–128.

Thalidomide—removed from widespread clinical use by 1962 because of severe teratogenicity—has anti-angiogenic and immunomodulatory effects, including the inhibition of TNF-alpha. It has returned to practice as an effective oral agent in the management of various disease states including erythema nodosum leprosum, for which it was FDA-approved in 1998, and more recently certain malignancies, including multiple myeloma. While the mechanism of action of thalidomide remains incompletely understood, considerable insight has been generated by extensive preclinical studies in multiple myeloma. Moreover, clinical trials both as a single agent and in combination have confirmed benefit in relapsed and refractory disease. Thalidomide's role in treating newly diagnosed patients is currently under study and it is now established as an important therapeutic option in the treatment of multiple myeloma.—Authors' Abstract

Russel, F., Starr, M., Hayman, J., Curtis, N. and Johnson, P. *Mycobacterium ulcerans* infection diagnosed by polymerase chain reaction. J. Paediatr. Child Health **38** (2002) 311–313.

Mycobacterium ulcerans infection is the third most important mycobacterial infection world-wide affecting immunocompetent individuals and causes chronic progressive skin ulcers. It has been described in many different regions world-wide. The diagnosis of *M. ulcerans* infection is often delayed because the diagnosis is difficult to make when cases appear outside known endemic areas. However, molecular methods are now available to diagnose and distinguish *M. ulcerans* from other mycobacteria, allowing rapid diagnosis. Presented here is the case of a previously well girl from Townsville, Queensland, with extensive *M. ulcerans* infection involving the elbow joint, triceps tendon and underlying bone. Rapid diagnosis by polymerase chain reaction confirmed *M. ulcerans* infection. This is the first known case of *M. ulcerans* infection from Townsville in over 25 years, highlighting the changing epidemiology of this disease.—Authors' Abstract

Schaller, A., Sun, Z., Yang, Y., Somoskovi, A. and Zhang, Y. Salicylate reduces susceptibility of *Mycobacterium tuberculosis* to multiple antituberculosis drugs. Antimicrob. Agents Chemother. **46** (2002) 2626–2639.

Salicylate induces multiple antibiotic resistance in various bacterial species. Here we investigated the effect of salicylate on the susceptibility of *Mycobacterium tuberculosis* to a range of antituberculosis (anti-TB) drugs. In the presence of salicylate, the killing effects of isoniazid (INH), rifampin (RMP), ethambutol (EMB), streptomycin (STR), and p-aminosalicylate (PAS) were reduced, as shown with a tetrazolium redox dye viability assay and a bacterial survival assay. Salicylate-induced resistance was more pronounced for PAS, STR, and EMB but was not apparent for INH and RMP when salicylate and the anti-TB agents were incorporated into 7H11 plates. The significance of these findings for TB treatment needs to be further evaluated *in vivo*.—Authors' Abstract

Triccas, J. A., Sun, L., Palendira, U. and Britton, W. J. Comparative affects of plasmid-encoded interleukin 12 and interleukin 18 on the protective efficacy of DNA vaccination against *Mycobacterium tuberculosis*. Immunol. Cell Biol. **80** (2002) 346–350.

Protective immunity against *Mycobacterium tuberculosis* infection requires the induction and maintenance of mycobacteria-specific, IFN-gamma-secreting CD4+ and CD8+ T lymphocytes. The development of Th1-like T cells is promoted by the early secretion and synergistic action of interleukin (IL)-12 and IL-18. This study compares the effects of plasmid-encoded IL-12 and IL-18 on the immunogenicity and protective efficacy of a DNA vaccine expressing the *M. tuberculosis*-secreted protein antigen 85B (DNA-85B). Co-immunization with either IL-12- or IL-18-expressing plasmids augmented the IFN-gamma-secreting

T-cell response, and the maximum effect was observed with plasmids encoding both cytokines. Further the IL-12, but not the IL-18-expressing plasmid, significantly increased the protective efficacy of DNA-85B against pulmonary *M. tuberculosis* infection. Therefore co-administration of plasmid-encoded cytokines provides a potential method for optimizing the protective efficacy of DNA vaccination against tuberculosis.—Authors' Abstract

Von Reyn, C. F. and Vuola, J. M. New vaccines for the prevention of tuberculosis. Clin. Infect. Dis. **35** (2002) 465–474.

Mycobacterium bovis, bacille Calmette-Guerin (BCG) is administered widely to newborns throughout the world and has been shown to be effective in preventing childhood tuberculosis but not reactivation pulmonary disease or human immunodeficiency virus-associated tuberculosis. Development of a more effective, better standardized, affordable vaccine with durable activity and fewer side effects is a major priority. Contemporary molecular techniques have identified promising immunodominant antigens and novel immunization strategies. Vaccine development has also been informed by an improved understanding of the role of nontuberculous mycobacteria in the efficacy of BCG and in the prevention of tuberculosis. Vaccines under investigation include attenuated or enhanced whole-cell live, whole-cell inactivated, subunit, DNA, and prime-boost vaccines. Several candidate vaccines have demonstrated activity in animal models that is equal to or superior to that of BCG, and human trials are under way. Because there is no identified surrogate marker for protection, identification of an improved vaccine will require long-term efficacy trials in humans.—Authors' Abstract