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

Joint Meeting with

U.S.–JAPAN IMMUNOLOGY BOARD

Cathedral Hill Hotel
San Francisco, California, U.S.A.
27–30 June 1999

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U.S. Tuberculosis-Leprosy Panel

CO-CHAIRS

Dr. Jerrold J. Ellner
School of Medicine
Case Western Reserve University
2109 Adelbert Road, Room E202
Cleveland, OH 44106

Dr. Patrick J. Bennan
Department of Microbiology
College of Veterinary Medicine
and Biomedical Sciences
Colorado State University
Fort Collins, CO 80523

MEMBERS

Dr. Thomas P. Gillis
Immunology Research Department
Laboratory Research Branch
GWL Hansen's Disease Center at LSU
P.O. Box 25072
Baton Rouge, LA 70894

Dr. Gilla Kaplan
Laboratory of Cellular
Physiology and Immunology
The Rockefeller University
1230 York Avenue
New York, NY 10021

Dr. Philip C. Hopewell
San Francisco General Hospital
University of California, San Franciso
1001 Potrero Ave., Room 5K1
San Francisco, CA 94110

Dr. David N. McMurray
Medical Microbiology and
Immunology Department
Texas A&M University
Mail Stop 1114
College Station, TX 77843–1114

Japanese Tuberculosis-Leprosy Panel

CO-CHAIRS

Dr. Masao Mitsuyama
Department of Microbiology
Graduate School of Medicine
Kyoto University
Yoshidakonoe-cho, Sakyo-ku
Kyoto 606-8501

Dr. Takeshi Yamada
Department of Oral Microbiology
School of Dentistry
Nagasaki University
1-7-1 Sakamoto
Nagasaki 852-8102

MEMBERS

Dr. Chiyoji Abe
Research Institute of Tuberculosis
Japan Anti-Tuberculosis Association
3-1-24 Matsuyama
Kiyose-shi
Tokyo 204-0022

Dr. Yasuo Fukutomi
Leprosy Research Center
National Institute of
Infectious Diseases
4-2-1 Aoba-cho
Higashimurayama-shi
Tokyo 189-0002

Dr. Kazuo Kobayashi
Leprosy Research Center
National Institute of Infectious Diseases
4-2-1 Aoba-cho
Higashimurayama-shi
Tokyo 189-0002

Dr. Kiyoshi Takatsu
Department of Immunology
Institute of Medical Science
University of Tokyo
4-6-1 Shirokanedai, Minato-ku
Tokyo 108-8639

U.S. Immunology Board Speakers

Dr. Philippa Marrack (Acting Chair)
Department of Medicine
National Jewish Center for Immunology
and Respiratory Medicine
1400 Jackson Street
Denver, CO 80206

Dr. Carl F. Nathan (ad hoc)
Department of Medicine
Cornell University Medical College
Box 57
1300 York Avenue
New York, NY 10021-4896

Dr. Richard Locksley
Department of Medicine
University of California, San Francisco
Box 0654
521 Parnassus Avenue, Room C443
San Francisco, CA 94143-0654

Dr. Michael B. Brenner (ad hoc)
Division of Rheumatology/
Immunology/Allergy
Department of Medicine
Brigham and Women's Hospital
Smith Building, Room 552
One Jimmy Fund Way
Boston, MA 02115

Japanese Immunology Board Speakers

Dr. Takehiko Sasazuki (Chair)
Medical Institute of Bioregulation
Kyushu University
3-1-1 Maidashi, Higasji-ku
Fukuoka 812-81

Dr. Hitoshi Kikutani
Department of Molecular Immunology
Research Institute for Microbial Diseases
Osaka University
3-1 Yamada-ka, Suita
Osaka 565

Dr. Hideo Nariuchi
Institute of Medical Science
Department of Allergy
Tokyo University
4-6-1 Shiroganedai, Minato-ku
Tokyo 108

Dr. Ko Okumura
Faculty of Medicine
Department of Immunology
Juntendo University School of Medicine
2-1-1 Hongo, Bunkyo-ku
Tokyo 113

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

The 34th Research Conference on Tuberculosis and Leprosy of the U.S.-Japan Cooperative Medical Science Program was held in the Cathedral Hill Hotel, San Francisco, California, U.S.A., 28–30 June 1999. The meeting was preceded by a one-day Joint Meeting with the U.S.-Japan Immunology Board. The Joint Meeting was organized by Dr. Philip C. Hopewell, San Francisco General Hospital, University of California, San Francisco, with the help of his staff and Dr. Ann Ginsberg, Tuberculosis/Leprosy Program Officer, NIAID, NIH, and Ms. Gail Jacobs, Assistant to Tuberculosis/Leprosy Program Officer, NIAID, NIH. Present were all members of both panels except for Dr. McMurray and Dr. Takatsu.

Dr. Hopewell opened the Joint Meeting with the Immunology Board and Dr. Philippa Marrack also made opening remarks on behalf of the Immunology Board. Dr. Mitsuyama made the closing remarks at the end of the four-day meeting. The abstracts of oral presentations in the area of leprosy research are presented below. Abstracts of papers on tuberculosis research will appear in *Tubercle and Lung Disease.*

ABSTRACTS*

Adams, L. B., Soileau, N. A. and Krahenbuhl, J. L. Induction of chemokine expression by *M. leprae.*

Mycobacterium leprae is an obligate intracellular pathogen. Depending on the level of immunity in the host, a distinct granulomatous infiltration is generated in an effort to contain the bacilli. We have previously reported that mice, which were genetically incapable of producing a functional inducible nitric oxide synthase (iNOS), exhibited a markedly enhanced granuloma formation upon infection with *M. leprae* compared to C57BL/6 (B6) mice. The cellular composition of these granulomas was primarily CD4+ cells and multinu-

* Some of these abstracts were not provided by the authors but are a synopsis prepared by the U.S. Co-Chair Dr. Brennan. Abstracts are alphabetical by first author.

cleated giant cells were often present. To determine if *M. leprae* induces a chemokine response, macrophages from B6 and iNOS knockout (KO) mice were infected with freshly harvested, athymic *nu/nu* mouse-derived *M. leprae*. After a 4-hour incubation, total RNA was extracted from the infected cells as well as from uninfected control cells and examined by reverse transcription polymerase chain reaction for the expression of chemokine mRNA. *M. leprae* induced elevated levels of MCP-1, MIP-1α, and MIP-1β expression in both strains. Interestingly, both *in vitro* in macrophages and *ex vivo* from liver tissue, *M. leprae*-infected iNOS KO mice expressed higher levels than B6 mice of MIP-1β, a chemokine which in the human system has been reported to be a chemoattractant for CD4+ lymphocytes. These results indicate that *M. leprae* can induce a notable chemokine response which may regulate granuloma formation.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Franzblau, S. G., Bardarov, S. S., Gillis, T. P. and Jacobs, W. R., Jr. Luciferase expression in *M. leprae* infected with phAE129.

Using a combination of freshly harvested, nude mouse-propagated *Mycobacterium leprae* which have been enriched for viable bacilli by Percol-gradient centrifugation and a modified D29 mycobacteriophage (phAE129) carrying the luciferase gene we have obtained the first evidence of both successful phage infection and foreign gene expression in the leprosy bacillus. *M. leprae* was incubated at 33°C in a Middlebrook-based medium in the presence of phAE129 at MOI ranging from approximately 1–800. Luciferase expression was monitored at time intervals by addition of luciferin and detection of light in a microplate luminometer. In four independent experiments, expression of luciferase in phAE129-infected *M. leprae* was detected at greater than 10 times background levels, reaching peak levels at 24–48 hr post-infection. At 1×10^7 and 1×10^8 *M. leprae* per assay, net luciferase production was in excess of 2500 and 4000 relative light units (rlu), respectively. Significant luciferase expression (2–3 × background) could be detected with as few as 1×10^6 *M. leprae* per assay. Luciferase expression was reduced by greater than 95% in the presence of 1 μg/ml rifampin or clarithromycin and was unaffected by 50 μg/ml ampicillin. Preliminary data indicate that exclusion of palmitic acid may enhance luciferase expression. Following peak light production the decay of light was very gradual and no increases in phage titer (determined by plaque assay on *M. smegmatis*) were observed, suggesting the absences of a phage-mediated lytic burst.

The ultimate goal of this line of investigation is the construction of shuttle cosmid vectors capable of stable expression of foreign DNA in *M. leprae* for the purpose of establishing *in vitro* growth competence. Such a vector could conceivably carry DNA libraries from slow-growing, cultivable mycobacteria. Alternatively, pending the completion of the *M. leprae* genome sequence and its comparison with that of *M. tuberculosis*, it may be possible to choose selected genes for introduction. The results of the current investigation suggest that these approaches will indeed be possible by demonstrating the ability to successfully introduce and express foreign DNA in the leprosy bacillus.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A. and Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, NY, U.S.A.]

Fukutomi, Y., Kimura, H., Toratani, S. and Matsuoka, M. Opposing effects of IFN-γ on TNF and IL-10 production in macrophages stimulated with *M. leprae*.

Leprosy is a chronic infectious disease caused by an obligate intracellular pathogen, *Mycobacterium leprae*. The disease manifests a wide range of clinical spectra according to immunity in the form of type 1 and type 2 cytokine responses. We investigated the role of macrophages in leprosy lesions as the cytokine producing cell. Mouse macrophages produced both TNF-α and IL-10 simultaneously in response to *M. leprae*. In addition *M. leprae*-induced TNF-α production by the macrophages was suppressed by exogenously added IL-10, and

the production was enhanced in the presence of neutralizing anti-IL10 antibody. Moreover IFN-γ had opposing effects on the macrophages to produce these cytokines, acting as co-stimulator for TNF-α and an inhibitor for IL-10. In these observations, the amount of cytokines released into the culture supernatants paralleled with the extent of cytokine mRNA expressions. Thus, in leprosy T-cell-derived IFN-γ, which has an important role in cell-mediated immunity, could regulate macrophage cytokine expression by upregulating type 1 cytokines and by downregulating one of the type 2 cytokines, IL-10.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Gillis, T. P., Torerro, M. N., Brennan, P. J., Huygen, K. and Krahenbuhl, J. L. Immune response and protective efficacy of DNA vaccines for leprosy.

Leprosy is a chronic infectious disease of man that afflicts 0.5 to 0.6 million people each year. A safe and effective vaccine could help control the spread of leprosy and provide the critical tool in the fight to eradicate the disease. In this study we have analyzed the protective efficacy and related immune responses in mice to two mycobacterial proteins delivered as DNA vaccines. Genes encoding GroES and Ag85A were cloned into plasmid vectors pcDNA 3.1 and VR1020, respectively. Recombinant constructs (pcDNA 10-1 and VRAg85A) to be used as vaccines expressed the appropriate mycobacterial protein antigen following transfection of COS-7 cells and were detected using specific monoclonal antibodies. BALB/c mice were injected either intramuscularly or intradermally with 100 μg of DNA/mouse, 4 times over a 2 month period. Immune responses were monitored by measuring lymphoproliferation and cytokine production of spleen and lymph node cells as well as serum antibody 3 weeks after the last injection of DNA vaccines. Vaccination of mice with pcDNA 10-1 resulted in weak IgG1 and IgG2a antibody responses and no demonstrable lymphoproliferation or cytokine production to GroEs. Intramuscular injection with VRAg85A resulted in strong IgG1 and IgG2a antibody responses consistent with potent vaccination. Cytokines (IFN-γ and IL-2) were produced at low levels but suggestive of a Th1-like response. Intradermal injection of VRAg85A resulted in the most potent antibody and cell-mediated responses as judged by lymphoproliferation, cytokine and isotype-specific antibody production. IFN-γ and IL-2 production suggested a strong Th1-like response. Antibody isotypes produced to Ag85A were suggestive of overlapping Th1 and Th2 responses. Protection studies in the mouse foot pad infection model showed only marginal protection with the Ag85A DNA vaccine delivered intradermally. Protection with Ag85A was much less significant than the heat-killed *M. leprae* vaccine which produced approximately a one log reduction in bacterial growth. All other vaccines showed no protection in the model infection.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.; Colorado State University, Fort Collins, CO, U.S.A; Pasteur Institute of Brussels, Brussels, Belgium]

Kashiwabara, Y., Matsuoka, M., Kai, M., Maeda, S., Nakata, N. and Maeda, Y. Mutations in *gyrA* gene of *M. leprae* isolates resistant to fluoroquinolones.

Fluoroquinolones, potent, broad-spectrum antibiotics, are increasingly used in the treatment of infections. Ofloxacin, one of the fluoroquinolones, has been introduced to the regimens of multidrug therapy (MDT) for leprosy. Acquired resistance to fluoroquinolones has commonly been observed for many bacterial species. However, it has not been reported in *Mycobacterium leprae* except for one case from a lepromatous leprosy patient in Mali.

The sequences of the quinolone resistance determining region (QRDR) of the *gyrA* gene, the mutational alteration of which has been reported to lead to resistance to quinolones, were analyzed for 13 clinical isolates of *M. leprae;* 8 of 13 showed mutations in this region, suggesting the development of fluoroquinolone resistance. Moreover, 5 of the 8 strains showed mutations in the *rpoB* gene, suggesting that these strains have acquired resistance to both rifampin and quinolones.

Our results suggest that such a survey for drug-resistant strains is required for the prevention of multidrug resistance in leprosy.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Matsumoto, S., Yukitake, H. and Yamada, T. Analysis of a novel DNA binding protein in mycobacteria.

Pathogenic species of *Mycobacterium* are extremely slow-growing intracellular bacteria. Slow growth is important for the parasitism of these organisms and chronicity of the disease, but the precise mechanism has not been elucidated. We analyzed a 28-kDa protein (MDP1) which was one of the abundant proteins expressed in BCG. This protein was highly polymerized and localized in the nucleoid, 50S ribosomal subunit, and cell surface. Analysis in *Escherichia coli* cell-free macromolecular biosynthesizing systems revealed that it interfered with replication, transcription, and translation. MDP1 transformed the rapidly growing bacterium *E. coli* to a slow grower. MDP1 was conserved in slow-growing *Mycobacterium* thus far examined, including *M. tuberculosis* and *M. leprae*. These data are suggestive of an important role of MDP1 for generating slow growth mycobacteria.—[School of Dentistry, Nagasaki University, Nagasaki, Japan]

Matsuoka, M., Maeda, S., Kai, M., Nakata, N., Chae, G.-T., Gillis, T. P., Kobayashi, K., Izumi, S. and Kashiwabara, Y. *M. leprae* typing by genomic diversity and global distribution of genotypes.

To find strain-specific markers of *Mycobacterium leprae* for epidemiological study, sequences of the *rpoT* gene were compared among isolates. Isolates were classified into two groups based on the number of tandem repeats composed of 6 base pairs in the *rpoT* gene. *M. leprae* isolates comprising one group showed 3 such tandem repeats of 6 bp (ACATCG) while another group showed 4 tandem repeats of the 6 bp sequence. Isolates from Japan, except Okinawa, and Korea belonged to the group of 4 tandem repeats, while almost all isolates from South-East Asian countries, Latin American countries and Okinawa belonged to the group of 3 tandem repeats. *M. leprae* obtained from two nonhuman sources, an armadillo and a mangabey monkey, demonstrated the latter genotype. Biased distribution of isolates with 4 tandem repeats in the main islands of Japan and Korea suggest that migration of this type of *M. leprae* into Japan might have accompanied the movement of one of the Mongolian tribes through the Korean peninsula.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan; Institute of Chronic Disease, Catholic University Medical College, Seoul, Korea; Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.; National Sanatorium Oshima Seishoen, Kagawa, Japan]

Ohyama, H., Matsushita, S., Hatano, K., Kato, N., Nishimura, F., Takashiba, S. and Murayama, Y. IL-12 production from monocytes induced by stimulus via HLA Class II molecules in humans with leprosy.

The objective of this study was to assess whether the difference in cellular immune responses against *Mycobacterium leprae* among clinical forms would be controlled by the balance between IL-12 from monocytes produced by stimulus via HLA class II molecules and IFN-γ from Th cells in humans with leprosy. Th cell lines reactive with *M. leprae* major membrane (MMP II) protein were used in this study. The results of this study were as follows: a) IL-12/IL-1β ratio of LL patients tended to be higher than that of TT patients. b) IFN-γ/IL-12 ratio of LL patients was significantly lower than that of TT patients. These results suggest that the clinical forms of leprosy are restricted by the IFN-γ/IL-12 ratio in humans with leprosy.—[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 Oka Komyo-En, Okayama, Japan]

Scherman, H., Mahapatra, S., Crick, D. C. and Brennan, P. J. Genetics and biosynthesis of peptidoglycan: effects of various antibiotics on peptidoglycan synthesis.

Peptidoglycan (PG) is an essential component of the bacterial cell wall, providing mechanical strength as well as shape. It is also the fulcrum for mycolylarabinogalactan. The structure of the peptidoglycan of mycobacteria was previously studied in the 1970s, and it was shown to be quite different from other gram-positive bacteria. By using antibiotics such as vancomycin, bacitracin and D-cycloserine against growing cultures, peptidoglycan precursors were shown to accumulate. These were then isolated and characterized to facilitate the study of peptidoglycan synthesis. The availability of the full genome sequence of *Mycobacterium tuberculosis* and most of the *M. leprae* sequence allows comparative genomics of the responsible gene clusters, further facilitating a rational approach to the study of this most important component of cell wall structure.—[Department of Microbiology, Colorado State University, Fort Collins, CO, U.S.A.]

Shimoji, Y. and Rambukkana, A. Identification and characterization of a novel surface protein of *M. leprae* that binds peripheral nerve laminin-2 and mediates Schwann cell invasion.

Nerve damage is the hallmark of *Mycobacterium leprae* infection which partly results from *M. leprae* invasion of the Schwann cell of the peripheral nervous system. We have recently shown that the laminin-2 isoform, especially the G domain of laminin α2 chain, in the basal lamina that surrounds the Schwann cell-axon unit serves as an initial neural target for *M. leprae* (Rambukkana, *et al.* Cell 88: 931–841, 1997). However, *M. leprae* surface molecules that mediate bacterial invasion of peripheral nerves are entirely unknown. By using human α2 laminins as a probe, a major 28-kDa protein in the *M. leprae* cell wall fraction that binds α2 laminins was identified. After N-terminal amino-acid sequence analysis, a PCR-based strategy was employed to clone the gene that encodes this protein. Deduced amino-acid sequence of this *M. leprae* laminin-binding protein predicts a 21-kDa molecule (ML-LBP21), which is smaller than the observed molecular size in SDS-PAGE. Immunofluorescence and immuno-electron microscopy on intact *M. leprae* using MAbs against recombinant (r) ML-LBP21 revealed that the protein is surface exposed. rML-LBP21 avidly bound to α2 laminins, the rG domain of the laminin-α2 chain and to the native peripheral nerve laminin. The role of ML-LBP21 in Schwann cell adhesion and invasion was investigated using fluorescent polystyrene beads coated with rML-LBP21. Although beads coated with rML-LBP21 alone specifically adhered and invaded primary Schwann cells, these functions were significantly increased when beads were preincubated with exogenous α2 laminins. Taken together the present data suggest that ML-LBP21 may function as a critical surface adhesin that facilitates the entry of *M. leprae* into Schwann cells.—[Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, NY, U.S.A.]

Williams, D. L., Spring, L., Harris, E., Roche, P. and Gillis, T. P. Dihydropteroate synthase of *M. leprae* and dapsone resistance.

The molecular mechanism of dapsone (DDS) resistance in *Mycobacterium leprae* is currently unknown. Circumstantial evidence predicts an association with the dihydropteroate synthase (DHPS), a key enzyme in the folate biosynthesis pathway encoded by *folP*. *M. leprae* possesses two *folP* homologs (*folP*1 and *folP*2). We have previously shown that DDS resistance was not associated with mutations in *folP*2 from two high-level DDS-resistant strains of *M. leprae*. In the present study, we show that the *M. leprae folP*1 homolog encodes a functional DHPS which is exquisitely sensitive to inhibition by DDS. In addition, mutations within a highly conserved region of *folP*1 were identified in two of three high-level (0.01%) DDS-resistant *M. leprae* clinical strains. These data further support earlier predictions that DDS resistance in *M.*

leprae is associated with alterations in folate metabolism, and that one possible mechanism of resistance is related to mutations in *folP*1.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.; Anandaban Leprosy Hospital, Kathmandu, Nepal]