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

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

The 35th Research Conference on Tuberculosis and Leprosy of the U.S.-Japan Cooperative Medical Sciences Program was held at the Holiday Inn Yokohama in Yokohama, Japan, from 19–21 July 2000. Dr. Yasuo Fukutomi and his staff from the Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan, organized the meeting. Further consolidation of the Panels and selection of new chairmen finds the U.S. Tuberculosis-Leprosy Panel comprised of Drs. Clifton E. Barry, III, Thomas P. Gillis, Gilla Kaplan and David N. McMurray. The new U.S. Panel Chairman is Dr. Philip C. Hopewell. Drs. Kazuo Kobayashi, Yasuo Fukutomi, Kiyoshi Takatsu and Hatsumi Taniguchi comprise the Japanese Panel with Dr. Masao Mitsuyama serving as Chairman.

The abstracts of oral presentations in the area of leprosy research are presented alphabetically by first author below. Abstracts of presentations on tuberculosis research will appear in *Tubercle and Lung Disease*.

ABSTRACTS

Adams, L. B., Scollard, D. M., Soileau, N. A., Cooper, A. M. Frank, A. A., Orme, I. M. and Krahenbuhl, J. L. *M. leprae* infection in IFN-γ gene disrupted mice.

Gamma interferon (IFN-γ) is a key cytokine in cell-mediated immunity against intracellular pathogens. To study its role in experimental leprosy, *Mycobacterium leprae* infection was evaluated in IFN-γ knockout (GKO) mice. In BALB/c control mice, growth of the bacilli in the foot pads reached a peak of ~10^5 at 3 months post-infection. In contrast, *M. leprae* continued to multiply in the GKO mice until ~4 months post-infection and then plateaued at ~10^6 bacilli per foot pad. Histopathologically, control mice exhibited mild lymphocytic and histiocytic infiltrates; whereas GKO mice developed large, unorganized infiltrates composed of epithelioid macrophages with small aggregates of lymphocytes. FACS analysis of popliteal lymph node cells demonstrated similar profiles of CD4 and CD8 cells, both of the naive and activated/memory phenotype; however, GKO cells exhibited an elevated proliferative response to *M. leprae* antigen. Peritoneal macrophages from both strains behaved similarly in that normal macrophages supported *M. leprae* viability, but macrophages that were activated *in vitro* inhibited bacterial metabolism and produced nitrite. Although GKO mice were deficient in an important TH1 cytokine, growth of *M. leprae* was not uncontrolled. Thus, GKO mice may reveal interesting compensatory mechanisms toward immune deficiency in experimental leprosy.—[Laboratory Research Branch, National Hansen's Disease Programs at LSU-SVM, Baton Rouge, LA; Mycobacterial Research Laboratories, Colorado State University, Fort Collins, CO, U.S.A.]

Fukutomi, Y., Kimura, H., Torantani, S., Matsuoka, M., and Kobayashi, K. Regulation of cytokine production by prostaglandin E2 in *M. leprae*-stimulated macrophages.

Mycobacterium leprae-stimulated macrophages produced both tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) simultaneously. Prostaglandin E2 (PGE2) suppressed TNF-α production by inhibiting its mRNA expression. Indomethacin, which suppresses PGE2 production, enhanced TNF-α production. On the contrary, IL-10 production was enhanced by PGE2 and was suppressed by indomethacin. IL-12 production could not be observed by *M. leprae* stimulation in contrast to the significant production stimulated by lipopolysaccharide (LPS). These results suggest that in lepromatous leprosy, which manifests TH2 cytokines, PGE2 produced by macrophages in response to a large amount of leprosy bacilli in the local lesion could enhance IL-10 production concomitantly with the suppression of TNF-α production. IL-10 subsequently appears to suppress T-cell production of TH1 cytokines, such as IFN-γ and IL-2.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo; Osaka City University, Osaka, Japan]

Goto, M., Nomoto, M., and Matsuoka, M. Presentation of *M. leprae* DNA in the nervous tissue of cured leprosy.

Pathogenesis of leprous neuropathy is not fully understood. The persistence of *Mycobacterium leprae*-specific antigen in the nervous tissue of clinically cured leprosy has been observed. However, the viability of these bacilli is difficult to evaluate. For this purpose, we tried to amplify *M. leprae*-specific DNA sequence by polymerase chain reaction (PCR) from the nerve tissue. Previously, using *M. leprae*-specific heat shock protein (hsp70) primers we were unable to amplify DNA. This year, we designed a *M. leprae rpoT* gene-specific, short-sequence primer set (122 base pair for Japanese strain), and re-tried the PCR. Among the 9 antigen-positive lepromatous leprosy specimens, 6 were PCR positive, and 3 of 5 antigen-negative tuberculoid leprosy also showed positive PCR. Viability of *M. leprae* in the nervous system is suspected by this study.—[Second Department of Pathology, Faculty of Medicine,

Kagoshima University, Kagoshima; National Hansen's Disease Sanatorium Hoshizuka-Keiai-En, Kagoshima; Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Kashiwabara, Y., Maeda, S., Kai, M., Nakata, N., Maeda, Y., Hashimoto, K., Agdamag, A. T., and Matsuoka, M. Mutations in the genes involved in drug resistance in *M. leprae.*

Molecular biological analysis focused on the mechanisms of drug resistance has revealed mutational changes in some important genes in *Mycobacterium leprae*. To search the emergence of drug-resistant *M. leprae*, the target regions of *fol*P, *rpo*B, *gyr*A and the 23S rRNA genes of clinical isolates of *M. leprae* were amplified and sequenced. Results showed the presence of *M. leprae* harboring mutations in more than two genes, suggesting the emergence of multidrug resistant *M. leprae.* Isolates with mutations in these genes were detected in isolates from endemic countries, suggesting the importance of the surveillance of drug-resistant *M. leprae* for control strategies of leprosy.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan. Dr. Jose N. Rodriguez Memorial Hospital, Kalookan City, The Philippines]

Maeda, S., Kai, M., Maeda, Y., Nakata, N., Hashimoto, K., Brennan, P. J., and Kashiwabara, Y. Phospholipid synthases in *M. leprae.*

Phosphatidylinositol (PI) is one of the important components of the mycobacterial cell envelope because PI has been reported to be an essential prominent phospholipid of mycobacterial membranes and also to function as an anchor of various constituents of the cell wall and membrane. Since little is known about the synthesis of PI, we examined the properties of phosphatidylinositol synthase (PIS) from *Mycobacterium leprae*. The genes with the consensus sequence of phospholipid synthase were selected from the *M. leprae* genome database, and the expression of four kinds of genes was attempted in *M. smegmatis*. PIS activity was detected in the membrane fraction when the gene encoding pgsA1 was expressed.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan. Colorado State University, Fort Collins, CO, U.S.A.]

Martelli, C. M. T., Stefani, M. M. A., Costa, M. B., Gomez, M. K., Rebello, P. F. B., Narahashi, K., Nery, J. A. C., Krahenbuhl, J. L. and Gillis, T. P. Single lesion paucibacillary leprosy: epidemiological and immunological profile of the Brazilian multicenter cohort study.

Baseline clinical and immunological parameters of patients with single skin lesion paucibacillary (SSL-PB) leprosy were examined to determine whether certain characteristics are important in the long-term outcome of disease following single-dose ROM (rifampin-ofloxacin-minocycline) therapy. The patients were classified as TT (33.6%), BT (33.6%) and I (29.7%) according to Ridley-Jopling criteria with 3.1% of the patients considered leprosy by clinical criteria alone. Twelve biopsies were diagnosed as other skin diseases and 7 were considered multibacillary (MB) cases, yielding a total of 19 false-positive SSL-PB, giving a positive predictive value of the clinical screening of 93.2%. The presence of a BCG scar varied from 30% to 62.7% among patients from different sites and 33.2% of the participants had knowledge of a previous leprosy case in the household; 67.1% of the participants reported that the lesion had been perceived within a time span shorter than 1 year. Children (<15 years) had smaller lesions than adults and, independent of the age-group, most skin lesions were described as macular hyperchromic or erythematous lesions (47.9%). Most of the skin lesions were detected on the limbs, and all patients had at least moderate sensory loss on the lesion with no disability. Mitsuda reactions were similar among patients from different geographical regions with 75% of the patients testing positive. Antibody responses to phenolic glycolipid-I were positive in only 17.3% of the patients. Correlations between patients showing signs of complete and incomplete healing during the follow-up period are discussed.—[Federal Universities of Goias

and Rio de Janeiro, Brazil; Alfredo de Matta Foundation, Manaus, Brazil; Secretariat of Health, Rondonia, Brazil; Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. National Hansen's Disease Programs, Laboratory Research Branch, National Hansen's Disease Programs at LSU-SVM, Baton Rouge, LA, U.S.A.]

Matsuoka, M., Kashiwabara, Y., Maeda, S., Kai, M., Nakata, N., Chae, G.-T., Yin, Y.-P., Wu, Q., and Agdamag, A. T. Distribution of *M. leprae* with different genotype in China and Korea.

Mycobacterium leprae isolates were divided into two groups according to the polymorphism of a 6 base tandem repeat in *the rpoT* gene. Genotypes with three 6 base tandem repeats and four 6 base tandem repeats were observed. The distribution of *M. leprae* with a different genotype in China, Korea, The Philippines and Bangladesh was investigated to verify the assumption that the biased distribution of the four copy type isolates in the main islands of Japan is based on migratory trends of people to Japan. All isolates from Bangladesh (N = 11) and The Philippines (N = 24) showed four copies of the 6 base tandem repeat. Among 36 samples from Korea, 26 samples showed four copies of the 6 base tandem repeat. In China the four-copy type was dominant in the northeast district, and most all isolates from the south region were of the three-copy type. The geographical distribution of the different genotypes strongly indicated that the spread of different *M. leprae* genotypes and the movement of various population groups may be related.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan. Institute of Hansen's Disease, Catholic University Medical College, Seoul, Korea. Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, China. Dr. Jose N. Rodriguez Memorial Hospital, Kalookan City, The Philippines]

Ng, V., Zanazzi, G., Salzer, J. A., Timpl, R., Brennan, P. J., and Rambukkana, A. Role of the cell wall phenolic glycolipid-I in the neural predilection of *M. leprae.*

Among the bacterial pathogens, neural predilection is unique to *Mycobacterium leprae*. However, the *M. leprae*-specific components that determine such tropism are unknown. The cell wall of *M. leprae* is endowed with a large amount of phenolic glycolipid-I (PGL-I) with a highly specific trisaccharide unit whose role in the disease pathogenesis has remained elusive. In this study, we determined the role of PGL-I in the neural affinity of *M. leprae*. Using a well-characterized Schwann cell-neuron co-culture system that mimics the *in vivo* situation, we showed that purified PGL-I binds specifically to the native laminin-2, a neural target of *M. leprae*, in the basal lamina that surrounds the Schwann cell-axon units. This binding is competitively inhibited by the synthetic terminal trisaccharide but not the lipid portion of PGL-I, suggesting the crucial role of the trisaccharide moiety in PGL-I-nerve interaction. In a PGL-I overlay assay with the lysates of Schwann cell-neuron co-cultures and laminin-2, we found that PGL-I binds to 80-kDa and ~300-kDa molecular-sized proteins which correspond to the naturally cleaved fragments of the laminin α2 chain. By using individual recombinant G modules (G1, G2, G3, G4 and G5) of the α2 chain, we provided evidence that PGL-I reactivity to 80-kDa and ~300-kDa proteins in nerve cultures is due to the specific binding of PGL-I to G4/G5 and G1 modules, respectively. We propose that the interaction of *M. leprae*-specific PGL-I with tissue-restricted laminin G modules on Schwann cell-axon units may determine the overall neural predilection of this bacterium.—[Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, NY; Department of Cell Biology and Department of Neurology, New York University Medical Center, New York, NY, U.S.A. Department of Protein Chemistry, Max-Plank-Institut Fur Biochemie, Marttinsried, Germany. Department of Microbiology, Colorado State University, Fort Collins, CO, U.S.A.]

Nomaguchi, H., Jahan, N., Yogi, Y., Kawatsu, K., Yoshizawa, Y., and Oka-

mura, H. Bactericidal activity of murine peritoneal cells against *M. leprae* after synergistic stimulation with IL-12 and IL-18.

Interleukin-12 (IL-12) and IL-18 synergistically induced damage to host cells through natural killer cell activation. Also, IL-12 and IL-18 induced bactericidal effects in the host cell. It appeared that gamma interferon, tumor necrosis factor-alpha and nitric oxide were not the main factors inducing host cell damages. The molecular mechanism for the observed damage of host cells infected with *M. leprae* and treated with IL-12 and IL-18 remains to be determined.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo; Saitama Medical Center, Saitama; Hyogo Medical College, Hyogo, Japan]

Ohara, N., Matsuoka, M., Nomaguchi, H., Naito, M., and Yamada, T. Inhibition of multiplication of *M. leprae* in mouse foot pads by recombinant BCG vaccination.

The components of antigen 85 (Ag85)—Ag85A, Ag85B, and Ag85C—are putative protective antigen candidates. Two recombinant *Mycobacterium bovis* Bacillus Calmette-Guerin (rBCG) were created which overproduce Ag85A alone (rBCG/85A) or together with the Ag85B and the MPB51 (rBCG/BA51). Immunization of mice with these recombinant rBCG reduced the multiplication of *M. leprae* in the foot pads of mice. The inhibition of *M. leprae* growth in mice immunized with rBCG/85A or rBCG/BA51 was more evident than that with parental BCG. Repeated rBCG/85A immunization significantly reduced *M. leprae* multiplication in mice. Therefore, rBCG may be useful for the development of more effective mycobacterial vaccines.—[Nagasaki University School of Dentistry, Nagasaki; Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Ohyama, H., Matsushita, S., Hatano, K., Meguro, M., Takeuchi, K., Nishimura, F., Takashiba, S., and Murayama, Y. Assessment of T-cell response to IL-12 in humans with leprosy.

The objective of this study is to assess T-cell responsiveness to interleukin-12 (IL-12) in leprosy patients, including LL and TT types, and in healthy donors. The amount of gamma interferon (IFN-γ) produced from T cells stimulated with PHA in the presence of IL-12 was measured. The degree of IL-12 receptorβ1 chains (IL-12Rβ1) expression on freshly isolated and PHA-stimulated T cells was compared by using flow cytometric analysis. The results of the study are as follows: 1) T cells isolated from LL patients produced small amounts of IFN-γ regardless of the presence of IL-12; whereas T cells from healthy donors produced a larger amount of IFN-γ in proportion to the IL-12 concentration. 2) No difference was found in the expression of the β1 subunit of IL-12 receptor between patients and healthy donors. We conclude that low IFN-γ productivity of T cells, which is induced by IL-12 in LL patients, is not caused by the degree of IL-12Rβ1 expression on T cells.—[Department of Periodontology and Endodontology, Okayama University Dental School, Okayama; Division of Immunogenetics, Department of Neuroscience and Immunology, Kumamoto University Graduate School of Medical Sciences, Kumamoto; National Leprosarium Oku Komyo-En, Okayama, Japan]

Williams, D. L., Pittman, T. L., Matsuoka, M., Kashiwabara, Y. and Gillis, T. P. Simultaneous detection of *M. leprae* and its resistance to dapsone directly from clinical specimens.

Comprehensive estimates of drug-resistant leprosy are difficult to obtain due to the cumbersome nature of the conventional drug-susceptibility testing method using mouse foot pad inoculation which requires at least 6 months to obtain results. Recently, we have determined that dapsone-resistant strains contained missense mutations in codons 53 or 55 of the folP1 gene of *M. leprae* and have provided evidence linking these mutations with dapsone resistance. We then developed a polymerase chain reaction (PCR)-based heteroduplex assay (HDA-DDS-ML) for the simultaneous de-

tection of *M. leprae* and its susceptibility to dapsone directly from clinical specimens. It relies on the amplification of a 231 bp folP1 fragment containing codons 53 and 55, annealing this fragment to a universal heteroduplex generator and separation of induced heteroduplexes and homoduplexes by minigel polyacrylamide electrophoresis. Using the profiles obtained in this manner, the presence of *M. leprae* and its susceptibility to dapsone can be identified from crude cell lysates of biopsies from leprosy patients. It is anticipated that this assay will contribute to the global goal of leprosy elimination by providing a tool for diagnosis of cases of leprosy, identifying drug-resistant cases early in the course of the disease, and providing a means to estimate the global burden of dapsone-resistant leprosy.—[Molecular Biology Research Department, Laboratory Research Branch, National Hansen's Disease Programs at LSU-SVM, Baton Rouge, LA, U.S.A. Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]