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ABSTRACTS

TWENTY-FOURTH JOINT LEPROSY RESEARCH CONFERENCE

Town and Country Hotel
San Diego, California, U.S.A.
23 and 25 August 1989

U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

My colleagues in leprosy research:

We are meeting here today for the 24th time under the aegis of the U.S.–Japan Cooperative Medical Science Program, a prestigious, bilateral program, which next year will celebrate a quarter of a century of existence.

The U.S.–Japan Cooperative Medical Science Program encompasses ten diverse areas of medical research: parasitic and viral diseases, leprosy, tuberculosis, cholera, hepatitis, AIDS, topics in environmental health, malnutrition and immunology, and it is sponsored and nurtured by the highest authorities of our respective governments.

We within the leprosy research component of the U.S.–Japan Cooperative Medical Science Program have been blessed by exemplary leadership during the course of these 24 years: Charles Shepard, Barry Bloom, Masahide Abe, Tonetaro Ito, Robert Hastings, Masahiro Nakamura, Ward Bullock, Zanvil Cohn, Mitsugu Nishura, among many others, have spearheaded these deliberations at one time or another during these past years. Dr. Darrel Gwinn, Leprosy Program Officer at the National Institutes of Health (NIH), has for 11 years facilitated these deliberations, in fact, directed them through his knowledge of the relevant personnel, both U.S. and Japanese, his keen insight into the work being pursued by individual investigators, and his marvelous tact and sensitivity.

At the higher echelons of the U.S.–Japan Cooperative Medical Science Program, at the level of the overall delegations, we have also been blessed with wise and experienced leadership, people who are conscious of and sympathetic to the special challenges of research in leprosy, among them Dr. Ivan Bennett, long-time chairman of the United States' delegation; Dr. Norio Suwa, Dr. Shiro Someya, Dr. Tadao Shimao, Dr. Edward Hook, Dr. Yuichi Yamamura, Dr. Charles Carpenter, and Dr. Harley Sheffield of NIH, Co-ordinator of the Program.

In view of the prestige of the Program through which we all assemble here today, we may ask ourselves whether we, as the

present-day inheritors, have maintained the standards of exemplary cooperative, bilateral research in leprosy. Surely we have, you may well respond. After all, these meetings in recent years have witnessed enormous progress, in the definition and manipulation of the genome of the leprosy bacillus; in the exhaustive definition of its antigenic components and in their expression through recombinant means; in the correlation of immunopathologic events with bacterial moieties; in the dissection of the cellular immune response within the dermal lesions of leprosy patients; in new specific diagnostic and immunological tools; in new animal models and chemotherapeutic regimens; and so on.

Given this heady and quite extraordinary progress in fundamental and applied research aimed toward the control of leprosy, and all within the gambit of the U.S.–Japan Cooperative Medical Science Program, should not we be content in the belief that we have carried the flag, that we have maintained the tradition, that we have matched the accomplishments of other components of the U.S.–Japan Cooperative Program, that we have earned the trust and respect of past and present leadership of the overall Program? Sadly, my colleagues, there is no justification for self-congratulation. In fact, we, the present-day U.S. participants in this prestigious, bilateral program, have been remiss in our adherence to one of the primary missions of the Program. While as individual researchers we may have excelled, we have been lacking in pursuing the principle of a close, interdependent, working relationship with our Japanese colleagues.

In reporting on progress under this Program for the year of 1988, not one U.S. participant recorded any joint work or exchange with a Japanese counterpart (although, in fact, in subsequent conversations and correspondence, it emerged that there are examples of some individual interactions). Accordingly, there is the fear that this relationship built by our predecessors over the past 24 years will become a mere annual get-together without any mutually beneficial, symbiotic interaction.

We in the U.S. often are not conscious of the outstanding field work being conducted by our Japanese colleagues, such as Dr. Yuasa, Dr. Ito, Dr. Kohsaka, and Dr. Izumi in endemic areas in The Philippines, in Thailand, and in other parts of Southeast Asia, including even Burma, in Micronesia, and other regions of the Western Pacific. We may have overlooked the fact that through our Japanese colleagues, through this very Program, we may be in a position to implement in a humanitarian fashion some of our outstanding fundamental findings in leprosy research of recent years. We may have overlooked the fact that one of the greatest proponents of the use of multiple-drug therapy combined with serology, for the eradication of pockets of leprosy in the Western Pacific region, is now Director General of the World Health Organization, namely, Dr. Nakamura. We may have overlooked the fact that at the National Institute for Leprosy Research in Tokyo is housed the finest resources and the finest personnel singularly devoted to research on leprosy.

Accordingly, I ask each of you U.S. participants on the eve of the 25th anniversary of the founding of the U.S.–Japan Cooperative Medical Science Program to re-dedicate yourself to the international, bilateral, cooperative aspects of this outstanding Program. I ask each of you to consider how you as an individual researcher can advance the humanitarian cause of leprosy control through interactive exercises with Japanese counterparts. I ask each of you to approach members of the Panel with your ideas on this matter; ideas on personnel exchange; exchange of materials, such as *M. leprae,* its native and recombinant products; exchange visits; cooperative laboratory and field work; and so on. My colleagues, I am telling you that the future of this marvelous Program surely depends on your efforts, here and now.

In closing, on behalf of the United States Panel; on behalf of Dr. Darrel Gwinn, our benefactor at the National Institutes of Health, who, in fact, has made this setting in San Diego possible; on behalf of Dr. Sheffield, Coordinator of the overall United States–Japan Cooperative Medical Science Program; I would like to welcome Dr. Mori, Dr. Izumi, Dr. Kohsaka, Dr. Nakamura, and Dr. Saito of the Japanese Panel, other Japanese participants and guests, all of our U.S. participants and guests, and Dr. Tim Henry of the Division of Research Grants,

National Institutes of Health. I wish for all of you a productive, stimulating meeting, and an enjoyable time in beautiful San Diego.

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

PROGRAM
TWENTY-FOURTH JOINT LEPROSY CONFERENCE

23 August, Wednesday

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

Session I

Co-Chairmen: Dr. Hajime Saito
Dr. Gilla Kaplan

Krahenbuhl, J. L. and Chae, G.-T. Study of the Turnover, Traffic and Function of Bone-marrow-derived Macrophages in the Granulomas of Experimental Lepromatous Leprosy

Salgame, P., Modlin, R. L. and Bloom, B. R. On the Mechanism of T-cell Suppression in Leprosy

Franzblau, S. G. and White, K. E. Comparative *in vitro* Activity of Fluoroquinolones Against *Mycobacterium leprae*

Gelber, R. H. The Activity of a Beta-lactamase Inhibitor, Augmentin®, Against *Mycobacterium leprae* in Mice: a Novel and Promising Target for Drug Development

Saito, H. and Tomioka, H. *In vivo* activities of New Quinolones Against *Mycobacterium leprae* Infection Induced in Mice

Session II

Co-Chairmen: Dr. Shinzo Saito
Dr. James Krahenbuhl

Sathish, M., Thole, J. E. R., Bosecker, B. and Clark-Curtiss, J. E. Cloned Antigenic Determinants of *Mycobacterium leprae* that React with Leprosy Patients' Sera: Their Characterization and Ability to Elicit Delayed-type Hypersensitivity Responses in Mice Following Immunization

Sela, S. and Clark-Curtiss, J. E. Isolation and Characterization of the Putative Promoter of the *Mycobacterium leprae* Ribosomal RNA Operon

Hunter, S. W. and Brennan, P. J. The Native Proteins of *Mycobacterium leprae*: Preliminary Results on Subcellular Location, Isolation and Chemical and Immunological Characterization

Modlin, R. L., Torigian, V., Bloom, B. R., Rea, T. H. and Brenner, M. B. Antigen-Reactive TCR$\gamma\delta$ Lymphocytes in Leprosy Lesions

25 August, Friday

Session III

Co-Chairmen: Dr. Kenji Kohsaka
Dr. Thomas Rea

Izumi, S., Fujiwara, T., Ikeda, M., Nishimura, Y., Kawatsu, K. and Sugiyama, K. Sensitivity, Specificity, Reliability of Positive and Negative Indirect ELISA and Gelatin Particle Agglutination Test for Serodiagnosis of Leprosy

Williams, D. L., Gillis, T. P., Booth, R. J., Looker, D. and Watson, J. D. Application of Polymerase Chain Reaction Amplification Technology for the Detection of *Mycobacterium leprae*

Kohsaka, K., Hirata, T. and Nakamura, M. Effect of Lyophilization on Viability of *Mycobacterium leprae* Grown in Nude Mice

Walsh, C. P., dela Cruz, E. C., Abalos, R. M., Fajardo, T. T., Guido, L. S., Cellona, R. V., Meyers, W. M., Gormus, B. J., Resuello, R. and Lange, F. W. Leprosy Studies in Philippine Cynomolgus Monkeys (*Macaca fasicularis*): Preliminary Results

Nakamura, K. and Yogi, Y. The MRL/*lpr* Mouse as an Experimental Leprosy Model (continued)

Session IV

Co-Chairmen: Dr. Kazunari Nakamura
Dr. Josephine Clark-Curtiss

Matsuo, E., Sasaki, N. and Skinsnes, O. K. On the Soluble Fraction of *Mycobacterium scrofulaceum* HI-75, Originally Separated from Human Leproma, Which Combines with Beta-glucuronidase

Hancock, G. E., Cohn, Z. A. and Kaplan, G. Generation of Antigen-specific, MHC-restricted Cytotoxic T Lymphocytes of the CD4+ Phenotype; Enhancement by Cutaneous Administration of IL-2

Fukutomi, Y., Inui, S. and Onozaki, K. Ia Antigen Expression in Macrophages After Phagocytosis of Mycobacteria *in vitro*

Rea, T. H., Cooper, C. L., Mueller, C., Bloom, B. R. and Modlin, R. L. Identification of Interferon-gamma mRNA and Human Serine Esterase mRNA in Spontaneously Occurring DTH Reactions in Leprosy Skin Lesions by *in situ* Hybridization

JOINT U.S.–JAPAN TUBERCULOSIS AND LEPROSY SYMPOSIUM

Town and Country Hotel
San Diego, California, U.S.A.

24 August, Thursday

Session I

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

Plikayetes, B. B. and Shinnick, T. H. Rapid, Sensitive, and Specific Detection of Mycobacteria Using Gene Amplification Techniques

Tanaka, T., Tamura, T., Yamamoto, Y. and Furuyama, J. Cloning and Expression of *Mycobacterium tuberculosis* Strain Aoyama B in *Escherichia coli*—a Gene Encoding 15 kDa and 60 kDa Antigens

Mizuguchi, Y., Udou, T., Taniguchi, H., Goto, Y. and Tokunaga, T. Establishment of a Host-vector System in Mycobacteria

Kaplan, G. and Cohn, Z. A. The Role of rIL-2 in the Immune Modulation of Leprosy

Tomioka, H. and Saito, H. Properties of Suppressor Macrophages Induced with *Mycobacterium leprae* Vaccine

Session II

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

Mehra, V., Barnes, P. F., Hunter, S. W., Brennan, P. J., Rea, T. H., Bloom, B. R. and Modlin, R. L. Characterization of Antigens Associated with the Cell Walls of *M. leprae* and *M. tuberculosis* Using T-lymphocyte Clones

Schlesinger, L. S. and Horwitz, M. A. Complement Receptors and Complement Component C3 Mediate Phagocytosis of *Mycobacterium tuberculosis* and *M. leprae*

Snapper, S. B., Jacobs, W. R., Jr., and Bloom, B. R. Development of a Recombinant BCG Vaccine Vector; Cloning and Expression of Foreign Antigen Genes in BCG

PANEL MEMBERS
U.S.-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

U.S. Leprosy Panel

Brennan, Patrick J. (*Chairman*), Professor, Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.

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Krahenbuhl, James L., Chief, Department of Immunology Research, Laboratory Research Branch, GWL Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.

Rea, Thomas H., Department of Health Sciences, Los Angeles County/University of Southern California Medical Center, 1200 North State Street, Room 8441, Los Angeles, California 90033, U.S.A.

Japanese Leprosy Panel

Mori, Tatsuo (*Chairman*), Director, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo 189, Japan

Izumi, Shinzo, Chief, Laboratory of Pathology, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo 189, Japan

Kohsaka, Kenji, Research Assistant, Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Yamadakami, Suita, Osaka, Japan

Nakamura, Kazunari, Director, First Research Department, National Institute for Leprosy Research, Higashimurayama-shi, Tokyo 189, Japan

Saito, Hajime, Professor, Department of Microbiology and Immunology, Shimane Medical University, Izumo-shi 693, Japan

ABSTRACTS

Krahenbuhl, J. L. and Chae, G.-T. Study of the turnover, traffic and function of bone-marrow-derived macrophages in the granulomas of experimental lepromatous leprosy.

Previous studies from our laboratory have demonstrated that although macrophages (Mϕs) isolated from the lepromatous foot-pad granulomas of *Mycobacterium leprae*-infected *nu/nu* mice share most phenotypic and functional characteristics with peritoneal Mϕs, because of their heavy intracellular burden of *M. leprae* these foot-pad Mϕs are refractory to activation by recombinant murine interferon-gamma (IFN-γ). Regardless of the IFN-γ dose, *M. leprae*-burdened foot-pad Mϕs cannot be activated (enhanced microbicidal capacity for the obligate intracellular protozoan *Toxoplasma gondii,* enhanced oxidative burst, or increased Ia expression). The defect is localized since peritoneal Mϕs from the same *nu/nu* mice are fully responsive to IFN-γ. Extrapolation of these findings to the localized skin lesions of lepromatous leprosy in man suggests that any killing, breakdown, and clearance of bacilli resulting from immunotherapeutic or chemotherapeutic measures must be accomplished by the arrival of new competent Mϕs at the site.

Autoradiography studies are being carried out to define the rate of ^{3}H-thymidine-labeled, bone-marrow-derived Mϕ turnover in the lepromatous foot pad itself, as well as in other anatomical compartments (blood monocytes, peritoneal Mϕs, popliteal lymph nodes) of untreated, *M. leprae*-infected *nu/nu* mice. To compare this steady-state level of Mϕ turnover with an experimental condition designed to induce clearance of bacilli or to enhance host Mϕ-mediated defense mechanisms, our present studies are also concerned with determining whether there is enhanced migration of bone-marrow-derived Mϕs into the lepromatous foot-pad lesions following immunotherapy (multiple treatments with IFN-γ) or chemotherapy (dietary rifampin).

Our initial studies of the traffic of Mϕs into the lepromatous foot pad revealed no apparent difference in the rate of entry of bone-marrow-derived Mϕs into the peripheral blood or peritoneal cavities of control and IFN-γ-treated, *M. leprae*-infected *nu/nu* mice. However, in untreated mice at 15%–20% of the foot-pad-granuloma Mϕs burdened with *M. leprae* were newly arrived, suggesting a relatively high rate of turnover. IFN-γ treatment appeared to enhance the influx of Mϕs into the foot-pad granuloma. The effects of treatment with rifampin are still being analyzed. The actual turnover (i.e., lifespan) of Mϕs in the foot-pad granuloma is currently being studied.

Regarding the function of these newly arrived Mϕs, in preliminary studies, although there was no clear evidence for breakdown and clearance of *M. leprae* in treated mice, foot-pad Mϕs from IFN-γ- or rifampin-treated mice were partially responsive to activation of IFN-γ *in vitro.* These data suggest that the defective Mϕ response to IFN-γ in experimental lepromatous granulomas may be reversible by inducing an increase in the rate of turnover of new Mϕs into the lesion.—[Immunology Research Department, GWL Hansen's Disease Center, Carville, Louisiana, U.S.A.]

Salgame, P., Modlin, R. L. and Bloom, B. R. On the mechanism of T-cell suppression in leprosy.

We have earlier provided evidence that T-suppressor (Ts) cells may be important regulatory cells determining T-helper (Th) cell unresponsiveness to *Mycobacterium leprae* antigens in lepromatous leprosy. The mechanism by which the Th activity is regulated by Ts cells remains unclear in leprosy as well as in most other experimental systems. To analyze the mechanism of suppression, Ts clones were established from lesions and peripheral blood of lepromatous patients. The phenotype of these clones are CD3+, CD4+, CD8+ and TCR$\alpha\beta$+. The induction of suppression *in vitro* by the Ts clones was specific for antigens of *M. leprae.* However, the effector function of the clones was antigen nonspecific.

In the present work we have explored several mechanisms by which antigen-specific Ts clones may downregulate CD4+ helper

responses *in vitro,* including a) killing of antigen-presenting cells (APC); b) killing of Th cells by idiotopic or antigen-specific recognition; c) functional inactivation of Th cells without killing. The Ts clones failed to show any evidence of cytotoxicity for four antigen-pulsed, MHC-matched target cells: a) an ori$^-$ SV40 transformed macrophage line; b) EBV transformed B-cell line; c) primary macrophages; and d) *M. leprae*-reactive CD4+ clones. These results exclude the possibility that the Ts clones are cytotoxic to either APC or CD4+ helper cells. The possibility of functional inactivation of the Th clones by Ts clones was investigated. *M. leprae*-reactive CD4+ clones were preincubated with Ts clones, APC, and *M. leprae* antigen for 16 hr. After this overnight incubation, the CD8+ Ts clones were removed from culture. The Ts-depleted CD4+ cells were further purified on Ficoll-paque gradient to remove dead cells and antigen and restimulated with fresh APC and *M. leprae* antigen. CD4+ clones preincubated with Ts clones responded poorly to restimulation with *M. leprae* antigen, although they were not killed and could respond well to interleukin 2 (IL-2). The presence of IL-2 in the preincubation or postincubation cultures neither prevented nor reversed the induction of unresponsiveness. The inability of the CD4+ cells to respond to antigen did not result from decreased expression of the CD3-TCR complex.

Earlier models of tolerance have suggested that receptor occupancy in the absence of second signals induces tolerance in B and T cells. Ts cells may functionally downregulate CD4+ Th cell responses through similar mechanisms requiring antigen presentation without appropriate second signals.—[Albert Einstein College of Medicine, Bronx, New York; University of Southern California School of Medicine, Los Angeles, California, U.S.A.]

Franzblau, S. G. and White, K. E. Comparative *in vitro* activity of fluoroquinolones against *Mycobacterium leprae.*

The *in vitro* activity of 19 fluoroquinolones against *Mycobacterium leprae* was evaluated using the BACTEC 460 system. *M. leprae* was incubated in BACTEC 128 medium at 33°C under reduced oxygen for 2–3 weeks in the presence of fluoroquinolones at 0.31–5 μg/ml. Activity was determined by a reduction in $^{14}CO_2$ evolution compared to drug-free controls. Of the commercially available agents, ofloxacin was most active while enoxacin and norfloxacin were inactive. However, a number of newer fluoroquinolones (OPC-17100 and 17066, AT-4140, PD-127391, 124816, and 127391), all containing a cyclopropyl group at R-1 and either a halogen or methyl group at R-8, were more active than ofloxacin *in vitro.* Further *in vivo* evaluations of these agents should help to determine their potential in leprosy.—[Laboratory Research Branch, GWL Hansen's Disease Center, Carville, Louisiana, U.S.A.]

Gelber, R. H. The activity of a beta-lactamase inhibitor, Augmentin®, against *Mycobacterium leprae* in mice: a novel and promising target for drug development.

Beta-lactam antibiotics are the agents of first choice for most bacterial infections, both gram-positive and gram-negative. They have not, however, found application in the therapy of mycobacterial disease to date, primarily as a result of their demonstrated lack of permeability for mycobacteria (Kasik, Biochem. J. *107,* 665, 1988; Mishra, Int. J. Clin. Pharmacol. *3,* 73, 1970). The lack of activity for mycobacteria of this class of compounds also appears a result of the unequivocal demonstration of the presence of beta-lactamases in various mycobacterial species (see Kasik and Mishra; Wallace, Am. Rev. Respir. Dis. *132,* 1093, 1985), and probably *Mycobacterium leprae* itself (Prabhakaran, ASM Abstr. D-23, 1986). Previously, however, Shepard (Am. J. Trop. Med. Hyg. *20,* 616, 1971) found that a cephalosporin, cephaloridine, was active and indeed bactericidal for *M. leprae* in the mouse model. Unfortunately, cephaloridine proved uniquely nephrotoxic and, for this reason, was removed from the commercial market. Shepard later (Int. J. Lepr. *55,* 322, 1987) found that of 12 beta-lactams tested in *M. leprae*-infected mice, only three were minimally active and none were comparable in activity to cephaloridine. Previously, we (Int. J. Lepr. *53,* 171, 1985) found that five

beta-lactams (250 mg/kg administered intraperitoneally five times weekly) were inactive against *M. leprae* in mice (cefoxitin, cephradine, cefamandole, cefotoxamine, and moxalactam), while cephradine 0.5% in diet was purely bacteriostatic. In this preliminary report, we found that Augmentin®, a beta-lactamase inhibitor, together with a beta-lactam antibiotic (amoxicillin/clavulanic acid) 100 mg/kg by gavage five times weekly, but not amoxicillin alone, resulted in some bactericidal activity for *M. leprae*. This present work confirms and extends that finding.

In our current studies we first evaluated the activity of Augmentin (4 parts amoxicillin/1 part clavulanic acid) by the kinetic technique of Shepard, the drug being administered from 60 to 150 days following infection of both hind foot pads with 5000 mouse-derived and logarithmically multiplying *M. leprae*. In this study, Augmentin was administered to groups of mice five times weekly by gavage in doses of 25 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg, and 600 mg/kg. Also, in this study, the activity of previously established active agents alone and in combination with Augmentin, 400 mg/kg by gavage five times weekly, were similarly studied. These active drugs included: dietary dapsone 0.0001%, rifampin 20 mg/kg by gavage monthly, and kanamycin 25 mg/kg intraperitoneally five times weekly. One-hundred-fifty days after *M. leprae* infection, the number of *M. leprae* per foot pad in untreated control mice plateaued at 5×10^5 *M. leprae* and remained relatively constant for 8 months thereafter. In the foot pads of groups of treated mice, *M. leprae* were enumerated at 150 days, and also at generally two or more separate intervals up to 11 months after the discontinuation of treatment. Augmentin in doses of 400 mg/kg and 600 mg/kg entirely prevented the multiplication of *M. leprae* for 6 months after drug discontinuation, while Augmentin 200 mg/kg was still active even for 11 months after therapy had been stopped. These three doses of Augmentin were found to be bactericidal for *M. leprae* and at least as active against *M. leprae* in mice as was found previously for cephaloridine. Even Augmentin administered at doses of 50 mg/kg and 100 mg/kg, though not entirely preventing *M. leprae* multiplication, were found active. Augmentin 25 mg/kg five times weekly was inactive. All combinations of Augmentin with other agents were found active, and Augmentin plus kanamycin was found to be consistently more active than either agent alone.

In a second study, utilizing the proportional bactericidal technique, we quantitated the bactericidal activity for *M. leprae* in mice of five times weekly Augmentin 400 mg/kg and Timentin® 1000 mg/kg intraperitoneally (30 parts ticaricillin/1 part clavulanic acid). In this technique, the hind foot pads of groups of mice were infected with 10^1, 10^2, 10^3, and 10^4 *M. leprae*; mice were untreated and treated with antimicrobials for the initial 60 days. One year after the completion of therapy, a sufficient period of time to detect multiplication of *M. leprae* from any bacilli surviving therapy, ten foot pads from each group of mice were harvested, and the *M. leprae* therein enumerated. From these results, the percentage of *M. leprae* killed is then quantitated by the method of Spearman and Karber. In these studies, Timentin was found to have no significant bactericidal activity for *M. leprae,* while Augmentin was found 80% ± 14% bactericidal, similar to dapsone itself (Colston, Lepr. Rev. *49,* 7, 1978; Gelber, Lepr. Rev. *57,* 347, 1986).

In summary, we have found that Augmentin is consistently bactericidal for *M. leprae* in mice, and to a degree that is similar to dapsone and cephaloridine. This activity is attained at levels easily achieved in the serum and tissues of man by this commercially available oral antibiotic. These results, thus, confirm that beta-lactam antibiotics and beta-lactamase inhibitors appear to offer potential for the therapy of leprosy. Further, these results suggest the need to study other beta-lactamase inhibitors, possibly with superior penetration and enzyme affinity, for even greater activity against *M. leprae.*—[Kuzell Institute, San Francisco, California, and GWL Hansen's Disease Center, Carville, Louisiana, U.S.A.]

Saito, H. and Tomioka, H. *In vivo* activities of new quinolones against *Mycobacterium leprae* infection induced in mice.

New quinolones, such as ofloxacin (OFLX) and ciprofloxacin (CPFX), have potent antimycobacterial activities, particularly against *Mycobacterium tuberculosis, M. kansasii, M. marinum,* and *M. fortuitum.* We also noted the appreciable antileprosy activity in OFLX. We examined *in vitro* and *in vivo* antimycobacterial activities of AM-1091 [7-(3-amino-1-pyrrolidinyl)-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid hydrochloride] and AM-833 [6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-piperazinyl)-4-oxo-3-quinolinecarboxylic acid], quinolones newly developed by the Kyorin Pharmaceutical Co., Tokyo, particularly against *M. leprae* and the *M. avium* complex (MAC).

When the minimal inhibitory concentrations of test agents against various mycobacteria were measured by the agar dilution method using 7H11 agar medium. AM-1091 and AM-833 had levels of antimycobacterial activity similar to those seen with OFLX and CPFX which exhibit a potent antimicrobial activity against *M. tuberculosis, M. kansasii,* and *M. fortuitum.* The order of antimycobacterial activity was CPFX ≥ AM-1091 > OFLX ≥ AM-833.

When murine peritoneal macrophages that phagocytosed the MAC were cultivated in RPMI 1640 medium containing 10% fetal bovine serum, with or without the addition of AM-1091, AM-833 or OFLX, these agents enhanced the antimicrobial activity of host macrophages against the MAC, and the efficacy was in the order of AM-1091 > OFLX > AM-833.

When AM-1091 and AM-833 were given to mice by gavage in the dose of 3 mg/mouse/day, once daily, six times per week for 50 days from day 31 to day 80, there was significant antileprosy activity. Neither norfloxacin (NFLX) nor CPFX exhibited antileprosy activity when given to mice in the dose of 2 mg/mouse/day. Treatment of mice with CPFX even at 3 mg/mouse/day induced no antileprosy activity, while NFLX given in the same dose had weak antileprosy activity. OFLX given at 3 mg/mouse/day caused a marked decrease in the number of acid-fast bacilli recovered from the infected foot pads of mice even on day 150. Other quinolones, such as enoxacin, pipemidic acid, and piromidic acid, had no antileprosy activity. It is noteworthy that the *in vitro* antimycobacterial activity of test quinolones did not always correlate with their antileprosy activity *in vivo,* although both activities generally correlated. The *in vitro* antimycobacterial activity of these agents was in the order of CPFX ≥ AM-1091 > OFLX ≥ AM-833, NFLX; antileprosy activity *in vivo* was in the order of AM-1091, OFLX > AM-833, NFLX > CPFX. CPFX showed no antileprosy activity *in vivo,* presumably because of unfavorable pharmacokinetics, such as very low absorption efficiency after oral administration and in subsequent tissue distribution.—[Department of Microbiology and Immunology, Shimane Medical University, Izumo, Japan]

Sathish, M., Thole, J. E. R., Bosecker, B. and Clark-Curtiss, J. E. Cloned antigenic determinants of *Mycobacterium leprae* that react with leprosy patients' sera: their characterization and ability to elicit delayed-type hypersensitivity responses in mice following immunization.

A diversity of avirulent *Salmonella typhimurium* strains have been developed; these derivatives are unable to cause disease, but have not lost their ability to attach to, invade, and persist for defined periods of time in the gut-associated lymphoid tissue (GALT) and thus elicit secretory, humoral and cellular immune responses. Several plasmid cloning vector systems have been developed for expression of colonization and virulence determinants of several pathogens in these avirulent *S. typhimurium* derivatives. Since there is strong evidence that *Mycobacterium leprae* invade the human body through nasal and oral mucosal membranes, an active secretory immune response might well be important as a first line of defense against infection and invasion by *M. leprae.* Thus, we have begun to immunize mice with avirulent *S. typhimurium* derivatives that contain plasmids expressing some of the antigenic determinants identified by their ability to react with antibodies in leprosy patients' sera, in order to determine whether such immunization elicits secretory, humoral and/or cellular

immune responses.—[Departments of Microbiology and Biology, Washington University, St. Louis, Missouri, U.S.A., and The Royal Tropical Institute, Amsterdam, The Netherlands]

Sela, S. and Clark-Curtiss, J. E. Isolation and characterization of the putative promoter of the *Mycobacterium leprae* ribosomal RNA operon.

Although several genes have been isolated from *Mycobacterium leprae*, regulatory transcription signals have not been studied. Expression of the 65-kDa heat-shock protein probably occurs from its own promoter, and the existence of sequences similar to the *Escherichia coli* consensus promoter sequences (−35 base and −10 base upstream of a translation start codon) strongly suggests that *E. coli* RNA-polymerase can recognize at least part of the mycobacterial promoters. The promoter region of the *M. leprae* ribosomal RNA (rRNA) genes was chosen for isolation and characterization since most of the operon has been recently cloned and the nucleotide sequences for the rRNA genes are highly conserved among eubacteria. Parts of the regulatory sequences of these genes were reported to be conserved in gram-negative as well as gram-positive bacteria.

The rRNA genes are usually organized in the bacterial genome in the order 16S-23S-5S, and transcription starts from the 16S gene. Since the whole 16S gene from *M. leprae* was not cloned previously, we initially isolated a DNA fragment which contains the beginning of the 16S gene (5′ end) as well as upstream flanking regions. Using a specific probe from *M. smegmatis* to screen the pHC79 :: *M. leprae* cosmid library (ALX-1), a cosmid clone designated pYA1100 was isolated. Restriction mapping of this clone revealed a *Pst*I fragment of about 5 (Kb) which contains the desired fragment. To test for the existence of promoter sequences in the *Pst*I fragment, pYA1100 was digested with *Hae*III, a restriction enzyme which recognizes four nucleotides. The resulting fragments were subcloned into a plasmid vector (pMH109) which contains a chloramphenicol acetyltransferase (CAT) gene without its own promoter. Insertion of a promoter-like sequence into pMH109 can be detected by selecting transformed *E. coli* host strains that are resistant to chloramphenicol (Cm). A 1.2 Kb DNA fragment from the *Hae*III digest inserted into the *Sma*I site of pMH109 (just before the CAT gene) gave rise to Cm colonies, suggesting that a promoter-like sequence had been cloned. The fragment was partially mapped by restriction enzyme digestions, and the promoter activity was narrowed to an *Xba*I fragment of about 700 base pairs. Partial sequencing of 440 bases of the 1.2-Kb fragment show 78 nucleotides with an 83% similarity to coding sequences of the beginning of the *E. coli* 16S gene. A portion of 24 nucleotides positioned 150 nucleotides upstream of the previous sequence showed a 75% similarity to sequences positioned downstream from a 16S gene promoter in *Bacillus subtilis* as well as in *Mycoplasma capricolum*. This sequence is known as a consensus signal for processing the precursor to 16S rRNA in gram-positive bacteria. Further sequencing of the upstream region will allow actual comparison of the promoter(s) sequences. Thus, functional analysis as well as partial sequencing data suggest that the cloned fragment contains a putative rRNA promoter(s) of *M. leprae*.—[Departments of Microbiology and Immunology and Biology, Washington University, St. Louis, Missouri, U.S.A.]

Hunter, S. W. and Brennan, P. J. The native proteins of *Mycobacterium leprae*: preliminary results on subcellular location, isolation and chemical and immunological characterization.

Mycobacterium leprae was subjected to a simple fractionation protocol to arrive at three major subcellular fractions, cell walls, cytoplasmic membrane and cytosol. The application of extensive Triton X-114 phase separations to membrane fractions allowed removal of lipoarabinomannan and phosphatidylinositol mannosides, and the recognition and purification of two major membrane proteins (MMP) of Mr 35 kDa (MMP-I) and 22 kDa (MMP-II); recovery of these was about 630 μg and 460 μg per g of *M. leprae*, respectively. MMP-I is N-blocked and is apparently the same as a 35 kDa protein, but not a 36 kDa protein

previously recognized with monoclonal antibodies. Three major cytosolic proteins (MCP) of Mr *ca.* 14 kDa (MCP-I), 18 kDa (MCP-II), and 28 kDa (MCP-III) were also recognized. MCP-I was fully purified. Recovery was about 10.1 mg/g *M. leprae*; it represents the single most abundant protein in *M. leprae*. N-group analysis of the first 30 residues of MCP-I and comparison with the like product from *M. tuberculosis* Erdman and *M. bovis* BCG shows near-perfect homology; two amino acid differences were noted. Thus, MCP-I is akin but not identical to the 10 kDa (BCG-a) protein of *M. tuberculosis*/*M. bovis*. The cell walls of *M. leprae* are dominated by a single polypeptide of Mr *ca.* 18 kDa, apparently different from MCP-II. The protocols allow the generation of sufficient amounts of the native proteins of *M. leprae* to enable comparison with recombinant products and assessment of the host immune response to the major bacterial somatic proteins in leprosy.—[Department of Microbiology, Colorado State University, Fort Collins, Colorado, U.S.A.]

Modlin, R. L., Torigian, V., Bloom, B. R., Rea, T. H. and Brenner, M. B. Antigen-Reactive TCR$\gamma\delta$ lymphocytes in leprosy lesions.

Antigen recognition by T cells is mediated by cell-surface, clonally variable structures termed T-cell receptors (TCRs) in association with the CD3 complex. The majority of T cells have a TCR heterodimer composed of α and β chains. Recently a smaller population of peripheral T cells has been identified which have a TCR composed of γ and δ chains. This population has potential for tremendous diversity in antigen recognition because of the diversity encoded by the D regions of the δ chains. However, the antigen reactivity and functional attributes of TCR$\gamma\delta$ lymphocytes are not known.

To determine whether TCR$\gamma\delta$ lymphocytes accumulate in the skin lesions of infectious disease, we performed immunoperoxidase staining of leprosy biopsy specimens using monoclonal antibodies against the TCR$\alpha\beta$ (anti-βF1) and TCR$\gamma\delta$ (anti-δ1) structures; βF1+ lymphocytes accounted for 70%–80% of the CD3+ cells infiltrating leprosy lesions across the spectrum. In peripheral blood of leprosy patients and in tuberculoid lepromatous and erythema nodosum leprosum leprosy, δ1+ lymphocytes accounted for < 10% of CD3+ cells. However, in both lepromin skin tests and reveral reactions, TCRδ1+ lymphocytes comprised 25%–35% of CD3+ cells in lesions.

We derived a T-cell line from a lepromin skin test site on a patient with tuberculoid leprosy. This line was expanded with interleukin 2 (IL-2) and sorted by flow cytometry to select for TCR$\gamma\delta$ lymphocytes. This line was found to proliferate in response to *Mycobacterium leprae* and PPD but not to the recombinant 65-kDa and 18-kDa proteins.

The finding of TCR$\gamma\delta$ lymphocytes in acute granulomatous responses such as reversal reactions and lepromin skin tests, but not the comparatively chronic tuberculoid lesions, suggests a role for these cells in granuloma formation and/or organization. The demonstration of reactivity of TCR$\gamma\delta$ lymphocytes derived from lesions to *M. leprae* provides an opportunity to study the role of these cells in the pathogenesis of leprosy.—[University of Southern California School of Medicine, Los Angeles, California, U.S.A.]

Izumi, S., Fujiwara, T., Ikeda, M., Nishimura, Y., Kawatsu, K. and Sugiyama, K. Sensitivity, specificity, reliability of positive and negative indirect ELISA and gelatin particle agglutination test for serodiagnosis of leprosy.

The serological study of *Mycobacterium leprae*-specific phenolic glycolipid (PGL-I) has reached a turning point from a laboratory phase to a field application phase. The techniques of ELISA and the gelatin particle agglutination test (MLPA) developed during the past few years could have widespread application in field studies in endemic countries. However, it is important to emphasize to field workers the significance of the sensitivity, specificity and reliability of these tests before employing them for the treatment and prevention of leprosy. Since leprosy is an infectious disease with a broad clinical, pathological and immunological spectrum, we cannot expect a homogeneously high antibody titer in leprosy patients. We, therefore, have to adopt different

cut-off values to meet the level of reliability.—[National Institute for Leprosy Research, Tokyo; Institute of Natural Science, Nara University, Nara; Fuji Rebio, Inc., Hachioji; National Ohshima Seisho-en Leprosy Hospital, Aji-cho, Japan]

Williams, D. L., Gillis, T. P., Booth, R. J., Looker, D. and Watson, J. D. Application of polymerase chain reaction amplification technology for the detection of *Mycobacterium leprae.*

A specific assay for the detection of *Mycobacterium leprae* from crude biological samples is presently not available. The major limitation for developing this type of assay has been the inability to obtain specific reagents which detect small numbers of *M. leprae.* Recently, we have developed and characterized an *M. leprae*-specific DNA probe which is capable of detecting picogram quantities of purified *M. leprae* DNA by dot-blot hybridization. This probe is a 36 bp fragment (LOGAN fragment) obtained from the 3.2 Kb cosmid fragment of *M. leprae* DNA. The specificity of this probe for *M. leprae* was defined using both dot-blot and Southern blot analysis using stringent and nonstringent hybridization conditions. The ^{32}p-labeled LOGAN fragment probe did not hybridize the DNA obtained from several other cultivable species of mycobacteria, nonmycobacteria, and human, armadillo or mouse tissues. Using the LOGAN fragment probe as a target nucleic acid sequence, a sensitive method for detecting *M. leprae* DNA from a variety of biological sources is being developed. This assay employs the use of polymerase chain reaction (PCR) amplification and subsequent detection of amplification products by DNA hybridization technology. Primers corresponding to 25 bp segments flanking the LOGAN fragment have been synthesized and used with PCR to successfully amplify the LOGAN fragment approximately 10^6 times from 1 nanogram of purified *M. leprae* DNA. In addition, the LOGAN fragment has been amplified from purified DNA obtained from *M. leprae* (human and sooty mangabey monkey) passed through the armadillo as well as DNA purified from *M. leprae* obtained from a wild-caught armadillo with a leprosy-like infection. The ability to identify *M. leprae* directly from tissue homogenates of human skin biopsies from patients with lepromatous leprosy, tissue homogenates from nude mouse foot pads previously infected with *M. leprae,* and tissue homogenates from a chimpanzee with a leprosy-like infection was examined using the PCR. The PCR method detected the *M. leprae*-specific LOGAN fragment sequence from all of the above samples. These data indicate that the amplification of *M. leprae*-specific DNA sequences using PCR provides a tool for the detection of *M. leprae* directly from a variety of biological sources (i.e., human skin biopsies, mouse foot pads, armadillo tissues) without the need for isolation and subsequent amplification of this microorganism. The specificity and sensitivity of PCR amplification of the LOGAN fragment for detection of small numbers of *M. leprae* is presently being evaluated.—[Laboratory Research Branch, GWL Hansen's Disease Center, Carville, Louisiana, U.S.A.; Somatogenetics International, Inc., Broomfield, Colorado, U.S.A.; University of Auckland, Auckland, New Zealand]

Kohsaka, K., Hirata, T. and Nakamura, M. Effect of lyophilization on viability of *Mycobacterium leprae* grown in nude mice.

In an effort to preserve *Mycobacterium leprae in vitro,* the effect of freezing and drying, i.e., lyophilization, on the viability of *M. leprae* was studied. The viability of the bacilli was quantitatively measured by foot-pad inoculation of dilutions of lyophilized *M. leprae* into the foot pads of nude mice. The results obtained demonstrate that the viability of *M. leprae* was reduced approximately 10^{-2} to 10^{-3} from that of the starting material during the process of lyophilization. No viable bacilli were detected in the lyophilized inocula containing $< 1.8 \times 10^3$ bacilli. On the other hand, the bacilli capable of multiplication in nude mouse foot pads were contained in the lyophilized samples containing $> 10^5$ bacilli. These results show that although it is possible to preserve *M. leprae in vitro* by means of lyophilization, the viability of the bacilli is remarkably reduced during the lyophilization procedure.—[National Institute for Leprosy Research, Tokyo; Coga Hospital Medical Research Institute, Kurume, Japan]

Walsh, G. P., dela Cruz, E. C., Abalos, R. M., Fajardo, T. T., Guido, L. S., Cellona, R. V., Meyers, W. M., Gormus, B. J., Resuello, R. and Lange, F. W. Leprosy studies in Philippine cynomolgus monkeys (*Macaca fasicularis*): preliminary results.

Leprosy studies were initiated in Philippine cynomolgus monkeys to determine their susceptibility to leprosy, their response to lepromin, and the prevalence of naturally acquired leprosy in wild-caught animals.

Ten cynomolgus monkeys born and raised in captivity were inoculated with *Mycobacterium leprae.* One group of four animals was inoculated in December 1988 with a suspension of *M. leprae* prepared from biopsy specimens from an untreated lepromatous (histoid) patient. The suspension contained 6.8×10^8 *M. leprae*/ml, and each animal received a total of 1.8×10^9 *M. leprae* given by a combined intravenous-intradermal route. At 2 months' postinoculation, small discrete lesions were apparent at several inoculation sites on the ears and extremities. Slit-skin smears of selected lesions showed numerous acid-fast bacilli (AFB) in three animals; the fourth animal remained asymptomatic. By 3 months after inoculation, the lesions in one of the three animals had enlarged while those in the other two animals remained unchanged or had regressed. At this time, the fourth animal showed small lesions at several inoculation sites on the ears and extremities. At 6 months' postinoculation, the lesions in one animal are progressing while those in the remaining three appear to be regressing. Histologic examination of biopsy specimens from selected lesions placed the lesions in the BB to BL region of the leprosy spectrum but with a cell-mediated reactive component. Beginning at 2–3 months, there were marked increases in serum IgM antibodies to PGL-I antigen in three of the animals.

An earlier group of six monkeys had been inoculated in November 1988. The inoculum was obtained from pooled biopsy specimens from three untreated lepromatous patients. The count of this suspension was 1.4×10^8 *M. leprae*/ml. Four of the animals were inoculated by the combined intravenous-intradermal route and two intranasally. None of the animals in this group are symptomatic 7 months after inoculation. This may be due, in part, to the lower count of the suspension used. The viability of the inocula is being assessed in mouse foot pads.

Lepromin studies have been done in four normal cynomolgus monkeys using lepromin prepared from two different sources, mangabey and armadillo. The mangabey lepromin had the standard count of 1.6×10^8, while three concentrations of armadillo lepromin were tested (1.6, 3.2, and 4.8×10^8). Each of the animals received four intradermal injections at well separated sites on the abdomen. The responses were read at 28 days, and biopsy specimens were taken from all sites for histologic evaluation. One of the four animals responded strongly to all four lepromins; a second animal was less reactive but still positive to all the lepromins; the third animal responded only to the two highest concentrations of armadillo lepromin; the fourth animal reacted only to the highest concentration of armadillo lepromin. Thus, in this study, lepromin reactivity varied among normal cynomolgus monkeys, a finding consistent with the heterogeneous response observed in the transmission study.

Sera from 396 wild-caught cynomolgus monkeys from Mindanao were examined for antibodies to PGL-I antigen and all were negative.

The results of our transmission studies and of lepromin reactivity in normal cynomolgus monkeys suggest that this species manifests a heterogeneous response to *M. leprae.* Further observation is needed to define the role of this species as a potential model for leprosy.—[Leonard Wood Memorial, Cebu, The Philippines; Armed Forces Institute of Pathology, Washington, D.C., U.S.A.; Delta Regional Primate Research Center, Covington, Louisiana, U.S.A.; Simian Conservation Breeding and Research Center, Manila, The Philippines]

Acknowledgment. This study was supported in part by the Sasakawa Memorial Health Foundation, the Simian Conservation Breeding and Research Center, and the Leonard Wood Memorial.

Nakamura, K. and Yogi, Y. The MRL/*lpr* mouse as an experimental leprosy model (continued).

Susceptibility of nude mice to *Mycobac-*

terium leprae has been studied with various mouse strains, and the importance of the genetic backgrounds in relation to the development of lepromatous lesions, especially in NFS/N and N:NIH(s) nude mice highly susceptible to *M. leprae,* has been reported. In addition, NOD hybrid nude mice developed by transfer of the Crj:CD-1 (ICR) nude gene were highly susceptible to *M. leprae,* while the susceptibility of their original NOD (non-obese diabetic) mice (developed as an experimental animal for the study of insulin-dependent diabetes mellitus) was confined to the multiplication of *M. leprae.*

In the present study, we have undertaken a comparison of the susceptibility to *M. leprae* of MRL/*lpr* mice, an animal model for systemic lupus erythematosus (SLE), with autoimmune NOD mice (an animal model for type I diabetes). Descriptions are made as to the high susceptibility to *M. leprae* of MRL/*lpr* mice as well as previously reported by using F_2(*lpr/lpr* +/+) mice of (MRL/*lpr* × *nu/nu*)M_1.

MRL/*lpr* and NOD mice were obtained from CLEA, Tokyo. Crj:CD-1 (ICR) mice were obtained from Charles River, Atsugi, Japan. Thirty MRL/*lpr* mice and 20 NOD mice 12–16 weeks old, and 20 Crj:CD-1 (ICR) 6-week-old mice were used. The inoculum size of *M. leprae* derived from the foot passage of nude mice was 5.8×10^6 bacilli. The inoculation was made at the right dorsal site of the hind foot. The MRL-*lpr* mice were maintained in a vinyl isolator under specific pathogen-free conditions, and were provided with a sterilized autoclavable commercial diet (CE-2, CLEA) and tap water *ad libitum.* NOD and Crj:1 (ICR) mice were housed in a conventional animal room. For identification, the harvested acid-fast bacilli (AFB) from the MRL/*lpr* mice 240 days after inoculation were inoculated into the right hind foot of Crj:CD-1 (ICR) mice in order to confirm the growth pattern (persistent infection) in the mice. The AFB contained were cultured on a 1% Ogawa's medium and a modified Nemoto's egg-yolk medium at 33°C and 37°C for 3 months. The smears of AFB were treated with pyridine as modified by us. In addition, the tissues obtained from infected MRL/*lpr* mice were fixed in buffered formaldehyde solution for Fite-Faraco and hematoxylin-eosin staining.

In this experiment we used 20 male and 10 female MRL/*lpr* mice with already developed, massive, generalized lymph node enlargement. We used 10 male and 10 female NOD and Crj:CD-1 (ICR) mice. The bacillary counts were carried out after multiplication of 105 days and 240 days after *M. leprae* were inoculated into the right hind feet of the MRL/*lpr,* NOD, and Crj:CD-1 (ICR) mice. At 240 days after inoculation, the bacillary counts of the inoculated site on some MRL/*lpr* mice, especially some male MRL/*lpr* mice, were increased to 10^8–10^9 bacilli per foot, while that of the NOD mice were approximately 10^4 bacilli per foot and showing a decrease in the number of *M. leprae.* Sections of the infected foot of the Mrl/*lpr* mice at that time showed a large number of bacilli and globi around blood vessels in subcutaneous tissue or muscular layer, and the mice were observed to have systemic degenerative vascular disease.

The AFB isolated from the MRL/*lpr* mice were distinguished from other mycobacteria. Results of reinoculation in Crj:CD-1 (ICR) mice showed multiplication limited to isolated AFB. Cultivation on 1% Ogawa's medium and modified Nemoto's egg-yolk medium at 33°C and 37°C for 3 months were all negative. The pyridine extraction test showed that the acid fastness of the bacilli disappeared immediately.

To summarize the results, MRL/*lpr* mice showed high susceptibility to *M. leprae,* while that of NOD mice was poor. In conclusion, the immunobiological characteristics of MRL/*lpr* mice, autoimmune mice bearing the *lpr* gene, had an effect on the multiplication of *M. leprae.* This mouse is a suitable multibacillary model for the study of leprosy.—[National Institute for Leprosy Research, Tokyo, Japan]

Matsuo, E., Sasaki, N. and Skinsnes, O. K.
On the soluble fraction of *Mycobacterium scrofulaceum* HI-75, originally separated from human leproma, which combines with beta-glucuronidase.

Our previous studies have shown that *Mycobacterium leprae* in lepra cells combine with human beta-glucuronidase (B-Gase) and seem to grow, utilizing glucuronic acid (GA) which is a split product produced due to the activity of this enzyme. On the

other hand, HI-75, which was originally separated from a leproma and utilized in this study, was identified as *M. scrofulaceum* by Pattyn. A reason for using this mycobacterium, in spite of that, is that we cannot culture *M. leprae in vitro.* As previously known, the enzyme B-Gase combines with HI-75 *in vitro.* This seems to support the immunohistopathologic finding of the combination of B-Gase with *M. leprae* in leprosy patients.

In this study, we tried to understand how to solubilize and to fractionate that fraction from HI-75 which combines with B-Gase.

HI-75 was grown in modified Ogawa's medium and was recovered by vigorous washing and sonication with phosphate buffered saline (PBS) containing 1 mM EDTA, sedimented by 68% ethanol, and dried after acetone treatment. It was rehydrated and sonicated in PBS containing 1 mM each of $CaCl_2$ and $MgCl_2$ (CM-PBS) and centrifuged to take a supernatant (specimen 1). To get specimen 2, trypsin digestion of specimen 1 was followed by the same procedure as that to get specimen 1. Specimen 2 was fractionated by column chromatography with Sephacryl S-200. The specimens thus obtained were analyzed by dot-ELISA. After blocking, bovine liver B-Gase was dissolved in CM-PBS and combined with the specimens.The presence of B-Gase bound to the fractions was visualized utilizing substrates. Specimens treated with either 6 M urea or SDS were also examined. According to the present study, the combinations of B-Gase with specimens except those treated with SDS were easily observed. Chromatography of specimen 2 showed two major peaks, the second of which seemed to be combined with B-Gase.—[Departments of Pathology, Kyorin University School of Medicine, Tokyo, Japan, and Sun Yat-Sen University of Medical Sciences, Guangzhou, China; Tohoku Shinsei-en, Miyagi, Japan]

Hancock, G. E., Cohn, Z. A. and Kaplan, G. Generation of antigen-specific, MHC-restricted cytotoxic T lymphocytes of the CD4+ phenotype; enhancement by cutaneous administration of IL-2.

During the course of a cell-mediated immune (CMI) response, effector mechanisms are induced that result in the selective destruction of parasitized host cells. In leprosy, the destruction of parasitized host cells can be observed by ultrastructural analysis of the paucibacillary tuberculoid leprosy lesions. In this instance, macrophages and epithelioid cells are destroyed in association with highly polarized lymphocytes of an unknown phenotype. This destruction is not observed in the multibacillary lesions of lepromatous leprosy. However, following the generation of an antigen-drived CMI response to the crossreactive mycobacterial antigen PPD, parasitized macrophages are destroyed in a milieu containing many newly emigrated mononuclear cells, including CD4+ and CD8+ T cells, monocytes and T6+ Langerhans' cells, and *Mycobacterium leprae* are liberated into the extracellular space. This cell-mediated response results in a striking local reduction in bacilli.

To investigate the nature of the cytotoxic cell(s) involved in the destruction of parasitized monocytes and their recognition mechanism(s), we have examined an *in vitro* system in which peripheral blood mononuclear cells from PPD-sensitized donors generated cytotoxic T lymphocytes (CTL) after activation with antigen. Our results demonstrated that the cytotoxic cells selectively destroyed mycobacterial-antigen-pulsed monocyte targets. Selection for T-cell subsets with monoclonal antibodies directed against CD4 or CD8 molecules demonstrated that the CTL were of the CD4+ phenotype. Our studies further demonstrated that the cytolytic activity was MHC class II restricted since monoclonal antibodies directed against HLA-DR determinants blocked cytotoxicity, while monoclonal antibodies directed against HLA-ABC determinants had no inhibitory effect. CD8+ T cells were poorly if not at all cytotoxic under similar conditions. Smaller numbers of peripheral blood mononuclear cells with properties of the natural killer and lymphokine-activated killer-cell lineage were also present in these cultures.

In addition, we investigated whether recombinant human interleukin 2 (IL-2), administered locally in small doses, had a systemic effect and was able to modulate antigen-specific cytolytic activity in lepromatous leprosy patients. Recombinant hu-

man IL-2 was injected into the skin of lepromatous patients at 10 μg doses, given at 48-hr intervals for three doses. We demonstrated that the peripheral blood mononuclear cells obtained 8–14 days after the initiation of the IL-2 injection had enhanced antigen-dependent destruction of monocyte targets.

Our data suggest that antigen-specific, CD4+ killer cells generated during a CMI response to mycobacterial antigens could play an important role in the local destruction of parasitized macrophages and the subsequent disposal of *M. leprae* from the dermis. Currently, we are investigating whether human monocytes infected *in vitro* in long-term culture with viable *M. leprae* are recognized and killed by similar cytotoxic mechanisms. In addition, their amplification by IL-2-dependent mechanisms suggests a potential therapeutic role for cytokines in lepromatous leprosy.—[The Rockefeller University, New York, New York, U.S.A.]

Fukutomi, Y., Inui, S. and Onozaki, K. Ia antigen expression in macrophages after phagocytosis of mycobacteria *in vitro.*

Production of interleukin 1 (IL-1) and tumor necrosis factor (TNF) was induced by murine peritoneal macrophages after phagocytosis of heat-killed *Mycobacterium lepraemurium, M. intracellulare,* or *M. gordonae in vitro.* These macrophages also showed enhanced consumption of glucose in medium much more than macrophages cultured with medium alone. These results indicate that macrophages were activated after phagocytosis of these mycobacteria. Similarly, lymphokine (supernatants of concanavalin A-stimulated spleen cells)- or IFN-γ-treated macrophages showed enhanced glucose consumption. However, by flow cytometry analysis, it was observed that although enhanced expression of Ia antigen was induced in macrophages treated with IFN-γ, decreased and not increased expression of Ia was induced in macrophages after phagocytosis of these mycobacteria. Moreover, if macrophages phagocytosed a large amount of the mycobacteria, almost all the cells lost the antigen in their surfaces. These results demonstrate that monokine production and the reduced expression of surface IA antigen are simultaneous phenomena after phagocytosis of these mycobacteria.—[National Institute for Leprosy Research, Tokyo; Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki, Japan]

Rea, T. H., Cooper, C. L., Mueller, C., Bloom, B. R. and Modlin, R. L. Identification of interferon-gamma mRNA and human serine esterase mRNA in spontaneously occurring DTH reactions in leprosy skin lesions by *in situ* hybridization.

The reversal reactions of leprosy are clinically destructive lesions of abrupt onset associated with clearance of bacilli. Because of a concomitant increase in *Mycobacterium leprae*-induced lymphocyte blastogenesis, reversal reactions represent an opportunity to study spontaneously occurring delayed-type hypersensitivity (DTH) reactions in skin. To define the cellular constituents of this DTH response, we have sought evidence of expression of mRNA for interferon-gamma (INF-γ) as a marker of T-helper cells, and of mRNA for human serine esterase (HSE) as a marker of T-cytotoxic cells, by *in situ* hybridization with cDNA-derived ^{35}S-labeled RNA probes, in these same lesions.

Frozen sections from 55 patients with leprosy were studied. The prevalence of cells expressing INF-γ mRNA in reversal reactions was 50-fold greater than in lepromatous lesions and 10-fold greater than in tuberculoid lesions. Also, the average number of grains in each positive cell, a measure of gene expression, was 45 in reversal reactions but only 10 in tuberculoid lesions. The prevalence of cells expressing HSE-mRNA in reversal reactions was 6-fold greater than in lepromatous and 2.5-fold greater than in tuberculoid tissues. The distribution of HSE-mRNA-positive cells was identical to that of CD4-positive T cells. The prevalence of these molecular phenotypes in reversal reaction lesions has been substantiated by finding increased numbers of monoclonal-antibody-defined, phenotypic T-helper cells and T-cytotoxic cells in these tissues.

The data suggest that reversal reactions represent hyperimmune or dysregulated DTH responses characterized by selective increases of $CD4^+$ INF-γ cells and T-cytotoxic cells which result in bacteriolysis at the expense of tissue damage.—[Albert Einstein College of Medicine, New York, New York; Stanford University, Palo Alto, California; University of Southern California, Los Angeles, California, U.S.A.]

Plikayetes, B. B. and Shinnick, T. H. Rapid, sensitive, and specific detection of mycobacteria using gene amplification techniques.

Bidirectional polymerase cascade reactions are powerful techniques capable of amplifying a specific nucleotide sequence several millionfold. We have applied this technology to detecting and identifying two medically important mycobacteria: *Mycobacterium tuberculosis* and *M. leprae.* Our initial target sequence for amplification has been the gene encoding the 65-kDa antigen. Oligonucleotide primers corresponding to various regions of this gene have been synthesized. The sequences of some of these regions are conserved between the two species, while others are unique to each of the species. Primers corresponding to the conserved sequences direct the amplification of the target sequence from both species. Primers representing sequences unique to either species direct the amplification of the gene only from the corresponding mycobacterium. Using these primers, we can specifically detect as few as 10^6 copies of the *M. tuberculosis* 65-kDa antigen gene and vice versa. This degree of sensitivity and specificity should allow the rapid detection and identification of small numbers of mycobacteria in clinical specimens.—[Division of Bacterial Diseases, Centers for Disease Control, Atlanta, Georgia, U.S.A.]

Tanaka, T., Tamura, T., Yamamoto, Y. and Furuyama, J. Cloning and expression of *Mycobacterium tuberculosis* strain Aoyama B in *Escherichia coli*—a gene encoding 15-kDa and 60-kDa antigens.

There has been an increasing demand to develop improved peptide antigens because the present purified protein derivatives (PPD) are a mixture of unidentified peptides, in spite of the wide diagnostic use for the tuberculous infection. A breakthrough in the genetic manipulation of PPD-associated genes is necessary in order to meet this demand.

Although a number of peptides from a mycobacterial source have been cloned in *Escherichia coli,* there has been little discussion on their relationship with PPD. To obtain recombinant peptides related to PPD, we constructed a genomic library from the DNA of *Mycobacterium tuberculosis* Aoyama B, a standard strain used in Japan to manufacture PPD, in *E. coli* and plasmid vectors pUC18, pUC181 or pUC182. Clones reacting with the anti-PPD rabbit serum were screened by immunoblotting from among 3×10^4 clones. Seven clones were selected and designated: pAT01, pAT101, pAT102, pAT103, pAT104, pAT105, and pAT201. On Western blotting, they produced a 15-kDa (pAT01, pAT101, pAT105), an 18-kDa (pAT103), and a 60-kDa (pAT102, pAT201) peptide.

Restriction endonuclease maps of each of these clones were composed and putative coding frames of the anti-PPD rabbit-serum-reactive peptides were deduced by analysis of the deletion derivatives. In all seven clones, the antigens were expressed regardless of an isopropyl thiogalactoside (IPTG) induction. In addition, a 15-kDa gene could still express the peptide in a vector from which the entire promotor had been removed. These facts suggest that the mycobacterial intrinsic promotor in the inserted fragment could functionally regulate in *E. coli* heterogeneously.

A nucleotide sequence and a putative ORF of pAT01 and pAT201, encoding 15-kDa and 60-kDa peptides, were determined. In the N-terminal of the 15-kDa peptide, a signal sequence was present, and it is possible that 15-kDa may be secreted in the culture filtrate. When 15-kDa and 60-kDa nucleotide sequences in this study were subjected to comparative database analysis (GENBANK), they revealed a striking level of homology in corresponding cloned mycobacterial peptides.

In order to analyze the immunological activity of the 15-kDa and 60-kDa peptides,

they were purified from *E. coli,* carrying pAT01 or pAT201, by using DEAE chromatography and Detoxi-Gel to remove LPS. Both 15-kDa and 60-kDa peptides showed a reaction similar to PPD, both in the DTH skin reaction and the lymphocyte proliferation response. Therefore, these peptides may be closely tied to PPD in immunogenicity.—[Departments of Bacteriology and Genetics, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan]

Mizuguchi, Y., Udou, T., Tangiguchi, H., Goto, Y. and Tokunaga, T. Establishment of a host-vector system in mycobacteria.

Recombinant plasmids were constructed from pMSC262 of *Mycobacterium scrofulaceum* and pACYC177 of *Escherichia coli.* The plasmids were capable of replication in both *E. coli* and *M. bovis* BCG. When introduced into BCG by electroporation, the plasmids conferred kanamycin resistance. These plasmids were quite stable in BCG; almost no drug-sensitive segregants were produced upon subculture of the transformants. The restriction endonuclease digestion pattern of the plasmid extracted from BCG showed a pattern identical with that used for transformation. Putative restrictionless mutants of BCG showed a higher frequency of plasmid transformation than did wild-type BCG. Deletion analysis revealed that about a 2.2 kb fragment of pMSC262 was necessary for the replication and maintenance in mycobacteria.—[Department of Microbiology, University of Occupational and Environmental Health, Japan School of Medicine, Kitakyushu, Japan]

Kaplan, G. and Cohn, Z. A. The role of rIL-2 in the immune modulation of leprosy.

In an attempt to circumvent T-cell unresponsiveness in lepromatous leprosy patients, we injected small doses of recombinant human interleukin 2 (rIL-2) intradermally and evaluated the local and systemic responses to the lymphokine. All patients responded to the lymphokine with local areas of induration that peaked at 24 hr and persisted for 4–7 days. Within 24 hr there was an extensive emigration of T cells and monocytes into the site, peaking at 4 days and persisting for > 15 days. Both $CD4^+$ and $CD8^+$ T cells entered the site. Initially, T cells of the $CD4^+$ phenotype predominated but by 11 days, $CD8^+$ cells were predominant. Considerable numbers of $T6^+$ Langerhans' cells appeared in the dermis. By 4 days, the thickness of the overlying epidermis had increased twofold, and keratinocytes were expressing MHC class II antigen and the IFNγ-induced peptide IP-10. Extensive destruction of mononuclear phagocytes that contained structurally intact or fragmented *Mycobacterium leprae* was observed at the electron microscope level. This was followed by marked reductions in the number of acid-fast organisms in the injected site. Thirteen of 15 patients exhibited a reduction of acid-fast bacilli ranging from 5-fold to 1000-fold, with a mean value of ~100-fold.

Further studies examined the ability of *M. leprae* and its soluble products to modify the cutaneous response to intradermal rIL-2. Neither the simultaneous injection of *M. leprae* and IL-2, the prior injection of *M. leprae* followed in 2 days by IL-2, nor the prior administration of IL-2 followed in 4 days by *M. leprae,* into the same skin site, modified the zone of induration generated by IL-2. In addition, the immunocytochemical and histopathological evaluations of biopsied skin sites were identical. We concluded that *M. leprae*-responsive suppressor-T cells, if they exist, do not influence the gross or microscopic responsiveness of a cell-mediated skin reaction to IL-2. IL-2 could, however, enhance the responsiveness of skin-test-positive tuberculoid patients and family contacts to *M. leprae* antigens by a synergistic effect on the zone of induration and local cell accumulation.

Single or multiple injections (1–3) of IL-2 did not modify the total number of circulating lymphocytes nor the number of T cells and the CD4/CD8 T-cell ratio. However, IL-2 had a pronounced influence on the ^{3}H-thymidine incorporation in response to various stimuli 4–8 days after intradermal IL-2. Stimulation indices of 3- to 7-fold above pre-IL-2 levels were observed with the polyclonal activator PHA, and enhanced thymidine incorporation occurred in the presence of antigens to which the patients were already sensitized, such as PPD and BCG. IL-2 had no effect on the unresponsive state of lepromatous leprosy patients' T cells to the antigens of *M. leprae.*

Thus, the administration of IL-2 leads to the generation of an effective cell-mediated immune response, recapitulating an antigen-driven event and leading to striking local reductions in *M. leprae.* In comparison with the PPD reaction, bacilli are cleared more promptly, although emigratory cells persist for a shorter time. Although a cellular immune response is reconstituted, there is no reversal of the anergy to *M. leprae* observed in these patients.—[The Rockefeller University, New York, New York, U.S.A.]

Tomioka, H. and Saito, H. Properties of suppressor macrophages induced with *Mycobacterium leprae* vaccine.

In experimental and clinical trials on leprosy vaccination, vaccines consisting of viable *Mycobacterium bovis* BCG alone or in combination with heat-killed *M. leprae* (HK-ML) were found to be efficacious. With advances in gene manipulation technology, recombinant BCG which produces *M. leprae*-specific protective antigens will eventually become available.To obtain superior results, it is important to regulate the generation of suppressor macrophages (Mϕs) induced by injection of the vaccines, particularly in the case of vaccines containing viable BCG because BCG has potent activity to induce suppressor Mϕs. Therefore, it is of interest to acquire knowledge of the detailed characteristics of suppressor Mϕs induced by *M. leprae* vaccine (ML vaccine) combined with viable BCG.

The ConA mitogenic response of spleen cells (SPCs) from mice given intravenous (i.v.) injections of ML vaccine, consisting of viable BCG and HK-ML, as well as those given viable BCG was markedly reduced compared to responses of the control mice. In the case of mice given HK-ML, ConA mitogenesis of the SPCs was enhanced over the controls at cell densities lower than optimal, but was reduced at higher cell densities.

A monolayer culture of splenic Mϕs was prepared on microtiter wells by seeding the prescribed number (2.5×10^5–1.6×10^7 cells/well) of the SPCs, and 1.5×10^5 of normal SPCs were cultured on the Mϕ monolayer in the presence of 2 μg/ml of ConA. Strong suppressive activity was seen for the Mϕ monolayer prepared by seeding 1×10^6 of SPCs from mice given ML vaccine. A similar level of suppression was noted in mice given BCG. On the other hand, the Mϕ monolayer prepared by seeding even 1.6×10^7 of SPCs from mice given HL-ML or control mice caused no appreciable suppression of blastogenesis of the splenic T cells.

When "per spleen" activity of suppressor Mϕs was calculated, the activity of the splenic Mϕs from mice given the ML vaccine was nearly the same as that of the BCG-injected mice but more than 300-fold higher than findings in the control and HK-ML-injected mice. In addition, in mice given ML vaccine or viable BCG, a remarkable splenomegaly and an increase in the number of SPCs were noted.

When the Mϕ monolayer culture was prepared on plastic culture dishes by seeding test SPCs, and the Mϕs from the dishes were measured for suppressive activity against the ConA mitogenic response of normal SPCs (1.5×10^5 cells/well), ML vaccine-induced Mϕs showed the most potent activity followed by the BCG-induced Mϕs. The former had about a twofold higher suppressor activity. In contrast, HK-ML-induced Mϕs showed no such activity, as in the case of normal Mϕs. Thus, the ML vaccine and the BCG injection augmented the suppressor activity of individual splenic Mϕ cells.

ML vaccine as well as BCG-induced Mϕs showed a potent chemoluminescence (CL) in response to phorbol myristate acetate (PMA), reaching a peak at around 90 sec. On the other hand, HK-ML-induced and normal Mϕs showed a very low CL. This indicates that the former two Mϕs had characteristics of "activated Mϕs," as determined on the basis of PMA responsiveness for respiratory burst. Separate experiments on peritoneal Mϕs induced by various agents, such as thioglycollate, zymosan A, streptococcal preparation (OK-432), viable BCG, and live *M. intracellulare,* showed a strong correlation between the suppressor activity and active oxygen-producing ability of a given Mϕ.

These findings indicate that i.v. treatment of mice with ML vaccine consisting of HK-ML and viable BCG induces potent suppressor Mϕs in the host spleen, and this was

mimicked by treatment with viable BCG alone but not by HK-ML. The expression of suppressor activity in ML vaccine- and BCG-induced splenic Mϕs was associated with an increase in the responsiveness to PMA for oxidative burst, indicating a close correlation of the suppressor activity of these Mϕs with their state of "activation."

When the ML vaccine was given subcutaneously to mice, no significant suppressive activity was induced in the host splenic Mϕs. Therefore, there is little possibility that, in the case of practical application of the ML vaccine containing viable BCG via the intradermal route, severe immune unresponsiveness would be induced in a systemic manner.—[Department of Microbiology and Immunology, Shimane Medical University, Izumo, Japan]

Mehra, V., Barnes, P. F., Hunter, S. W., Brennan, P. J., Rea, T. H., Bloom, B. R. and Modlin, R. L. Characterization of antigens associated with the cell walls of *M. leprae* and *M. tuberculosis* using T-lymphocyte clones.

Both *in vivo* and *in vitro* studies suggest that resistance to *Mycobacterium leprae* and *M. tuberculosis* infection is mediated by T cells rather than by antibodies. Thus, to design new vaccines and skin-test reagents there is the need to identify antigens relevant for induction of T-cell responses that are likely to be important in protection.

We have explored the possibility that the cell walls of *M. leprae* and *M. tuberculosis* may contain important antigens required for mounting cell-mediated immune (CMI) responsiveness since they have long been known to evoke delayed-type hypersensitivity (DTH) responses and to have strong adjuvant activity. Our earlier studies demonstrate that highly purified cell walls of *M. leprae* stimulate proliferation of T cells from tuberculoid but not lepromatous leprosy patients and elict DTH skin reactions in guinea pigs, tuberculoid patients, and contacts sensitized to *M. leprae.* Analysis of the precursor frequency of antigen-reactive, human, peripheral T cells revealed that there are as many T cells reactive to antigens associated with purified cell walls as to intact *M. leprae.* The immunological reactivity of the cell wall was destroyed by protease treatment, indicating that cell-wall-associated protein(s) are a major contributor to CMI reactivity to *M. leprae.*

In order to identify immunoreactive protein determinants associated with purified cell walls, we have developed T-helper cell lines and clones reactive to the cell walls of *M. leprae/M. tuberculosis* from the peripheral blood lymphocytes of tuberculoid leprosy patients and individuals immunized to *M. leprae/M. tuberculosis.* T-cell lines/clones thus established were used to identify the individual proteins of mycobacteria separated by SDS-PAGE, using the T-cell Western blot procedure. The proteins recognized with highest frequency by T-cell lines reactive to *M. leprae* cell walls were in the 7-kDa, 16-kDa, and 28-kDa molecular size range. On the other hand, *M. tuberculosis* cell-wall-reactive T-cell lines proliferated primarily to protein antigens of mol. wt. 10 kDa, 30 kDa, and 40-45 kDa.

In order to assess the relationship of cell-wall-associated antigens to the proteins secreted in the early culture filtrates of actively growing *M. tuberculosis,* cell-wall-reactive T-cell clones were tested for their ability to recognize early culture filtrates. At least three cell-wall-associated proteins of mol. wts. 10 kDa, 23 kDa, and 30 kDa were detected in the early culture filtrates. Studies are in progress to isolate and characterize some of these immunodominant antigens further in order to determine which of them are good candidate antigen(s) for vaccine development or skin-test antigen.—[Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York; Department of Microbiology, Colorado State University, Fort Collins, Colorado; Section of Dermatology and Departments of Medicine and Pathology, University of Southern California School of Medicine, Los Angeles, California, U.S.A.]

Schlesinger, L. S. and Horwitz, M. A. Complement receptors and complement component C3 mediate phagocytosis of *Mycobacterium tuberculosis* and *Mycobacterium leprae.*

Mycobacterium tuberculosis and *M. leprae,* the causative agents of tuberculosis and leprosy, respectively, are intracellular bacterial pathogens that invade and multiply

within host mononuclear phagocytes. To gain a better understanding of phagocytosis of these bacteria by host mononuclear phagocytes, we have studied monocyte receptors and bacterium-bound ligands mediating uptake of these organisms.

To study monocyte receptors mediating uptake, we assayed the adherence of bacteria to monocytes in the presence of monoclonal antibodies (mAbs) directed against various monocyte surface antigens. We incubated monocytes with either *M. tuberculosis* Erdman strain or armadillo-derived *M. leprae,* stained monocyte-adherent bacteria with auramine-rhodamine, and enumerated the bacteria by fluorescence microscopy. MAbs against complement receptors CR1 and CR3 significantly inhibited the adherence of both bacteria in the presence of nonimmune serum. In contrast, isotypic control mAbs against HLA-DR, the transferrin receptor, LFA-1, and CR2 did not inhibit adherence.

A combination of 1 mAb against CR1 and 1 against CR3, or a combination of 2 mAbs against CR3, resulted in much stronger inhibition of adherence (up to 1 log) than did single mAbs against these receptors. Combinations of isotypic control mAbs did not significantly inhibit adherence. Similarly, mAbs against CR1 and CR3 but not other monocyte surface antigens inhibited adherence of preopsonized *M. tuberculosis* in the presence of heat-inactivated serum. MAbs against CR1 and/or CR3 did not reduce monocyte viability or capacity to phagocytize polystyrene beads.

The modulation of monocyte Fc receptors on antigen-antibody complexes had little or no effect on monocyte adherence of either bacterium; nor did inhibition of the B-glucan inhibitable receptor with laminarin. Adherence was also not significantly affected by depleting serum of fibronectin or by adding excess fibronectin.

We studied ingestion of the bacteria by electron microscopy. Monocytes ingested all *M. tuberculosis* and *M. leprae* that were adherent to them in the presence of nonimmune serum. As in the adherence assay, mAbs against CR3 but not isotypic control mAbs markedly inhibited ingestion (> 1 log). Monocytes ingested both *M. tuberculosis* and *M. leprae* by conventional phagocytosis.

Complement component C3 in serum served as a bacterium-bound ligand for both *M. tuberculosis* and *M. leprae.* Adherence was serum-dependent, markedly reduced in heat-inactivated serum (> 70%), and markedly enhanced by preopsonization (threefold). Adherence was also dependent upon C3 and Factor B of the alternative pathway. Purified C3 restored the capacity of C3-depleted serum to mediate adherence and purified Factor B restored the capacity of Factor B-depleted serum to mediate adherence. C3 was fixed to *M. tuberculosis* and *M. leprae* by the alternative pathway of complement activation, as demonstrated by an enzyme-linked immunosorbent assay (ELISA).

This study demonstrates that CR1 and CR3 on monocytes and C3 in serum mediate phagocytosis of *M. tuberculosis* and *M. leprae* by human monocytes. Complement receptors appear to play a general role in mediating uptake of intracellular bacterial pathogens.—[Division of Infectious Diseases, Center for the Health Sciences, UCLA School of Medicine, Los Angeles, California, U.S.A.]

Snapper, S. B., Jacobs, W. R., Jr., and Bloom, B. R. Development of a recombinant BCG vaccine vector; cloning and expression of foreign antigen genes in BCG.

We have recently developed systems to stably introduce recombinant DNA into mycobacteria using phage- and plasmid-based shuttle vectors. Gene cloning into plasmid vectors has been greatly facilitated by our isolation of chromosomal mutations in mycobacterial host strains that electrotransform at 4 to 5 logs higher efficiency than the parent strain. These mutant hosts have facilitated subsequent plasmid cloning into the mycobacteria and permitted us to determine the minimum DNA sequence necessary for plasmid replication in mycobacteria. We have utilized plasmid vectors to be used as vehicles in the development of a multivalent recombinant DNA vaccine, and have successfully cloned into the shuttle plasmid pYUB12 the gene encoding the immunodominant *Mycobacterium leprae* 65-kDa heat-shock protein. These recombinant plasmids have been successfully introduced into both *M. smegmatis* and

BCG. Western analysis, using a monoclonal antibody that recognizes the *M. leprae* immunospecific epitope IIIE9, demonstrates that this foreign antigen is expressed in both of these mycobacterial hosts. Experiments to assess the immune response elicited following immunization of mice with this first recombinant BCG vaccine are in progress.—[Albert Einstein College of Medicine, Bronx, New York, U.S.A.]

CLOSING REMARKS

Ladies and Gentlemen:

I am very pleased to say a great thank you to our moderators, Dr. Gwinn and Dr. Brennan, and to various other members who assisted in this meeting. I also wish to thank all of the chairmen, speakers, and other participants for their valuable contributions.

On behalf of all the Japanese participants, I would like to thank you that such a beautiful place in San Diego was chosen to hold the meeting this year. We have had many excellent speeches and discussions in this meeting. I hope the Joint Conference develops more and more, with reports of our good works appearing frequently.

The U.S.–Japan Cooperative Conference will have been in existence for a quarter century next year, and our conference will be held in the city of Sapporo on Hokkaido. We look forward to seeing you again in Sapporo.

I now declare the 24th Joint Conference closed. Thank you!

—Tatsuo Mori, *Chairman*
Japanese Leprosy Panel