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ABSTRACTS

TWENTY-FIRST JOINT LEPROSY RESEARCH CONFERENCE

International Hotel
Osaka, Japan
25–27 September 1986
U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

Ladies and Gentlemen:

It is again a great privilege for me to have the honor of welcoming you to a U.S.–Japan Joint Conference on Leprosy Research. It is also a pleasure to welcome our friends from the United States and to renew our friendship since we met in Bethesda last year. Since this conference is again held together with the Joint Conference on Tuberculosis, it is also our particular pleasure to have an opportunity of understanding recent knowledge in both diseases more deeply and of renewing our friendship with the participants in the Tuberculosis Panel. Recent advances in research methodology, especially those in immunology, molecular biology, and gene technology, enable us to talk with common words and to discuss common topics at the joint symposium on tuberculosis and leprosy held tomorrow. This is, of course, very favorable for mutual understanding and for promoting further research activities in our respective fields. However, we must always keep in mind how different the problems are between tuberculosis and leprosy. Recognition of the differences should be a basis of good partnership.

In 1978, the Thirteenth Joint Conference on Leprosy Research was held in Osaka. At that time, for example, we had no information on a phenolic glycolipid antigen of *Mycobacterium leprae* or on leprosy in the mangabey monkey. How many or how big advances or changes could we see during these 8 years? I expect that the answer to this question will be found not only in this Conference but also in our experiences in this second largest city of Japan.

Finally, I would like to express our deep appreciation to Professor Ito and his colleagues for organizing and supporting this conference again here in Osaka. Thank you very much.

—Masahide Abe, *Chairman*
Japanese Leprosy Panel

PROGRAM
TWENTY-FIRST JOINT LEPROSY CONFERENCE

25 September, Thursday

Opening Remarks: Dr. Masahide Abe, Chairman, Japanese Leprosy Panel

Session I—Microbiology and Antileprosy Drugs

Co-Chairmen: Dr. Patrick J. Brennan
Dr. Tonetaro Ito

Mori, T., Miyata, Y., Ito, T. and Abe, M. Collection method of *Mycobacterium leprae* from infected armadillo liver compared with Draper's method

Nakamura, M. and Hastings, R. C. Cord-like formation of nude mouse-passaged *M. leprae* in a cell-free liquid medium containing tryamine HCl

Hirata, T. Re-examination of pyridine extractability of acid-fastness from leprosy bacilli

Franzblau, S. G. A rapid primary screen for anti-leprosy compounds in an axenic medium

Tsutsumi, S. and Gidoh, M. Effects of several anti-leprotic agents on severe adjuvant arthritis induced in female DA rats

Gelber, R. H. Minocycline: studies in mice of a promising agent for the treatment of leprosy

Session II—Experimental Leprosy

Co-Chairmen: Dr. Thomas H. Rea
Dr. Tatsuo Mori

Matsuoka, M. Different bacillary growth and host reactions among mouse strains after *M. leprae* infections

Nakamura, K. and Yogi, Y. *M. leprae* growth following combined infections of forefoot, hindfoot, upper lip, and base of tail in SHR and WM nude rats

Ito, T., Kohsaka, K. and Subowo. Effect of Lamprene on experimental leprosy in the nude mouse

Kohsaka, K., Miyata, Y., Makino, M., Mori, T. and Ito, T. Therapeutic effect with thymus transplantation in *Mycobacterium leprae*-infected nude mice

Hastings, R. C., Sibley, L. D., Ramasesh, N., Franzblau, S. G. and Krahenbuhl, J. L. Effect or function of macrophages on experimental leprosy

Izaki, S., Hsu, P. S., Hibino, T. and Izaki, M. Characterization of elastin-degrading enzymes during development of murine leprosy

27 September, Saturday

Session III—Antigens and Antibodies

Co-Chairmen: Dr. Zanvil Cohn
Dr. Kazunari Nakamura

Fujiwara, T. and Izumi, S. Synthesis of the trisaccharide related to the phenolic glycolipid of *Mycobacterium leprae* and preparation of sugar-protein conjugate

Izumi, S., Fujiwara, T. and Sugiyama, K. Serological property of semi-synthetic trisaccharide antigen NT-P-BSA and serodiagnosis of leprosy

Hunter, S. W., Chatterjee, D., Cho, S.-N., McNeill, M., Gaylord, H., Fujiwara, T. and Brennan, P. J. The cell envelope of *Mycobacterium leprae*

Abe, M., Ozawa, T., Minagawa, F. and Yoshino, Y. Immunoepidemiological studies on subclinical infection with *M. leprae*. IV. Five years' observation on school children by means of FLA-ABS and lepromin tests

Minauchi, Y., Tokunaga, H., Suzuki, M. and Matsubara, H. Effects of autoantibodies against peripheral nerve tissues—second report

Matsuo, E., Sasaki, N. and Skinsnes, O. K. On the immunohistologic staining of β-glucuronidase in paraffin-embedded leprous tissue; requirement of the tissue extracts or probably the mycobacterial extracts to deparaffinize and cardiolipin and lecithin for staining

Session IV—Cell-mediated Immunity

Co-Chairmen: Dr. Thomas M. Buchanan
Dr. Shinzo Izumi

Maeda, T., Ohta, Y., Nozawa, K. and Someya, K. Study of peripheral lymphocyte subsets in leprosy patients

Mohagheghpour, N., Engleman, E. G. and Gelber, R. H. T-cell defect in lepromatous leprosy is reversible *in vitro*

Nomoguchi, H., Dohi, Y., Ohno, N., Fujiwara, T. and Ito, T. Inhibition of the proliferative response of lymphocytes to ConA or PHA by unique *M. leprae* glycolipid

Modlin, R. L., Bloom, B. R., Nelson, E. E., Shen, J.-Y., Gunter, J. R. and Rea, T. H. *In situ* and *in vitro* characterization of T-helper lymphocytes in leprosy

Modlin, R. L., Mehra, V., Nelson, E. E., Kato, H., Pattengale, P. K., Rea, T. H. and Bloom, B. R. T-lymphocyte clones derived from leprosy skin lesions

Cohn, Z. A. and Kaplan, G. Macrophage activation and the secretory repertoire

Closing Remarks: Dr. Robert C. Hastings, Chairman, U.S. Leprosy Panel

JOINT U.S.–JAPAN TUBERCULOSIS AND LEPROSY SYMPOSIUM
International Exposition '70 Guest House
Osaka, Japan

26 September, Friday

Session I—Molecular Biology of Mycobacteria

Co-Chairmen: Dr. Mayer Goren
Dr. Masahide Abe

Makino, M., Suzuki, Y., Nagata, A., Nakata, A. and Ito, T. Construction of genomic library of *Mycobacterium leprae* and its expression in *Streptomyces lividans*

Anderson, D. C., Young, R. A. and Buchanan, T. M. Synthesis of two epitopes that react with monoclonal antibodies to the 65 kD protein of *Mycobacterium leprae*

Session II—Special Lectures

Co-Chairmen: Dr. Robert C. Hastings
Dr. Toru Tokunaga

Mehra, V., Sweetser, D. and Young, R. Fine mapping of epitopes in 65 kD protein antigen of *M. leprae* using recombinant DNA

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ABSTRACTS

Mori, T., Miyata, Y., Ito, T. and Abe, M. Collection method for *Mycobacterium leprae* from infected armadillo liver compared with Draper's method.

The outline of Draper's collection method protocol 1/79 is as follows: Infected armadillo liver is homogenized in a blender, and the bacterial fraction is collected by centrifugation 2 times. The sediment is treated with DNase, filtered through stainless steel mesh, and the sediment after centrifugation is suspended in 30% Percoll solution and centrifuged. The bacterial band fraction is separated by a two-polymer system, such as polyethyleneglycol and dextran, and the leprosy bacilli are concentrated in the upper phase.

In our method (Int. J. Lepr. 52:41–43, 1984) a homogenous leprosy bacilli suspension is made by filtering through two sheets of gauze to prevent an agglutination of the bacilli in the starting materials. A Percoll gradient is made with 90% Percoll 4 ml, 80% 5 ml, 70% 5 ml, 60% 10 ml, 50% 5 ml, and then 8 ml of the bacterial suspension is applied. These centrifuge tubes are run with a Beckman Swinging Bucket SW 27 rotor at 27,000 rpm (about 100,000 × *g*) for 1 hr. The top clear red zone is composed of a soluble tissue fraction and hemoglobin. The next white zone is an insoluble tissue fraction, and the middle white zone is leprosy bacilli. Since the black liver tissue contaminants cannot separate from this bacterial fraction collected by Draper's method, the leprosy bacterial fraction in the bottom of the centrifuge tube collected by Draper's method is more brown than our fraction. The findings of both in Ziehl-Neelsen stain smear preparations are not much different. Every bacterial fraction obtained only by centrifugation or a two-polymer system is contaminated with liver tissue components which are stained by anti-armadillo liver rabbit serum and a second fluorescent antibody. The host liver tissue contaminant is absent in our alkali-treated fraction by the antibody staining. Since the armadillo liver components adhere to the leprosy bacilli fraction, we cannot obtain pure leprosy bacilli without drastic treatment, such as 1 N sodium hydroxide treatment.

The yield of leprosy bacilli by Draper's method is 8%; 56.8% of the leprosy bacilli is collected by our method. There is more loss in the two-polymer system; moreover, this two-polymer system is troublesome, expensive, and has the potential of exposing the operator to infection. In some armadillo livers, brown granules concentrate in the same place in the Percoll gradient as the leprosy bacilli. These armadillos may have some other liver disease but, in any case, each armadillo liver must be treated individually.

Our collection method is superior to Draper's method in yield, purity, brevity, safety, and minimum damage to the bacilli without enzyme treatment.—[National Leprosarium Tama Zenshyo-en, Tokyo, Japan]

Nakamura, M. and Hastings, R. C. Cord-like formation of nude mouse-passaged *M. leprae* in a cell-free liquid medium containing tyramine HCl.

We previously reported that growth-like features of *Mycobacterium leprae* in a cell-free liquid medium containing catalase and folic acid (NH-2 medium) were occasionally observed when the medium was adjusted to pH 7.1–7.2 and a large inoculum was used (II Congress of Hansenology of the Endemic Countries, Baton Rouge, Louisiana, U.S.A., 1985). However, this phenomenon was not reproducible. Accordingly, to make a reproducible condition, various factors affecting this phenomenon, such as variation of modification of culture medium and addition of chemicals presumably related to the physiology of *M. leprae,* were studied.

It was found that cord-like formation of *M. leprae* was more consistently observed when tyramine HCl, one of the compounds related to *o*-diphenoloxidase, was added at a final concentration of 0.1% to the base medium (NH-9) which was a simplified NH-2 medium. We refer to this medium as NH-10 (NH-9 containing tyramine HCl). There is a close relationship between doses of tyramine and the appearance of cord-like formation. A large inoculum size is still essential for reproducing this phenomenon, and a neutral medium is better than an alkaline medium. As controls, no such findings took place a) when plain Dubos medium was used as the base, b) when heat-killed *M. leprae* were employed as an inoculum, and c) when the incubation was carried out at 4°C. On the other hand, it is curious that no cord-like formation was observed with *M. leprae* derived from armadillos or in the slide-culture system. Of the antileprosy drugs, dapsone (2 μg/ml) did not inhibit this phenomenon, but slight inhibitory effects were found with rifampin (2 μg/ml) and streptomycin (2 μg/ml). No effect was seen with penicillin (100 U/ml). Bacillary quantitation demonstrated a twofold increase in the number of *M. leprae* derived from nude mice as well as armadillos in NH-10 medium compared to those in the medium without tyramine.

At present, it is not clear whether this enhancement of cord-like formation of *M. leprae* in the presence of tyramine is an initial growth phase or just a biochemical aggregation resembling the growth pattern of mycobacteria.—[GLW Hansen's Disease Center, Carville, Louisiana, U.S.A.]

Acknowledgment. This work was supported by a grant from the Baton Rouge Area Foundation.

Hirata, T. Re-examination of pyridine extractability of acid-fastness from leprosy bacilli.

We report on the microscopic observations of the acid-fastness after pyridine treatment of *Mycobacterium leprae* in human skin and nasal mucosal lepromas, and of cultured atypical mycobacteria. Pyridine acid-fast extractability of *M. leprae* in the human skin leproma, nasal mucosa and nasal washings, and cultured atypical mycobacteria was re-examined several times on the basis of the method reported by Convit and Pinardi. Acid-fastness of the materials was not constant in every experiment. The extractability could be comparatively distinguished in the summer season but not in the winter season. Under the conditions leading to complete loss of acid-fastness of *M. leprae* in direct smears from the skin leproma, the number of acid-fast rods after pyridine extraction of *M. leprae* in the nasal mucosal leproma and of most of atypical mycobacteria was only slightly smaller than in control preparations. However, it must

be emphasized that the smears from pure cultures in Dubos media of *M. fortuitum, M. phlei,* and *M. vaccae* displayed a greater number of non-acid-fast cells after pyridine treatment.—[National Institute for Leprosy Research, Tokyo, Japan]

Franzblau, S. G. A rapid primary screen for antileprosy compounds in an axenic medium.

Primary screening for new antileprosy drugs as well as susceptibility testing of clinical isolates is conducted using a mouse foot pad technique. This method is expensive, cumbersome, and requires 6–12 months to complete. Measurement of intracellular adenosine triphosphate (ATP) has been used as an index of the metabolic status of *Mycobacterium leprae* isolated from armadillos, from human biopsies following drug treatment, and to evaluate the potential of *in vitro* culture media (Dhople and Green, IRCS Med. Sci. 13:779, 1985 and Lee and Colston, J. Gen. Microbiol. 131:3331, 1985). A close correlation between ATP levels and viability in the mouse foot pad has been noted in a number of these studies. The present paper evaluates the potential of an *in vitro* incubation system and ATP analysis as a rapid primary screen for identifying compounds with antileprosy activity.

Foot pads of athymic nude mice, infected 6–12 months previously with 5×10^7 *M. leprae,* were surface decontaminated and then homogenized in Dubos broth base without polysorbate 80 (Gibco) (adjusted to pH 7.2 before autoclaving), and 20% v/v Difco Dubos medium albumin (DA). Bacilli were partially purified by differential centrifugation and resuspended in DA. The suspension was held at 4°C overnight to allow for a contamination check on various media. Following dilution in fresh DA to a density of $1.5–8 \times 10^7$/ml, 1 ml aliquots were distributed to sterile 1.5 or 2.0 ml screw-capped microfuge tubes. Drugs were prepared at 100 × concentrations, filter sterilized, and added in a volume of 10 μl. Tubes were incubated at 33°C. At appropriate time intervals, replicate samples were analyzed for intracellular ATP content using a luciferin-luciferase system.

In at least 10 distinct experiments, control cells (in drug-free media) have consistently demonstrated a decay in intracellular ATP over a 3–4 week period. While this rate has varied among individual experiments, it has been sufficiently slow to allow for detection of accelerated decay rates in the presence of antileprosy drugs. Rifampin-treated cells usually showed an initial dose-related (but nonsignificant) increase in ATP pools at 7 days, but thereafter exhibited an accelerated rate of ATP decay compared to controls at concentrations ranging from 0.2–20 μg/ml. The latter effect was observed in 5 of 5 individual experiments. In contrast to rifampin, dapsone failed to elicit such a change in ATP pools at any concentration in 3 of 3 experiments. Ethionamide-treated cells showed an accelerated ATP decay as early as 7 days post-incubation in 3 of 3 experiments, but only at ≥20 μg/ml. *M. leprae* responded in a similar manner to clofazimine (B663), showing an early accelerated ATP decay but, in this case, cells were sensitive to ≥2 μg/ml.

Two clofazimine analogs, B3785 and B826, were synthesized by and obtained from Dr. J. F. O'Sullivan, Trinity College, Dublin. These analogs were previously found to be the most active among a number of compounds when evaluated against clofazimine-sensitive and -resistant *M. smegmatis in vitro,* and also showed roughly equivalent activity to B663 against *M. leprae* in a single mouse foot pad experiment.

In a 3-week kinetic study using the *in vitro*-ATP system, both analogs were significantly more active than B663 at one or more concentrations at each time point. These results were confirmed in a second experiment in which cells were analyzed at a single time point, 21 days post-incubation.

A number of antimicrobial agents were screened for activity in the *in vitro* system by measuring ATP levels of control and drug-treated cells at a single time point, 20–21 days post-incubation.

Among the protein synthesis inhibitors, gentamicin showed significant activity at 20 μg/ml, while streptomycin and kanamycin appeared inactive. However, these results were not obtained in a second experiment, and thus must be considered very preliminary. Tetracycline showed only marginal activity at 20 μg/ml, while minocycline appeared to be considerably more active.

Chloramphenicol was highly active but only at 20 μg/ml, while erythromycin showed strong activity at 2 μg/ml.

Penicillin G, ampicillin, methicillin and cloxacillin were all inactive, while cephalothin and cycloserine showed activity at 200 μg/ml. Bacitracin was only marginally active at this concentration. At 20 μg/ml INH, trimethoprim, griseofulvin, nalidixic acid, and polymyxin B were all inactive, while ciprofloxacin and the gramicidins demonstrated weak, but significant activity.

The sensitivity of the system to agents with various modes of action is encouraging. The failure of dapsone to show activity may represent the major disadvantage of this system—an insensitivity to compounds affecting specific enzymes involved in pathways which would not be expected to affect ATP levels within the available time frame.

This system would appear to be most useful as a primary screen for testing large numbers of new compounds or for comparing analogs of compounds known to have activity in the *in vitro*-ATP system. This might be followed by testing in a macrophage system, using radiolabeled precursors to phenolic glycolipid-I or nucleic acids to eliminate from further consideration compounds which do not affect phagocytosed *M. leprae.* Of course, compounds would have to be ultimately evaluated in a whole animal system.—[GWL National Hansen's Disease Center, Carville, Louisiana, U.S.A.]

Tsutsumi, S. and Gidoh, M. Effects of several antileprotic agents on severe adjuvant arthritis induced in female DA rats.

Rat adjuvant-induced arthritis (AIA) is known to be a reaction in which suppressor T-cell (Ts) function is lowered in contrast to the increments covering B, helper-inducer, and natural-killer (NK) cells. Although it seems to be contrary to the symptoms of lepromatous cases, on the other hand, no clear conclusion has been found in Ts function during acute or chronic erythema nodosum leprosum (ENL). AIA is also known to be an undesirable side effect of acute immunostimulation. Immune complexes (IC), including antibodies against the inoculated tubercle bacilli and endogenous antigens such as collagen II, are said to be formed during AIA. Referring to a finding that thalidomide suppressed antibody formation (Shannon, *et al.,* Scand. J. Immunol. 13: 553, 1981), it was examined whether thalidomide suppressed AIA by using female DA rats. These rats were obtained from Australia, and are said to be related to rats of the COP strain in which thyroiditis can be induced. The results were: a) Thalidomide (10 mg/kg) neither suppressed nor appeared to sedate the rats. b) Dapsone or DDS (1 mg/kg) stimulated AIA in aged male SD rats and at 2 mg/kg increased the swelling of inoculated hindfoot pads in female DA rats. c) B663 (20 mg/kg) suppressed AIA, but not as strongly as in male SD rats. d) Dexamethasone (0.2 mg/kg) strongly suppressed severe AIA. e) This AIA was induced by 0.05 mg (dry weight) of the tubercle strain Aoyama B (TB); whereas SD-AIA was not even induced by 0.2 mg. The nodulation of the tail was typical of DA-AIA.

So far as AIA responses are concerned, female DA rats were presumed to be comparable to female Lewis rats. On the other hand, the suppression of AIA by immunomodulative drugs is said to be generally weaker than the suppression caused by drugs more appropriate for anti-inflammation. Realizing that the response of female DA rats to AIA seems to be too high to examine the anti-AIA effect of immunomodulative drugs, we utilized this high response for comparing the arthritogenetic activity of leprosy bacilli (LB) purified from the foot pads of BALB/c nude mice to that of *M. tuberculosis* (TB). In several TB groups, a second inoculation with an arthrogenic dose (5×10^8 or 3×10^9 bacilli) was performed during a secondary reaction induced by a primed inoculation (5×10^8 bacilli) or 4 weeks later than a primed subarthrogenic inoculation (3×10^7 bacilli). Even though the leprosy bacillus (LB) is an *in vivo* microorganism and its content of peptidoglycans such as MDP may be different from that of cultivated mycobacteria, the arthritogenetic activity of LB was found to be extremely mild and not enough induction of the secondary reaction of AIA was observed. The result of this simple experiment suggests no serious side effect due to alleged immunostimulation by LB. In TB groups, the fact that the primed subarthrogenic inoculation suppressed AIA coincides

with a finding by Tsukano, *et al.* in which the induction of surface immunoglobulin-negative Ts by 3×10^7 TB to female DA rats was mentioned. Moreover, following the second inoculation with an arthrogenic dose (5×10^8 bacilli) during a second reaction of AIA, the animals' symptoms gradually remitted. As for the suppressive action of thalidomide on ENL, if this drug suppresses ENL by its immunomodulative action (Moncada, *et al.*), it is an exceptional case, because immunomodulative drugs can suppress AIA. In order to explain this contradiction, a delicate affinity to its possible receptor or a delicate selectivity for a cell population were suggested. The suppression mechanism of B663 to ENL was presumed to be simpler and due to its immunomodulative activity. The stimulation mechanism of DDS to AIA is unknown; its direct antibacterial one is as yet unsolved.

For an additional example, a peanut-oil suspension of finely powdered DADDS (4.5 mg/kg) was twice (on days 1 and 13) injected intramuscularly into the dorsa of female DA rats, and its influence on AIA was compared to the daily dose of DDS (1 mg/kg/day). The start of the secondary reaction was delayed, and the severity lowered also in the DADDS group; whereas it was stimulative in the DDS group. This result is similar to a clinical finding by Ramu, *et al.* (Int. J. Lepr. 51: 207, 1980) who noticed an enhancing effect of an oily suspension of DADDS injected once intramuscularly on the lepromin reaction in TT and BT patients in contrast to a suppressive effect of DDS.—[National Institute for Leprosy Research, Tokyo, Japan]

Gelber, R. H. Minocycline: studies in mice of a promising agent for the treatment of leprosy.

Minocycline is a commercially available oral tetracycline which has proved safe on chronic administration. Our studies demonstrate that, among the tetracyclines, minocycline is unique in being active against *Mycobacterium leprae.* We have established that the minimal inhibitory concentration of minocycline for *M. leprae* is exceedingly low and considerably less than levels easily obtained in plasma and tissues of patients treated with customary doses. Furthermore, in our studies minocycline has proved to be consistently bactericidal for *M. leprae.*

Others (Gaugas, Lepr. Rev. 38:225, 1967 and Shepard, Int. J. Lepr. 39:340, 1971) have previously found tetracycline itself inactive against *M. leprae.* In our studies doxycycline (0.02% in mouse chow) also was inactive. In three separate studies we found minocycline bactericidal against *M. leprae*-infected mice: in two studies by the kinetic technique, 0.04% dietary minocycline resulted in prevention of *M. leprae* multiplication for 270 days and 200 days after therapy was discontinued, and in another study by the proportional bactericide technique, 0.04% dietary minocycline was found to be 99% bactericidal. Of the drugs used to treat leprosy, only rifampin has proved more bactericidal. This impressive activity found for minocycline is likely the result of it being at neutral pH the most lipid-soluble tetracycline derivative, allowing for its penetration of *M. leprae*'s largely lipid outer capsule and cell wall to its ribosomal site of action. We have established minocycline's minimal inhibitory dietary concentration for *M. leprae* in mice to be 0.004–0.01% and, by analysis of resultant mouse plasma by an agar disk diffusion method utilizing the minocycline-sensitive *Bacillus cereus* strain ATCC 1178, established the mouse minimal inhibitory plasma level to be <0.2 μg/ml. Also, we have demonstrated that activity against *M. leprae* increases with increasing dietary concentration from 0.02% to 0.04%, which yield plasma concentrations from 0.5 μg/ml to 0.9 μg/ml. It is noteworthy that in man following usual therapeutic doses 2–4 μg/ml plasma levels are attained, skin levels exceed plasma levels, and minocycline appreciably penetrates nerves. Furthermore, we found minocycline additive or synergistic with dapsone, rifampin and kanamycin against *M. leprae.*

The armamentarium of drugs effective against *M. leprae* in clinically achievable concentrations without serious organ toxicity is exceedingly small. Since the introduction of rifampin in 1970, no new agents have been introduced. Because frequently patients are intolerant to currently available agents used to treat leprosy and because antimicrobial resistance, particularly to dapsone but also to all the currently available agents used to treat leprosy, is an in-

creasingly emerging problem, minocycline should prove useful in the therapy of leprosy.—[GWL Hansen's Disease Center, Carville, Louisiana; Seton Medical Center, Daly City, California, U.S.A.]

Acknowledgment. This work was supported by clinical studies grant no. 84-13-85 from the GWL Hansen's Disease Center.

Matsuoka, M. Different bacillary growth and host reactions among mouse strains after *M. leprae* infection.

The bacillary multiplication of *Mycobacterium leprae* in the mouse foot pad and concomitant host reactions with infection were compared among mouse strains to develop an experimental model reflecting the spectrum of leprosy in man.

Among seven strains of mice infected into the foot pads with 1×10^4 bacilli, KK mice showed the highest bacillary number and C57BL/6 mice yielded the lowest bacillary number at 30 and 40 weeks. Bacillary yield of C57BL/6 mice was about one tenth of that of KK mice.

The strain distribution of the trait of *M. leprae* growth in the foot pad is quite distinct from the strain variation to *M. lepraemurium* infection. It was proved that the difference on bacillary multiplication occurs at 15 weeks between C57BL/6 and KK mice.

Marked swelling of inoculated foot pads and enlargement of draining lymph nodes were shown in C57BL/6 mice infected with 1×10^7 bacilli. However, these reactions were significantly less in KK mice infected simultaneously and not conspicuous in both strains infected with 1×10^4 bacilli. Prominent proliferations of lymphocytes and macrophages were noted in the inoculated site of C57BL/6 mice infected with a large dose. Slighter cellular reactions composed of same cells were also recognized in C57BL/6 mice infected with small dose but not in KK mice.

To elucidate the underlying mechanisms of the strain difference, the growth of the organisms in KK-*nu/nu* mice, C57BL/6-*nu/nu* mice, and other nude mice and control mice were compared with their genetic backgrounds. The bacillary number in KK-*nu/nu* mice is not different from the yields in the other strains of nude mice. However, conventional KK mice exhibited a higher bacillary number than did other conventional mice. The results indicate that the different manifestation on bacillary growth in conventional mice is closely related to cellular immunity, which involves a T-lymphocyte function.

An experiment on superinfection was conducted to prove this hypothesis. The mice previously infected with 1×10^4 bacilli in the right foot pads were challenged in the left foot pads 5 or 10 weeks after the primary infection. C57BL/6 mice strictly suppressed the challenge infection in both examinations, but in the KK mice, the bacilli challenged at 5 weeks multiplied until 15 weeks at the same rate as in the control mice. The results indicate a more prompt development of host immunity in C57BL/6 mice than in KK mice.

This study clearly revealed different susceptibilities to *M. leprae* infection among mouse strains. The similarity to each type of human leprosy will be discussed in future immunological studies on mice.—[National Institute for Leprosy Research, Tokyo, Japan]

Acknowledgment. This study was supported by a grant from the Sasakawa Memorial Health Foundation.

Nakamura, K. and Yogi, Y. *M. leprae* growth following combined infections of forefoot, hindfoot, upper lip, and base of tail in SHR and WM nude rats.

We have previously established an animal model for experimental lepromatous leprosy using the SHR nude rat which gave excellent results in the development of a heavy lepromatoid formation. The formation of the lepromatoid lesion in nude rats was influenced by the genetic background gene of the athymic animals, similar to the nude mice experiments we previously described. In this report, we compared the susceptibility of the SHR nude rat with that of a "resistant" WM nude rat by using the multi-infection method.

We have established the nude rats of SHR background by mating the cross-intercross system with F344 male nude rats and normal SHR female rats. The WM nude rats were obtained from CLEA, Tokyo, and then bred and maintained in our laboratory. Ten each of the SHR and WM nude rats 3–5

weeks old were used. Inoculations of *Mycobacterium leprae* derived from foot passage of nude rats were made into four subcutaneous sites: forefoot, hindfoot, upper lip, and root of tail on the right side. Each inoculum was 1.9×10^5 bacilli. The nude rats were maintained in vinyl isolators under specific pathogen free (SPF) conditions.

Following inoculation with *M. leprae* at each of the four cutaneous sites, a marked nodular lesion in each infected site was observed in male SHR nude rats 305 days after injection. In contrast, only a slight lesion was seen in the WM nude rats. Severe lepromatoid lesions with ulceration of the injected upper lip and having a lepromata weight of 6.3 g were seen in male SHR nude rats. Lesions advanced to the eyelids. With forefoot infection, erythematous lesions developed on the lower legs and the leproma there weighed 5.2 g. Heavy swollen lesions with redness were observed in the right hindfoot, and lesions developed in the lower legs. The lepromata there weighed 7.1 g. With infection at the root of the tail, swollen lesions advanced to scrotal skin in the male SHR nude rat. The lepromata weighed 4.7 g. Total lepromata weights were 2.3 g. Marked nodular lesions in other SHR nude rats were observed at each of the four injection sites, having a total weight of 24.9 g 305 days after infection. At that time, the bacillary counts at each of the four lesions were over approximately 10^{10} bacilli/g in erythematous and abscessed tissues. When leprosy bacilli were injected at each of the four cutaneous sites in the WM nude rats, slight lesions were observed 305 days after inoculation. At that time, bacillary counts were approximately 10^8 bacilli/g in slightly thickened tissues. We have reconfirmed that WM nude rats are a "resistant" strain, showing no advanced lesions at each of the four injection sites, and showing no spread to neighboring sites. These results are similar to those previously reported by using a single injection method.

In summary, we have reconfirmed that the WM nude rat is a "resistant" strain for use as an experimental lepromatous leprosy model as compared with the SHR nude rat. In the SHR nude rat, infection with leprosy bacilli at each of four subcutaneous sites, forefoot, hindfoot, upper lip and root of tail, produced a tremendous development of heavy swelling due to *M. leprae* in injected sites which advanced to neighboring sites, such as the eyelids, nose, ears, upper and lower lips, fore- and hind lower legs, dorsolumbar site, perianal and genital sites, and both testicular sites, 305 days after inoculation. This combined multi-infection was useful for obtaining numerous bacilli at an early stage after infection as a source of *M. leprae* in the SHR nude rats by using rats reared in vinyl isolators under SPF conditions. This source would have an advantage over leprosy bacilli from the nine-banded armadillo because of the lack of problems which could arise due to naturally occurring leprosy-like disease and the individuality for susceptibility to the growth of *M. leprae*.—[National Institute for Leprosy Research, Tokyo, Japan]

Ito, T., Kohsaka, K. and Subowo. Effect of Lamprene on experimental leprosy in the nude mouse.

The effect of Lamprene (B663, clofazimine) on experimental leprosy in the nude mouse was tested by 0.003% Lamprene in the mouse diet. BALB/c-*nu/nu* female mice were inoculated with 1.2×10^6 *Mycobacterium leprae* in both hindfoot pads. From 18 weeks after the inoculation, half of the infected animals were given Lamprene in the diet for 25 or 29 weeks; the other half of the infected nude mice were left untreated as controls. Each group consisted of six mice. Both groups of infected mice were killed 43 or 47 weeks after *M. leprae* infection. The right hindfoot pad was used for bacillary counting, and the left hindfoot pad was used for histopathological examination.

There was remarkable suppression of the multiplication of *M. leprae* in the foot pads of the treated group. The number of *M. leprae* in all foot pads of the treated group was $<10^8$; the maximum number was 7.0×10^7. In the control group, all foot pads contained $>10^8$ of *M. leprae*. Three out of six control mice contained $>10^9$; the minimum number was 3.2×10^8. Histopathological findings also supported the effectiveness of Lamprene on experimental leprosy in the nude mouse.—[Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Kohsaka, K., Miyata, Y., Makino, M., Mori, T. and Ito, T. Therapeutic effect with thymus transplantation in *Mycobacterium leprae*-infected nude mice.

At the 19th Joint Leprosy Research Conference, we reported the results of the effect of thymus transplantation on *Mycobacterium leprae* in nude mice. The possibility of the immunotherapy was suggested by the results of thymus transplantation. Both a prophylactic effect on the growth of *M. leprae* and a therapeutic effect on experimental leprosy in nude mice was seen. The histopathological findings revealed the induction of a reversal reaction by thymus transplantation in experimental leprosy in nude mice. In the previous experiment, however, the mice in the treated group were repeatedly transplanted with thymus for a long period, 12 times for 12 months in the prophylactic experiment and seven times for 7 months on established experimental leprosy in the therapeutic experiment.

This time, instead of repeated transplantation, a similar experiment with a one-time transplantation of the thymus into *M. leprae*-infected nude mice was carried out as a therapeutic experiment.

Two strains of *M. leprae* were used as inoculum. Infected nude mice inoculated with *M. leprae* 9 months earlier were used for immunotherapy. They were divided into two groups. The mice of the treated group were transplanted with thymus intraperitoneally by a one-time transplantation. The number of organisms gradually increased in the untreated group. In the treated group, however, the bacillary population was significantly reduced compared with the untreated control group.

The results indicate that a one-time thymus transplantation was also effective suppressively on the growth of *M. leprae* in nude mice, and was effective as immunotherapy in experimental leprosy. Histopathological findings support the bacillary count data.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Hastings, R. C., Sibley, L. D., Ramasesh, N. and Krahenbuhl, J. L. Role of the mononuclear phagocyte in host resistance to leprosy.

Cells from the foot pads of *Mycobacterium leprae*-infected nude (nu/nu) mice were characterized and employed to explore the microbicidal capacity and response to lymphokines of lepromatous macrophages heavily parasitized with *M. leprae.* Foot pad granulomas were excised, the tissue gently minced, and the granuloma cells dispersed with an enzyme solution consisting of DNase and collagenase. Two types of adherent cells were obtained in about equal numbers: type 1 cells were fibroblast-like and often contained a few acid-fast bacilli; type 2 cells were tightly adherent, rounded up mononuclear cells gorged with acid-fast bacilli. Centrifugation of these cells through Nycodenz resulted in a band of cells highly enriched for type 2 cells. Type 2 cells were characterized as macrophages by the following criteria: tight adherence to plastic, resistance to removal by trypsin, phagocytic capacity for *Candida,* positive staining for nonspecific esterase, possession of Fc receptors, ability to reduce nitroblue tetrazolium, and staining with the universal macrophage monoclonal antibody marker, MAC-1. In spite of a heavy intracellular load of *M. leprae,* these foot pad macrophages phagocytized and supported the growth of the intracellular protozoan, *Toxoplasma gondii,* at a rate similar to that observed in normal peritoneal macrophages. However, unlike peritoneal macrophages from nu/+ or nu/nu mice, the foot pad granuloma macrophages could not be activated by lymphokines to kill intracellular *Toxoplasma.* Enhanced microbicidal activity could not be induced in foot pad macrophages with either crude macrophage activating factor (MAF) or with recombinant mouse interferon-gamma. These findings indicate that lepromatous tissue macrophages appear normal in many of their functions but are defective in their response to macrophage activating signals.

In other studies, the biosynthesis of the *M. leprae*-characteristic phenolic glycolipid-I (PGL-I) antigen by leprosy bacilli in tissue cultures of mouse peritoneal macrophages was studied quantitatively. Freshly harvested bacilli from *M. leprae*-infected nu/nu mice were fed to mouse peritoneal macrophages and the monolayers pulsed with ^{14}C-labeled palmitic acid at appropriate intervals. The cells were harvested, the lipids

extracted, and the glycolipid fraction concentrated and assayed by thin-layer chromatography. The PGL-I spot was stained with orcinol-sulfuric acid for identification, scraped off and ^{14}C palmitic acid uptake quantitated in a liquid scintillation counter. Formalin-treated *M. leprae* failed to synthesize PGL-I and the addition of rifampin to the culture medium also inhibited PGL-I synthesis. In a preliminary experiment, normal unstimulated mouse peritoneal macrophages and activated macrophages from mice chronically infected with *Toxoplasma* were infected with *M. leprae.* The activated macrophages clearly inhibited the incorporation of radiolabeled palmitic acid into *M. leprae* PGL-I. This technique shows promise as a means of evaluating macrophage microbicidal capacity against *M. leprae* and may be of value in studying the effects of antileprosy chemotherapeutic agents.—[Laboratory Research Branch, Gillis W. Long Hansen's Disease Center, Carville, Louisiana, U.S.A. Supported by grants AI 22442 and AI 22492 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health]

Izaki, S., Hsu, P. S., Hibino, T. and Izaki, M. Characterization of elastin-degrading enzymes during development of murine leprosy.

Elastin-degrading enzymes were investigated in two polar types of murine leprosy induced in resistant (C57BL/6N) and susceptible (CBA/N) mice. Both types of murine lepromas were sequentially extracted: firstly, with 0.05 M Tris-HCl, pH 7.5, containing 0.14 M NaCl to obtain the soluble fraction and, secondly, with 2 M KSCN containing 0.1% Triton X-100 to obtain the bound fraction. The enzyme activity was assayed with [H^3]-elastin solubilization, as well as synthetic chromogenic peptide substrates such as L-pyroGlu-Pro-Val-p-nitroanilide (S-2484) and succinyl-$(Ala)_3$-p-nitroanilide (AAApNA). Inhibitor activity for porcine pancreatic elastase was assayed by using AAApNA.

In the soluble fraction of tissue extracts of both C57BL/6N and CBA/N mice, only a minimum elastin-degrading activity was observed; whereas elastase inhibitor activity was demonstrated. In the bound fraction, on the other hand, enzyme activity was revealed while elastase inhibitor activity was negligible. In C57BL/6N mice elastin-degrading activity for [H^3]-elastin was approximately 25,000 CPM/24 hr/mg protein, and this enzyme activity was unchanged during the early (5–7 weeks after infection), middle (8–15 weeks), and late (>15 weeks) stages of development of hypersensitivity granulomas. In CBA/N mice, the elastin-degrading activity was significantly ($p < 0.05$) low, showing 12,000 CPM/24 hr/mg protein. The enzymatically active bound fraction of both types of murine lepromas fractionated with Sephacryl S-200 gel filtration column showed its major elastase activity at approximately Mr 20,000. The following DEAE anion exchange column chromatography of C57BL/6N mice separated three fractions (E-I, -II, and -III). E-I in a pass-through fraction showed hydrolytic activity only for [H^3]-elastin but not for either S-2484 or AAApNA. This activity was inhibited by 3.3 mM iodoacetamide, a thiol proteinase inhibitor. E-II eluted by 0.5 M NaCl showed the highest hydrolytic activity for all substrates. This activity was inhibited by 3 mM DFP, a serine proteinase inhibitor, and soybean trypsin inhibitor. No inhibition was obtained by aprotinin, elastatinal, antipain, chymostatin, iodoacetamide, or 1,10 phenanthroline. E-III eluted by 1 M NaCl showed similar characteristics to E-II. The bound fraction of CBA/N mice showed a lower activity in the E-I as well as E-II fractions, but not in the E-III fraction.

In summary, the present study demonstrated a serine proteinase-type elastase activity in the bound fraction of hypersensitivity-type murine lepromas in C57BL/6N mice. The activity was lower in CBA/N mice in which no granulomatous hypersensitivity develops. The regulating inhibitor activity for elastase was separately shown in the soluble fraction. Experiments to test the effects of various proteinase inhibitors revealed that properties of the granuloma-associated elastase were most similar to membrane-bound elastase of monocytes (Lavie, *et al.*, J. Immunol. 125:175–180, 1980; Senior, *et al.*, J. Clin. Invest. 69:384–393, 1982) but not identical to neutrophil or pancreatic elas-

tase. No metallo-proteinase-type elastase was observed.—[Department of Dermatology, Iwate Medical College, Iwate, Japan]

Fujiwara, T. and Izumi, S. Synthesis of the trisaccharide related to the phenolic glycolipid of *Mycobacterium leprae* and preparation of sugar-protein conjugate.

Chemical synthesis of the trisaccharide part of the phenolic glycolipid of *Mycobacterium leprae* which contained the linker arm *p*-hydroxyphenylpropionate and the preparations and activities of the trisaccharide-BSA conjugates are reported.

The trisaccharide was synthesized by the coupling of reducing-end monosaccharide with linker arm *p*-(2-methoxy-carbonylethyl)phenyl 4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (I) and nonreducing-end disaccharide derivative 1-bromo (II)- or 1-chloro (III)-4-*O*-(2,4-di-*O*-acetyl-3,6-di-*O*-methyl-β-D-gluco-pyranosyl)-2,3-di-*O*-methyl-L-rhamnopyranose. Compound I was synthesized from benzyl 4-*O*-benzyl-α-L-rhamnopyranoside through specific allylation of 3-OH by using phase transfer catalyst, methylation, rearrangement of allyl group to prop-1-enyl group, hydrolysis with TFA, acetylation, bromination, coupling with methyl *p*-hydroxyphenylpropionate and deacetylation of 2-*O*-acetyl group. Coupling reaction of the bromide (II) gave the expected trisaccharide but the yield was very poor because of the poor stability of the bromide (II). In order to overcome this, chloride was tried. The chloride (III) was stable and gave the trisaccharide by the Königs-Knorr reaction with compound I in dichloromethane in the presence of mercury bromide and molecular shieve 4A in good yield (over 70%). But synthesized trisaccharide was the anomer (β-linkage) of natural trisaccharide at Rha-Rah linkage. This trisaccharide was de-derivatized and coupled to BSA, giving βT-P-BSA. The chloride (III) was coupled with compound I in dichloromethane in the presence of silver triflate and 1,1,3,3-tetra-*N*-methylurea to give the expected trisaccharide derivative. The yield of this reaction was about 35%. The yield was not very good but this reaction could be used for the mass preparation of the trisaccharide. It was then de-derivatized and coupled to BSA, giving NT-P-BSA.

Serological activities of NT-P-BSA and βT-P-BSA were tested. Two conjugates showed very good correlation of r = 0.960 and βT-P-BSA = 0.968 × (NT-P-BSA)-14 against IgM antibody. The activity of βT-P-BSA was slightly lower than NT-P-BSA against IgG antibody [βT-P-BSA = 0.797 × (NT-P-BSA)-14] but showed very good correlation of r = 0.968. These data show that two conjugates can be used for the serodiagnosis of leprosy.—[Institute for Natural Science, Nara University, Nara; Leprosy Research Institute, Kyoto University School of Medicine, Kyoto, Japan]

Izumi, S., Fujiwara, T. and Sugiyama, K. Serological property of semi-synthetic trisaccharide antigen NT-P-BSA and serodiagnosis of leprosy.

The successful synthesis of the trisaccharide epitope of the phenolic glycolipid-I (PGL-I) is one of the most important developments in research on *Mycobacterium leprae*-specific antigens. We recently synthesized a new semi-synthetic glyco-protein antigen, NT-P-BSA, by conjugation of a chemically synthesized trisaccharide with bovine serum albumin (BSA) via parahydroxyphenylpropionate as the linker arm. The seroreactivity of the antigen was tested and compared to those of the natural PGL-I and disaccharide-based antigen, ND-P-BSA, by the indirect ELISA and competitive ELISA inhibition tests. It was found that this new antigen is highly specific for leprosy, and the affinity to IgG-class antibody is much higher than PGL-I or ND-P-BSA. NT-P-BSA will be a powerful new tool in the seroepidemiological study of leprosy or monitoring of chemotherapeutic effect of antileprosy drugs.—[Leprosy Research Laboratory, Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto; Department of Natural Science, Nara University, Nara, Japan]

Hunter, S. W., Chatterjee, D., Cho, S.-N., McNeil, M., Gaylord, H., Fujiwara, T. and Brennan, P. J. The cell envelope of *Mycobacterium leprae.*

In order to arrive at a comprehension of the major agents of immunogenicity and of pathogenicity within the leprosy bacillus, an image of the architecture of the cell wall is of the essence. Foremost and outermost among the defined components is phenolic glycolipid-I (PGL-I) and the related dimycocerosyl phthiocerol. Recent interest in PGL-I centers on synthesis of the entire triglycosyl unit on its own and also as part of a neoglycoprotein, the so-called natural trisaccharide-octyl-bovine serum albumin (NT-O-BSA). Comprehensive inter-laboratory serological studies were conducted in order to compare NT-O-BSA with the monosaccharide- and disaccharide-containing neoglycoproteins prepared earlier [the so-called monosaccharide-octyl-BSA (M-O-BSA) and natural disaccharide-octyl-BSA (ND-O-BSA)]. In addition, the parallel products synthesized by Fujiwara and associates [the so-called natural disaccharide-phenylpropionyl-BSA (ND-P-BSA) and the natural trisaccharide-phenylpropionyl-BSA (NT-P-BSA)] were included in the comparative study. The sensitivity rate of NT-O-BSA and NT-P-BSA was appreciably higher than that of any of the other semi-synthetic or natural antigens; for instance, of 199 serum specimens from patients with mixed classifications, 106 were seropositive to NT-O-BSA and NT-P-BSA versus 97 when PGL-I was used. More importantly, NT-O-BSA and NT-P-BSA showed a lesser propensity to react nonspecifically with sera from an asymptomatic control population; only 3 (1.8%) of 169 sera reacted positively to NT-O-BSA and NT-P-BSA compared to 7 (4.1%) for PGL-I. The study thus showed that the trisaccharide-containing neoglycoproteins showed appreciably greater sensitivity and specificity than all other available antigens and, moreover, the correspondence between NT-O-BSA and NT-P-BSA was near perfect, a gratifying fact since the products were synthesized by different investigators on different continents.

The second major cell-wall antigen of the leprosy bacillus that has been described in considerable detail is the so-called lipoarabinomannan (LAM). Previously, we had indicated that besides arabinose and mannose, LAM contains glycerol and phosphate and is acylated by lactate, succinate, palmitate, and 10-methyloctadecanoate. About half of the phosphate was released by mild alkali treatment, and this was identified as consisting of inositol-1-phosphate side chains. Recent evidence indicates that the remainder of the phosphate, that which is alkali stable, also exists as inositol-1-phosphate, probably as part of glycosidically bound phosphatidylinositol mannoside. Thus, we now have sufficient information to propose a complete structure for LAM for *Mycobacterium leprae*. The substance previously called LAM-A is, in fact, lipoarabinomannan (LM). It is nonantigenic due to the absence of 5-linked arabinofuranose units; it also contains the inositol-1-phosphate units.

The remaining major antigenic segment of the cell wall of *M. leprae* is the bound arabinogalactan and associated peptidoglycan. Draper has indicated that the basic peptidoglycan subunit of the cell wall of *M. leprae* contains features that are both characteristic of mycobacteria and specific for *M. leprae*. Thus, N-glycolylmuramic acid is present rather than N-acetylmuramic acid. However, unlike all other mycobacteria, the amino acid at the $-NH_2$ terminus of the tetrapeptide side chain is L-glycine rather than L-alanine. In addition, the nature of the cell-wall mycolic acids has been examined in detail. However, other than those two moieties, nothing is known of the basic composition of *M. leprae* cell walls or of the basis of its inherent immunogenicity. As a prelude to such an examination, we have recently established that the cell-wall-associated polysaccharide of *M. leprae* is composed almost solely of arabinose (Ara) and galactose (Gal), and, in this respect, it is like all other mycobacteria. Conventional linkage analyses in which the partially *O*-methylated sugars were examined by gas chromatography/mass spectrometry (GC/MS) showed the expected terminal Ara-*f* and 2-linked ara-*f* units. However, methylation analysis could not distinguish between 5-linked Ara-*f* and 4-linked Ara-*p*, 3,5-linked Ara-*f* and 3,4-linked Ara-*p*, and 5-linked Gal-*f* and 4-linked Gal-*p*. To resolve these questions, the permethylated cell wall was subjected to partial acid hydrolysis, reduced, ethylated, and the products subjected to GC/MS. It was at once obvious

that all of the Ara was in the furanose form and, indeed, so also was all of the Gal, and, in fact, no Gal-*p* was present in the cell walls of *M. leprae*. We now believe that this is a feature of all mycobacteria rather than of *M. leprae* only, and that the literature is incorrect in attributing a →4)-β-D-Gal-*p* to the cell wall of mycobacteria; rather, Gal appears consistently as →5)-D-Gal-*f*.

We have referred to the cell wall skeleton of *M. leprae* as the bacilli's "last immunogenic frontier." Undoubtedly, as we explore its basic immunochemistry, other novel features will emerge that may be of the essence in explaining the immunological and other reactional responses of the human host to the cell wall of *M. leprae*.—[Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A. and Laboratory of Chemistry, Institute of Natural Science, Nara University, Horai-cho 1230, Nara, Japan 631]

Acknowledgments. Our research has been supported by Contract (NO1 AI-52582) and Grant (AI 21051) from the U.S.–Japan Cooperative Medical Science Program, National Institute of Allergy and Infectious Disease, National Institutes of Health, and funds from the Leonard Wood Memorial/American Leprosy Foundation.

Abe, M., Ozawa, T., Minagawa, F. and Yoshino, T. Immunoepidemiological studies on subclinical infection with *M. leprae*. IV. Five years' observation on school children by means of FLA-ABS and lepromin tests.

School children in three districts of the Miyako Islands, Okinawa, were followed up once a year from 1978 to 1984. All of the children 5 or 6 years old in the first grade of primary school and older children with suspicious symptoms (such as enlargement of the peripheral nerve or depigmentation of skin, both of which had no sensory loss) were tested with the fluorescent leprosy antibody absorption (FLA-ABS) test and the lepromin (Dharmendra's antigen) test. The FLA-ABS test was positive in 22.1% of 684 school children 5 or 6 years old examined during the 5 years since 1980. The percentage showed a significant difference by school districts, but no difference by sex. An association between the percentage of positive FLA-ABS tests and the number of new leprosy cases detected in each year was seen in two districts. The percentage of positive FLA-ABS tests was significantly higher in children with neural symptoms than in those without; whereas dermal symptoms showed no correlation with the FLA-ABS test results. These symptoms were most frequently found in the year when the largest number of new leprosy cases was reported during the 5 years. These facts support previous findings that both a positive FLA-ABS test and neural symptoms may be induced by subclinical infection with *Mycobacterium leprae*.

Delayed-type hypersensitivity against Dharmendra's antigen was examined in a total of 694 children, among which 648 were also FLA-ABS tested. The lepromin test was positive in 61% of the children; the percentage was higher in girls than in boys and was significantly different depending on the school district. The highest percentage was seen in the school district with the largest number of new leprosy cases. However, the percentage of positive lepromin tests was significantly higher in school children vaccinated with BCG than in those unvaccinated. Among 648 school children both FLA-ABS and lepromin tested, 6.8% of the children were FLA-ABS positive but lepromin non-positive responders. They were considered to have a high risk of leprosy because they have been infected with *M. leprae* without acquisition of cell-mediated immunity. Fortunately, however, no case with leprosy was found among those examined so far.

During a 7-year period, the FLA-ABS test was given twice for 232, three times for 48, and four or more times for 25 school children. Among 232 children examined twice, both neural symptoms and positive FLA-ABS tests persisted in 35 children. Another 71 children showed coincident changes or persistent negativity in both findings. Among the 48 children examined three times, neural symptoms persisted in 18 children, 12 of whom showed various changes or persistent negativity in the FLA-ABS test. Another 19 children showed persistent positivity or coincident changes in both findings. Among 25 children examined four or more times, persistently positive FLA-ABS

tests were seen in four children. Conversion of this test from negative to positive was observed in five children; from positive to negative was seen in nine. Conversion of the lepromin test from positive to negative accompanied by an increase in the FLA-ABS test grade was seen in three children. Moreover, persistently non-positive lepromin tests accompanied by positive FLA-ABS tests were seen in four children. Although further follow up of these children is necessary, the majority of the other children who were positive in both tests were considered to have almost no risk of the disease, because they have already acquired both types of immunity against *M. leprae* or because they have spontaneously recovered from its subclinical infection.—[National Institute for Leprosy Research, Tokyo, Japan]

Minauchi, Y., Tokunaga, H., Suzuki, M. and Matsubara, H. Effects of autoantibodies against peripheral nerve tissues—second report.

In 1984, we reported the autoantibodies against peripheral nerve tissues, through immunohistochemical technique, in patients with lepromatous leprosy and also suggested that slowly progressive sensory neuritis, which occurs in the lepromatous type regardless of the condition of the disease and of the existence of *Mycobacterium leprae* in the skin, might be partly due to those antibodies.

In this presentation, the results of further immunohistochemical study using purified IgG of whole sera from the patients as the primary antisera and the data of antibody against neutral glycolipid, known to be located predominantly in the myelin sheath, in human sciatic nerves by the ELISA method are reported.

Materials and Methods

Peripheral nerves were all from autopsied cases. For cryostat fresh-frozen sections, nerves were quick-frozen with dry ice in acetone, and kept in a −80°C freezer until used. Eighty sera were from patients with lepromatous leprosy, 22 were from tuberculoid leprosy patients, and 10 were from normal controls. The patients' average age was 64 and the mean period with leprosy was 33 years; most patients had so-called arrested disease.

IgGs were isolated from the sera by ion-exchange column chromatography with DEAE-Sephacel. The IgG solutions were concentrated and confirmed to be mono-peak fractions by paper electrophoresis, quantified by using immunodiffusion plates, and then kept in the freezer until used as primary antisera in immunochemistry. The indirect immunofluorescent technique was mainly used. IgGs were diluted with PBS. Biotinyl-IgG $F(ab')_2$ and Avidin-FITC were diluted to adequate content (ABC method). Each fresh-frozen 8-μm section of different kinds of nerves were placed on the same glass slides to be in the same conditions and were processed immunohistochemically. As further control, IgGs absorbed with acetone-treated PNS powder were used.

Crude glycolipid was extracted from the human sciatic nerves by the choloroform-methanol method, and the solution was separated by DEAE-Sephadex A-25. A neutral glycolipid fraction produced through a latro-beads column was applied as an antigen to detect its antibody in each 20 sera of the lepromatous and tuberculoid type and of normal controls by an ELISA method.

Results

The sera from the tuberculoid type and normal controls did not work. Lepromatous sera worked at the areas of axis cylinder (57/80; 72.5% in spinal anterior roots and 12/80; 15% in posterior roots) and at the areas of myelin sheath (5/80; 6.2% in anterior roots and 25/80; 31.3% in posterior roots). The IgG sensitivity was higher than whole sera. The mean maximum diluting ratio with IgG was 1:28 (80 mg/dl of IgG), which was nearly the same as with whole sera.

No titers of anti-neural glycolipid antibody were detected by ELISA in normal controls. Increased levels of antibody titer were found mainly in the sera of lepromatous patients (more than 200; 6/20), and all of the titers of the tuberculoid leprosy patients showed less than 100.

Conclusion and Discussion

From the two results, it is clear that autoantibodies against the peripheral nervous system exit in the IgG fraction mainly in

lepromatous leprosy, although Eustis-Turf, *et al.* (1985) reported evidence of antineural antibody (38%) in sera from patients regardless of the type of leprosy. Titers are apparently lower than those which are thought to be responsible for peripheral neuropathies associated with other neurological/immunological diseases, and their role in the pathogenesis of the progressive neuritis in leprosy is still obscure.

Our pathological data of commonly finding the *M. leprae* fragments in autopsied peripheral nerves of lepromatous leprosy suggest that these low titers might be due to the continuous release of debris or components of nerve tissue directly or indirectly destroyed by *M. leprae* into the bloodstream which may lead to the production of the antibodies.

The pathogenesis of leprous progressive sensory neuritis in accordance with autoantibodies and the permanent existence of *M. leprae* in peripheral nerves is briefly discussed.—[National Leprosarium Hoshizuka Keiai-en, Kanoya; Third Department of Internal Medicine, Kagoshima University, Kagoshima, Japan]

Matsuo, E., Sasaki, N. and Skinsnes, O. K. On the immunohistologic staining of β-glucuronidase in paraffin-embedded leprous tissue; requirement of the tissue extracts or probably the mycobacterial extracts to deparaffinize and cardiolipin and lecithin for staining.

The present study is concerned with developing methodology to detect immunoreactive β-glucuronidase (B-Gase) in leprosy tissues which have been processed to make paraffin sections. The immunoreactive component of B-Gase tends to be extracted during routine processing. We noticed that deparaffinization with a mixture of peanut oil and xylene was somewhat helpful. More recently, further experiments have led to improvements beyond the use of peanut oil.

In the present study we have used sections from lepromatous leprosy, tuberculoid leprosy, and tuberculosis. Paraffin was removed from the section with xylol containing: a) autoclaved HI-75 (a mycobacterium originally isolated from a leproma and more recently cultivated in Ogawa's medium), b) acetone power of human kidney, or c) peanut oil. Anti-human B-Gase was prepared by immunizing rabbits with a chromatographically homogeneous enzyme preparation extracted from human kidney. Anti-HI-75 was prepared by immunizing a rabbit with a solubilized fraction prepared from the bacilli by sonication. The antisera were adsorbed and purified by treating the original antisera with chloroform, methanol, and saline treated materials, respectively. The avidin-biotin complex (ABC) method of Hsu, *et al.* was used for immunohistologic stain throughout the study. The antisera were diluted with phosphate buffered saline (PBS) according to the method of the FLA-ABS test of Abe.

The respective antisera showed their corresponding immunoreactive sites in the sections treated as outlined above. Without these procedures insufficient immunoreactivities were demonstrable. Deparaffinization with xylene containing peanut oil showed relatively much weaker immunoreactivities in the sections than sections deparaffinized by the other techniques. Appropriate adsorptions of the respective antisera nullified background stainings and resulted in improved resolution in localizing the immunoreactivities in the tissue.

Overall, the results suggest the possibility of immunohistologic staining for B-Gase of human and possibly of mycobacterial origin in leprosy tissues which have been embedded in paraffin.—[Departments of Pathology, Kyorin University School of Medicine, Tokyo; National Institute for Leprosy Research, Tokyo, Japan; Zhongshan Medical College, Guangzhou, Guandong, People's Republic of China]

Maeda, T., Ohta, Y., Nozawa, K. and Someya, K. Study of peripheral lymphocyte subsets in leprosy patents.

Helper T cells, suppressor T cells, NK/K cells, and activated T cells in peripheral blood of leprosy patients were measured using monoclonal antibodies Leu3a, Leu2a, Leu11, and Leu HLA-DR in order to evaluate their cell-mediated immunity (CMI); peripheral IL-2R was also measured. Thirty-one patients who were treated for a long time were grouped as follows: 7 leprosy patients with the complication of malignant

tumors, 9 LL patients with the recurrence of positive *Mycobacterium leprae,* 10 LL patients with negative *M. leprae,* and 5 tuberculoid (T) patients.

Patients in whom positive *M. leprae* recurred during the course of long-term treatment showed a small number of suppressor T cells and a large number of helper T cells and IL-2R positive cells. These results were the same as those found in LL patients who responded well to a single treatment with dapsone (DDS) and who showed no positive *M. leprae* thereafter. This suggested that the proliferation of this organism affected CMI. Leprosy patients with the complication of malignant tumors showed high values of activated T cells, indicating a decrease in NK/K cells during the active phase of malignant tumors. Patients having a past history of erythema nodosum leprosum (ENL) demonstrated a high number of helper T cells and a low number of both suppressor T and NK/K cells.

Leprosy patients in Japan are becoming old; the mean age is now over 60 years. The most troublesome problems in our sanatorium were thought to be the recurrence of positive *M. leprae* among patients treated for a long time and rapid increases in the occurrence of malignant tumors. The present study suggests that CMI was changed in leprosy patients and could be used as a very helpful tool for the determination of the clinical status of leprosy patients.—[Suruga National Leprosarium, Gotemba, Shizuoka; Third Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan]

Mohagheghpour, N., Engleman, E. G. and Gelber, R. H. T-cell defect in lepromatous leprosy is reversible *in vitro.*

T lymphocytes from patients with lepromatous leprosy (LL) characteristically fail to respond to *Mycobacterium leprae.* This specific immunologic defect is thought to contribute to the aggressive clinical course that typifies patients LL. We report that while fresh CD4+ T cells from most LL patients are specifically unresponsive to *M. leprae,* following culture in medium alone for 48 hr the same cells respond to *M. leprae* antigens. The recovery of T-cell function is specific for *M. leprae,* occurs at the level of responder CD4+ T cells, and is not affected by monocytes or CD8+ (suppressor) T cells. Recovery of T-cell activity is blocked by the presence of *M. leprae* bacilli in the pre-culture medium. These findings indicate that despite the apparent specific energy seen in patients with LL, the T cells of most LL patients can respond to *M. leprae.* Their failure to do so, *in vivo,* may be due to the persistence of antigen, which renders antigen-reactive T cells nonresponsive, either directly or via activation of CD4+ suppressor cells. Experiments are in progress to determine the effects of antigens from leprosy bacilli on the subsequent proliferative response of *M. leprae*-responsive CD4+ T-cell clones and lines.—[Stanford Medical School Blood Center and Department of Pathology, Stanford University School of Medicine, Stanford, California; Seton Medical Center, Daly City, California, U.S.A.

Nomaguchi, H., Dohi, Y., Ohno, N., Fujiwara, T. and Ito, T. Inhibition of the proliferative response of lymphocytes to ConA or PHA by unique *M. leprae* glycolipid.

In order to analyze cellular mechanisms underlying the profound immune suppression in leprosy, it is desirable to develop mouse systems, even though *Mycobacterium leprae* is nonpathogenic for mice. We attempted to experimentally induce the suppression of lymphocyte proliferation to ConA or PHA by phenolic glycolipid-I (PGL-I) in mice and to study the preliminary analyses of the cellular mechanisms of the suppression.

Spleen cells of control and PGL-I-injected cells response to ConA (5 μg/ml) or PHA (1:1000) were determined by thymidine incorporation. Injection of PGL-I into mice induced suppression of the ConA and PHA response of the spleen cells. Furthermore, the ConA response of the control mice was reduced remarkably (79%) by the presence of PGL-I *in vitro,* and the PHA response of the control mice was completgely abolished by the presence of PGL-I. Restimulation of PGL-I-injected mouse spleen cells with PGL-I *in vitro* induced much more profound suppression in the ConA response.

Thus, PGL-I induced strong suppression of proliferation of mouse spleen lymphocytes *in vivo* and *in vitro*.

It is well known that α-methylmannoside binds to ConA and inhibits ConA responses. Since PGL-I bears a terminal trisaccharide on the molecule, we examined the possible involvement of these terminal saccharides in the suppression. The chemically synthesized preparations (40 μg/ml) of the terminal natural disaccharide (ND), natural disaccharide phenylpropionate (ND-P), or natural trisaccharide phenylpropionate (NT-P) showed no suppression on the conA or PHA responses. These data indicate that there was no involvement of the terminal carbohydrate portions in the inhibition.

To examine the possibility that the PGL-I-injected mice may generate suppressor cells which inhibit the ConA or PHA response, the control spleen cells were co-cultivated with the same numbers of PGL-I-injected spleen cells. The PGL-I-injected spleen cells did not inhibit the response of control spleen cells. Thus, active suppressor cells were not detected in PGL-I-injected spleen cells.

We attempted to restore the impaired spleen-cell response of PGL-I-injected mice with the help of fresh peritoneal macrophages. The normal peritoneal cells restored the impaired responses efficiently. These data together with the data showing an absence of suppressor cells suggest that the nonspecific suppression of the ConA response of the PGL-I-injected mice may be due to the impairment of macrophages. In addition to the nonspecific suppression of ConA or PHA response, PGL-specific suppressor cells also were induced by stimulation with PGL-I or NT-P.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University; Department of Bacteriology, Osaka University Medical School, Osaka; Department of Natural Science, Nara University, Nara, Japan]

Modlin, R. L., Bloom, B. R., Nelson, E. E., Shen, J.-Y., Gunter, J. R. and Rea, T. H. *In situ* and *in vitro* characterization of T-helper lymphocytes in leprosy.

We have been interested in characterizing the state of activation of T-helper lymphocytes in leprosy lesions. However, no marker for activated T cells, including interleukin-2 (IL-2) receptor or HLA-DR, has been shown to correlate with the predicted or actual response to lepromin.

Tal, a 105 kD molecule and distinct from the IL-2 receptor, is expressed strongly on activated, antigen-specific, helper T cells, but only weakly on a small fraction of resting T cells.

We used a monoclonal antibody directed against the Tal antigen in conjunction with an immunoperoxidase technique to identify cells in frozen sections of leprosy lesions. In tuberculoid tissues, lepromin skin tests, and reversal reactions, Tal antigen was expressed on the cell surface of both small and large nucleated cells. In contrast, most of the Tal expressed in lepromatous and erythema nodosum leprosum (ENL) tissues was on small lymphocytes. When comparing the percentages of Tal positive cells in the various types of leprosy, we found approximately 15–20% of the cells stained positively in tuberculoid specimens, lepromin skin tests, and reversal reaction skin lesions. In contrast, less than 10% of the cells stained positively in lepromatous and lepromatous with ENL lesions, and the differences were statistically significant ($p < 0.01$). Double immunostaining showed that Tal was expressed mainly on helper/inducer T lymphocytes.

Thus, of the four activation markers we have studied, only Tal positive staining correlates with the lepromin response or clinical status of the patient.

Modlin, R. L., Mehra, V., Nelson, E. E., Kato, H., Pattengale, P. K., Rea, T. H. and Bloom, B. R. T-lymphocyte clones derived from leprosy skin lesions.

We have previously reported on the characteristics of cells in the skin lesions of patients with leprosy. T-helper cells are abundant in tuberculoid lesions. In contrast, T-suppressor cells are the predominant lymphocyte phenotype in lepromatous lesions. These differences are not present in the peripheral blood. We sought to study the functional properties of these cells present in the tissue compartment. Since the

lymphocytes present in the skin lesions are activated *in situ* (Tac+) and should be concentrated for antigen reactivity, they could be cloned in the presence of interleukin-2 (IL-2), yielding homogeneous populations of cells for functional study.

Skin biopsy specimens from five lepromatous and four tuberculoid patients were extruded through surgical mesh, labeled with FITC-conjugated T4 (helper) and T8 (suppressor) monoclonals, and sorted with a fluorescent activated cell sorter FACS IV. Cell lines were obtained by seeding 1–10,000 T cells in the presence of IL-2. T8 cells were then cloned from lines by limiting dilution in the presence of IL-2, and T4 cells were similarly cloned in the presence of IL-2 and lepromin. T8 clones were assayed for lepromin-induced suppression of ConA responses of normal PBMC and suppression of lepromin-responsive T4 clones. T4 clones were examined for proliferative response to lepromin, PPD, and tetanus.

Nine T8+ lines from lepromatous lesions exhibited lepromin-induced suppression of ConA responses of normal PBMC; in contrast five T8+ lines from tuberculoid lesions did not. Two T8+ clones from lepromatous lesions showed lepromin-induced suppression of three HLA-DR matched PBMC but did not suppress three mismatched PBMC. In addition, these clones suppressed a HLA-DR matched lepromin-responsive T4 clone but not a mismatched T4 clone. A T4+ lepromin-nonreactive clone was added to control for the addition of cells and did not show suppression.

T4+ clones from tuberculoid lesions but not lepromatous lesions were reactive to lepromin. Four types of T4+ clones were present in tuberculoid lesions—lepromin specific, lepromin-PPD crossreactive, PPD reactive, and nonreactive IL-2 dependent.

These data indicate that T8-cell suppression is restricted by MHC class II antigens in man, and suggest that the unresponsiveness of LL patients may be related to the presence of these suppressor cells within lesions. Both lepromin-specific reactive T4 cells and lepromin-PPD crossreactive T4 cells appear to contribute to the *in situ* immune response of tuberculoid patients and may be absent from lepromatous lesions.—[Section of Dermatology and Department of Pathology, University of Southern California School of Medicine, Los Angeles, California; Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, U.S.A.]

Cohn, Z. A. and Kaplan, G. Macrophage activation and the secretory repertoire.

Using the interaction of *Mycobacterium leprae* and a selected number of bacterial and protozoan obligate intracellular parasites, we trace their interactions with the plasma membrane and elements of the vacuolar apparatus of mammalian mononuclear phagocytes. Particular emphasis is placed on their interactions with defined glycoprotein receptors, their ability or inability to stimulate antimicrobial and inflammatory elements of the secretory repertoire, their interactions with the endosome to either promote or inhibit phagosome-lysosome fusion, and the hydrolytic potential of macrophages to degrade microbial antigens.

Since macrophage activation is an essential part of the host response to these pathogens, we compare and distinguish the activation process as it proceeds *in vivo* to that occurring in the culture vessel. This process involves structural, metabolic receptor and membrane changes which alter both the endocytic and secretory responses of macrophages in both a positive and negative fashion. These findings emphasize the temporal significance of the activation process and the irreversibility of many of its parameters.—[The Rockefeller University, New York, New York, U.S.A.]

Makino, M., Suzuki, T., Nagata, A., Nakata, A. and Ito, T. Construction of genomic library of *Mycobacterium leprae* and its expression in *Streptomyces lividans.*

Shuttle vector pSN463, which is replicable in both *Escherichia coli* and *Streptomyces lividans,* was used as a vector for gene cloning of *Mycobacterium leprae.* A genomic library of *M. leprae* was constructed with this vector and kept in plasmid form. pSN463 with DNA of *M. leprae* was transformed into *S. lividans* using the character of shuttle vector. Transformants were grown

and the expression of *M. leprae*-specific proteins was examined by Western blotting with enzyme immunoassay. *M. leprae*-specific monoclonal antibodies which were provided by the World Health Organization were used as reference sera. Polyclonal antibody against *M. leprae* was also used as a reference serum.

It has been very difficult to obtain proof that the promotor of the *M. leprae* gene works in *S. lividans,* but by the polyclonal serum tests, one of the major proteins was detected on nitro-cellulose membrane. By the recent experiments, among these transformants, two strains reacted with *M. leprae*-specific monoclonal antibody (IV E 9).—[Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Anderson, D. C., Young, R. A. and Buchanan, T. M. Synthesis of two epitopes that react with monoclonal antibodies to the 65,000 dalton protein of *Mycobacterium leprae.*

The gene coding for synthesis of the 65,000 dalton protein of *Mycobacterium leprae* has recently been sequenced (Mehra, *et al.*, Proc. Natl. Acad. Sci. U.S.A. 83:7013–7017, 1986). Comparison of the sequence of subclones of this gene which expressed the epitopes recognized by IIC8 or IIIC8 monoclonal antibodies (MOAbs) as contrasted with those which did not express these same epitopes allowed prediction of the amino acid sequence of these specific antigenic domains. The IIC8 epitope was predicted to be 12 amino acids in length and the IIIC8 epitope was located to a 13 amino acid region at the carboxy-terminus of the 65,000 dalton protein. The accuracy of these predictions was further analyzed by solid-phase peptide synthesis (SPPS). The predicted IIC8 epitope sequence was EYEDLLKAGVAD. Synthetic peptides of both this sequence and YEDLLKAGVAD retained maximal antigenicity and 3 ng of each peptide was sufficient to inhibit by 40% an immunoassay employing IIC8 MOAb and polystyrene plates coated with *M. leprae* sonicate or IIC8 12mer peptide. The smallest peptide retaining detectable antigenicity was a 9mer with a sequence of EDLLKAGVA, and 850 ng of this peptide were required to produce 40% inhibition in the same immunoassay. The predicted sequence for the IIIC8 epitope was ASDPTGGMGGMDF. Synthetic peptides with this structure had equal antigenicity to peptides with the sequence SDPTGGMGGMDF (12mer) and DPTGGMGGMF (11mer) with each requiring 8–16 ng to produce 40% inhibition of a IIIC8 immunoassay. Some antigenicity was retained even by a 10mer with the sequence DPTGGMGGMD and 500 ng of this peptide were required to produce 40% inhibition in the same immunoassay. The oxidation state of the methionines in the IIIC8 epitope proved critical for antigenicity. The 12mer containing methionines oxidized to the methionine sulfoxide state required 3500 ng to produce 40% inhibition, as compared to 14 ng of the reduced peptide to produce 40% inhibition in the same immunoassay. Peptides prepared by SPPS allow definitive characterization of the epitope sequence and should allow determination of which residues are critical for antigenicity. This approach may also prove useful for the elucidation of the sequences of T-cell and antibody reactive epitopes specific for leprosy or tubercle bacilli which might prove helpful for the development of new reagents potentially useful for skin tests, vaccines or diagnostic assays.—[University of Washington, Seattle, Washington; Whitehead Institute for Biomedical Research, Cambridge, Massachusetts; Gillis W. Long Hansen's Disease Center, Carville, Louisiana, U.S.A.]

Mehra, V., Sweetser, D. and Young, R. Fine mapping of epitopes in 65 kD protein antigen of *M. leprae* using recombinant DNA.

We have previously isolated the genes encoding the five most immunogenic protein antigens of *Mycobacterium leprae* from a recombinant DNA expression library constructed using bacteriophage lambda gt11 vector. Polypeptides encoded by the foreign insert DNA in lambda gt11 phage were produced in *Escherichia coli,* and the recombinant DNA phage clones encoding the relevant polypeptides were identified and isolated using monoclonal antibodies di-

rected against 65 kD, 36 kD, 28 kD, 18 kD, and 12 kD antigens (Young, *et al.*, Nature 316:450–452, 1985).

We have further used the same approach of isolating genes using monoclonal antibodies as probes and mapped epitopes in 65 kD antigen of *M. leprae.* The strategy involves isolating a recombinant DNA clone that produces positive signals with each of the six anti-65 kD monoclonal antibodies individually and is most likely to contain the entire coding sequence for the antigen. It is then subjected to DNA sequence analysis and epitope mapping. To map precisely the epitope coding sequences within 65 kD antigen, a lambda gt11 sub-library containing fragments of the gene was constructed from the foreign insert DNA of the clone of interest and screened with each of the six anti-65 kD antigen monoclonal antibodies. The appropriate recombinant DNA subclones were isolated, and the nucleotide sequences of the cloned DNA fragments were determined. Minimum nucleotide sequence shared by all the subclones that produce the positive signals with a particular antibody encodes for the epitope. The amino acid sequences of all the six epitopes in 65 kD antigen can then be deduced from the DNA sequences. These sites are contained within 13 to 35 amino acid residues.

The ability of this strategy to identify accurately antigenic determinants is demonstrated by showing that the predicted synthetic peptide is bound specifically by the respective antibody. Studies are in progress to determine if any of these antigenic determinants are recognized by T cells.—[Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York; Whitehead Institute for Biomedical Research, Cambridge, Massachusetts; Massachusetts Institute of Technology, Cambridge, Massachusetts, U.S.A.]

CLOSING REMARKS

Ladies and Gentlemen:

It is time once again to close another U.S.–Japan Leprosy Research Conference. Once again we have been privileged to learn of the latest exciting findings in leprosy research from our two countries. Again we have been able to meet with our colleagues in tuberculosis research, to learn from them, and to share ideas with them.

On behalf of all U.S. participants, I would like to thank Dr. Abe, Professor Ito, and their colleagues for another superbly organized conference, and another conference characterized by incomparable Japanese hospitality and much enjoyment.

This, the 21st Joint Conference, marks my last as Chairman of the U.S. Leprosy Panel. Next year Dr. Patrick Brennan will ably assume this role.

May I say on this occasion that serving on the U.S. Leprosy Panel since 1977, and the honor of serving as Chairman of this panel since 1980 have been the highest privilege to me. May I thank you all very much for your support and hard work in making the U.S.–Japan Leprosy Research Program a resounding success over the last 6 years.

I look forward very much to the continued accomplishments of the U.S.–Japan Leprosy Program in the coming years.

Thank you very much.

—Robert C. Hastings, *Chairman*
U.S. Leprosy Panel