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ABSTRACTS

TWENTY-FIFTH JOINT LEPROSY RESEARCH CONFERENCE

Hokkaido University
Sapporo, Japan
27 and 29 August, 1990

U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

Good Morning, Ladies and Gentlemen:

It is a great pleasure to open the Twenty-Fifth Joint Conference on Leprosy Research today. We are particularly grateful to the United States scientists who have traveled far distances in order to participate in this conference. The Hokkaido district is a north distant dreamland in Japan. I would like to deeply thank Dr. Azuma who, as Director of the Institute of Immunological Science, Hokkaido University, arranged this meeting place.

This year is the 25th anniversary of the U.S.–Japan Cooperative Medical Science Program, the great Silver Jubilee was held in Tokyo in July and was attended by His and Her Imperial Hitachi Highness. When we remember the 25 years of our Leprosy Panel, many historic and splendid achievements were born from this working panel. We also remember many excellent leaders, such as Drs. Yoshie, Shepard, Namba, Bloom, Abe, and Hastings, and other excellent workers such as Drs. Kirchheimer, Nakamura, Ito, Buchanan, Bullock, Nishiura, Matsuo, Urabe, Nishimura, Binford, and Hanks.

As we begin the Joint Conference, I must now inform you of very sad news. Dr. Ivan Bennett, who was for a long time chairman of the United States delegation, fell ill suddenly while attending a meeting the day before the Silver Jubilee. He was taken to Nisseki Hospital. After a few days he died, despite excellent treatment by President Dr. Oka and other doctors. It is an extremely mournful situation for our participants. We have lost a great doctor who was a member of the U.S.–Japan Cooperative Medical Science Program for 25 years. It will be a great return to Dr. Bennett for us to continue to develop the Joint Conference with splendid achievements.

I wish to declare open the Twenty-Fifth Joint Conference on Leprosy Research.

Thank you.

—Tatsuo Mori, *Chairman*
Japanese Leprosy Panel

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PROGRAM
TWENTY-FIFTH JOINT LEPROSY CONFERENCE

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Co-Chairmen: Dr. Thomas H. Rea
Dr. Hajime Saito

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Gelber, R. H. and Rea, T. H. Minocycline Has Impressive Activity Against Lepromatous Leprosy

Osawa, N. and Akiyama, T. Effect of Antimicrobial Agents on *Mycobacterium leprae* in Murine Macrophages

Tanaka, M. and Tanaka, Y. Effect of Cyclosporin A on Immunological Responsiveness in Early Stages of Infection with *Mycobacterium leprae*

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Co-Chairmen: Dr. Gilla Kaplan
Dr. Kenji Kohsaka

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Goto, M. and Izumi, S. Light and Electron Microscopic Immunochemistry Using Anti-PGL-I Antibody Specific for *Mycobacterium leprae*

Matsuo, E., Komatsu, A., Sasaki, N. and Skinsnes, O. K. On the Possible Cross-immunoreactivity of the Beta-Glucuronidase Receptor of *Mycobacterium scrofulaceum* HI-75 with that in Globi in Lepromas; an Immunohistologic Study

Session III

Co-Chairmen: Dr. Josephine E. Clark-Curtiss
Dr. Yoshinori Tanaka

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Clark-Curtiss, J. E., Bosecker, B., de Carvalho, E. and Sela, S. Expression of Antigenic Determinants of *Mycobacterium leprae* in an Avirulent *Samonella typhimurium* Vaccine Delivery System

Ganjam, K., Bloom, B. R. and Jacobs, W. R., Jr. Isolation of Auxotrophic Mutants of Mycobacteria Using Shuttle Mutagenesis

Nomaguchi, H., Park, I. and Kohsaka, K. Characterization of a 65-kDa Heat-shock Protein of Mycobacteria

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Session IV

Co-Chairmen: Dr. James Krahenbuhl
Dr. Sinzo Izumi

Uyemura, K., Sano, S., Deans, R., Band, H., Morita, C., Rea, T. H., Bloom, B. R. and Modlin, R. L. T-Cells Bearing $\gamma\delta$ Antigen Receptors in Leprosy

Adams, L., Franzblau, S. G., Taintor, R., Hibbs, J. and Krahenbuhl, J. Role of L-Arginine-dependent Pathway of Activated Macrophages on the Metabolic Activity of *Mycobacterium leprae*

Kaplan, G. and Cohn, Z. Role of Recombinant Human IL-2 in Immune Modulation of Leprosy

Fujiwara, T., Izumi, S. and Sakamoto, Y. Correlation Between the Chemical Structure of the Carbohydrate Part of *Mycobacterium leprae* PGL-I and its Activity to the Monoclonal Antibodies

Izumi, S., Hua, C. Y., Amiruddin, M. D., Kawatsu, K., Choudhury, A. M., Mendes, M. F. and Fujiwara, T. Distribution of Anti-PGL-I Antibody in Household Contacts from Endemic Countries

Closing Remarks: Dr. Patrick Brennan, Chairman, U.S. Leprosy Panel

JOINT U.S.-JAPAN TUBERCULOSIS AND LEPROSY SYMPOSIUM
Hokkaido University
Sapporo, Japan

28 August, Tuesday

Session I

Co-Chairmen: Dr. Patrick J. Brennan
Dr. Tatsuo Mori

Matsuo, K., Yamaguchi, R., Yamazaki, A., Nagai, S., Tozuka, M. and Yamada, T. Progress in Establishing a Recombinant BCG Secreting System

Pane, L., Belisle, J., Ganjam, K., Brennan, P. J., Bloom, B. R. and Jacobs, W. R., Jr. Analysis of *Mycobacterium leprae, M. tuberculosis* and *M. avium* Genomic Libraries in *M. smegmatis* and *E. coli* Using Shuttle Cosmid Vectors

Nishimura, Y., Sudo, T., Inamitsu, T., Nomaguchi, H. and Sasazuki, T. Application of the HLA-DQ Transgenic Mice for the Analysis of Genetic Control of the Immune Response to *Mycobacterium leprae* Antigen

Session II

Co-Chairmen: Dr. Frank M. Collins
Dr. Ichiro Azuma

Venkataraman, S. and Barker, R. H., Jr. Detection of Mycobacteria Using PCR and Specific DNA Probes

Plikaytis, B. B., Mundayoor, S. and Shinnick, T. M. Detection and Identification of *Mycobacterium* Species Using Gene Amplification Techniques and PFLP Analysis

Brennan, P. J., Hunter, S. W., Chatterjee, D., McNeil, M., Rivoire, B. and Bozic, C. M. Toward a Thorough Resolution and Structural Definition of the Major Antigens of *Mycobacterium leprae*

Shinnick, T. M. Heat-Shock Proteins as Antigens of Pathogenic Microorganisms

ABSTRACTS

Saito, H., and Tomioka, H. Antileprosy activity of a newly developed quinolone, fleroxacin.

We previously reported that newly developed quinolones, ofloxacin and AM-1091, had appreciable therapeutic activity against *Mycobacterium leprae* infection. In the present work, we examined the therapeutic efficacy of a new quinolone, fleroxacin (FLRX) (6,8-difluoro-1[2-fluoroethyl]-1,4-dihydro-7-[4-methyl-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid), against *M. leprae* infection induced in mice. A therapeutic experiment was performed as follows: The left hind foot pads of female BALB/c nude mice (5 weeks old) were infected subcutaneously (s.c.) with 1×10^6 *M. leprae*. The drugs (FLRX, OFLX, CPFX and NFLX), emulsified in 0.1 ml of distilled water, were given to mice by gavage once daily six times per week for up to 50 days, from day 31 to day 80. At day 150 and day 365, foot-pad swelling and the number of acid-fast bacilli (AFB) in the left hind foot pad were examined.

FLRX exhibited appreciable antileprosy activity, and FLRX treatment of mice at the dose of 3 mg/mouse gave 0.4 and 1.7 log decreases in the number of AFB on day 150 and day 365, respectively, when compared to untreated mice. FLRX also reduced foot-pad swelling associated with the progression of the *M. leprae* infection. In separate experiments with respect to dose dependency, we found that FLRX was efficacious at the does of 3 mg/mouse but not at doses lower than 1 mg/mouse. On the other hand, OFLX treatment inhibited the growth of organisms at the site of infection, and the numbers of AFB recovered from the infected foot pad on day 150 and day 365 were decreased by 1.9 and 1.0 logs in OFLX-treated mice, respectively, when compared to untreated mice. There was a remarkable delay in foot-pad swelling at the site of infection. It is noted that FLRX caused a more prolonged effect against the growth of *M. leprae* than did OFLX. Differing from OFLX and FLRX, CPFX and NFLX virtually lacked antileprosy activity. The numbers of AFB recovered from the foot pads of CPFX-treated mice on day 150 and day 365 were somewhat larger than those from control mice. There is a possibility that CPFX may enhance the growth of the organism. Although NFLX slightly retarded the growth of *M. leprae* during the first 150 days (0.6 log decrease in the number of AFB), this efficacy disappeared on day 365.

Although CPFX has a higher *in vitro* antimycobacterial activity than does OFLX, it is disdvantageous when given orally because of its slow adsorption and low tissue distribution. The fault of CPFX in its pharmacodynamics may have resulted in its obscure therapeutic effect against *M. leprae* infection. Thus, the present findings support the importance of pharmacodynamics of a given antimicrobial agent in its expression of *in vivo* activity against *M. leprae* infection. This is also the case for therapeutic efficacy of FLRX, since the agent exhibited appreciable antileprosy activity, although its MIC values against cultivable mycobacteria are somewhat larger than those of CPFX.—[Department of Microbiology and Immunology, Shimane Medical University, Izumo 693, Japan]

Gelber, R. H. and Rea, T. H. Minocycline has impressive activity against lepromatous leprosy.

There is an urgent need for new antimicrobials to treat leprosy. This is due to the fact that there are only a limited number of available active agents, each with certain undesirable side effects and toxicities, an increasing problem of drug resistance, and the requirement for multidrug therapy. Furthermore, the key to short-course chemotherapy of pulmonary tuberculosis has been the use of two bactericidal agents. Because, unlike pulmonary tuberculosis, lepromatous leprosy is associated with a serious immunological host-defense defect and a considerably greater number of bacilli, this requirement appears even more compelling for the success of short-course therapy for lepromatous leprosy. Although in mice dapsone, clofazimine and rifampin all have been demonstrated to have some bactericidal ac-

tivity, unfortunately only rifampin appears to be bactericidal for *Mycobacterium leprae* in man. Thus, the finding that in this clinical trial minocycline was found impressively active, both clinically and microbiologically, has major implications for the future treatment of leprosy.—[Kuzzell Institute, San Francisco, California; GWL Hansen's Disease Center, Carville, Louisiana; U.S.C. Medical Center, Los Angeles, California, U.S.A.]

Osawa, N. and Akiyama, T. Effect of antimicrobial agents on *Mycobacterium leprae* in murine macrophages.

There are similar defense mechanisms in leprosy, tuberculosis and salmonellosis. For instance, during the course of *in vivo* and *in vitro* infections, the responsible bacteria grow in phagocytic cells. In the present study, we tested the effects of antimicrobial agents entrapped in liposomes on *Mycobacterium leprae* or other bacteria in murine macrophages.

Mice of the CBA and DNN strains, 8–10 weeks old, of both sexes, weighing 28 g, were used for *M. leprae* and *Salmonella* infection. *M. leprae* Thai-53 and *S. enteritidis* 116-54 were used. *M. leprae* were obtained by nude mice inoculation. Peritoneal macrophages were cultured in a TD-15 culture chamber with 4 glass cover slips (9 × 12 cm). Culture medium consisted of 20% fresh horse serum and 80% Waymouth solution. At appropriate time intervals after phagocytosis, the cover slips to which macrophages adhered were removed, dried, fixed and stained. Infected macrophages were observed microscopically. Liposomes were prepared by standard methods. Clofazimine was soluble in chloroform.

Multiplication of *Salmonella* was observed in macrophages in the presence of penicillin, streptomycin, or tetracycline in the medium. On the other hand, growth of *Salmonella* was inhibited by the presence of ofloxacin and chloramphenicol in the medium, and growth of all bacteria was inhibited by streptomycin- or tetracycline-entrapped liposomes.

Cultured macrophages were infected with *M. leprae* to yield a phagocytic index of 90; 2–3 bacteria were phagocytized in a cell. The bacterial numbers in the phagocytic cells were increased 3 weeks after infection. On the other hand, the macrophages of mice which were treated with clofazimine-entrapped liposomes inhibited intracellular multiplication of *M. leprae*. Previously, we have reported on the efficacy of protamine or rifampin-entrapped liposomes against *M. leprae*. These methods are applicable to the problem of testing the susceptibility of *M. leprae* to antimicrobial agents in general.—[Department of Microbiology, School of Medicine, Kitasato University, Sagamihara 228, Japan]

Tanaka, M. and Tanaka, Y. Effects of cyclosporin A on immunological responsiveness in early stages of infection with *Mycobacterium leprae*.

Mycobacterium leprae were inoculated into BALB/c mice in conjunction with an orally administered dose of 8 mg/kg of cyclosporin A (CsA). The number of organisms was significantly higher ($p < 0.05$) after 19 months as compared with mice in which the dose of CsA gradually decreased after 8 months and was discontinued after 12 months. In order to explore features of the immune response in *M. leprae*-infected mice, we then investigated the effects of CsA on the lymphocyte transformation test (LTT) in the early stage of infection.

LTT of the spleen cells was measured in the CsA-treated mice 1, 4, and 15 weeks after infection with *M. leprae*. At week 1, the lymphocytes from CsA-untreated mice inoculated with *M. leprae* responded to sonicated supernatant of *M. leprae* suspension (SS), *M. leprae* suspension (Ml), and concanavalin A (ConA) greater than the responses from uninfected mice. When a dose of 100 μg CsA per kg body weight was administered to mice for 7 days, lymphocytes took up almost the same amount of ^{3}H-thymidine as those from the CsA-untreated mice. In contrast, the response to ConA in lymphocytes from CsA-treated mice decreased remarkably as compared with those from CsA-untreated mice infected with *M. leprae*. At week 4, immune responses to all three stimulants in the lymphocytes from *M. leprae*-infected mice decreased as a result of CsA treatment. However, at week 15 the LTT responses to SS and Ml in the cells from *M. leprae*-infected mice exceeded that

of control mice, and the response to ConA in *M. leprae*-infected mice was less than that of normal mice. Thus, if CsA treatment was discontinued for longer periods, the T-cell function was partially activated. These results indicate that *M. leprae* would grow in CsA-treated mice only if dosing intervals did not exceed 3 weeks.—[Department of Bacteriology, Tottori University School of Medicine, Yonago, Japan]

Hirata, T. Fine structures of macrophages and/or leprosy bacilli in lepromatous leprosy lesions of human skin.

An electron-microscopic study of macrophages and leprosy bacilli in the lesions of lepromatous leprosy was performed. Bacteria-containing macrophages were divided into three groups: macrophages containing either one or two bacilli, or higher numbers of bacilli, or a great number of bacilli. Results from ultrastructural analysis of the cells and the bacilli are presented.—[National Institute for Leprosy Research, Tokyo, Japan]

Goto, M. and Izumi, S. Light- and electron-microscopic immunohistochemistry using anti-PGL-I antibody specific for *Mycobacterium leprae.*

In spite of numerous serological studies of anti-phenolic glycolipid-I (PGL-I) antibodies, experiments on the immunohistochemical localization and turnover of PGL antigen in the tissue have not been conducted. We have now successfully demonstrated immunohistochemical localization of PGL antigen in formalin-fixed, paraffin-embedded, lepromatous leprosy skin biopsies from various clinical stages with polyclonal and monoclonal anti-PGL antibodies generated using a synthetic relative of the glycolipid natural trisaccharide phenylpropionate, NTP. The results have been compared with those obtained with cross-reactive BCG antibody and conventional Fite's acid-fast staining. Positive PGL staining was observed in not only active lesions but also in regressive stages where Fite's staining was negative. The staining pattern was different according to stages, i.e., solid or granular staining in fresh lesions and foamy or vacuole-like staining in regressive stages. BCG staining was also positive in lepromatous leprosy, showing similar staining patterns. Tuberculosis sections and BCG smears were positively stained by BCG antibody and Fite's staining, but not by PGL antibody. The immunogold method applied to ultrathin sections of lepromatous leprosy demonstrated that PGL staining was ultrastructurally localized in rod-shaped bacilli and intracellular opaque structures. BCG staining was positive in the rod-shaped bacilli and intercellular spaces. These methods are quite useful for the specific diagnosis of leprosy and in the understanding of the turnover of serum PGL antibody and antigen.—[National Leprosarium Hoshizuka-Keiaien, Kagoshima; National Institute for Leprosy Research, Tokyo, Japan]

Matsuo, E., Komatsu, A., Sasaki, N. and Skinsnes, O. K. On the possible cross-immunoreactivity of the beta-glucuronidase receptor of *Mycobacterium scrofulaceum* HI-75 with that in globi in lepromas; an immunohistologic study.

Recently, we have come to realize that *Mycobacterium scrofulaceum* HI-75 (HI-75), originally isolated from a leproma, binds beta-glucuronidase (β-Gase) *in vitro.* In earlier studies we recognized the presence of a receptor for β-Gase in HI-75. We also reported on the solubilization and testing of the activity of the β-Gase receptor *in vitro.* However, HI-75 itself did not seem to form β-Gase but, instead, was enhanced to grow in Ogawa's medium in the presence of glucuronic acid (GA). We have considered the possibility that in lepromas, GA might be produced in lepra cells from abundant hyaluronic acid by hydrolysis in which β-Gase participates. In other words, *M. leprae* might combine β-Gase of human origin and grow, utilizing GA which is a split product produced due to the activity of this enzyme. The present study, designed to examine the question of immunological crossreactivity between the β-Gase receptor of HI-75 and *M. leprae,* further addresses the role of the β-Gase receptor in the biology of lepromas.

HI-75 was grown in modified Ogawa's medium containing both GA and *N*-acetylglucosamine (NAG). Autoclaved HI-75 was trypsin digested. The 68% ethanol sedimented and rehydrated fraction containing the β-Gase receptor was separated further

by utilizing submarine preparatory polyacrylamide gel electrophoresis. β-Gase receptor was recovered as a homogeneous solitary fraction in the cathode side before the sharp brown band in the gel. The buffer was Tris-borate (pH 8.3) containing 7 M urea and 1 mM Na_2EDTA. Anti-β-Gase receptor antiserum was produced in three male albino rabbits by intramuscular immunization with incomplete adjuvant for 3 consecutive months, followed by intravenous injection of 20 mg each time for 5 consecutive weeks. The production of antibody in rabbits was tested using a modified Ouchterlony double-diffusion method on nitrocellulose sheets, dot-ELISA, and immunohistologic staining of a leproma. The immunoreactivity of *M. leprae* in human lepromas was evaluated and confirmed by staining of paraffin-embedded skin sections with avidin-biotin, using anti-β-Gase receptor rabbit antiserum. Two rabbits among three survived the immunization. The anti-β-Gase receptor rabbit antibody was detected by immunohistologic staining of lepromas and dot-ELISA (more than 10×2^{12}), but not by immunoprecipitin reaction by the modified Ouchterlony method. The antibody immunohistologically stained globi of lepromas. The results suggest the monovalency of β-Gase receptor as antigen.—[Departments of Pathology, Kyorin University School of Medicine, Tokyo, Japan; Sun Yat-Sen University of Medical Sciences, Guangzhou, China; Tohoku Shinseien, Miyagi, Japan]

Hatfull, G. F., Donnelly, M., Lee, M. H., Peterson, L. and Oyaski, M. Molecular genetics of mycobacteriophage L5.

We are in the process of characterizing the temperate mycobacteriophage L5 with a view to constructing mycobacterial cloning vectors, understanding mycobacterial gene expression and regulation, and characterizing the process of site-specific integration. In this paper we review our current understanding of L5, present a restriction map of the phage genome, and show that the L5 genome has ligatable cohesive ends with 9-base single-strand 3′ extensions. We briefly discuss our attempts to locate the gene coding for the integrase function and a gene that confers immunity to superinfection. We hope to apply these approaches to the study of *Mycobacterium leprae* gene function in cultivatable mycobacteria.—[Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, U.S.A.]

Clark-Curtiss, J. E., Bosecker, B., de Carvalho, E. and Sela, S. Expression of antigenic determinants of *Mycobacterium leprae* in an avirulent *Salmonella typhimurium* vaccine delivery system.

Using the approach described previously by Sathish, *et al.*, we have identified two λgt11::*Mycobacterium leprae* clones that specified fusion proteins of 132 and 125 kDA, respectively, that reacted with anti-cell-wall protein (CWP) antibody. Neither of the proteins specified by these clones reacted with monoclonal antibodies against the 65-kDa protein, nor did they react with antibodies in patients' sera (either LL or BT/TT). The strong reacting clones have been subcloned into a plasmid cloning vector, pYA292, and the subclones have been introduced into the avirulent Δ*cya* Δ*crp* strain of *Salmonella typhimurium* in order to use this recombinant avirulent *S. typhimurium* vaccine delivery system to determine whether or not any of these *M. leprae* antigenic determinants is capable of eliciting a delayed-type hypersensitivity reaction to *M. leprae* cell components.—[Departments of Molecular Microbiology and Biology, Washington University, St. Louis, Missouri 63130, U.S.A.]

Ganjam, K., Bloom, B. R. and Jacobs, W. R., Jr. Isolation of auxotrophic mutants of mycobacteria using shuttle mutagenesis.

The analysis of the genes encoding virulence determinants of mycobacteria will require both the ability to isolate clones of mycobacterial cells possessing single mutations and the ability to transfer DNA between these genetically characterized clones. The isolation of mycobacterial cells possessing single mutations can be greatly facilitated by using an insertional mutagenesis strategy. Such a strategy generates mutations by inserting a DNA element contain-

ing a selectable marker gene, e.g., a transposon, into a DNA fragment on the genome. The selection for the expression of the marker gene selects for the growth of an insertionally mutagenized cell and against unmutagenized cells. In the absence of a defined transposon that functions in mycobacteria, we have developed a shuttle mutagenesis system employing the gram-negative transposon Tn*5* and its derivative, Tn*5 seq1* in *Mycobacterium smegmatis.*—[Albert Einstein College of Medicine, Bronx, New York 10461, U.S.A.]

Nomaguchi, H., Park, I. and Kohsaka, K. Characterization of a 65-kDA heat-shock protein of mycobacteria.

The 3.6-kb DNA fragment flanked with *EcoR*I sites from a phage strain Y4178 was recloned into the *EcoR*I site on a plasmid vector, pUC8. The recombinant plasmids, pUC-N5, were introduced into *Escherichia coli* strain JM83. Transformant cells (JM83/pUC-N5) grown in Luria-Bertani broth were harvested by centrifugation, and the lysate was used as crude 65-kDa protein. The crude 65-kDa protein was subjected to immunoaffinity chromatography on monoclonal antibody-3A IgG-Sepharose for purification. Female BALB/c mice were immunized intradermally (i.d.) in the chest with intact 10^7–10^8 *Mycobacterium leprae* in 0.1 ml of phosphate buffered saline. Four months after immunization, the immunized and control mice were challenged in the foot pad with an i.d. injection of 0.05 ml sterile saline, 5 μg of *M. leprae* crude lysate, 5 μg of the synthesized epitope for the monoclonal antibody ML-IIIE9, or 5 μg of affinity-purified 65-kDa protein. Swelling of the foot pad was observed when the *M. leprae* lysate or 65-kDa protein challenges were used, but no swelling was observed when the synthesized epitope or sterile saline were applied. Control mice did not show any foot-pad swelling when challenged with these antigens. Thus, the whole 65-kDa protein induced delayed-type hypersensitivity responses in mice infected with *M. leprae.*

To test the role of these antigens in protective immunity against *M. leprae* infection, the mouse foot pad model of Shepard was applied. BALB/c mice were immunized (i.d.) in the chest with *M. bovis* BCG, a mixture of BCG and *M. leprae, E. coli* carrying pUC8 or pUC-N5 without adjuvant, or affinity-purified 65-kDa protein with Freund's incomplete adjuvant. Three weeks after the immunization, 5×10^3 *M. leprae* were injected into the right hind foot pad of the mice. Nine months later, the number of *M. leprae* in the right hind foot pad was counted by Shepard's method. Live BCG or the mixture of live BCG and *M. leprae* gave significant protection, but the others were not effective in this method.

High antibody titer against the 65-kDa protein of *M. leprae* was observed in the serum of mice immunized with the affinity-purified 65-kDa protein of *M. leprae.* Thus, under our conditions as shown here, the protein itself did not induce protective immunity, although a high antibody titer against the 65-kDa protein of *M. leprae* was obvious.

When five monoclonal antibodies to mycobacteria isolated in our laboratory were tested for their ability to bind to tissue-culture cells infected with *M. lepraemurium* by the immunofluorescent technique, positive staining was high in the infected cells. However, binding has not yet been associated with any of the individual bacterial products.—[Research Institute for Microbial Diseases, Osaka University, Osaka; National Institute for Leprosy Research, Tokyo, Japan]

Uyemura, K., Sano, S., Deans, R., Band, H., Morita, C., Rea, T. H., Bloom, B. R. and Modlin, R. L. T-Cells bearing γδ antigen receptors in leprosy.

Within the skin lesions of leprosy, there is a complex array of immune responder cells. Present within the dermal granulomas are CD4+ cells which bear the T-memory and T-naive markers, CD8+ cells which bear the T-cytotoxic and T-suppressor markers, macrophages and CD1a+ cells. Recently, TCR γδ cells have been identified within the granulomas of lepromin skin tests. The present study demonstrates that these TCR γδ cells are of limited receptor diversity, both in use of Vδ and Jδ segments and also in nucleotide diversity at the V-J junction. Immunohistochemical analysis reavealed the presence of both Vδ1 and Vδ2 receptor cells in the dermal granulomas;

whereas, TCR δ cells in the epidermis were invariably Vδ1 positive. It is interesting that there is a specific Vδ gene segment associated with intra-epidermal cells, since murine γδ Thy-1+ epidermal cells are restricted to a particular V gene usage. Thus, it appears that the expression of certain V genes may influence their tissue microdistribution.

Polymerase chain reaction (PCR) analysis of these lesions revealed a restricted rearrangement of Vδ1 with Jδ1 and Vδ2 with Jδ1. In contrast, in the blood of the same individuals, these rearrangements plus Vδ2 and Jδ3 were common. Therefore, there is limited diversity of V-J rearrangements in tissue relative to blood. Furthermore, junctional analysis revealed limited nucleotide diversity in lesions relative to blood. The apparent limited diversity in both V-J rearrangement and junctional sequences in lesions would suggest that a limited number of antigens is recognized by the TCR γδ repertoire in leprosy. The presence of γδ T cells with limited receptor diversity may also suggest that immune surveillance in the skin involves T cells which detect common or pathologic antigens. Consistent with this hypothesis, γδ T cells may recognize heat-shock or stress proteins, a highly conserved family of proteins that may be induced by a variety of cellular injury mechanisms. Thus, limited receptor diversity would permit γδ T cells to recognize and respond to these antigens on mycobacteria, or homologous proteins present on autologous cells stressed by intracellular infection.—[U.S.C. School of Medicine, Los Angeles, California; Dana Farber Cancer Institute, Boston, Massachusetts; Albert Einstein College of Medicine, Bronx, New York, U.S.A.]

Adams, L., Franzblau, S. G., Taintor, R., Hibbs, J. and Krahenbuhl, J. L. Role of the L-arginine-dependent pathway of activated macrophages on the metabolic activity of *Mycobacterium leprae.*

In contrast to normal mouse macrophages (Mφ), activated Mφ exhibit a potent microbial effect on a broad spectrum of phylogenetically unrelated intracellular pathogenic microorganisms. Reactive oxygen intermediates produced in the respiratory burst have been implicated as a major, but not the sole, mechanism of the Mφ antimicrobial effector function. Recent reports described the importance of a single amino acid, L-arginine, as a necessary substrate for activated Mφ-mediated cytostatic activity for tumor target cells as well as microbiostatic function against *Cryptococcus neoformans* and *Leishmania major.* We have recently characerized the arginine-dependent pathway in Mφ-mediated microbiostatic activity against the obligate intracellular protozoan *Toxoplasma gondii.*

These Mφ-mediated cytotoxic functions were dependent upon a novel biochemical pathway synthesizing L-citrulline and inorganic nitrogen oxides from a terminal guanidine nitrogen atom of L-arginine. Nitric oxide, a paramagnetic, stable, free radical appears to be the effector molecule synthesized from L-arginine by cytotoxic activated Mφ. The nitrite and nitrate measured in the culture medium are stable products of nonenzymatic oxidative degradation of nitric oxide in aqueous solution. In addition, N^Gmonomethyl-agrinine (N^GMMA) was discovered to be a specific competitive inhibitor of the synthesis of inorganic nitrogen oxides and L-citrulline from L-arginine as well as activated Mφ cytostasis for tumor cells, and microbiostasis, *Cryptococcus, Leishmania,* and *Toxoplasma.*

In previous studies, we have employed a variety of procedures to demonstrate that activated Mφ have a deleterious effect on the obligate intracellular bacterium, *Mycobacterium leprae.* In the present study, we have examined the role of the arginine-dependent pathway of activated Mφ on *M. leprae.*—[GWL Hansen's Disease Center, Carville, Louisiana 70721; Veterans Administration Medical Center, Salt Lake City, Utah 84148, U.S.A.]

Kaplan, G. and Cohn, Z. A. Role of recombinant human IL-2 in immune modulation of leprosy.

The basic defect in lepromatous leprosy is the selective unresponsiveness of T cells to the antigens of *Mycobacterium leprae.* This defect may be partial or complete, and does not appear to change with prolonged chemotherapy. A lack of T-cell-derived lymphokines, such as IFN-γ and Il-2, could account for the inability of macrophages and

other cells to eliminate *M. leprae*. Attempts to circumvent T-cell unresponsiveness have already been initiated in both *in vitro* systems and in the confines of the cutaneous lesions. Initial observations have been reported on the efficacy of crossreacting antigens such as purified protein derivative of tuberculin (PPD) or the lymphokine rIFN-γ. Intradermal administration of PPD or IFN-γ has led to a long-lived emigratory mononuclear leukocyte response, the local destruction of parasitized macrophages, and a striking reduction in the number of *M. leprae* within 21 days. These responses and the accompanying modification of cellular subsets are evidence of a vigorous cell-mediated immune reaction in the environment of a lepromatous lesion. We have now extended these observations to the use of human rIL-2. We report results on the use of small doses of IL-2, in the skin of lepromatous patients, and the reconstitution of cutaneous cellular immunity.—[The Rockfeller University, New York, New York, U.S.A.]

Fujiwara, T., Izumi, S. and Sakamoto, Y. Correlation between the chemical structure of the carbohydrate part of *Mycobacterium leprae* PGL-I and its activity to the monoclonal antibodies.

Correlation between the chemical structure of the carbohydrate part of the *Mycobacterium leprae* phenolic glycolipid (PGL-I) and its activity to the monoclonal antibodies was studied by using synthetic carbohydrate antigen. All monoclonal antibodies tested (mAb 24, mAb 25, ml 6A2, ml 8A2, ml 8B2, FH-21, SF-1) did not recognize the carbohydrates without the terminal 3,6,di-*O*-methylglucose. The regions recognized by these antibodies were terminal 3,6-di-*O*-methylglucose for mAb 24; the outer disaccharide for ml 6A2, ml 8A2 and ml 8B2; the outer disaccharide and some part of the inner monosaccharide for FH-21; the whole trisaccharide, including some part of the phenyl ring, for SF-1. These results indicate that recognition of the antigen had the fixed direction from outer to inner carbohydrate. This conclusion was confirmed by a competitive ELISA inhibition assay and the result of immunization with inner disaccharide-KLH conjugate. A high positive rate to the inner disaccharide was found in healthy sera and in leprosy and tuberculosis sera. However, these activities were not absorbed by the trisaccharide antigen and, also, the activity to trisaccharide antigen was not absorbed by the inner disaccharide antigen. These results suggest the possibility of the presence of the PGL antigen, common to most mycobacteria, and that the specificity of the PGL was obtained by adding 3,6-di-*O*-methylglucose to the common structure of PGL.—[Institute of Natural Science, Nara University, Nara; National Institute for Leprosy Research, Tokyo, Japan]

Izumi, S., Hua, C. Y., Amiruddin, M. D., Kawatsu, K., Choudhury, A. M., Mendes, M. F. and Fujiwara, T. Distribution of anti-PGL-I antibody in household contacts from endemic countries.

In order to know the utility of phenolic glycolipid (PGL)-related serodiagnosis of leprosy for epidemiological investigation and consequent prevention of leprosy, we are now conducting household contact surveys in endemic areas of South Sulawesi, Indonesia, and Parana, Brazil. The anti-PGL-I antibody titer was measured by the newly established *Mycobacterium leprae*-specific gelatin particle agglutination test, SERODIA-LEPRAE. It was found that the seropositive rates in contacts from South Sulawesi and Parana were significantly higher than in contacts from Japan, suggesting that the seropositive rate in contacts has a positive correlation with the endemicity of leprosy. The differences in seropositive rates among the blood relatives of contacts were tested by using South Sulawesi sera. There were no statistically significant differences between the blood relatives and contacts themselves.

The differences in seropositive rates and distribution of antibody titers among the index cases with different types of leprosy were significant in South Sulawesi. Generally, the positive rate and the titer were significantly higher in the contacts of multibacillary leprosy (LL, BL, BB) than in the contacts of those with paucibacillary leprosy (BT, TT, Indeterminate). The differences in the positive rates in the contacts of patients with different types of leprosy in Parana, Brazil, however were not signifi-

cant. The reasons for the differences between the Indonesian and Brazilian studies in this respect remains obscure. In both South Sulawesi and Parana, 5%–6% of the contacts were strongly positive. These contacts are known to be at high risk of developing clinical disease. Chemoprophylaxis for these contacts is being considered.—[National Institute for Leprosy Research, Tokyo, Japan; Institute of Dematology, Chinese Academy of Medical Sciences, Nanjing, China; Hasanuddin University, South Sulawesi, Indonesia; Leprosy Control Institute and Hospital, Dhaka, Bangladesh; Sociedade Filantropica Humanitas, Parana, Brazil; Nara University, Nara, Japan]

Matsuo, K., Yamaguchi, R., Yamazaki, A., Nagai, S., Tozuka, M. and Yamada, T. Progress in establishing a recombinant BCG secreting system.

Two-dimensional elecrophoretic analysis of the proteins of the culture filtrate of *Mycobacterium bovis* BCG revealed approximately 200 individual spots. Four antigenic proteins—α, MPB64, MPB70, MPB57—out of these proteins were purified. The *N*-terminal amino acid sequences were determined, and complementary oligonucleotide probes were synthesized and then used for cloning of the genes for these antigens. The characterization of both α antigen and MPB64 had already been reported at the last symposium. In this paper the study of MPB70 and MPB57 of *M. bovis* BCG and α antigen of *M. kansasii* is reported.

MPB70 possessed a signal peptide consisting of 30 amino acids, and the mature protein consisted of 163 amino acids with a molecular weight of 16,305. The signal peptide displayed a characeristic Ala-rich property which provides for efficient secretion. A putative SD sequence is located 9 nucleotides upstream of the initiation condon. *Escherichia coli* promoter consensus sequences -10 and -35 were seen. The *N*-terminal amino-acid sequence of MPB57 was identical to that of BCG-α except that one amino acid, Glu-15 of BCG-α, was replaced by Gln-15 in MPB57. The DNA sequence contained ORF beginning with GTG, ending with the double stop condons, and lacked a signal peptide. The gene for the extracellular α antigen of *M. kansasii* was cloned by using the α-antigen-gene fragments of *M. bovis* BCG as probes. Gene analysis revealed that this gene encodes 40 amino acids for the signal peptide followed by the mature protein. The amino-acid homology between *M. kansasii* and *M. bovis* is 89.1%. The hydropathy profiles of the proteins from these two species were similar, but two highly changed regions were observed which might acount for the antigenic diversity of this antigen.

The application of these studies toward the construction of recombinant BCG for the purpose of protecting against leprosy is discussed.—[Central Laboratories of Ajinomoto Co., Inc., Kawasaki City; Osaka City University School of Medicine, Osaka City; Nagasaki University School of Dentistry, Nagasaki City, Japan]

Pane, L., Belisle, J., Ganjam, K., Brennan, P. J., Bloom, B. R. and Jacobs, W. R., Jr. Analysis of *Mycobacterium leprae, M. tuberculosis,* and *M. avium* genomic libraries in *M. smegatis* and *E. coli* using shuttle cosmid vectors.

We have shown that representative mycobacterial genomic cosmid libraries can be used to express, identify, and clone mycobacterial genes. The main advantage of our shuttle cosmid cloning strategy is that 40 kb of contiguous mycobacterial genome, which should contain approximately 20 to 40 genes, can be analyzed in a mycobacterial host. We are currently exploring the utility of the shuttle cosmid cloning strategy in the identification of clusters of mycobacterial genes responsible for complex phenotypes, such as the biosynthesis of cell-surface determinants, and expression of virulence determinants. Preliminary evidence suggests that we have identified a cosmid clone involved in the biosynthesis of the polar glycopeptidolipid of *Mycobacterium avium* serovar 2.—[Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York; Department of Microbiology, Colorado State University, Fort Collins, Colorado, U.S.A]

Nishimura, Y., Sudo, T., Inamitsu, T., Nomaguchi, H. and Sasazuki, T. Application of HLA-DQ transgenic mice for the analysis of genetic control of the immune

response to *Mycobacterium leprae* antigen.

We have reported previously the strong association between HLA-DR2-DQw6 and lepromatous leprosy (LL) and the low immune responsiveness specific to *Mycobacterium leprae* antigen in patients with LL. Furthermore, a strong immune response was restored in LL patients by the addition of anti-HLA-DQ monoclonal antibody (mAb) *in vitro,* suggesting a HLA-DQ-linked genetic control of low responsiveness to *M. leprae* antigen.

There are obvious limitations to the immunobiological investigation of leprosy in human subjects. Therefore, we have generated the HLA-DQw6 transgenic mice in an attempt to establish a mouse model suitable for the investigation of genetic control of the immune response to *M. leprae* antigen by HLA-DQ. The tissue-specific expression of DQw6 molecules was much the same as those of mouse I-A^b molecules, and DQw6-B6 acquired a tolerance to the DQw6 molecule, at least at the T-cell level. Surprisingly, DQw6-B6 responded to streptococcal cell-wall antigen (SCW), and this response was completely inhibited by an anti-HLA-DQ mAb; whereas B6 mice behaved as low responders to SCW. A SCW-specific T-cell line established from DQw6-B6 mice responded to a mouse L-cell transfectant expressing the HLA-DQw6 molecule in the presence of SCW, proving that the SCW-specific T cells of DQw6-B6 recognize SCW in the context of DQw6 molecule. Both DQw6-B6 and B6 responded to crude antigen extract derived from *M. leprae.* Partial purification of the 65-kDa heat-shock protein of *M. leprae* (HSP65-ML) was accomplished by ion exchange chromatography of the *Escherichia coli* lysate, and complete purification was conducted by affinity chromatography using anti-HSP65-ML mAb. DQw6-B6 exhibited low responsiveness to partially purified HSP65-ML; whereas B6 responded well to this antigen. However, when completely purified HSP65-ML was used as immunogen, both B6 and DQw6-B6 exhibited equally small but definite responses to HSP65-ML. Because partially purified HSP65-ML contains proteins derived from *E. coli* itself, these data indicate that the DQw6-B6 acquired low responsiveness to some unknown antigen derived from *E. coli.*

In these experiments, we could not observe any change in the immune response to *M. leprae* by the expression of HLA-DQw6 genes in B6 mice. However, our observations clearly indicate HLA-DQ-linked genetic control of immune responses in the transgenic mice.—[Department of Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812, Japan]

Venkataraman, S. and Barker, R. H., Jr. Detection of mycobacteria using PCR and specific DNA probes.

At present, diagnosis of tuberculosis depends upon observation of acid-fast bacilli (AFB) in clinical specimens, while definitive speciation requires culturing organisms. The latter procedure is cumbersome, taking between 1–6 weeks to complete. The sensitivity of the AFB test is between 10^3–10^4 cells per sample; therefore clinical specimens containing fewer organisms may be falsely considered negative. Similarly, methods for speciation depend upon the ability to culture organisms from clinical samples. If organisms fail to grow out in culture, speciation is not possible. It is therefore not surprising that considerable attention has been focused upon the development of nucleic acid probes for identification of mycobacterial species. The specificities of such probes can be selected according to the requirements of the user (i.e., species- vs genus-specific), and assays utilizing DNA hybridization can be accomplished in a matter of hours.

One such approach utilizes cDNA probes to detect bacterial rRNA sequences. However, the sensitivity of this method is about 10^5 organisms, and therefore culture of organisms from clinical specimens is still required. An alternate approach has been to identify repeated DNA sequences for use as genomic DNA probes. In the case of *Mycobacterium leprae,* Clark-Curtiss and Docherty developed a probe capable of detecting 1 pg of purified DNA, corresponding to approximately 4000 bacilli. This probe has also been used to detect organisms directly in skin biopsy material, further demonstrating the potential of this approach. Similarly, Patel and coworkers developed a

probe for repeated sequences found in *M. tuberculosis* complex. Sequence analysis has shown that this probe consists of two nearly identical 508 bp repeats which show no sequence homology with DNA from other species, while hybridization studies indicate that homologous sequences are found in *M. tuberculosis* isolates obtained from widely dispensed geographical areas. However, when used in direct assays, sensitivity was only about 10^5 organisms. Patel and coworkers therefore initiated a series of experiments to examine the use of this probe in conjunction with PCR-amplified target sequences. These studies showed that oligonucleotide primers derived from this clone could be used to specifically amplify as little as one fg of purified *M. tuberculosis,* which is equivalent to less than a single organism. Even more important, these studies showed that treatment of cultured organisms by freeze-thaw either alone or in the presence of NP 40 caused sufficient disruption of the cell wall to permit direct amplification of mycobacterial DNA. This last observation was especially important, since it could represent the basis for developing a clinically applicable diagnostic procedure.

In the present studies, we examine a variety of methods for disrupting bacilli prior to PCR amplification, and have further analyzed the use of different PCR products as species-specific probes. Experiments currently in progress are examining the use of this method for the detection of mycobacteria in clinical specimens.—[Harvard University School of Public Health, Department of Tropical Public Health, Boston, Massachusetts, U.S.A.]

Plikaytis, B. B., Mundayoor, S. and Shinnick, T. M. Detection and identification of *Mycobacterium* species using gene amplification techniques and RFLP analysis.

Two rapid and sensitive polymerase chain reaction (PCR)-based assays have been developed to detect and identify mycobacteria. In one assay, nested sets of primers are used to amplify specifically target sequences from either *Mycobacterium tuberculosis* or *M. leprae.* Such specific assays should be useful in situations that simply require detection of *M. tuberculosis* or *M. leprae* but not other *Mycobacterium* species. In the second assay, common primers are used to amplify sequences from many *Mycobacterium* species followed by a RFLP analysis to identify the species. Such an assay should be useful in situations that require detection and identification of any *Mycobacterium* species, as is the usual situation in a clinical laboratory. Overall, the sensitivity and specificity of these two PCR-based assays are quite encouraging for their potential use in the rapid detection and identification of small numbers of mycobacteria in clinical specimens.—[Hansen Disease Laboratory, Division of Bacterial Diseases, Centers for Disease Control, Atlanta, Georgia 30333, U.S.A.]

Brennan, P. J., Hunter, S. W., Chatterjee, D., McNeil, M., Rivoire, B. and Bozic, C. M. Toward a thorough resolution and structural definition of the major antigens of *Mycobacterium leprae.*

The leprosy bacillus has now been successfully fragmented into its major subcellular compartments and the major antigens of each resolved and, at times, structurally elucidated. Thus, the major B-cell-reactive cell-wall antigens of *Mycobacterium leprae* are phenolic glycolipid-I, described *ad infinitum,* and the unique arabinofuranosyl (Ara*f*)-containing unit [β-D-Ara*f*-(1→2)-α-D-Ara*f*-(1→]$_2$→(3 and 5)-α-D-Ara*f*→, which constitutes the terminal segments of arabinogalactan. The araginogalactan is covalently attached at its nonreducing terminus to peptidoglycan by the novel bridge, L-Rha*p*-(1→3)-D-GlcNac-1→P (where Rha*p* is rhamnopyranose and GlcNac is N-acetylglucosamine). The dominant T-cell-reactive protein antigens of *M. leprae* cell walls are the peptidoglycan-bound 14-kDa protein, described previously, and a free 17-kDa polypeptide apparently not described before. The plasma membrane of *M. leprae* is dominated by the highly antigenic lipoarabinomannan. It contains a phosphatidylinositol anchor at the reducing end. The nonreducing antigen-determinant end is characterized by the same Ara*f*-containing motifs found on arabinogalactan. The membranes of *M. leprae* contain two major proteins, major membrane protein (MMP)-I of Mr 35 kD, identified previously with monoclonal antibodies, and a new protein,

MMP-II, of Mr 22 kD. Two major cytosolic proteins (MCP) of Mr 14 kD (MCP-I) and 28 kD (MCP-II) were also recognized and isolated. MCP-I is the single most abundant protein in *M. leprae,* representing about 1% of the bacterial mass. It shows similarities to the BCG-α/10.8 kDa protein of *M. tuberculosis/M. bovis.* MCP-II is apparently the superoxide dismutase of *M. leprae* described by others. Thus, the major antigens of *M. leprae,* protein and carbohydrate alike, are now nearing complete definition.—[Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.]

Shinnick, T. H. Heat-shock proteins as antigens of pathogenic microorganisms.

It is clear that heat-shock proteins (HSP) are immunologically active components of many pathogens. For several pathogens, the humoral immune response to the HSP is directed predominantly toward nonconserved epitopes. Perhaps this is a parasite survival strategy. Here, the nonconserved epitopes may serve as an immunological smokescreen to divert the host's immune response away from conserved epitopes, which may be regions required for functional activity. Of course this is not a general survival strategy, since some HSP in some pathogens seem to be targets of a protective immune response. On the other hand, investigators have taken advantage of the specificity of the humoral immune response to develop immunodiagnostic tests. Although assays involving the whole protein are usually too crossreactive to be diagnostic, epitope-specific assays may yet turn out to be useful. Finally, HSP of pathogenic microorganisms can elicit humoral and cellular immune responses to epitopes that are shared with their hosts. Such immunoreactivity could play important roles in pathogenicity and autoimmune consequences of infections.—[Hansen Disease Laboratory, Division of Bacterial Diseases, Centers for Disease Control, Atlanta, Georgia 30333, U.S.A.]

CLOSING REMARKS

Dr. Someya, Chairman of the Japanese Delegation, Dr. Mori, Dr. Izumi, Dr. Kohsaka, Dr. Tanaka, Dr. Saito, fellow members of the U.S. Panel, guests and friends:

As we bring this 25th Joint U.S.-Japan Conference on Leprosy Research to a close, there are sentiments of joy and sadness within each of us.

Deep sadness that Dr. Ivan Bennett is no longer with us. He was a great visionary American, who almost single handedly conceived of and guided the U.S.–Japan Cooperative Medical Science Program through the 25 years of its existence. He had a deep appreciation of the importance of close U.S.–Japan relationships and interaction at the scientific level, and we will sorely miss his leadership within this great cooperative program as it continues to develop during the coming years. We are grateful to Dr. Someya for his sensitive tribute to a great man and a great scientist.

My friends, we are also saddened by the loss of colleagues who contributed to these deliberations in years past, and I am mindful in particular of Dr. Chapman Binford, Dr. John Hanks, and now I also hear of the death of Dr. Yamamura, who contributed hugely to our knowledge of the composition of mycobacteria and who, just a few short years ago in Osaka, contributed beautifully to our joint symposium.

We are also a little saddened, my friends, by the end of a conference that was so successful and that we had looked forward to with great anticipation. We are also a little saddened by our imminent departure from this beautiful university, beautiful city, beautiful island, country and people.

Yet, the dominant emotion within all of us is one of joy; joy that we were witness and party to a most successful joint conference on leprosy research and joint symposium. In my experience, yesterday's joint symposium was the most sophisticated,

most enlightened symposium that has ever been conducted under the aegis of this program.

Dr. Mori and friends, we are also heartened and joyful by what we all perceive as a rather spectacular advancement in the ease of personal communication between Japanese and U.S. participants. I was personally most impressed with the ease with which we can communicate with each other on most occasions. This we can attribute to the extraordinary diligence of Japanese participants in mastering the English language. As one who is trying to learn Japanese, I am most impressed with this development.

And we are also joyful and appreciative of the changes and metamorphosis that we have seen at this conference, such as the emergence of young people of extraordinary talent and one singles out for special mention in this respect, the two Dr. Gotos, Dr. Osawa, Dr. Hatfull, Dr. Guido from Cebu, Dr. Nishimura, Ms. Lisa Pane and Ms. Kalpana Ganjam, among the presentors, and too many to mention among the participants. Dr. Mori and Japanese colleagues, we also take great joy in witnessing the growth and expansion of leprosy research within Japan, and in this context we note, in particular, your new training programs for scientists from endemic countries and your vigorous involvement in leprosy research and control in India, Bangladesh, Indonesia, Malaysia, Thailand, The Philippines, China, and other countries in the western Pacific region. Our interactions of the last few days clearly demonstrated a renewed vigor and growth in leprosy research within Japan.

Finally, Dr. Mori and Japanese Panel members, our primary emotion is one of gratitude and thankfulness. Gratitude to Dr. Azuma and colleagues here at Hakkaido University for exceptional hospitality, for the outstanding conditions here at the conference center, for the elegant and spacious rooms at Kosei Nenkin Kaikan, for the sumptuous banquets and for the warm personal relationships.

On behalf of the U.S. Panel members (Drs. Clark-Curtiss, Kaplan, Krahenbuhl, Modlin and Rea), on behalf of our guests from Japan itself, from the U.S., from China, from The Philippines, from India, from Bangladesh; on behalf of Dr. Darrel Gwinn and Dr. Tim Henry of the National Institutes of Health, we thank Dr. Azuma and his colleagues and you, Dr. Mori, and your Panel and all of you, our Japanese colleagues and friends, for a truly marvelous meeting and a marvelous, wonderful time. You truly did your part in ensuring the continuation of growth of this wonderful program.

Arigatoo; Doomo Arigatoo.

—Patrick J. Brennan, *Chairman*
U.S. Leprosy Panel