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ABSTRACTS

FIFTEENTH JOINT LEPROSY RESEARCH CONFERENCE

Shiroyama Kanko Hotel
Shin-Shoin Cho, Kagoshima, Japan
9–11 October 1980

U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

Good morning ladies and gentlemen! You are welcomed to Kagoshima, the deep south of Japan! Last year, in New Orleans, I talked about the volcano of this place. Thereafter, in Washington state, Mount St. Helens burst into a big eruption, and so I became deeply concerned about your safety in being here in Kagoshima, a city that just stands on the volcano. But as you can see, a column of smoke, the ejecta from the volcano, comes out at all times, and that may be the safety valve. I hope you will make your minds quite easy.

Now let us return to our subject, leprosy. Kagoshima was once an endemic area in Japan, and now leprosy is almost eradicated. Owing to our members' efforts, many subjects have been studied and elucidated, and great progress has been achieved. However, in Southeast Asia, leprosy is still a hard problem in every country. Many problems are awaiting solution, and troublesome questions, such as primary resistant leprosy, which is being reported at this meeting by Dr. Jacobson, come out one after another. We are proud of our American friends for their great efforts in every field of leprosy research.

At the very beginning of our U.S.–Japan Cooperative Program, the U.S.–Japan Cooperative Therapy Program of the Leonard Wood Memorial in 1952, we had only a few research workers, letting us feel lonely. Since then, the number of American and Japanese scientists has been getting to be more and more. We have worked together shoulder to shoulder in our U.S.–Japan Cooperative projects, and we have so many friends now. As we can see in the Review Committee, it is hoped that our Joint Conference will be continued for some time more. I hope we would continue our cooperation more and more vigorously from now on.

On the other hand, we must pay attention not to interfere with the policy of each country. Our fundamental policy is supposed to be limited to basic fields of research only, but we should like to make our U.S.–Japan Cooperative project an international one with our Conference as the nucleus. From this point of view, we succeeded in getting approval from Professor Ma from the People's Republic of China to make a special speech at our Conference. I regret to say that we have just received a telegram from Professor Ma, telling us he was forced to give up his arrangements, owing to some urgent official business. I have asked Dr. Cheng, who came to Japan just the year before last from Peking, to take the place of Professor Ma and speak on leprosy in China.

I hope we will have active discussions on our research work during these three days.

Thank you.

—Masashi Namba, *Chairman*
Japanese Leprosy Panel

Kagoshima, site of the Fifteenth Joint Leprosy Research Conference

Mt. Minamidake, on Sakurajima island, near Kagoshima.

The participants at the Fifteenth Joint Leprosy Research Conference

Drs. Barry R. Bloom and Masashi Namba

PROGRAM OF THE FIFTEENTH JOINT LEPROSY RESEARCH CONFERENCE

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Dr. Masahide Abe

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ABSTRACTS OF LEPROSY CONFERENCE

Nakamura, K. and Yogi, Y. The nude mouse as an experimental lepromatous leprosy model (continued): The lepromatoid lesions in mystacial vibrissae located site of injection.

We have successfully established an animal model for experimental lepromatous leprosy using the SPF nude mouse and the germ-free nude mouse. We have developed a "foot injection" method, by oneself, in the vinyl isolator. This method involves injection through the dorsal aspects of fore and/or hind paws without passing through the plantar aspect (foot pad) by handling with the right hand through the globe, using a two-step needle. We have developed a combined method, which involves a second injection in the mystacial vibrissae located site, by oneself, in the vinyl isolators. This combined method was useful for obtaining numerous bacilli (10^{10}–10^{11}) at an early stage after infection as a source of *M. leprae*.

In this report, long term observation was performed to determine whether or not heavy lepromatoid lesions occur in the mystacial vibrissae located site (swelling) using a "virulent" *M. leprae* strain.

The injected site was the right upper lip (mystacial vibrissae located part), and the control group was injected in the right dorsal site of the hind paw. The strain used was "virulent" because it had caused slight swelling in infected hind paws of other nude mice at 6 to 7 months after the foot injection with 10^5–10^6 bacilli. In addition, we used the nude mouse to develop the congenitally vibrissaeless nude mouse. The inoculum size was 5.0×10^5 bacilli.

At 10 months after infection introduced into the foot, all tested nude mice were observed to have swelling of the injected foot (thickness: less than 2 mm). In contrast, in the case of the animals injected in the upper lip, only a small amount of swelling of the injected site was observed. At 11–13 months after the infection, the upper lip site was swollen in four of seven nude mice tested. At that time, bacillary counts were approximately 5.0×10^{11} bacilli per gram. In addition, systemic infection had occurred. At 16–17 months after inoculation, three of the nude mice were completely swollen in the injected lip and began to develop swelling in neighboring parts such as the ear, nose, eye lid, rhinarium, left lip, uninoculated lip, skin over the diastema, dentine of incisors, skin of muzzle and nasal septum, and skin of sites on the mandible. In addition, systemic infection occurred as evidenced by lesions in the paws, tail, liver, lung, spleen, testis, and swelling of the epididymis. Long-term survival included only one mouse at 25.5 months after the infection.

In summary, we have established severe lepromatoid lesions of the injected upper lip, including neighboring tissues, and systemic lesions in the vibrissaeless nude mouse. These occurred after injections into the upper lip as well as after hind foot pad injections. However, in spite of severe nose lesions at an earlier stage, the intra-upper lip infection could not accelerate lung lesions or lesions involving dentine, skin of the mandible site, and tongue. These findings led to the conclusion that the preferred injection sites in the nude mouse, especially in the vinyl isolators by oneself, were reconfirmed. That is to say, our simple combined method by which the dorsal sites of the fore and hind paws and intra-upper lips were injected for experimental lepromatous leprosy was useful for obtaining numerous *M. leprae* at an early stage after the infection. In addition, we have confirmed that whether or not lepromatoid swelling occurs in injected sites in the paws and/or upper lips in nude mice, can serve as a marker for "virulent" strains of *M. leprae*. This marker can be used to compare different strains of *M. leprae* as well as previously reported differences in morphology.—[National Institute for Leprosy Research, Tokyo, Japan]

Hastings, R. C., Chehl, S. P. K., Morales, M. J., Shannon, E. J. and Kirchheimer, W. F. Multiplication of acid-fast bacilli in nude mice inoculated with armadillo-derived *M. leprae*.

M. leprae have been shown to multiply well in athymic nude (nu/nu) mice (Colston, J. J. and Hilson, G. R. F. Nature **262** [1976]

399–401; Kohsaka, K., Mori, T. and Ito, T. La Lepro **45** [1976] 177–187; Nakamura, K. and Yogi, Y. LSM **L-784** [1976]). The present investigation was undertaken to establish more detailed growth patterns of *M. leprae* in nude mice and compare these patterns with the established, limited, and generally localized multiplication of *M. leprae* in immunologically intact BALB/c mice (Shepard, C. C., J. Exp. Med. **112** [1960] 445–454). Freshly harvested *M. leprae* from the spleen of an infected armadillo were inoculated into the plantar surfaces of the left hind foot pads of 160 nude mice and 160 BALB/c mice. Sixty animals in each group received 1×10^6 *M. leprae*; 50, 1×10^5 *M. leprae*; and 50, 1×10^4 *M. leprae*. Nude mice were housed under sterile conditions in a laminar flow environment. BALB/c mice were conventionally housed. Six or seven mice from each group were sacrificed 5, 90, 180, 270, 365, and 565 days after inoculation. One animal from the 1×10^6 inoculated groups was autopsied at each time interval. Bacterial enumerations were performed on the remaining animals by counting acid-fast bacilli (AFB) in the left hind foot pads, right hind foot pads, pools of each animal's liver and spleen, and homogenates of the remainder of the carcass. The bacilli multiplied in the inoculated foot pads with a generation time of 10.6 to 16.0 days. The *M. leprae* inoculated foot pads from the 1×10^6 inoculated group contained $1.3\ (\pm 0.4) \times 10^{10}$ AFB (mean [±S.D.] N = 6) per gram of tissue and weighed 0.12 ± 0.03 grams 272 days after inoculation; $1.9\ (\pm 2.5) \times 10^{10}$ AFB per gram and weighed 0.84 ± 0.30 grams at 365 days; and $3.74\ (\pm 1.71) \times 10^{10}$ of AFB per gram and weighed 1.5 ± 0.6 grams 565 days after inoculation.

At the 365 day harvests the AFB were subjected to standard identification tests for *M. leprae*. Dopa oxidase testing, pyridine extraction tests, *in vitro* cultivation, and growth patterns in BALB/c mice are compatible with the AFB being *M. leprae*. Lymphocyte blast transformation studies are in progress at the time of this writing.

To date the most striking findings have been 1) tremendous bacterial loads in inoculated foot pads of the nude animals, 2) an apparent "ceiling" in bacterial density in the inoculated foot pads of the nude mice after which further bacterial increases are accompanied by gross enlargements, and 3) the relative lack of bacterial dissemination from the inoculated foot pads in the nude mice despite documented bacteremia. —[U.S. Public Health Service Hospital, Carville, Louisiana 70721, U.S.A.]

Fieldsteel, A. H., Colston, M. J. and Sato, N. Comparison of the neonatally thymectomized Lewis rat (NTLR) and the congenitally athymic nude rat with respect to infection with *Mycobacterium leprae*.

Over the last several years we have developed the NTLR as a model of multibacillary leprosy and have utilized the model both for chemotherapy studies and as a means of detecting persisting viable organisms in the tissues of patients undergoing therapy. It has been our experience that a varying percentage of NTLR, although thymectomized within 16 hours after birth, respond to infection with *M. leprae* in much the same fashion as intact rats, yet show no evidence of residual thymus when killed at up to 2 or more years of age. It is important to recognize and eliminate these animals before they are placed in long-term experiments. Since we have found that the gestation period of Lewis rats varies between 21 and 25 days, it is possibie that those rats born more than 21 days after conception may have developed a greater degree of immunocompetence by the time they are born.

In this study we have produced highly specific, absorbed antithymocyte serum and have used it in a direct fluorescent antibody test to determine whether there is a correlation between growth of *M. leprae* in NTLR and the remaining population of T-cells. In addition, we have carried out lymphocyte stimulation tests to compare the response of splenic lymphocytes from NTLR, intact Lewis rats, and athymic rats. The results of both studies indicate that NTLR do possess circulating T-cells, albeit at a lower than normal level, and we have attempted to correlate numbers of T-cells with susceptibility to infection with *M. leprae*.

We have also compared the NTLR and

the athymic rat with respect to both foot pad and intravenous infection with *M. leprae*. Preliminary results indicate that while both animals develop an enhanced and disseminated infection, the infection in the athymic rat shows a greater degree of uniformity between individual animals and a more rapid and extensive dissemination.—[Life Sciences Division, SRI International, Menlo Park, California 94025, U.S.A.]

Nakamura, K. and Yogi, Y. The experimental inoculation with *M. leprae* in the asplenic mouse.

We have previously observed that neonatal or adult splenectomy has no significant effect on the multiplication of *M. leprae* in mice. Additionally, creating abnormal hind paws with a mixed infection with *M. leprae* and *Corynebacterium kuscheri* or mixing oil (adjuvant such as Drakeol No. 6, liquid paraffin, and vegetable oil) with *M. leprae* caused either no growth or reduced multiplication of *M. leprae* in mice, as compared to *M. lepraemurium* infections. In 1959, congenitally asplenic mice were discovered by Searle. The homozygous Dh/Dh mice die after birth due to visceral anomalies, and heterozygotes (Dh/+), besides lacking spleens, have abnormalities of the hind paws and toes and/or shorter femurs than normal.

The present report describes studies to determine the suitability of the congenitally asplenic mouse as a model for leprosy. Relationships between the spleen and the hind paw abnormalities were determined, using foot pad inoculation techniques.

The study was performed to determine the effects of a lack of spleen and the presence of foot abnormalities on the multiplication of *M. leprae* in Dh/+ mice in comparison with +/+ mice and normal mice. Congenitally asplenic mice (B6C3DH) were obtained from Dr. Suzuki, and then an inbred colony of asplenic mice was maintained by sib-mating 6 to 10 week-old Dh/+, and +/+ mice. C3H/He, C57/BL/6, BALB/c, and ICR mice were used as controls. Inoculum sizes were 4.0×10^4 bacilli/hind foot and 1.2×10^4 bacilli/fore foot.

After inoculating *M. leprae* in the hind paws, multiplication was not observed at 3, 6, or 12 months after inoculation. After injecting *M. leprae* into the fore paws, multiplication was observed at 5, 8, and 10 months after infection. On the other hand, there were no significant differences in the number of *M. leprae* found among Dh/+, +/+, and normal mice with infections in the fore foot, and there were no differences between +/+ mice and normal mice infected in the hind feet. Dh/+ mice did not develop systemic infections with *M. leprae* such as nude mice do.

In summary, it may be concluded that the congenital asplenic mouse represents a unique model for the study of leprosy and the relationships among the spleen, bone marrow, and thymus, as compared to the nude mouse. The multiplication of *M. leprae* in the abnormal hind paws could not be seen. Thus the infectivity of *M. leprae* was tremendously weak compared to *M. lepraemurium* infections. There was no enhancing effect of immunobiologically deficient characteristics such as B and T cells from Dh/+ mice. In addition, this lack of multiplication of *M. leprae* in the abnormal hind paws was similar to that seen after treatment with oil. Therefore, we are investigating this mechanism further in the case of the normal fore paws (Dh/+). In the injected site only, persistent resistant infections were established in the normal fore paws of Dh/+ mice as well as normal mice without an enhancing effect.—[National Institute for Leprosy Research, Tokyo, Japan]

Shepard, C. C. Statistical tests of significance of results obtained by the kinetic method and the proportional bactericidal method.

Three methods have been available for testing the activity of drugs against *Mycobacterium leprae*. The continuous method, for technical reasons, is now used largely for the testing of individual patients' strains for drug resistance. The kinetic method and the proportional bactericidal method are now the chief ones used for testing new drugs and regimens. A comparison of the advantages of the latter two methods is complicated, but it can be said that the kinetic method serves better when detection and measurement of even bacteriostatic activity is important, and the proportional

bactericidal method when measurement of higher degrees of bactericidal activity is important. A disadvantage of both of these methods, however, has been that satisfactory methods for the assessment of the statistical significance of the results were not in hand. The problem was seen especially with some published kinetic method results where not enough harvests were carried out to delineate the logarithmic phases in the control and treated groups. Beyond this, the needs for quantitation are greater now that quantitative structure-activity relationships (QSAR) are playing an important role in the design of new drugs.

In the kinetic method, the growth curve in the drug-treated group is compared to that in the control groups in order to measure the drug-caused delay in the appearance of the logarithmic phase of growth. On the basis of experience, 60 days seemed to be the shortest significant delay that could be detected. Our present protocol calls for four control groups and harvests (on pools of four mice) carried out every 28 days beginning early in the logarithmic phase (usually 126 days after infection). When the control groups first become positive, the count in the treated group will be lower if the drug is active. The probability for this arrangement is 0.2; the probability that it will also occur at the next harvest is 0.04, etc. A delay of 20 days can ordinarily be detected with statistical significance of <0.04. In the case of purely bactericidal activity, this would be equivalent to a reduction in numbers of viable *M. leprae* of about 0.4 $\log_{10}$.

In the proportional bactericidal method, a series of tenfold dilutions of *M. leprae* is inoculated into groups of mice, and some groups are treated with drug. After 12–18 months, counts of *M. leprae* in individual mice are carried out. On the assumption that one or more viable *M. leprae* will produce an infection and that the distribution of viable *M. leprae* is random in control mice at 0 days and in treated mice at the end of treatment, the most probable numbers of viable *M. leprae* are calculated with the use of published tables. The results obtained, however, especially in treated groups, may not reveal whether the distribution is truly random. A random distribution of positive cultures can be expected when dilutions of cultivable bacteria are inoculated into favorable medium, but the results in many experimental infections do not fit such a model. Consequently, methods for the estimation of the dose that infects 50% of the animals (ID_{50}) have been developed. The Spearman-Kärber calculation allows the estimate of the standard deviation of the result. This calculation requires that the titration extend over a range from 100% to 0% infection so one or two more dilutions than usual of the inoculum may be necessary to reach 0% infection. To reach 100% infection in drug treated groups may be impossible, but some simple assumptions allow a conservative estimate of the significance. We find that with counts on five mice per dilution, standard deviations of about ± 0.25 logs are usually observed and that a difference between groups of about 0.7 logs is usually significant at the <0.05 level.—[Center for Disease Control, Atlanta, Georgia 30333, U.S.A.]

Nishiura, M., Fukunishi, Y., Okada, S., Abe, M., Kohsaka, K., Yoneda, K., Mori, T., Ito, T., Bretana, A., Convit, J., Walsh, G. P., Meyers, W. M. and Binford, C. H. A joint study on the naturally acquired leprosy-like disease of armadillos.

Four nine-banded armadillos from Louisiana with a naturally acquired leprosy-like disease were studied jointly by investigators in Japan, Venezuela, and U.S.A.

Electron microscopic studies of freeze-etch replicas of the lesions in these armadillos revealed structures that closely resembled the foamy structures seen in experimental leprosy in armadillos and in human lepra cells.

The FLA-ABS test failed to detect *M. leprae*-specific antigens in the bacilli in Armadillo No. 1. However, the FLA-ABS test gave positive results in other armadillos with the naturally occurring leprosy-like disease. The reasons for the negative results in Armadillo No. 1 are not yet clear. Bacilli from tissues of Armadillo No. 1 were inoculated into nude mice. These bacilli multiplied in the tissues of the nude mice, but only two of 52 of the mice survived as long as 8 months. Although the

macrophages of these mice contained many bacilli, no foamy structures were observed. Usually, nude mice inoculated with *M. leprae* produce foamy structures; therefore the inability of the bacilli from this armadillo to produce typical foamy structures in nude mice is at variance with typical *M. leprae*. The significance of this variation is as yet unclear.

Electron microscopic studies demonstrated small numbers of abnormally shaped bacilli, especially in lymph nodes and spleen of some armadillos. The significance of these forms is unknown, but they may represent deformed *M. leprae* or contaminating bacteria. Small numbers of cultivable mycobacteria identified as *M. intracellulare* and *M. scrofulaceum* were isolated from the spleen of Armadillo No. 1.

In summary, all four armadillos with the naturally acquired leprosy-like disease had lesions typical of those seen in experimental leprosy. However, the mycobacteria in one armadillo did not produce typical foamy structures in nude mice and did not stain typically in the FLA-ABS test.—[Leprosy Research Laboratory, Kyoto University, Kyoto, Japan; National Institute for Leprosy Research, Tokyo, Japan; Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; Instituto Nacional de Dermatologia, Caracas, Venezuela; Armed Forces Institute of Pathology, Washington, D.C. 20306, U.S.A.]

Fukunishi, Y., Meyers, W. M., Walsh, G. P., Johnson, F. B., Binford, C. H., Okada, S. and Nishiura, M. Ultrastructural features of the multiplication of leprosy bacilli in macrophages of armadillos inoculated with *M. leprae*.

Lesions of experimental armadillo leprosy (leproma, lymph node, spleen, liver, and lung) from 7 nine-banded armadillos (*Dasypus novemcinctus*) have been studied electronmicroscopically with freeze etching, ultrathin sectioning, and shadow casting techniques.

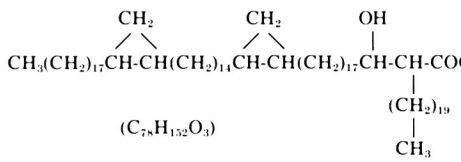
In all organs with lepromatous-like lesions, distinct intracytoplasmic foamy structures composed of the aggregation of tiny spherical droplets around bacilli in phagolysosomes of macrophages were found. This finding of foamy structures in the macrophage is very similar to the findings in human lepra cells and in nude mice lesions caused by the inoculation of *M. leprae*, as observed by freeze etching.

The three dimensional appearance of the bacilli in the experimental armadillo lesions was also examined by freeze etching. As observed by freeze etching, the bacilli were most natural with an artifact-free morphology lying in the host cells, and they showed mostly slender and long forms. The surface of the cell wall of the bacilli was usually smooth, and band structures were observed on the outermost surface of the cell wall. Each band structure is composed of two thin strings.

All these morphological features of the bacilli in the experimental armadillo leprosy lesions are quite similar to those of *M. leprae* in human lepra cells.—[Armed Forces Institute of Pathology, Washington, D.C. 20306, U.S.A.; Leprosy Research Laboratory, Kyoto University, Kyoto, Japan]

Kusaka, T., Fukunishi, Y., Akimori, N., Walsh, G. P., Meyers, W. M. and Binford, C. H. Analysis of mycolic acids in mycobacteria isolated from the lesions of armadillos with naturally acquired leprosy-like disease and experimental armadillo leprosy.

We showed last year (Int. J. Lepr. **47** [1979] 667) that high performance liquid chromatography (HPLC) is an essential tool for isolating mycolic acids in the highly purified form from a very restricted amount of mycobacteria grown both *in vivo* and and *in vitro*. Two series of mycolic acids could be isolated from only one foot pad of a nude mouse inoculated with *Mycobacterium leprae* using HPLC. One of these mycolic acids was demonstrated mass spectrometrically to belong to α-mycolate as shown below:



With the hope of gaining further evidence concerning the structure of mycolic acids in *M. leprae*, mycolic acids were isolated

this time directly from bacilli, which had been previously separated from the livers of armadillos with experimental armadillo leprosy and from bacilli from livers of armadillos with naturally acquired leprosy-like disease.

Methods used for isolating mycolic acids from the bacilli were about the same as previously reported (Int. J. Lepr. **47** [1979] 667). Exceptions were that the length of the gel-permeation column of the HPLC was elongated 2.5 times more than before in order to fractionate more precisely the fatty acids into various sized molecules. Additionally, a thin layer chromatography (TLC) using silicagel-G with a solvent system (n-hexane:diethylether:acetic acid 80:30:1) was preferred instead of the HPLC with an absorption column in order to save time. No differences were observed in the chromatographic data (HPLC using columns of gel-permeation and of reversed phase as well as the TLC) during isolation of mycolic acids from the two kinds of mycobacteria separated from the livers of armadillos with experimental armadillo leprosy, on the one hand, and with naturally acquired leprosy-like disease, on the other. A more definite analysis of these mycolic acids should be achieved by mass spectrometry, which is to be carried out very shortly. At that time, we hope to obtain a valuable result in order to identify the species of microorganism causing the naturally acquired leprosy-like disease.—[Department of Biochemistry, Kawasaki Medical School, Kuro-shiki City, Japan; Leprosy Research Laboratory, Kyoto, Japan; Armed Forces Institute of Pathology, Washington, D.C. 20306, U.S.A.]

Meyers, W. M., Walsh, G. P., Brown, H. L., Fukunishi, Y., Binford, C. H., Gerone, P. J. and Wolf, R. H. Naturally acquired leprosy in a mangabey monkey (*Cercocebus* sp.).

In December 1979 a histopathologic diagnosis of leprosy was made at the Armed Forces Institute of Pathology (AFIP) on a biopsy specimen of skin obtained from an adult female mangabey monkey (*Cercocebus* sp.) housed at Gulf South Research Institute, New Iberia, Louisiana. This monkey was imported from Central Africa and was under study in investigations unrelated to leprosy and had never been experimentally inoculated with *Mycobacterium leprae*. In January 1980 this monkey was transferred to the Delta Regional Primate Research Center (DRPRC), and a collaborative study of the disease in this animal was initiated between the AFIP and the DRPRC.

The clinical findings in the monkey included diffuse and nodular infiltrations of the ears, face, and forearms. Infiltrations over the muzzle were extensive and blocked the nares.

Biopsy specimens from the ears, face, and forearm contained up to 3.9×10^{10} acid-fast bacilli (AFB) per gram of tissue. Histopathologic study revealed an extensive infiltration of histiocytes into the dermis. There were large numbers of AFB in the histiocytes and in dermal nerves. The AFB were in clumps and globi and stained more intensely with the Fite-Faraco procedure than with the Ziehl-Neelsen method. The acid-fastness of the AFB in tissue sections was abolished on exposure to pyridine using the Convit-Pinardi procedure. Purified suspensions of AFB from lepromata oxidized D-Dopa. Suspensions of fresh lepromata inoculated onto Löwenstein-Jensen and 7H10 media have not shown any growth of mycobacteria after 8 months incubation at room temperature, 32°C, and 37°C.

The electron microscopic findings on freeze-fracture and thin section preparations were like those seen in lepromatous leprosy in humans, armadillos, and nude mice. There were well developed foamy structures in phagolysosomes of macrophages, and these foamy structures contained banded bacteria and spherical droplets.

The Fernandez and Mitsuda responses in the monkey to Lepromin-H were nonreactive. The skin test to PPD was likewise nonreactive. Fresh suspensions of the AFB isolated from lepromata have been inoculated into armadillos (*Dasypus novemcinctus*), foot pads of BALB/c mice, and mangabey monkeys, and these animals are under observation. Lepromins prepared from infected tissues of the monkey are now being tested in leprosy patients.

The results of all studies completed to

date are consistent with a diagnosis of leprosy in this mangabey monkey and consistent with the identification of the causative agent as *M. leprae*. Lesions were histopathologically similar to the subpolar lepromatous to borderline-lepromatous area of the spectrum of leprosy.—[Armed Forces Institute of Pathology, Washington, D.C. 20306, U.S.A. and Delta Regional Primate Research Center, Covington, Louisiana 70433, U.S.A.]

Tsutsumi, S. and Gidoh, M. A trial by high performance liquid chromatography method for the simultaneous analysis of DDS, RFP, B663, and their main metabolites.

The analytical methods of dapsone (DDS) and its metabolites have rapidly been improved by Peters, *et al.* and Burchfield, *et al.* A favorable monitoring method of DDS was developed by Ellard, *et al.* A fluorophotometric determination method for clofazimine (B663) was reported by Glazko, *et al.* Vlasáková, *et al.* and Peters, *et al.* reported analytical methods for rifampin (RFP) and desacetylrifampin (DARFP) or the hydrogenated derivatives of RFP by high performance liquid chromatography (HPLC). However, no simultaneous separation method for determining all of DDS, B663, RFP, and their main metabolites has been reported. On the other hand, combination drug therapy for DDS-resistant patients is gradually becoming more important at present. For this reason we have examined the simultaneous analysis of all three of these drugs in a pooled guinea pig serum by HPLC, using a μBondpack C18 column linked to a UV spectrophotometer. The elution patterns were always monitored at 287, 300, 310, or 320 nm fixed or under switchings of detection wave lengths. As a universal extraction solvent for all of these drugs from serum, a deproteinative redissolution system, H_2O-$CHCl_3$-$HCONMe_2$ (1:1:5) was recommended especially because $HCONMe_2$ can strongly stabilize RFPs. Except the mutual separation of water-soluble conjugates of DDS, the individual analysis of DDS, monoacetyl DDS (MADDS), Promizole, and s-DDS as the internal standard of sulfones is possible when the extract is first developed with acetonitril-water (20:80). After development with this developing solvent, RFPs and clofazimine with their internal standard PTH (ε-PTC)lysine (PEPL) remained inside the column and were eliminated by the successive development with THF-0.5% acetic acid (40:60). This developing solvent enabled the separation of RFPs, PEPL, and clofazimine. By the use of THF-water (50:50) containing PIC B-5, the rapid measurement of B663 isolated from the others is also possible. The practical minimum measurable quantities were 15 pM for sulfones, 30 pM for RFPs, and 5–15 pM for clofazimine when they were detected at 287 nm fixed or under switchings of detection wave lengths.—[National Institute for Leprosy Research, Tokyo, Japan]

Kohsaka, K., Yoneda, K., Mori, T. and Ito, T. The study of the chemotherapy of leprosy with the nude mouse. Effect of DDS on nude mice experimentally infected with *Mycobacterium leprae*.

We are attempting to utilize experimental leprosy in nude mice for studies on the chemotherapy of leprosy. We carried out two experiments on the effect of dapsone (DDS) on *M. leprae* infection of nude mice; one dealt with prevention, and the other dealt with therapy.

1) Preventive effect of DDS on *M. leprae* infection in nude mice. A suspension of *M. leprae* was prepared from a nude mouse with experimental leprosy infected with *M. leprae* derived from a previously untreated lepromatous leprosy patient. *M. leprae* (2.7×10^7) were inoculated into both hind foot pads, and the mice were divided into two groups. In the control group, 8.5×10^8 organisms were recovered after 8 months. In the treated group, the number of bacilli harvested was 5.2×10^8 despite 5 months' administration of 0.01% DDS in the diet. Five months later, 1.0×10^{10}, 2.1×10^9, and 1.0×10^9 bacilli were recovered from three mice, respectively. The foot pad swelling indicated remarkable bacillary growth, and lepromatous lesions with remarkable multiplication of bacilli were seen on histopathological examination in addition to the bacillary counting.

2) Effect of DDS treatment on experimental leprosy of nude mice. A bacillary sus-

pension was made with *M. leprae* obtained from a leproma of a previously untreated patient. *M. leprae* (1.7×10^7) were inoculated into both hind foot pads, and the mice were divided into two groups. Seven months after infection, slight swelling of the foot pad was observed, and 4.0×10^7 bacilli were recovered from the foot pad. Although the number of bacilli recovered was not much greater than the inoculum, this yield shows that the bacilli were multiplying because the retention rate of the inoculum is usually less than 30%. From this time onward, the experimental group of infected mice received 0.01% DDS in the diet for 10 months. In the untreated control, 5.2×10^8 and 5.1×10^8 *M. leprae* per foot pad were harvested at 9 months after the inoculation, and the visual swelling of the foot pads became very conspicuous in both groups. In the treated group, though bacillary counting was not done, the foot pad swelling indicated growth of *M. leprae*. Ten months have elapsed since the DDS treatment was started, but no sign of the swelling diminishing has been detected.

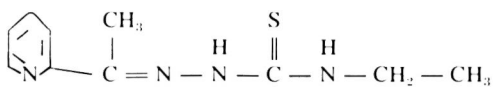
The results of both the preventive and the therapeutic experiments indicated that treatment with 0.01% DDS in the diet for 10 months was not effective in experimental leprosy of the nude mouse.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan]

Morrison, N. E., Klayman, D. L. and Collins, F. M. The 2-acetylpyridine thiosemicarbazones: A new series of compounds with antileprosy activity.

At the 14th Joint Conference on Leprosy the antimycobacterial activity of a new series of 2-acetylpyridine thiosemicarbazones was reported. The MICs for *M. tuberculosis* plus a group of atypical mycobacteria correlated with the property of thiosemicarbazone lipophilicity. It was concluded that the high concentration of lipid in the mycobacterial cell wall-membrane complex was a significant factor in the penetration rate of the 2-acetylpyridine thiosemicarbazones in order to attain adequate MICs to inhibit mycobacterial growth. Analysis of data obtained from *M. smegmatis*, ATCC 607, however, revealed a complex set of biphasic lipophilicity (log P) correlates with MICs. This data tended to separate the 607 organism away from the other mycobacteria in terms of responses to the thiosemicarbazones and raised the question as to what type of response to thiosemicarbazone structure would be given by *M. leprae*. We now wish to report on the type of *M. leprae* response to thiosemicarbazone structure.

A series of nine 2-acetylpyridine thiosemicarbazones plus thiacetazone were tested in the continuous mouse foot pad assay of Shepard. The compounds were blended in the powdered diet at maximum tolerated dose levels, and inhibitory effects on the multiplication of *M. leprae* were determined by microscopically counting the numbers of acid fast organisms growing in the foot pad throughout a 6 month period.

Four 2-acetylpyridine thiosemicarbazones, namely compounds PP, A, LL, and X, along with thiacetazone were found to reduce the multiplication of *M. leprae* to 30% or less below that of the control mice counts. Compound PP was the most active at 0.05% (w/w) in the diet giving acid-fast bacilli (AFB) counts that were less than 5% of the controls. The structure of compound PP



indicates that alkyl-substitution on the N^4 nitrogen enhances antileprosy activity. However, there are indications that further increases in alkyl chain length on the N^4 nitrogen leads to a reduction in antileprosy activity. The optimum log P calculations indicate that log P_{max} is in the range +2.3 to +2.5 for anti-*M. leprae* activity. This value is considerably less than that found for *M. tuberculosis* plus the atypicals. When assayed *in vitro* these latter organisms gave a log P_{max} of +4.0 for optimum lipophilicity.

It was particularly noteworthy that none of the *M. leprae* active 2-acetylpyridine thiosemicarbazones had significant growth, inhibitory effects on *M. tuberculosis* or the atypicals; they were, however, fully active against *M. smegmatis*, ATCC 607. For this particular series of thiosemicarbazones it is now clear that the 607 organism can be used as a preliminary screen to predict activity

against *M. leprae* dividing in the mouse foot pad.

Although very little is known about the mode of action of the 2-acetylpyridine thiosemicarbazones, it was observed in the mouse foot pad experiments that with the exposure of sufficient numbers of slowly dividing *M. leprae*, a subpopulation of organisms emerged that were no longer acid fast in their Ziehl-Neelsen staining response. These organisms, while retaining their rod-like morphology, i.e., presumably an intact peptidoglycan layer, counterstained with methylene blue or Nile blue. This loss of acid fastness, which is reminiscent of the mode of action of INH, may result from the inhibition of mycolic acid synthesis.

Further thiosemicarbazone structure-activity experiments are at present being undertaken using the mouse foot pad assay; these experiments also involve determination of blood levels of the thiosemicarbazones.

A patent has been filed with the U.S. Patent Office for the 2-acetylpyridine and the 2-propionylpyridine series of thiosemicarbazones.—[John Hopkins University School of Hygiene & Public Health, Baltimore, Maryland, U.S.A.; Walter Reed Army Institute of Research, Washington, D.C., U.S.A.; and Trudeau Institute, Inc., Saranac Lake, New York, U.S.A.]

Jacobson, R. R. and Hastings, R. C. Primary sulfone resistant leprosy.

Since 1972, routine mouse foot pad drug sensitivity studies have been done on new, previously untreated cases of leprosy admitted to Carville. An average of two to three cases per year with foot pad evidence of infection with sulfone resistant *Mycobacterium leprae* have been found. Additionally, two cases were observed in whom clinical evidence for primary sulfone resistance exists although a mouse foot pad study was not done in one and is not yet complete in the other. Growth observed in the foot pads of dapsone treated mice in these instances has usually been appreciably less than that obtained in untreated controls, has usually occurred only at the lowest level of dapsone (0.0001%), and with one exception, clinically, those cases treated with dapsone monotherapy have generally had a satisfactory response. Thus it seems inappropriate to abandon dapsone therapy in these cases just on the basis of mouse foot pad results, and it should probably continue to be a part of any treatment regimen proposed for them. The fact that dapsone may remain a useful antileprosy drug in those Third World countries where large numbers of cases of primary sulfone resistance are being found mostly on the basis of similar mouse foot pad studies is potentially of great economic importance since lifetime therapy with alternate drugs may not be feasible.—[U.S. Public Health Service Hospital. Carville, Louisiana 70721, U.S.A.]

Matsuo, Y. Attempts at cultivation of *Mycobacterium leprae* in cell culture.

Mycobacterium leprae obtained from untreated LL patients or nude mice with lepromatoid lesions were used to infect established mouse foot pad cells (MFP) or mouse peritoneal resident macrophages *in vitro*. Based on the findings with the growth pattern of *Mycobacterium lepraemurium* in the Nakamura system, reduced glutathione, DL-cysteine hydrochloride, and dithiothreitol were added to the cell culture medium in order to reduce redox potentials in the environment. The depth of the medium was varied from 5 to 25 mm to see the influence of oxygen tension. In addition, agar suspension cell cultures were tested.

Although a slight increase in the number of acid-fast bacilli was observed in some of the cell cultures, this probably did not represent true multiplication since the bacilli recovered from the cultures failed to multiply in mouse foot pads *in vivo*. No overt multiplication of *M. leprae* was demonstrated in any of the cell culture systems tested during the past 5 years.—[Department of Bacteriology, Hiroshima University School of Medicine, Hiroshima 734, Japan]

Nagata, Y. and Ito, T. The effect of clinically equivalent doses of dapsone and rifampin on antibody production and delayed-type hypersensitivity to sheep red blood cells in mice.

Since 1948, many leprosy patients in the world have been cured by the bacteriostatic sulfone compound, 4,4′-diaminodiphenyl-

sulfone (dapsone, DDS). However, it was reported a few years ago that dapsone took a long time to cure leprosy patients, and the slow therapeutic effect might be caused by a side-effect of the drug of depressing the host's immune system. Rifampin is another widely used drug for leprosy. It is a bactericidal antibiotic which exerts a remarkable effect on *Mycobacterium leprae*. Short periods of treatment with the drug bring very good results to patients. Rifampin is also described as an immunosuppressive agent in several reports. It is an important problem in the therapy of leprosy if the drugs cause immunosuppressive side-effects at normal clinical doses.

We investigated the effect of dapsone and rifampin at doses equivalent to those used in humans (one clinical dose or one CD) (based on body weight) on humoral antibody production and delayed-hypersensitivity reactions to sheep red blood cells (SRBC) in mice. SRBC were chosen as a thymus-dependent antigen instead of *M. leprae* because the experimental method using SRBC is established, and a short period is needed for the experiment. We also examined the three times higher dose (3CD) (i.e., a dose three times higher than the human equivalent dose based on body weight) in order to confirm the effects at the clinical dose.

Eight to 9 week old BALB/c, C57BL/6, and C3H/He mice of both sexes were used for the plaque forming cell (PFC) assay, and 8 to 9 week old female BALB/c mice were used for the delayed-type hypersensitivity (DTH) reaction. The daily dose of dapsone, 1CD and 3CD, was taken to be 1.7 mg/kg and 5.1 mg/kg, respectively, and 1CD and 3CD of rifampin corresponded to 10 mg/kg and 30 mg/kg, respectively. The drugs were orally administered by means of a spatula once daily, starting 7 days before the first injection with SRBC and continued through the entire experimental period. Mice were immunized ip with 4×10^8 SRBC in saline 4 days before the PFC assay was carried out according to Cunningham's method. The DTH reaction was performed as follows. Mice were sensitized with 2×10^8 SRBC in saline by sc injection into the left hind foot pads 4 days before challenge with the same amount of SRBC in the right hind foot pads. Swelling of the right hind foot pads was measured 24 hr after the challenge.

No effect was detected with either 1CD or 3CD of either drug in antibody production and DTH reaction to SRBC. Among many contradictory reports on the side-effects of rifampin, the present study may support administration of the drug to leprosy patients supposed to be treated for a short time. Experiments of longer duration are necessary to answer Sengupta's question that dapsone depresses host immunity resulting in delayed recovery from the disease.—[Department of Leprosy, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan]

Tsutsumi, S., Gidoh, M., Narita, M., Fukushi, K. and Yokomuro, K. The use of the rabbit red blood cell rosette formation method using thymectomized guinea pigs in immunopharmacological studies on leprosy.

We have used a favorable method for examining cell-mediated immunity (CMI) in guinea pigs named the rabbit red blood cell rosette method (RRBC). The principle of RRBC is to measure the percent of multicellular rosettes between rabbit red blood cells and guinea pig lymphoid cells (GPL). We improved the responsiveness of RRBC to the variation of CMI by using thymectomized guinea pigs instead of normal animals. Utilizing this improved RRBC, the influence of some antileprosy drugs on the CMI of guinea pigs has been examined by determining the *in vitro* effect of these drugs on the percent of rosettes. At the same time, the influence of these drugs on humoral immunity in mice was examined by an improved Cunningham plaque forming method (PF). The rapid eradication of leprosy bacilli never means the complete elimination of dead bacilli, the bacterial components and immune complexes *in vivo*, all of which are presumed to be injurious to immunities. The treatment of patients by excessively effective immunopotentiators before sufficient elimination of bacilli has occurred, may induce an exacerbative reaction. This may especially be the case in patients in whom the possibility of the occurrence of erythema nodosum leprosum (ENL) is seriously feared. In or-

der to find a promising, mild, and nonspecific immunopotentiator to be used in combination with chemotherapy in stages prior to more acute immunopotentiation therapy, studies on polysaccharides (PSs) have continued. In view of the results of PSs in bamboo grass, PSs possessing a definite range of molecular weights and a presumable structure were examined. For this purpose, four amphotric dextrans (ADXs) were synthesized with the anticipation that ADXs may show both immunopotentiative and DDS-type antiexudative effects. Together with the antileprosy drugs, the influences of PSs and ADXs on immunological states in guinea pigs and mice were examined. The results were as follows:

1) DDS clearly depressed CMI but seemed to have no influence on humoral immunity even in the dosage of 10 mg/kg.

2) Clofazimine (B663) potentiated CMI while it suppressed humoral immunity. Levamisole (LV) potentiated both immunities. *In vivo*, B663 prevented the decreases in CMI associated with thymectomy.

3) Neither rifampin (RFP) nor sulfadimethoxine (SD) seemed to have an influence on CMI in thymectomized guinea pigs.

4) Based on the resemblance between B663 and a new, novel antirheumatic agent named CCA as to their antiinflammatory spectra and their effects on immunities, the possibility of CCA as a substance without pigmentation for the prevention and therapy of ENL was proposed.

5) A fungal source PS named ATSO potentiated both humoral and cell-mediated immunities; thus the possibility was again proposed. On the other hand, a bacterial source PS named B512 F exhibited no effects on either type of immunity.

6) Among the ADXs, only one compound seemed to potentiate humoral and CM immunities although very slightly.

7) ATSO, the ADXs, and a taurine derivative of BHP-activated dextran named BHP-T all strongly inhibited the acute edema induced by carrageenan in rats. The order of potencies was ADXs, ATSO, BHP-T, and indomethacin in a dosage $^1/_{10}$ that of the polysaccharides. The possible mechanism of this inhibition will be discussed.—[National Institute for Leprosy Research, Tokyo, Japan; National Leprosarium Tama-Zenshoen, Tokyo, Japan; Nihon Medical College, Bunkyo-Ku, Sendagi-cho, Tokyo, Japan]

Koseki, M., Sanada, K. and Ozawa, T. Immunological differences among active, inactive, and relapsed patients in leprosy.

This study on the relation between relapse and clinical laboratory findings in lepromatous leprosy has been done to estimate the pre-relapse condition. In almost all of our, or other, immunological reports on leprosy patients, lepromatous leprosy patients have been classified into only two groups, active and inactive. For the purpose of immunological comparisons in lepromatous leprosy patients, this classification is not so good since a patient's condition is sometimes changed by time. A strict classification should be found which would accurately reflect their immunological condition.

In an attempt to accomplish this, a subclassification was applied to each of the active and inactive groups based on their maximum Bacterial Index (BI) on skin smear and the duration in years of their bacteriologic negativity, respectively. All 186 lepromatous leprosy patients at Tama-Zenshoen were subclassified by this method.

By the series of immunological examinations, some valuable results were found to estimate the pre-relapse condition at points which were in the normal range or under the normal range. The standard for judgments as to the pre-relapse condition or not will be done by results of CH50, C3, peripheral lymphocyte percentage, albumin/globulin (A/G) ratios, alpha-2-globulin, beta-globulin, gamma-globulin, IgG, IgA, and IgM.—[National Leprosarium Tama-Zenshoen, Tokyo, Japan and National Institute for Leprosy Research, Tokyo, Japan]

Abe, M., Ozawa, T., Minagawa, F. and Yoshino, Y. Immuno-epidemiological studies on subclinical infection with *M. leprae*. I. The sensitivity and specificity of the FLA-ABS test in the inhabitants of three villages in the Miyako Islands.

The fluorescent leprosy antibody absorption (FLA-ABS) test was used for detecting

subclinical infection with *M. leprae* in three villages in the Miyako Islands which were known to be leprosy hyperendemic areas in Japan. One hundred and fifty-six of 1559 schoolchildren in these areas and 77 of 574 adults in one village had one or more relevant clinical findings such as being registered leprosy cases, household contacts of leprosy, having suspicious skin eruptions or having enlargements of peripheral nerves without loss of sensation. The frequency of neural symptoms tended to increase with age in middle school boys and in adults more than 50 years old. It showed some regional differences in both the school children and adults.

The FLA-ABS test was positive in 102 (65.4%) of 156 schoolchildren with clinical findings. On the other hand, this test was positive in 13 (14.1%) of 92 schoolchildren without any clinical signs. Among 77 adults with clinical findings, 47 (61%) showed positive results in the FLA-ABS test, whereas it was positive in 23 (16.9%) of 136 adults without any clinical signs. The serological specificity of these positive reactions was checked by testing for cross-reactivity with other mycobacteria. Only 16 out of 183 positive sera showed some cross-reactions. *M. smegmatis* and *M. avium* were the most frequently cross-reactive bacteria. However, after additional absorption of the sera with these bacteria, positive reactions against *M. leprae* were not affected at all. Therefore, the results of FLA-ABS test in these inhabitants were found to be specific to *M. leprae*, and the above-described symptoms, especially the enlargements of peripheral nerves without loss of sensation, were considered to be subclinical signs caused by *M. leprae* infection.

Presuming that the percentage of positive FLA-ABS tests represents the infection rate, this rate in the general population in the Miyako Islands is estimated as at least 15% and is approximately a thousand times higher than the leprosy incidence rate in this area.—[National Institute for Leprosy Research, Higashimurayamashi, Tokyo, Japan]

Nelson, K. E., Speck, S. M., Suprasert, S. and Smith, T. A study of cellular immunity in clinically healthy children of parents with leprosy in Northern Thailand.

Patients with lepromatous and borderline leprosy are known to have depressed cell mediated immunologic (CMI) responses. Whether the pathogenesis of the deficiency in CMI is related to host or organism characteristics or immunological perturbations of the infectious process is not clear. The purpose of our study was to determine whether defects in CMI responses could be detected in clinically healthy children whose parent(s) had leprosy. A total of 302 children were studied. None had current or past evidence of leprosy. They were divided into three groups based upon the clinical status of their parent(s): 1) both parents were clinically normal, 2) one or both parents had lepromatous or borderline leprosy, 3) one or both parents had tuberculoid leprosy. The children were skin tested with PPD-tuberculin and *Candida albicans* (oidiomycin) antigens. The prevalence of PPD and Candida positivity was similar in the three groups. The PPD-negative children ($N = 143$) were then given a standard intradermal dose of BCG, and skin tests were repeated 8 weeks later. The tuberculin conversion rate following BCG in children with a lepromatous parent(s), (38.9%), was significantly lower than the conversion rate in children with tuberculoid parent(s), (77.7%), ($p < 0.05$) or in those with normal parents, (63.9%), ($p < 0.01$). There was no statistically significant difference between the children with tuberculoid parent(s) and those with normal parents. Among children initially negative to both Candida and PPD, the PPD conversion rates after BCG were significantly lower in the group with lepromatous parent(s) (0%) than in those with tuberculoid or normal parents (80%), ($p < 0.05$).

Our data suggest that clinically normal children of parents with lepromatous leprosy may have subtle defects in the cell-mediated immunologic responsiveness. Whether these defects are determined primarily by genetic or environmental factors is not entirely clear. However, further studies to define subtle perturbations in CMI responses among genetically related and unrelated contacts of patients with various forms of leprosy are needed. Studies such as these could lead to a better understanding of the immunopathogenesis and ultimately to an effective strategy for the pre-

vention of leprosy.—[Departments of Preventive Medicine and Medicine, University of Illinois, Chicago, Illinois, U.S.A.; Preventive and Social Medicine, Chiang Mai University, and McKean Rehabilitation Institute, Chiang Mai, Thailand]

Sugiyama, K., Izumi, S., Matsumoto, Y., Ohkawa, S., Matsumoto, H., Miyazaki, T., Juji, T. and Maeda, H. Analysis of the immunogenetic background of Japanese leprosy patients by HLA and serum protein allotypes.

To investigate the immunogenetics of leprosy, 408 Japanese leprosy patients (331 lepromatous and 77 tuberculoid) have been typed for HLA antigens by the NIH standard microlymphocytotoxicity test and compared with 111 healthy controls who were born in the same districts as the patients.

The frequencies of HLA-A did not show any significant differences between patients and controls. However, the gene frequency of B7 was significantly high in lepromatous leprosy compared with controls (corrected $p = 0.014$), and those of BW22 and BW54 were significantly low in lepromatous patients compared with controls (corrected $p = 0.046$ and 0.018). The gene frequency of CW1 is significantly low in lepromatous leprosy too.

HLA-DR specificities were examined by Terasaki's microlymphocytotoxicity method in 94 lepromatous patients, 30 tuberculoid patients, and 54 healthy controls. It was found that HLA-DR2 was significantly high in both lepromatous and tuberculoid patients compared with controls (corrected $p = 0.034$ and 0.054). The gene frequency of HLA-DRW9 was significantly low in both lepromatous and tuberculoid patients (corrected $p = 0.002$ and 0.049, respectively).

The association between certain HLA haplotypes and leprosy were tested by the formulas of Porta and McHugh, and it was found that the A26-BW54 haplotype is a relatively resistant haplotype to tuberculoid leprosy, and the A2-B7 haplotype is relatively resistant to the lepromatous form of the disease. As the relatively susceptible haplotypes to lepromatous leprosy, A9-B7, A26-B15, B5-CW2, B7-CW2 and BW35-DR4 haplotypes were found.

The frequencies of Gm, Km, and Gc allotypes in leprosy patients were examined to investigate the possibility of genetic control of susceptibility to leprosy by the genes linked to these markers, but we could find no associations in the population studied.—[National Ohshima Seishoen Leprosy Hospital; Leprosy Research Laboratory, Kyoto University School of Medicine; Department of Legal Medicine, Osaka Medical College; Blood Transfusion Service, Tokyo University Hospital, Tokyo, Japan]

Mehra, V., Mason, L., Convit, J. and Bloom, B. R. Evidence for *in situ* activation of suppressor T-cells in lepromatous leprosy patients.

While both *in vivo* and *in vitro* studies strongly suggest an impairment of cell-mediated immune responses in lepromatous leprosy, the mechanisms underlying the selective unresponsiveness to antigens of *M. leprae* remain unclear.

Our previous studies have indicated that Dharmendra lepromin induces *in vitro* suppression of the Con A response of mononuclear cells from lepromatous and borderline leprosy patients but not tuberculoid patients or normal donors. Cell fractionation studies indicated that two cell populations contribute to the lepromin induced suppressor activity: an adherent cell, presumably a macrophage, and T-cells.

In an attempt to define the nature of the *M. leprae* induced suppressor T-cell more precisely, human T-cells were separated into TH_2^+ and TH_2^- subsets, using absorbed anti-human T-cell antiserum and fluorescence activated cell sorting. These T-cell subsets from leprosy patients and normal individuals were admixed with normal mononuclear cells and tested for their ability to suppress the mitogenic response of normal mononuclear cells to Con A in the presence of lepromin. The TH_2^+ subset, constituting approximately 20% of the T-cells, when obtained from lepromatous and borderline leprosy patients, induced marked suppression of normal donors' lymphocyte response to Con A in the presence of lepromin. In contrast, no significant suppression was produced by the TH_2^+ subset obtained from patients with tuberculoid leprosy or normal individuals. The

TH_2^- subset, constituting approximately 80% of the T-cells, failed to induce suppression in any group. Thus the *M. leprae* induced suppressor T cells all belong to the TH_2^+ subset.

While resting T-cells from normal donors fail to exhibit Ia antigens controlled by Ir gene products, and probably Fc receptors for IgG, activated T-cells are known to display Fc receptors and Ia determinants. In order to test for the possibility that the TH_2^+ suppressor cells obtained from leprosy patients might be activated *in situ*, the T-cells obtained from the patients were examined for the presence of Ia antigen on their surface using heteroantisera and monoclonal antibodies to Ia determinants. Fewer than 5% of the T-cells from normal individuals exhibited Ia antigen, and they were distributed among the TH_2^+ and TH_2^- subsets. In contrast, T-cells obtained from lepromatous patients have elevated levels of Ia^+ T-cells, and most of these Ia^+ T-cells were found in the TH_2^+ subset. The range of Ia^+ TH_2^+ cells was 15–45%. We infer from these results that the degree of Ia positivity of the TH_2^+ subset may serve as a useful index of the degree of suppressor cell activation or function *in vivo*.—[Albert Einstein College of Medicine, Bronx, New York 10461, U.S.A; Institute for Dermatology, Caracas, Venezuela]

Tokunaga, T. and Nakamura, R. M. Suppressor T cells induced with mycobacterial infection in mice.

Delayed-type hypersensitivity (DTH) to *Mycobacterium bovis* BCG was remarkably different in two inbred strains of mice, SWM/Ms and C3H/He; SWM/Ms mice are high responders, and C3H/He mice are low responders to BCG when measured by the foot pad reaction (FPR) to purified protein derivative (PPD) 2 weeks after subcutaneous injection of BCG. Spleen cells from BCG-infected SWM/Ms mice reacted well to PPD *in vitro* whereas those from BCG-infected C3H/He mice did not. The induction of DTH to BCG was specifically inhibited by suppressor cells in C3H/He mice. The existence of these suppressor cells was confirmed by an adoptive transfer of spleen cells of BCG-injected mice into cyclophosphamide(CY)-treated recipients. The suppressor cells which appeared in the spleens were sensitive to anti-Thy 1.2 serum and complement and did not adhere to Sephadex G-10. These suppressor cells effectively inhibited the induction of DTH to BCG but showed only weak effect on the expression of it. PPD-pulsed macrophages of SWM/Ms mice stimulated BCG-primed lymphocytes of F1 hybrid mice well *in vitro*, but those of C3H/He mice did not. Significant difference in DTH to sheep red blood cells (SRBC) and BCG was observed also in SJL/J ($H\text{-}2^s$) and A.SW ($H\text{-}2^s$) strains, supporting our previous report that DTH to either SRBC or BCG is regulated by gene(s) which is not linked to H-2.—[Department of Tuberculosis, National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo, Japan]

Akiyama, T. and Yamaura, N. Does *Mycobacterium leprae* behave as an intracellular parasite in mice?

In the present studies we wish to discuss the validity of taking self-limiting lesions caused in mice by infection with *M. leprae* for a pertinent model of human leprosy.

Previously, our macrophage migration inhibition tests (MITs) have confirmed that cells of animals infected with intracellular parasites acquire altered antigen which is composed of microbial species specific and cross-reactive determinants, as a result of having allowed the microbes to multiply in them. This finding would imply that because of multiplication of a specific species of microorganisms in a host's phagocytic cells, the antigenicity of the infected host cells is altered so as to make them autoantigenic.

Subsequent studies have demonstrated that the antigenic alteration on the infected cells develops when primary culture cells as well as cells of established lines are infected *in vitro* either with intracellular parasites or with their microbial DNA. According to our hybridization tests and reassociation kinetic analyses, nucleotide sequence homology between the host's DNA and the DNA from the bacteria which can grow intracellularly in the host was significantly greater than that between the DNA of bacteria growing only extracellularly and their host's DNA.

These findings suggest the possibility that the altered self-antigen is an expression of genetic recombination between the genome brought in by the microbes and that of the host.

In the present experiments, responsiveness of the sensitized lymphocytes of mice, which had been infected with an attenuated strain of *Salmonella enteritidis* or strain BCG of *Mycobacterium bovis*, was examined by MIT against the liver cell extracts from two euthymic and five athymic mice which had been infected with *M. leprae*.

According to MITs, no positive result was obtained, without exception, with the liver cell antigens from the seven mice in spite of positive responsiveness with lepromal extracts from *M. lepraemurium*-infected mice and liver cell extracts isolated from sensitized lymphocyte donors.

According to results of the present studies, human leprosy bacilli do not seem to behave as intracellular parasites in mice so we would like to claim that it may be difficult to develop the murine lepromatoid infection due to *M. leprae* into a pertinent model for a broad investigation of human leprosy.—[Department of Microbiology, Kitasato University School of Medicine, Sagamihara-shi, Kanagawa-ken 228, Japan]

Krahenbuhl, J. L., Humphres, R. C. and Ferraresi, R. W. Effects of adjuvant immunotherapy on infection with *M. leprae* and *M. marinum*.

To determine the potential efficacy of adjuvant immunotherapy for leprosy, two types of studies were carried out in mouse foot pad models for infection with *M. leprae* and *M. marinum*: the first, employing the synthetic adjuvant muramyl dipeptide (MDP), the second, employing preparations of killed *Corynebacterium parvum*.

MDP (N-acetyl-muramyl-L-alanyl-D-isoglutamine) is a synthetic compound which, when administered in oil, appears to be the smallest chemical unit that can replace the effects of the mycobacterial cell wall in Freund's complete adjuvant. When administered in an aqueous solution, toxic side effects are few and minimal, and MDP can markedly enhance both antibody formation and cell mediated immunity to unrelated antigens. In addition, treatment with MDP can enhance resistance to a variety of infectious agents such as *Klebsiella pneumoniae*, *Trypanosoma cruzi*, *Pseudomonas aeruginosa*, and *Candida albicans* by a mechanism(s) of action which is not clearly understood. To determine the effects of MDP on *M. leprae* infection, BALB/c mice were treated subcutaneously (SC) prior to and at varying intervals following foot pad (FP) infection. As shown by comparison with the number of bacilli per FP in controls at the plateau phase, treatment with MDP failed to protect against *M. leprae* infection or alter the growth of an ongoing infection regardless of dose (0.8 to 80 mg/kg) or timing of administration. In additional studies, clearance of the leprosy bacilli from the FP tissues was not enhanced by local or SC treatment with MDP initiated after the growth of the organism had plateaued. Similar studies with *M. marinum* also failed to show that pretreatment with MDP afforded any protection to growth of the organism in the FP. Further studies which employed a wide dose range of two analogs of MDP (N-acetyl-desmethylmuramyl-L-alanyl-D-isoglutamine, and N-acetyl-muramyl-D-alanyl-D-isoglutamine) also failed to afford protection against *M. leprae* or *M. marinum*.

While the above studies were in progress, parallel studies were carried out with more convenient models employing other intracellular pathogens to evaluate certain variables of MDP treatment. Regardless of dose, route, timing, or choice of analog of MDP, no detectable resistance was afforded against the facultative intracellular bacteria *Listeria monocytogenes*. In contrast, a single treatment afforded remarkable protection to a virulent strain of the obligate intracellular protozoan *Toxoplasma gondii*. As resistance to Listeria is effected solely by the enhanced microbicidal properties of activated macrophages (Mϕ), these data suggest that Mϕ activation is not the means whereby MDP protects against infection. Further studies, involving more aggressive treatment with MDP (twice weekly for 16 weeks), are underway to complete our evaluation of its potential as an immunotherapeutic measure for leprosy.

A second series of experiments was designed to evaluate the potential of immunotherapy for leprosy employing local or

systemic administration of preparations of killed *C. parvum*. Because treatment with *C. parvum* continues to hold promise of becoming a potent weapon in the arsenal being brought to bear against cancer, a wealth of information is existent in the literature, including the effects of treatment on infection with a variety of agents. Enhancement of the nonspecific microbicidal and cytotoxic capacity of macrophages as a result of the specific immunologic interaction between T-lymphocytes and *C. parvum* appears to underlie the capacity of this preparation to enhance both resistance to infection and to cancer. In our own preliminary studies, systemic treatment with *C. parvum* before infection or even during infection, markedly reduced the growth of *M. leprae* and *M. marinum* in the mouse foot pad. Local treatment was even more effective. In mice infected in both hind feet, an initial treatment in one foot pad followed by a booster treatment in the opposite foot pad further enhanced the inhibitory effect on *M. leprae* growth. Current studies are exploring, among other goals, whether these effects of *C. parvum* are bacteriostatic or bactericidal and whether clearance of acid-fast bacilli from the tissues can be enhanced.—[U.S. Public Health Service Hospital, San Francisco, California 94118, U.S.A. and Syntex, Incorporated, Palo Alto, California 94304, U.S.A.]

Gillis, T. P. and Buchanan, T. M. Characterization of *M. leprae* "specific" antigens.

Twenty-one species of mycobacteria were identically extracted with lithium acetate (LiAc), and the antigenic extracts were tested in immunodiffusion precipitation for reactivity with a reference sera from human leprosy patients. This reference system (ARLS) was adsorbed with *M. vaccae, M. bovis,* and cardiolipin/lecithin to make it specific for *M. leprae* in an indirect immunofluorescence test. The ARLS produced a strong single precipitin line with LiAc extracts of *M. leprae* when low protein concentrations of extract were used or two precipitin lines with extracts of higher protein concentrations. One of these two immunoprecipitin lines showed cross-reactivity with *M. lepraemurium* (MLM) and with *M. bovis* (BCG). The other precipitin line was not shared with *M. bovis* and showed partial or no cross-reactivity with extracts of MLM. In addition, slight reactivity was observed between ARLS and antigen extracts of *M. flavescens, M. gastri, M. gordonae,* and *M. nonchromogenicum.* None of the LiAc extracts of the other 15 species reacted with ARLS, and these species, *M. tuberculosis* $H_{37}Rv$, *M. tuberculosis* $H_{34}Ra$, *M. kansasii, M. scrofulaceum, M. intracellulare, M. microti, M. smegmatis, M. vaccae, M. phlei, M. diernhoferi, M. marinum, M. triviale, M. thamnopheos, M. perigrinum, M. terrae,* and *M. duvali.* The LiAc extracts of *M. leprae,* MLM, and *M. bovis* were partially purified by ultracentrifugation and the 100,000 × *g* pellet of each extract was examined by immunodiffusion precipitation and SDS-slab polyacrylamide gel electrophoresis (SDS-SPAGE). Each of the pellet preparations in protein concentrations of 70–100 μg/ml gave comparable immunoreactivity to the original extract (≥3 mg/ml) when tested against ARLS. The *M. leprae* pellet contained two major and three minor proteins, and the pellets of MLM and *M. bovis* contained one major and one to three minor proteins as analyzed by SDS-SPAGE. A protein with a subunit molecular weight of approximately 36,000 daltons was a major component of the pellet preparations from each of the three species. This protein may contain shared antigenic determinants among the three species since it was absent from the ultracentrifugation supernate preparation of *M. bovis,* a preparation that showed no reactivity with ARLS. Three other proteins were present in large excess in the ultracentrifugation pellet of LiAc extracts of *M. leprae* as compared to the other two species. These were a major protein with a subunit molecular weight of approximately 23,000 daltons and two minor proteins with subunit molecular weights of approximately 85,000 and greater than 100,000 daltons. Any of these proteins may contain *M. leprae* specific antigenic determinants, and further characterization of them is needed.—[Immunology Research Laboratory, U.S. Public Health Service; University of Washington, Seattle, Washington 98114, U.S.A.]

Harada, K. and Kasai, T. The pathogenesis of the skin lesion in leprosy, with application of the periodic acid-carbol pararosanilin and the periodic acid-methenamine silver stains.

We have examined leprosy skin lesions of varying types with periodic acid carbol pararosanilin and periodic acid methenamine silver stains. In the leprosy infection, the target organ is the Schwann cell. There might be two ways for the subsequent spread of bacilli: a) the bacilli may spread from the Schwann cells of terminal (distal) nerves proximally to the same large nerves and b) from the nerves to be distributed to surrounding tissue macrophages; and via blood vessels, the bacilli may disseminate to be phagocytosed by other nerves and become distributed to surrounding macrophages. Therefore, leprosy could be classified according to bacillary distribution as either neural localized or disseminated.

Epithelioid transformation of macrophages is characteristic of human tuberculosis. In leprosy, Schwann cells showing epithelioid change have the ability to destroy the organisms in tuberculoid, borderline, and reversal reactions. In contrast, in lepromatous leprosy, the bacilli are engulfed by Schwann cells and macrophages, which have a marked deficiency in their ability to destroy the organism. Epithelioid transformation of macrophages is not seen in all types of leprosy.—[National Tamazenseiyen Sanatorium, Higashimurayamashi, Tokyo 189, Japan]

Okada, S. Electron microscopic study of tuberculoid lesions and a proposal of subpolar tuberculoid leprosy.

Two aspects of tuberculoid leprosy are reported. The first aspect deals with electron microscopic findings in tuberculoid lesions. Biopsies of skin lesions of four patients with tuberculoid leprosy were performed. A portion of the skin biopsy was fixed in formalin and studied with conventional light (optical) microscopy. The remaining part was fixed with osmium tetroxide and studied with the electron microscope. Additionally, semithin sections were made from the specimen embedded in resin for electron microscopy, stained with toluidine blue, and observed with the optical microscope in order to compare with the electron microscopic findings in the corresponding ultrathin sections. Under the electron microscope, most epithelioid cells are large cells having a large nucleus. Their cytoplasmic membrane is uneven, having microvilli-like or pseudopod-like projections. These cells have many mitochondria. In addition to the above type of epithelioid cell, there is another type of epithelioid cell which has many granules of various sizes. These granules are mostly lysosome-like. Some of the granules have lamellar structures in them, being similar to the granules of mast cells. Some of the large granules have an onion-like structure. If the semithin section is stained with toluidine blue, these granules are stained blue-violet, and the epithelioid cells having these granules can be readily distinguished from the epithelioid cells which do not have them. The former, ordinary epithelioid cell is named A-cell, and the latter, having the granules, is named B-cell. In A-cells, fibrillar formation is occasionally observed, and this fact suggests to us that the A-cells originate from the monocyte-macrophage system. Fibrillar formation is not observed in typical B-cells, but it can be seen in a transitional cell in which granules are seen although the number of granules is small. This fact suggests to us that the B-cell also originates in the monocyte-macrophage system.

The second aspect of the present study is the proposal of subpolar tuberculoid leprosy. LL_s, namely subpolar lepromatous or LI, indefinite lepromatous, is established in LL in the present classification of leprosy. LL which shifted from borderline is called LL_s. But in TT, TT_s is not yet established. Because I had the experience of cases which could be regarded as TT_s, they are reported here. The detailed clinical signs of those two cases are shown. The characteristics common to these two cases are as follows: the considerable number of skin lesions, a symmetrical distribution of skin lesions, the presence of a borderline-like patch having central healing, the presence of small skin lesions, the presence of satellite lesions, not so marked hypesthesia of skin lesions, diffuse enlargement of nerves, bacillary negativity in the smears from the skin lesions, positive lepromin reactions,

and the typical histological features of TT in the biopsy specimens. In short, the macroscopic features of the skin lesions make us think that the case is of the borderline group, but the microscopic features of the skin lesions are those of typical TT. The following process is thought to account for the formation of such cases as these. Such cases had lowered cell-mediated immunity when leprosy bacilli invaded into the body. Because of this, some multiplication of the leprosy bacilli and hematogeneous dissemination of the organism occurred to form the symmetrical lesions. Afterwards, the patients' cell-mediated immunity recovered, and the typical TT lesions were formed. Thus these patients should be regarded as the cases that moved from the borderline group to TT. They should be classified into TT_s. If TT_s is not established, such cases as these cannot be classified into any group in the present classification.—[Leprosy Research Laboratory, Kyoto University School of Medicine, Kyoto, Japan]

Cheng, Y. T. The effect of bases of nucleic acid on the growth of *M. bovis*, BCG, and *M. lepraemurium*.

Bases of nucleic acid are the most important components of nucleic acid. So far, however, very little has been known of the effect of nucleic acid bases on the growth of microorganisms, especially on the growth of mycobacteria.

I found that nucleic bases, especially in combination, possessed a growth promoting effect on BCG and *M. lepraemurium* at very low concentrations. The experiments were carried out with several kinds of semifluid media: modified Sauton media, Cheng's cholesterol media, egg yolk hydrolysate media, cholesterol-Tween 80 media and hemolyzed blood media, to which various amounts of nucleic bases, singly or in combination, were added. It was found that UG_5 and $(AT_{35} + GC_{65})_6$ were the best for the growth of both BCG and *M. lepraemurium*. The concentration was very low, such as in UG_5: U: 1.8 μg; G: 3 μg; and $(AT_{35} + GC_{65})_6$: A: 0.4 μg; T: 0.3 μg; G: 0.95 μg; C: 0.75 μg per ml.

The nutritionally fastiduous *M. lepraemurium*, when 0.1 mg per ml medium was inoculated, could grow in cholesterol medium, although not very abundantly. The ratio of positive cultures was low, but there were visible colonies suspended in the middle layer of the semifluid medium. When the nucleic bases, UG_5, AU_4, and $(AT_{35}+GC_{65})_6$, #21 were added, the ratio of positive cultures increased, and the time to detect the initial growth was shortened. When the pH was adjusted to 6.0, instead of 6.8, all cultures became positive, and the growth turned out to be more abundant. No growth was obtained in the inocula of 10^{-2} mg per ml of media. Subcultures have not been tested yet.—[National Leprosarium Tamazenshoen, Tokyo, Japan]

Nakamura, M. Effects of soluble starch lecithin and vitamins on *in vitro* growth of *M. lepraemurium* and *M. leprae*.

Hanks and Dhople obtained continuous growth of *M. lepraemurium* (*Mlm*) by replacing unstable supplements in the Nakamura system by stable compounds. In addition to their investigations, we have searched for growth stimulators to achieve continuous growth of *Mlm* without great modifications of the original Nakamura system. These growth stimulators presumably act as carbon sources as well as sources of nitrogen and vitamins.

Results obtained indicated that soluble starch stimulated the growth of *Mlm*. From the results obtained in analyzing the effect of the soluble starch, it was found that dextran (MW 100,000) and α-cyclodextrin also stimulated growth of *Mlm*, but dextrin and maltose had no effect.

Additional studies demonstrated that lecithin, cholesterol, and liposome had stimulating effects on the growth of *Mlm*. These results confirmed the data reported by Kato.

Among the vitamins tested, it was noted that vitamin K_3 as well as vitamin B_{12} at final concentrations of 0.005 μg/ml and 0.16 μg/ml, respectively, significantly enhanced the growth of *Mlm*.

Cultivation trials of *M. leprae* (*Ml*) were undertaken, using these stimulators, which were effective for *Mlm*.

An experiment with *Ml* suggested that there might be possible multiplication of *Ml* when dextran, liposome, vitamin K_3, and

vitamin B_{12} were added to the base of the Nakamura system. In the future, however, the same experiment should be repeated whether the results obtained here are reproducible or not.—[Department of Microbiology, Kurume University School of Medicine, Kurume 830, Japan]

Nomaguchi, H., Kohsaka, K., Yoneda, K. and Mori, T. Studies on *M. leprae* in tissue culture.

We tried to cultivate *M. leprae* in tissue cultures applying monolayer and agar suspension techniques.

In monolayer culture, *M. leprae* in A31 cells exhibited a fourfold increase in bacterial count after 50 days of cultivation. However, the A31 cells as host cells of the bacilli did not remain in good condition for more than 2 months without transfer of the cells. For this reason we attempted the agar suspension culture technique, as will be discussed below. *M. leprae* in nude mouse foot pad cells showed globi-like formation of bacilli after 50 days of cultivation. The host cells were damaged at this time, however, and the organisms appeared to be extracellular. With *M. leprae* in normal and nude mouse spleen cells, a similar phenomenon was seen as that which was the case with *M. leprae* in the nude mouse foot pad cells. In the next trials, some chemical compounds such as cysteine, ascorbic acid, glutathione, catalase, superoxide dismutase, vitamin E, and an adrenaline derivative (methyl epinephrine hydrochloride, R. M. 7868) were added to the cultures. Good growth of bacilli was not observed with the addition of these chemical compounds.

In agar suspension culture, the host cells were well preserved for longer periods of time. By applying the agar suspension culture technique, *M. leprae* in A31 cells exhibited a 16-fold increase in the number of AFB after 123 days of cultivation in the first experiment. On the other hand, the *M. leprae* did not increase in number in a second and third experiment. *M. leprae* in spleen cells showed 1.2 to 4.4-fold increases in the number of AFB. This increase in number of bacilli was suppressed by adding streptomycin in a final concentration of 100 μg per ml to the culture medium.

In summary, elongation and globi-like formations of *M. leprae* were observed in A31 cells in almost all of the infected cells, even in the cells infected at a ratio of only one bacillus per cell. However, on cultivation of *M. leprae*, globi-like formations were not observed in all of the infected cells but rather in only a small number of them. The frequency of the appearance of globi-like formations seemed to depend on the source of the bacilli. These results suggest the possibility that the different *M. leprae* samples contained varying proportions of viable bacilli. In the mouse foot pad cell cultures in which many kinds of cells are mixed, the epithelial cells showed the globi-like formations. In spleen cell cultures, some special cells showed the globi-like formations, but, to date, we have not been able to determine what kind of cell it is. In any case, the organisms in the globi-like formations appeared to be extracellular, and therefore the organisms seemed to be easily damaged.

We intend to determine the viability of these bacilli cultured in tissue culture by inoculation in nude mice.—[The Research Institute for Microbial Diseases of Osaka University, Yamada-kami, Suita, Osaka, Japan]

CLOSING REMARKS

As we conclude another very useful and enjoyable meeting, I have to say that this is the last meeting of which I will serve as Chairman of the U.S. Leprosy Panel. I have received enormous amounts of help from my colleagues in the United States and also from my colleagues in Japan. I probably know less about leprosy than anyone else in this room, and it is only with their help that I have been able to function at all adequately as Chairman of this panel. My hope is that I will be able to serve the cause of leprosy in other capacities in the future, but I have already developed what you call in Japan, "*Natsukashii*," a kind of nostalgia for the people whom I have become fond of and who have been so kind to me.

This is an occasion, perhaps, to reflect on my feelings about the field of leprosy. Perhaps the best way I can express those feelings is to recall a poem written by my favorite Japanese poet, Issa. In English, it goes, "this world of dew; Is a world of dew; And yet . . .; And yet . . . ." The field of leprosy is a world of dew; it's a bad disease. It's hard to find; it's hard to study; it's hard to treat, and it takes a long time and a lot of commitment. At a second level, it is a "world of dew" because very few people have any real interest in supporting either research on leprosy or care for leprosy. If they did, perhaps a long time ago leprosy would have been eliminated. The people in this room I take great pride in knowing because it is very easy to become a famous scientist in many other areas where research is much more rapid and much more visible. The people in this room are in the field of leprosy research because they believe in the cause, and they are truly dedicated people, who, I do not think, have received the appreciation that they truly deserve.

In the last part of that poem, at least in my understanding in English, is the "and yet, and yet" part, which suggests that there is much to be done. It is possible and hopeful. I think in reflecting on the progress that has been made by these panels under the leadership of Dr. Yoshie, Dr. Namba, and Dr. Shepard, the U.S.–Japan Leprosy Panel has become a world focus for information on progress in leprosy research. I would only recall that the first presentations and the early presentation of data on preliminary results for discussion and for dissemination that have come through the focus of the leprosy panel include the first specific antibody test for the leprosy bacillus, the growth of *M. leprae* in the armadillo, the first reports on the cultivation of *Microbacterium lepraemurium*, and the work that we heard today on the progress from there. Presented at these meetings were experimental chemotherapeutic protocols used in animals, the development of the nude mouse and rat, the refinements and generalization of the mouse foot pad test, which is now universal for studying the growth and chemotherapeutic efficacy of drugs in leprosy, and, more recently, interesting possibilities, I think, in the immunology of the leprosy bacillus, identification of specific antigens that may be more useful ultimately in the area of epidemiology and skin testing and perhaps some understanding of the immunological basis for resistance and susceptibility in lepromatous or tuberculoid leprosy. I think this is a strong record and one to be proud of, and I think the people in this room deserve a great deal of recognition for the efforts that they have made over these 15 years.

The last point to make is that because this has been an important focus of research, I personally welcome the visitors to this meeting, Dr. Kim from Korea, Dr. Cheng from China, and Dr. Vicharn Vithayasai from Thailand, and would hope in the future that the focus could be broadened to include people interested in the various aspects of leprosy from other parts of the world, and particularly from the region of Asia, which has such an important need.

Finally, it only remains for me, on behalf of the United States delegation, once again to express our deep appreciation for the enormous efforts that went in to making possible this meeting in this wonderful location and for the hospitality, warmth, and kindness of all of the Japanese colleagues and their associates who have done more than one could expect to make this a wonderful and productive meeting.

We thank you very much.

—Barry R. Bloom, *Chairman*
U.S. Leprosy Panel