INTERNATIONAL JOURNAL OF LEPROSY

Volume 66, Number 3
Printed in the U.S.A.
(ISSN 0148-916X)

THIRTY-THIRD U.S.-JAPAN TUBERCULOSIS AND LEPROSY RESEARCH CONFERENCE

KKR Hotel
Osaka, Japan
8–10 July 1998

sponsored by the
U.S.-Japan Cooperative Medical Science Program
National Institute of Allergy and Infectious Diseases
National Institutes of Health

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THIRTY-THIRD U.S.-JAPAN TUBERCULOSIS-LEPROSY RESEARCH CONFERENCE

The 33rd Research Conference on Tuberculosis and Leprosy of the U.S.–Japan Cooperative Medical Sciences Program was held at the KKR Hotel, Osaka, Japan, 8–10 July 1998. It was organized by Dr. Izuo Tsuyuguchi of Osaka Prefectural Habikino Hospital. Present were members of the Japanese (Drs. I. Tsuyuguchi, T. Yamada, C. Abe, K. Kobayashi, Y. Fukutomi, and M. Mitsuyama) and U.S. (Drs. P. J. Brennan, J. J. Ellner, T. P. Gillis, G. Kaplan, P. C. Hopewell, and D. N. McMurray) panels. Also present was Dr. Ann Ginsberg, Tuberculosis and Leprosy Program Officer, NIAID, NIH, and several of the U.S.–Japan CMSP Delegates (Drs. T. Shimao, S. Someya, and G. H. Cassell) and invitees and presenters from the U.S. and Japan.

Dr. Tsuyuguchi opened the three-day meeting and Dr. Brennan made closing comments. The abstracts of papers in the area of leprosy research are presented below. Abstracts of papers on tuberculosis research will appear in *Tubercle and Lung Disease.*

ABSTRACTS

Fukutomi, Y., Toratani, S., Matsuki, G. and Matsuoka, M. Mechanisms of nitric oxide production by macrophages that express anti-*M. leprae* response.

We previously reported that tumor necrosis factor (TNF) released by macrophages following the phagocytosis of *Mycobacterium leprae* was indispensable for the induction of anti-*M. leprae* response of the macrophages themselves as a second signal with gamma interferon (IFN-γ) priming. The extent of the response was in proportion to the production level of nitric oxide (NO). In this study we investigated further the mechanisms of NO production in cytokine-stimulated macrophages. Mouse peritoneal macrophages were cultured in the presence of IFN-γ and *M. leprae*. Supernatants were collected to measure the amount of NO. A significant increase in NO was observed after 24 and 48 hr, compared with little or no significant elevation of NO in supernatants of macrophages cultured with *M. leprae* or IFN-γ only. Interestingly, IFN-γ, *M. leprae*, and IFN-γ and *M. leprae* treatment all induced the inducible nitric oxide synthase (iNOS) mRNA expression. Moreover, iNOS protein was detected in macrophages cultured with only IFN-γ in addition to IFN-γ and *M. leprae* treatment. Thus, IFN-γ stimulation induced iNOS protein in macrophages, although it was still insufficient to obtain NO production. Processing and modification of the protein could be necessary to acquire its enzyme activity for the expression of anti-*M. leprae* response. TNF, as a second signal, could participate in the processing and modification mechanisms.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Goto, M., Matsuoka, M. and Kitajima, S. Animal model of leprous neuritis; comparison of transfer of CD4 and CD8 lymphocytes into *M. leprae*-inoculated nude mice.

In order to establish animal models of leprous neuritis and analyze its pathogenesis, *Mycobacterium leprae*-inoculated BALB/C-nu/nu mice were challenged with *M. leprae*-immunized BALB/C mouse whole spleen cells, spleen-derived CD4+ cells, or spleen-derived CD8+ cells. The results were compared with a naive BALB/C mouse spleen-derived cell challenge model. Following cell transfer, nude mouse foot pads showed increased swelling. Histological examination of foot pad granuloma showed a degree of inflammatory cell infiltration; whole spleen cell transfer > CD4+ cell transfer ≥ CD8+ cell transfer > non-transfer mice, and immunized mouse cell transfer > nonimmunized mouse cell transfer. In the nerve fascicles, infiltration of inflammatory cells and a decrease of myelinated fibers was noted in immunized whole spleen cell transfer mice. These results suggest that not only CD4+ lymphocytes but also CD8+ cells may play an important role in the reversal reaction and experimental leprous neuritis.—[National Hansen's Disease Sanatorium Hoshizuka-Keiai-En, Kagoshima, Japan; Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Hagge, D., Spring, L., Gillis, T. P. and Williams, D. L. Effects of *M. leprae* infection on gene expression in cultured Schwann cells.

The effects of *Mycobacterium leprae* infection on Schwann cell gene expression were analyzed using a rat Schwannoma cell line (33B), purified mouse foot pad-derived *M. leprae*, reverse transcription/polymerase chain reaction (RT/PCR) and differential display (RNA fingerprinting). RT/PCR analysis of gene transcripts encoding the myelin proteins and associated proteins demonstrated that peripheral myelin protein, neural cell adhesion molecule, glial fibrillary acidic protein and transforming growth factor-β1 were present in significant amounts in 33B cells. There was no detectable difference in the expression of these gene transcripts in 33B cells following 24 or 48 hr infection with *M. leprae*. Myelin basic protein was not expressed in

33B cells but was expressed in total RNA obtained from the sciatic nerves from 17-day-old rats. Differential display analysis demonstrated that several gene transcripts were differentially transcribed in *M. leprae*-infected 33B cells. These genes have not yet been identified. Our results suggest that *M. leprae* infection alters the expression of some Schwannoma cell genes as early as 24 hr postinfection. There does not appear to be a direct affect on the expression of myelin protein genes and myelin-associated protein genes up to 48 hr postinfection in the 33B cell model, and myelin basic protein (a major myelin protein) is not produced in these cells. Therefore, this model has limited use for the study of *M. leprae* effects on myelin protein gene expression and regulation. In addition, since 33B cells replicate every 20 hr, only early events of *M. leprae* infection can be studied.—[Biological Sciences Department, Louisiana State University, Baton Rouge, LA, U.S.A.; Molecular Biology Research Department, Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Izumi, S., Matsuoka, M., Budiawan, T., Nakata, N. and Saeki, K. An epidemiological study of *M. leprae* infection and prevalence of leprosy in an endemic district of North Maluku, Indonesia.

One of the most important unsolved problems in the epidemiology of leprosy is the heterogeneous geographic distribution of the disease. There are many highly endemic districts known as "hot spots" in endemic countries. We conducted an epidemiological survey to ascertain the reasons why leprosy is so common in such areas using serological and molecular biological techniques. It was found that approximately one third of the general inhabitants of hot spots are seropositive to the leprosy-specific phenolic glycolipid-I antigen, and a considerable number of the villagers carry the leprosy bacillus on the surface of their nasal cavities, suggesting that they inhale leprosy bacilli floating in the air with dust particles. It was also found that 13 out of 27 water samples from the sources in the villages contained leprosy bacilli, and the users of contaminated water for bathing and washing have more chances to get the disease. New tools will be needed for achieving global elimination of leprosy as a public health problem.—[National Leprosarium Oshima Seisho-En, Aji-cho, Kagawa, Japan; Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan; Ternate Leprosy Hospital, Ternate, North Maluku, Indonesia]

Job, C. K., Jayakumar, J., Williams, D. L. and Gillis, T. P. Role of polymerase chain reaction in the diagnosis of early leprosy.

Definitive diagnosis of paucibacillary (PB) leprosy continues to be a major obstacle in early diagnosis, confounding management of this chronic mycobacterial disease. Early lesions in leprosy are often characterized histologically by nonspecific inflammation of the dermis with no acid-fast bacilli (AFB). We have developed and report here the use of a sensitive and specific polymerase chain reaction (PCR) assay for *Mycobacterium leprae* which complements routine methods for diagnosing early leprosy. Thirty-nine skin lesions were biopsied for evaluation of suspected early leprosy by histopathology and PCR. The diagnosis of leprosy was made clinically in 14 patients and in another 12 patients by histopathologic exam. Whereas all suspect patients were smear negative and only 2 showed AFB in histopathologic exam, *M. leprae* DNA was detected in 9 specimens by PCR. Of 13 biopsies showing histological features consistent with nonspecific chronic inflammation, 2 tested positive by PCR; 1 of these patients was diagnosed with early PB leprosy on a follow-up biopsy. These results will be discussed with relationship to implementing PCR for *M. leprae* as an adjunct test in diagnostic methods for PB leprosy.—[St. Thomas Hospital and Leprosy Center, Chettupattu, India; Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Kaplan, G. Role of cytokines in the pathogenesis of ENL.

Erythema nodosum leprosum (ENL) is a serious, debilitating complication of leprosy. This inflammatory reaction occurs in approximately 40%–50% of lepromatous leprosy patients, usually while undergoing treatment with antileprosy drugs. These lesions are not necessarily associated with the pre-existing leprosy lesions. Systemic manifestations of ENL include fever, malaise, lymphadenopathy, arthralgia and progressive nerve damage. The addition of thalidomide (α-phthalimido-glutarimide) to the anti-mycobacterial drug regimen results in diminished clinical symptoms and clearance of the ENL skin lesions. We and others have subsequently shown that the efficacy of thalidomide in ENL is associated with a rapid reduction in the circulating level of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α). In addition, we have demonstrated that thalidomide exerts a specific inhibitory effect on the production of TNF-α by lipopolysaccharide-stimulated monocytes *in vitro*. These results suggested that inhibition of this cytokine is a possible mechanism for the effects of thalidomide therapy in ENL.

There has been much interest in the possibility that thalidomide might not only affect monocyte cytokine production, but also may influence the functions of T cells. We, therefore, examined the effects of thalidomide on primary human T-cell activation *in vitro*. Initially, we studied the effects of thalidomide on TNF-α production by T cells, but found no effect of the drug. Unexpectedly, when purified T cells were stimulated by antibodies to CD3, a component of the T-cell receptor (TCR) complex, we found that thalidomide could provide an essential co-stimulatory signal necessary for T-cell proliferation and lymphokine production. Treatment of T cells with thalidomide results in increases in production of the TH1 cytokines IL-2, IFN-γ and IL-12. The CD8+ subset of T cells was more responsive to the co-stimulatory effect of thalidomide. The drug also increased the primary CD8+ cytotoxic T-cell response which is induced by allogeneic dendritic cells in the absence of CD4+ T cells. In our future studies, we plan to examine whether thalidomide has a stimulatory effect on the T cells of patients undergoing treatment for ENL.

Our present approach focuses on examining our hypothesis that thalidomide may modulate the host response to mycobacterial infection not only by inhibiting TNF-α production but also by stimulating T cells to produce more of the TH1 type cytokines IL-2, IFN-γ and IL-12. Thus, the effectiveness of the drug in ENL would result from inhibition of TNF-α production and concomitant stimulation of T cells to produce TH1 cytokines and stimulate the host immune response to clear *M. leprae* more efficiently. These possibilities are currently under investigation.—[The Rockefeller University, New York, NY, U.S.A.]

Kobayashi, K., Gidoh, M., Kai, M., Singh, R. P., Hashimoto, K. and Saito, H. Rational basis of interleukin-12 immunotherapy for established mycobacterial infection in mice.

Cell-mediated immunity participates in the host defense against mycobacterial infection. Both interleukin-12 (IL-12) and interferon-gamma-inducing factor (IGIF/IL-18), produced mainly by macrophages, play a critical role in expression of cell-mediated immunity. Deficiency of IL-12, IGIF/IL-18, and gamma interferon has been found in susceptible mice infected with mycobacteria. Both strains of mice did not show expression of IL-4. Replacement IL-12 therapy resulted in the reduction of bacterial burdens in mice with established mycobacterial infection. In addition, the combination of antimicrobial chemotherapy and immunotherapy provided more effective anti-infective therapy than either alone. Obviously, the host and infective organisms are therapeutic targets in infectious disease. It is reasonable to assume that the most rational way to eradicate infectious diseases is by combination therapy including immune-based intervention and pathogen-specific chemotherapy.—[Departments of Host Defenses and Microbiology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan; Shimane Medical School, Izumo, Japan]

Moody, D. B., Guy, M. R., Muhlecker, W., Besra, G. S. and Porcelli, S. A. Fine structural requirements for T-cell recog-

nition of glucose monomycolate presented by human CD1b.

Mycobacteria-specific T cells are well known to recognize peptide antigens bound in the groove of MHC-encoded antigen-presentation molecules. Recently, we have demonstrated that human CD1 proteins mediate T-cell recognition of mycobacterial lipid antigens. These observations expand the dogma that T cells respond solely to peptide antigens, and reveal a fundamentally new pathway for immune recognition of mycobacteria. Here we report that CD1b-restricted T cells isolated from a donor exposed to *Mycobacterium leprae* show fine specificity for each structural component of a model glycolipid antigen, glucose monomycolate (GMM). The structure of the carbohydrate, carbohydrate linkage, mycolate stereochemistry and acyl chain length all affected T-cell recognition. These results provide further support for the molecular model of presentation of lipid antigens such as GMM and mycolic acids in which the branched acyl chain lies within the hydrophobic groove of CD1b, resulting in presentation of the hydrophilic cap of the lipid for specific interactions with the T-cell receptor. Furthermore, T cells did not recognize analogs lacking any single feature of the natural structure of GMM. This supports the hypothesis that these human T cells arose as a result of *in vivo* immunization of GMM, and strengthens the hypothesis that CD1b-restricted T cells participate in the human immune response to mycobacteria.—[Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, U.S.A.; Department of Microbiology, Colorado State University, Fort Collins, CO, U.S.A.; Mass Spectroscopy Resource, Boston University School of Medicine, Boston, MA, U.S.A.]

Naito, M. and Yamada, T. Biological activities of α-antigen; humoral immunity and fibronectin binding.

Sera from leprosy and tuberculosis patients were examined for their reactivities against the proteins in the culture filtrate (CF) of BCG separated by two-dimensional electrophoresis (2-DE). The sera were obtained from patients who were untreated. *Mycobacterium leprae* antigens that are analogous to BCG Ag85 (α-antigen) and MPB51 have been suggested as the main targets for humoral immunity in untreated patients. The reactivities of sera with newly identified antigens may provide the potential of predicting the severity and the progress of diseases. We further studied Ag85 for the ability to bind to human fibronectin. Two fibronectin-binding epitopes were defined on 27 amino acids from 84 to 110 and 20 amino acids from 211–230. Binding inhibition of fibronectin to intact α-antigen molecules was observed with peptide 84–110 but not with peptide 211–230. The peptide 84–110 also could inhibit binding of fibronectin to all components of the Ag85 complex of BCG. Further study with synthetic peptides defined 11 residues from 98 to 108 as the minimum motif. The motif revealed no homology with other known prokaryotic and eukaryotic fibronectin-binding proteins. The defined motif of α-antigen is novel and unique for mycobacteria.—[Nagasaki University School of Dentistry, Nagasaki, Japan]

Ohyama H., Matsushita, S., Hatano, K., Kato, N., Oyaizu, K., Makino, M., Nishimura, F., Takashiba, S. and Murayama, Y. T-Cell responses to major membrane protein II (MMP II) derived from *M. leprae* in leprosy patients.

Major membrane protein II (MMP II) is one of the major membrane protein species derived from *Mycobacterium leprae*. We have hypothesized that immune responsiveness to MMP II might reflect the immune system to *M. leprae* in leprosy patients since all leprosy patients have been reported to show a higher IgG titer to this protein than healthy subjects. The objective of this study was to evaluate the T-cell responses against MMP II, including recognition sites and lymphokine production patterns. We have established T-cell lines showing specific proliferative responses against MMP II from TT and LL patients, and evaluated T-cell responses to MMP. We found that: a) There were no differences between LL and TT patients in the T-cell epi-

topes of their own T-cell lines. b) Many T-cell lines specific to MMP II were restricted by HLA-DRB1 molecules in their proliferative responses. c) All of the T-cell lines induced gamma interferon except one line from a LL patient. As for type 2 lymphokines, many T-cell lines induced IL-5 but not IL-4. d) The T-cell line not inducing any lymphokines from the LL patient showed IL-5 production when cultured with sufficient amounts of anti-HLA-DQ and -DP monoclonal antibodies.

We were not able to distinguish the LL or TT type of leprosy based on the pattern of T-cell epitopes and lymphokine production in the responses against MMP II. However, there was one patient whose T cells showed unique responses against MMP II. These T-cell responses might be involved in the immunological features of leprosy. These observations raised the necessity to analyze individual T-cell responses against MMP II.—[National Leprosarium Oku Komyo-En, Okayama, Japan; Department of Periodontology and Endodontology, Okayama University Dental School, Okayama, Japan; Division of Immunogenetics, Department of Neuroscience and Immunology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan]

Okamura, H. and Nomaguchi, H. Roles of IL-18 and IL-12 in the defense mechanism against bacterial infection.

Interleukin-18 (IL-18), a cytokine produced by activated macrophages, exhibits antimicrobial action against various microbes, such as *Cryptococcus neoformans, Yersinia enterocolitica,* and *Plasmodium berghei.* In the present study, the effect of IL-18 on the defense mechanism against bacteria using *Listeria monocytogenes* was studied. While low doses of IL-18 or IL-12 failed to inhibit the growth and survival of this bacterium both *in vivo* and *in vitro,* the combination of these cytokines exerted a strong inhibitory effect on the growth. This effect was dependent upon gamma interferon (IFN-γ) and nitric oxide (NO) synthesis. It was also shown that IL-18 is able to induce NO production in macrophages in the presence of IFN-γ. Thus, IL-18 together with IL-12 play important roles against infection. However, repeated administration of IL-18 and IL-12 to mice caused a serious multiple organ disorder, indicating that IL-18 is a double-edged sword.—[Laboratory of Host Defenses, Institute for Advanced Medical Science, Hyogo College of Medicine, Nishinomiya, Japan; Institute for Leprosy Research, Tokyo, Japan]

Rambukkana, A., Yamada, H., Zanazzi, G., Salzer, J. L., Yurchenco, P. D., Campbell, K. P. and Fischetti, V. A. Molecular mechanism of neural targeting of *M. leprae*: role of the G domain of laminin-2 and its receptor α-dystroglycan.

Nerve damage is the hallmark of leprosy which results from *Mycobacterium leprae* invasion of the Schwann cell of the peripheral nerve. Although multidrug therapy is effective in bacteriological cure, it does not reverse nerve function loss in leprosy patients. There are about 3 million leprosy patients worldwide with significant disabilities and deformities as a result of nerve damage. Therefore, understanding the molecular basis of *M. leprae*-peripheral nerve interaction offers new insights toward the events leading to nerve damage. We previously reported that the neural tropism of *M. leprae* is due to its binding to the G domain of the α2 chain of the laminin-2 isoform (LN-α2G) in the basal lamina surrounding the Schwann cell-axon unit. Here we investigate Schwann cell receptors that mediate *M. leprae* interaction and invasion of peripheral nerves. *In vivo*, laminin-2 in the endoneural basal lamina anchors to Schwann cells via membrane laminin receptors. α-Dystroglycan is a novel laminin receptor and a central component of the dystrophin-glycoprotein complex which plays a critical role in a variety of muscular dystrophies. Using a bacterial adherence assay with immobilized native α-dystroglycan purified from peripheral nerves, we identified that the recombinant (r) human LN-α2G domain specifically mediates *M. leprae* binding to α-dystroglycan. By using a cell adhesion and invasion assays with primary Schwann cells purified from sciatic nerves, we demonstrated that native α-dystroglycan competitively inhibits rLN-α2G-

mediated *M. leprae* binding to and invasion of Schwann cells. This suggests that *M. leprae* interact with α-dystroglycan on the Schwann cell membrane via a LN-α2G bridge, and both LN-α2G and α-dystroglycan are sufficient for bacterial invasion of Schwann cells. These data indicate a mechanism by which *M. leprae* invade peripheral nerves, and raise the possibilities of new therapeutic strategies to interrupt *M. leprae*-nerve interactions. We also describe a novel function of α-dystroglycan as a receptor for a human pathogen.—[Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, NY, U.S.A.; Howard Hughes Medical Institute Department of Physiology and Biophysics, Department of Neurology, University of Iowa College of Medicine, Iowa City, IA, U.S.A.; Departments of Cell Biology, Neurology and the Kaplan Cancer Center, New York University Medical Center, New York, NY, U.S.A.; Department of Pathology, Robert Wood Johnson Medical School, Piscataway, NJ, U.S.A.]

Suzuki, Y., Katsukawa, C., Tamaru, A., Makino, M. and Matsuoka, M. Immunological characterization of five recombinant *M. leprae* proteins produced in *Escherichia coli.*

We have constructed an overexpression system for the *Mycobacterium leprae* 85 complex in *Escherichia coli.* The inhibition of the multiplication of *M. leprae* by vaccination with the purified recombinant 85 complex and other proteins was examined. We used 100 μg of recombinant proteins. The recombinant proteins used were the ribosomal L7/12, the *M. leprae* homolog of MPB51 (MPL51) and the 85 complex A, B, and C. The multiplication of *M. leprae* after immunization with these recombinant proteins was compared to controls without immunization. Considerable inhibition of growth through immunization with the recombinant proteins was seen when we used the recombinant 85 complex (A, B, C, and MPL51). The most potent individual protein in this experiment was antigen 85B.—[Departments of Pathology and Microbiology, Osaka Prefectural Institute of Public Health, Osaka, Japan; National Leprosarium Oku Komyo-En, Okayama, Japan; Leprosy Research Center, National Institute for Infectious Diseases, Tokyo, Japan]

CLOSING REMARKS

Thoughts on the Future of Leprosy Research in the Post-Elimination Era: Research Priorities and Exploitation of the *M. leprae* Genome

According to recent estimates provided by the World Health Organization (WHO) Action Programme for the Elimination of Leprosy, there were 1,150,000 leprosy sufferers worldwide in 1997, compared to 10–12 million just 14 years ago in 1984. Of these, 890,000 cases are registered and, hence, by definition are under treatment, and the estimated number of patients not detected is, at most, 300,000. Yet, in what appears to be a contradiction, over 570,000 new cases were detected in 1997, a figure that is practically unchanged in 10 years. WHO insists that this figure is not reflective of the incidence of leprosy but is due more to the very vigorous efforts to detect and treat all existing leprosy patients. Although the current prevalence rate in India, as a whole, is about 5.9 cases per 10,000 (553,783 registered cases), Brazil 6.6 (105,744 registered cases), Indonesia 1.7 (33,739 registered cases), Myanmar 4.1, Nepal 5.8, Mozambique 6.1, and Madagascar 2.9 (the total number of cases in some of these latter countries is relatively small), the WHO Action Programme still believes that the goal of 1 case in 10,000 population, even at a subcountry regional level, is achievable. The major factors in these extraordinary developments were, of course, the advent of multiple-drug therapy (MDT) in the 1980s, spearheaded by the old WHO/

TDR/THELEP Programme, the availability of considerable resource from ILEP and, in recent years, political determination and commitment on the part of regional and national governments in the relevant countries. It is now believed that with the huge infectious load drastically diminished, sociological factors, such as those that led to the eradication of leprosy from European and Mid-Eastern countries in the mid-1800s, will intervene and, just in case, the flexible back-up ROM (single dose of rifampin, ofloxacin and minocycline) regimen has been developed and is proving as effective as 6-months' treatment with standard WHO/MDT for paucibacillary leprosy against 1–5 lesion leprosy, i.e., the majority of the *ca.* 600,000 "new cases."

Throughout all of these magnificent developments, basic biological research on leprosy has suffered. Although the successful WHO Action Programme has provided us with a clear research agenda ("accurate case detection; diagnosis of sub-clinical infection; monitoring leprosy in the community and the effects of intervention; research/treatment for pathogenesis/nerve damage"), there has been a massive efflux of basic leprosy researchers to tuberculosis research, and there is a worrisome disappearance of leprosy specialists and specialized leprosy laboratories.

The villains in this massive brain drain are individual researchers and laboratories in that, on the verge of conquering and eliminating a disease of enormous historical proportions, many of us have fled what is believed to be a sinking research ship. Yet, despite the villains, there are heroes who are responsible for the preservation of a modicum of biomedical research on leprosy. Foremost among these must be the Joint Committee of the U.S.–Japan Cooperative Medical Sciences Program, which saved the Leprosy Panel in the context of the joint Tuberculosis and Leprosy Panel, with co-chairs and equal representation from the tuberculosis and leprosy research communities, and thereby provided stability and recognition for leprosy research within the U.S.A. and Japan. Also, the National Institute of Infectious Diseases (Japan) and relevant government agencies should be acknowledged for the incorporation of the old National Institute of Leprosy Research as the Leprosy Research Center within the National Institute of Infectious Diseases, thereby giving leprosy research a broader forum in which to work; and Indian government agencies for their vigorous support of institutions such as JALMA (Agra), Karigiri and Chengalpattu at a time when specialized research/treatment institutions are closing down worldwide. Also, the National Institutes of Health in the U.S.A. for its support of the early genome research on *M. leprae*, for contracts and grants to individual investigators; and the Heiser Program for Research on Leprosy and Tuberculosis and its parent body, the New York Community Trust, for providing funds for completion of the *M. leprae* genome project when all else failed; and Dr. Stewart Cole (Pasteur Institut, Paris) for continuing to sequence the *M. leprae* genome cosmids during the doldrums.

Accordingly, leprosy research is relatively healthy. The area is crucial as we plan for the "post-elimination era" and the problems that may emerge once the campaign of intensive coverage, free drugs and political commitment abates, and once leprosy is fully integrated into general health systems. Good research leadership is now in existence. There is still a solid core of laboratories and centers devoted to fundamental leprosy research, such as the National Hansen's Disease Center at Louisiana State University in the U.S.A.; laboratories at The Rockefeller University, University of California–Los Angeles, Colorado State University; the National Institutes of Infectious Diseases' Leprosy Research Center in Tokyo; Chengalpattu and JALMA in India; centers in Karachi; the Anandaban treatment and research center in Kathmandu; the Armauer Hansen Research Center within ALERT in Addis Ababa; the Institut Pasteur, Paris; the London School of Hygiene and Tropical Medicine; Yonsei University in Seoul; the Royal Institute of Tropical Medicine in Amsterdam; Instituto Oswaldo Cruz, Rio de Janeiro, etc. There is good cooperation among these laboratories and a healthy sharing of resources. And now, as a result of key recent meetings (such as the meetings of the WHO Expert Committee on Leprosy, the WHO Leprosy Elimination Advisory Group, the Joint WHO/ILEP Workshop on Reaching Undetected Lep-

rosy Patients in Endemic Countries, the Sasakawa Memorial Health Foundation/ WHO-sponsored International Workshop on Leprosy Research, the deliberations of the Medical Commission of ILEP, and the recent WHO-sponsored meeting in Addis Ababa on the Future Role of Biomedical Research in Leprosy), a global research plan is emerging that should find full definition at the forthcoming International Leprosy Congress in Beijing, China, in September 1998.

Priorities for leprosy research should be identified in both the short term (the next 5 years) and long term. Even in 10 years' time leprosy is still expected to exist, although perhaps confined to areas where control programs have not yet reached their targets. There is still, therefore, a need to train new people who will contribute to the elimination of leprosy as a public health problem. Our lack of understanding about the transmission of *M. leprae* and whether it has an environmental reservoir means that, even as the number of cases of leprosy decrease, there is a real need for continued surveillance.

New leprosy research is not necessarily required for the immediate leprosy elimination program, but it can and should play an important role in the post-elimination era by providing new field-applicable tools to identify areas or groups at high risk, and in monitoring progress toward the eradication of this debilitating disease. These may include new dipstick assays for antibodies or leprosy antigens, whole blood assays for cellular responses, specific skin-test reagents, and the detection of nasal carriers of *M. leprae* by polymerase chain reaction. The new knowledge available from the *M. leprae* genome will also lead to new insights into why *M. leprae* behaves as it does, with its predilection for nerves, and its capacity to induce anergy in a proportion of those it infects. Understanding why some individuals develop reactions and how these reactions can be controlled will also provide new information on the molecular interactions occurring in immunopathology. Thus, leprosy research still has enormous potential to generate important findings for leprosy. In addition, leprosy research can provide for a wider understanding of immunity and infection. In this respect, the genome sequence will again come to the fore. A comparison of the genomes of *M. tuberculosis* and *M. leprae* will allow us to identify, for the first time, the "minimal gene set" for an obligate intracellular pathogen; a comparison with the genome of *Mycoplasma genitalium* will help in this respect. Moreover, the genes beyond this set, many of which have already been identified as *M. leprae*-specific and devoted to secretory proteins, including glycoproteins and lipoproteins, will provide us with new diagnostic tools. The potential in the exploitation of the *M. leprae* genome is enormous and, in my opinion, will help to again galvanize leprosy research to the forefront.

—Patrick J. Brennan

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