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ABSTRACTS

NINETEENTH JOINT LEPROSY RESEARCH CONFERENCE

Takebashi Kaikan
Ohtemachi, Chiyodaku, Tokyo, Japan
27–28 August 1984

U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

Good morning, Ladies and Gentlemen:

It is a great pleasure to have the privilege of welcoming you to the 19th Joint Conference on Leprosy Research. This conference has been planned and organized in cooperation with the Japanese Tuberculosis Panel so that the leprosy and tuberculosis conferences are overlapped by a Joint Symposium on the Immunology of Tuberculosis and Leprosy. As you can see from the program, several papers regarding immunological studies on both diseases are concentrated at the sessions before and after the joint symposium. This means that many topics in this field will be presented and discussed for three days. Fortunately, it is a great pleasure to see many U.S. delegates from both the leprosy and tuberculosis panels. We eagerly look forward to learning of their findings and to beginning or renewing our friendships. It is also a great pleasure to see several guests from Asian countries. We extend a warm welcome and look forward to your participation in the conference.

Since the beginning of the U.S.–Japan Cooperative Medical Science Program, the leprosy panel has maintained four guidelines in the research area without substantial modification. These are: microbiology of *Mycobacterium leprae,* experimental leprosy, antileprosy drugs, and immunology of leprosy. These subjects are also used for the titles of four sessions at this conference, and we will happily learn and discuss the most recent topics in the respective fields. Since almost all areas of leprosy research are still in progress, some papers presented at this conference may be preliminary or informal. Therefore, any suggestions or recommendations for developing further studies or preparing formal papers would be greatly appreciated. This is the reason why each presentation is limited to 15 minutes, followed by 15 minutes for discussion. I would like to ask all participants to be punctual at the presentation, and I also ask the co-chairman of each session to take the chair for useful discussions.

Finally, I would like to express our deep appreciation to Dr. Darrel Gwinn, Dr. Ichiro Toida, and the staff of my Institute for splendidly organizing this conference.

Thank you very much.

—Masahide Abe, *Chairman*
Japanese Leprosy Panel

The Tokyo Tower as seen across the moat surrounding the Imperial Palace.

PROGRAM
NINETEENTH JOINT LEPROSY RESEARCH CONFERENCE

27 August 1984, Monday

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

Session I. Microbiology of *M. leprae*

Co-Chairmen: Dr. Zanvil Cohn
Dr. Yoshiyasu Matsuo

Nomaguchi, H., Kohsaka, K., Miyata, Y., Mori, T. and Ito, T. Adaptation of *Mycobacterium leprae* and the attenuated *M. lepraemurium*

Mori, T., Miyata, Y. and Kohsaka, K. Catalase and peroxidase activities of *Mycobacterium leprae* grown in nude mice

Matsuo, Y., Tsukiyama, F. and Katoh, M. Modification of the fluorescent staining method for mycobacterial cells

Kusaka, T. and Mori, T. Analysis of mycolic acids in *Mycobacterium leprae* using proton (H^+)-NMR and mass spectrometry

Fukunishi, Y. Spherical droplets around *M. leprae*

Franzblau, S., Takeda, T. and Nakamura, M. Plasmids in mycobacteria: A preliminary report

Session II. Experimental Leprosy and Therapy

Co-Chairmen: Dr. Robert H. Gelber
Dr. Kazunari Nakamura

Matsuoka, M., Kawaguchi, Y. and Kawatsu, K. Multiplication of *M. leprae* in nude mice after infection through different routes and suitable site for growth

Nakamura, K. and Yogi, Y. The athymic rodent as an experimental lepromatous leprosy model (continued): The SHR nude rat as a new model and effect of host in the formation of the lepromatoid lesion in nude mice

Gormus, B. J., Martin, L. N., Wolf, R. H., Baskin, G. B., Meyers, W. M., Walsh, G. P. and Binford, C. H. Experimental leprosy in monkeys

Kohsaka, K., Miyata, Y., Mori, T. and Ito, T. Reversal reaction by thymus transplantation in experimental leprosy of the nude mouse

Hastings, R. C. and Chehl, S. R. An anomalous response of *Mycobacterium leprae* to dapsone chemotherapy in nude mice

Ito, T., Kohsaka, K. and Miyata, Y. Effect of INAH on experimental leprosy of the nude mouse

28 August 1984, Tuesday

Session III. Drugs and Immune Responses

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

Gidoh, M. and Tsutsumi, S. Analysis of hydrophilic conjugates of DDS by high performance liquid chromatography

Tsutsumi, S. and Gidoh, M. Possible role of immunomodulators in the control of ENL

Collins, F. M. and Orme, I. M. Immunocompetence of the heavily infected host

Lefford, M. J. *Mycobacterium lepraemurium* infection of T cell-depleted mice: Implications concerning the immune response in intact mice

Ohkawa, S. Activation of human monocytes

Rea, T. H., Modlin, R. L., Fullmer, M. A., Dugan, F. and Taylor, C. R. Anti-interleukin 2 positive and anti-TAC positive cells in leprosy lesions, lepromin reactions, and tuberculin reactions

Session IV. Tissue Damage and Antibodies

Co-Chairmen: Dr. Thomas M. Buchanan
Dr. Takehisa Akiyama

Minauchi, Y., Tokunaga, H., Goto, M. and Suzuki, M. Immunocytochemical study of leprous neuritis—a trial of whether an immunological mechanism exists or not in progressive sensory leprous neuritis

Matsuo, E., Yamada, K., Sasaki, N. and Skinsnes, O. K. Possible significance of β-glucuronidase of *M. leprae* on its permeation from blood stream to peripheral nerve. A histopathologic study of leprous neuropathy and an experimental one to support that utilizing perfusion of *E. coli* to rat iliac artery

Levis, W. R., Meeker, H. C., and Schwerer, B. Serum IgM antibodies to *Mycobacterium leprae*-derived phenolic glycolipid: Relationships to bacterial index and erythema nodosum leprosum

Izumi, S., Sugiyama, K., Fujiwara, T. and Brennan, P. J. A novel qualitative complement fixation test for detection of anti-phenolic glycolipid I antibodies

Abe, M., Minagawa, F., Yoshino, Y., Miyaji, I., Ozawa, T. and Sanada, K. Anti-*M. leprae* antibody response induced by lepromin testing

Hirata, T., Shimizu, K., Ohi, S., Sanada, K., Ramasoota, T., Sampoonachote, P., Kongoebchart, K., Sampatavanich, S., Ochasanond, P. and Rungruang, S. A short preliminary report of the teichoic acid antibody in the sera of leprosy patients

29 August 1984, Wednesday

Combined Leprosy and Tuberculosis Symposium
Session II. Immunology of Leprosy

Co-chairmen: Dr. Masahide Abe
Dr. Robert C. Hastings

Kikuchi, I., Ozawa, T., Sanada, K., Koseki, M. and Sasazuki, T. Immunogenetic analysis of leprosy in the Japanese (second report)

Mohagheghpour, N., Sasaki, D. T., Brennan, P. J., Engleman, E. G. and Gelber, R. H. Effects of recombinant IL2 on the *Mycobacterium leprae*-induced T cell: Response in lepromatous leprosy

Brennan, P. J. Diagnosis of leprosy based on the 3,6-di-*O*-methyl-β-D-glucopyranosyl epitope; assay of antibodies with natural and synthetic probes; assay of antigen by chemical and immunological means

Fujiwara, T., Izumi, S. and Brennan, P. J. A correlation between the structure of phenolic glycolipid and its antigenic activity and synthesis of the derivatives for development of serodiagnosis of leprosy

Douglas, J. T. and Steven, L. M. Comparison of natural and synthetic antigens for early detection of leprosy

Young, D. B., Khanolkar, S. R., and Buchanan, T. M. Multiple strategies for the identification of antigen and species specificity of monoclonal antibodies reactive with *Mycobacterium leprae*

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

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ABSTRACTS OF LEPROSY CONFERENCE

Nomaguchi, H., Kohsaka, K., Miyata, Y., Mori, T. and Ito, T. Adaptation of *Mycobacterium leprae* and the attenuated *M. lepraemurium* to tissue culture cells.

Relative growth abilities of *Mycobacterium lepraemurium-in-vivo* (MLM-*in vivo*) and *M. lepraemurium*-Ogawa strains in tissue culture cells were studied. There was a consistent 5.7- to 20.6-fold increase in yields of MLM-*in vivo*, MLM-Ogawa 2nd, and MLM-Ogawa 5th isolated from a rough colony after 26–30 days of cultivation, giving a doubling time of 7 to 13 days. However, no increase in the yield of MLM-Ogawa 10Y isolated from a smooth colony was observed.

After long periods of time after inoculation in tissue culture cells, even MLM-Ogawa 10Y began to multiply as well as the original MLM-*in vivo* (MLM adapted to tissue culture cells).

Next, the pathogenicity of MLM-10Y and the MLM-10Y adapted to tissue culture cells was studied. MLM-10Y did not produce lepromas on the chest of CBA mice after subcutaneous inoculation, but MLM-Ogawa 10Y-A31 4M (MLM-Ogawa 10Y cultivated in A31 cells for 4 months) produced a small leproma in CBA mice. That leproma was passaged again in CBA mice. The leproma which was produced became larger and larger with time after inoculation. The leproma became even larger after passage into nude mice. Thus the pathogenicity of these attenuated bacilli is restored with time in passage in tissues and is more easily restored by passage in immunodeficient hosts, such as tissue culture cells or nude mice.

Next, we tried to make *M. leprae* adapt to tissue culture cells by long-term inoculation of bacilli in A31 cells. *M. leprae* strains Oki-4th, Oki-5th, and Naha-7th showed a bacterial increase in A31 cells, but strains Izumi and Thai-53 did not. However, only with Oki-4th was it possible to passage the bacilli into new A31 cells; with the other strains this was not possible.

Characterization of the Oki-4th strain was undertaken as follows: Ninety percent of the bacilli lost their acid-fastness by the Ziehl-Neelsen method after pyridine extraction. Transfer into normal CBA mice was impossible. Colony formation in Ogawa egg yolk medium was impossible after one-year cultivation. DOPA-oxidase activity was negative. Thus, these bacilli have not yet been identified as *M. leprae*.—[Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Mori, T., Miyata, Y. and Kohsaka, K. Catalase and peroxidase activities of *Mycobacterium leprae* grown in nude mice.

Wheeler and Gregory (J. Gen. Microbiol. **121**: 457–464, 1980) reported that catalase activity in purified *Mycobacterium leprae* isolated from infected armadillo liver was not detected and peroxidase and superoxide dismutase were only recognized on the protein band developed in acrylamide gel. Since we have now devised an effective isolation method for *M. leprae* from leprosy-infected nude mouse foot pad leproma, catalase and peroxidase were tested with purified leprosy bacilli from this source.

The leprosy bacillus was the Thai 53 strain grown in nude mice foot pads and was collected by the Ficoll gradient and 1 N alkali treatment method. Catalase activity was determined by Andreae's scopoletin horseradish peroxidase method (Nature **175**: 859–860, 1955). Peroxidase was determined by a diminution of absorption at 340 nm of NADH in the presence of hydrogen peroxide.

The inactivation of catalase activity by 1 N alkali treatment was tested in cultivated *M. lepraemurium*. Since the surface catalase enzyme protein may be degradated by 1 N alkali treatment, catalase activity of alkali-treated bacilli was lower than that of non-treated bacilli. High catalase activity was detected in nontreated *M. leprae* Ficoll fraction Fr. 1 to Fr. 5 which contained small amounts of tissue contaminants. On the other hand, after 1 N alkali treatment no catalase activity was seen. Therefore, *M. leprae* may be negative for catalase activity, the high activity of catalase being dependent on contamination by tissue debris.

In our previous report (Mori, Jpn. J. Bacteriol. **16**: 808–813, 1961), we determined

the degree of contamination with tissue debris by AMPase activity in our bacillary fraction collected from murine lepromas. AMPase activity was tested to detect tissue contaminants in each fraction of *M. leprae* collected from foot pad lepromas by the Ficoll gradient method. Alkali treatment was effective in purifying the leprosy bacilli, but the AMPase activity test showed poor sensitivity compared to the catalase activity test. Detection of tissue contaminants in *M. leprae* fractions may be most effective with the catalase activity test.

Cell-free extracts of 1 N alkali-treated *M. lepraemurium* and *M. leprae* were prepared by grinding with quartz sand. Catalase activities of cell-free extracts of *M. lepraemurium* and *M. leprae* were then tested. Very high catalase activity was detected in *M. lepraemurium,* but no catalase activity was seen in *M. leprae* despite a relatively high protein content of 196 μg protein per ml. Particulate fractions which were collected by centrifugation (40,000 rpm for 60 min) did not show any catalase activity. NAD-peroxidase activity was not detected in cell-free extracts of *M. leprae.*

Leprosy bacilli, having no catalase and no peroxidase activities, may be killed by hydrogen peroxide which is produced by an oxidation system inside the cell when the bacilli are in contact with oxygen in an aerobic culture medium. We have already reported (Mori, Int. J. Lepr. **39**: 796–812, 1971) that *M. lepraemurium* do not have glyceraldehyde-3-phosphate dehydrogenase which is a key enzyme in the glycolysis system. Now we have tested the dehydrogenase activities in cell-free extracts of *M. leprae.* Malate and succinate dehydrogenases which are contained in the tricarboxylic acid (TCA) cycle were positive, but glyceraldehyde-3-phosphate dehydrogenase and α-glycerophosphate dehydrogenase which are part of the glycolysis enzyme system were negative. Glyceraldehyde dehydrogenase activity which is also part of the glycolysis system was positive, but the aldehyde dehydrogenase was not specific for glyceraldehyde. Since mycobacteria may have a defect in the glycolysis enzyme system, it may be impossible to culture mycobacteria under anaerobic conditions; in fact, there are no reports on the anaerobic growth of mycobacteria. Moreover, it is not known that mycobacteria lack catalase, so it is very important that *M. leprae* lacks catalase activity. Leprosy bacilli lacking catalase may be difficult to grow under aerobic conditions. On the other hand, the leprosy bacillus which may lack the ability to produce ATP under anaerobic conditions, may be unable to grow under anaerobic conditions as well. This paradox might make the cell-free cultivation of *M. leprae* impossible.—[Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Matsuo, Y., Tsukiyama, F. and Katoh, M. Modification of the fluorescent staining method for mycobacterial cells.

The fluorescein diacetate-ethidium bromide (FDA/EB) staining method for determining the viability of mycobacterial cells by Kvach and Veras (Int. J. Lepr. **50**: 183–192, 1982) was investigated by applying the method to *Mycobacterium leprae* and *M. lepraemurium.* We modified the method as follows: a) concentrations of FDA and EB in the working solution were increased 50 times and 2 times, respectively, as high as the original method, b) staining was performed at 37°C for 60 min, and c) Dubos Tween-albumin liquid medium was used throughout the preparation of the cell suspensions. Our method could clearly differentiate the green-stained cells from the red-orange ones with either of the mycobacterial species. The original fluorescence produced could be maintained beyond 4 min under ultraviolet illumination. This permitted calculating the percent of green-stained cells in specimens and taking color photographs.—[Department of Bacteriology, Hiroshima University School of Medicine, Hiroshima 734, Japan]

Kusaka, T. and Mori, T. Analysis of mycolic acids in *Mycobacterium leprae* using proton (H^+)-NMR and mass spectrometry.

Studies on the analysis of *Mycobacterium leprae*'s mycolates seem of importance not only for identification but also for the taxonomy of this enigmatic bacillus. About five years ago, we isolated two series of mycolates from the foot pads of nude mice infected experimentally with *M. leprae.* One series of them was found to have a quite

similar structure to that of α-mycolates of *M. tuberculosis,* except for the characteristic carbon chain length (C_{20}) of the α-branch for *M. leprae*'s mycolates. Before and after that time, others have studied the structure of *M. leprae*'s mycolates from human and armadillo materials, but no one except us has used a nude mouse for this study. As the source of *M. leprae* for such studies, we feel that the nude mouse is more suitable than other animals which are often infected by other species of mycobacteria together with *M. leprae.* In this report, we present some mass spectrometric information about the structure of the second series of *M. leprae*'s mycolates and confirmation of some chemical groups, for example, cyclopropane group, etc., in both series of *M. leprae*'s mycolates using proton (H^+)-NMR.

The source for *M. leprae* used was foot pads of nude mice, sacrificed one year after inoculation with this microorganism. Methods for isolating mycolates from the foot pads were the same as previously reported, using high-performance liquid chromatography with three different modes of columns, such as gel-permeasion, absorption, and reverse phase. The instrument used for H^+-NMR was Varian's XL-200 type with 200 MHz and mass spectrometry was carried out with Hitachi's 80 M-B type of mass spectrometer.

1) Analysis of *M. leprae*'s α-mycolates: Signals specific for the cis-cylopropane group could be clearly observed when the α-mycolate fraction of *M. leprae* was applied to the H^+-NMR. The electron-impact (EI) mass spectrometry of the above fraction gave just the same result as previously reported. Based upon these results, the structure of *M. leprae*'s α-mycolates already proposed by us was reconfirmed with more certitude. 2) Analysis of the second series of *M. leprae*'s mycolates: Signals of H^+-NMR, specific for the cis-cyclopropane group could be observed also in this case. The EI-mass spectrometry of this series of mycolates indicated a somewhat different structure in the meromycolal part as compared to the series of α-mycolates. Based upon these results as well as on infrared spectrometric information, this series of mycolates appeared to have a structure quite similar to that of the γ-mycolates of *M. tuberculosis,* containing an oxo-group along with the cyclopropane group in the meromycolal part except for the characteristic carbon chain length (C_{20}) of the α-branch for *M. leprae*'s mycolate.

It seems too early to decide at present that *M. leprae* contains only two series of mycolates, because the origins of *M. leprae* used up to the present for mycolate analysis have been fairly restricted, and it is rather usual for almost all other species of mycobacteria to contain more than two series of mycolates. We propose to approach this problem by completing the analysis of *M. leprae*'s mycolates.—[Department of Biochemistry, Kawasaki Medical School, Kurashiki City, Okayama, Japan]

Fukunishi, Y. Spherical droplets around *Mycobacterium leprae.*

The main purposes of the present report are a) to elucidate the process of producing the small spherical droplets around *Mycobacterium leprae* by the freeze-etching technique, and b) to describe the isolation of the components of the acetone soluble lipids of lepromas in which a large amount of small spherical droplets were included by HPLC analysis, and c) then to examine the mass spectrometric characteristics of the two peaks (molecular weights 2000 and 1600) found in HPLC.

According to the freeze-etching findings of lepra cells of nude mouse, armadillo, and monkey leprosy, it is considered that the multiplication (by transverse fission) of *M. leprae* is done inside the phagolysosomes of the lepra cells.

According to the results of HPLC, GC, and mass spectral analyses, GPC peak I lipid at 2000 D was identified as phenolic glycolipid, and GPC peak II lipid at 1600 D, as phthiocerol dimycocerosate.

It is thought that GPC peak I lipid (phenolic glycolipid) and GPC peak II lipid (phthiocerol dimycocerosate) are included in the small spherical droplets around *M. leprae.*—[National Sanatorium Oshima Seisho-en, Kagawa Prefecture, Japan]

Franzblau, S., Takeda, T. and Nakamura, M. Plasmids in mycobacteria: A preliminary report.

Screening for mycobacterial plasmids was undertaken in order to obtain potential vectors for a) transfer of genetic information

from readily cultivable mycobacteria to *Mycobacterium leprae* for the purpose of producing an *in vitro* competent variant of this organism, and b) the reverse process; expression of *M. leprae* genes in a foreign mycobacterial host. The former was considered due to the lack of a confirmed method for cultivation of *M. leprae,* suggesting that cultivation by physico-chemical manipulation is very difficult or possibly that this bacterium is genetically incapable of *in vitro* growth. The latter (b) would facilitate genetic studies of *M. leprae* (and other mycobacteria).

Our proposal for producing an *in vitro* competent recombinant of *M. leprae* involves: 1) isolation and physical characterization of a variety of mycobacterial plasmids, 2) construction of recombinant plasmids containing random chromosomal DNA fragments from readily cultivable mycobacteria, 3) uptake of the recombinant plasmids by *M. leprae* using the cold $CaCl_2$ method, 4) inoculation of an animal host with the treated bacterial suspension and incubation for approximately 1–2 months (2–3 generations) to allow for expression of foreign DNA, 5) harvesting of *M. leprae* and inoculation of a variety of media used for cultivation of mycobacteria, 6) incubation to select for *in vitro* competent recombinants, and 7) confirmation of plasmid DNA.

Since it is impossible to predict which, if any, plasmids will replicate in *M. leprae,* we are seeking to obtain a variety of plasmids, including those previously described as well as newly discovered ones via our own screening process. We are particularly interested in those coding for resistance to an antibiotic(s) to which *M. leprae* is sensitive, such as dapsone, rifampin, or clofazimine, since this would allow for *in vivo* selection of plasmid-containing *M. leprae* (during step 4).

We employed a micro-adaptation of the procedure originally described by Kado and Liu for plasmid screening. Thus far, we have screened 33 clinical isolates of *M. avium-intracellulare,* 1 ATCC strain each of *M. avium* and *M. intracellulare,* a Hawaiian strain of *M. lepraemurium,* 2 *in vitro*-competent mycobacteria isolated from *M. leprae*-experimentally infected animal tissues, 5 acid-fast environmental isolates, and 5 strains of Dr. V. R. Khera's "*in vitro* adapted *M. leprae*," including drug-sensitive and drug-resistant isolates. One to three plasmids were detected in approximately 50% of the *M. avium-intracellulare* isolates. Overall, 7 or 8 different plasmids were observed. Plasmid DNA was not detected in any of the other isolates screened.

The clinical *M. avium-intracellulare* isolates were subsequently tested for antibiotic sensitivity and colonial morphology. A strong, but not absolute, correlation was found among plasmid-positive strains, high-level resistance to rifampin, kanamycin, and streptomycin, and translucent colony formation. Conversely, plasmid-negative strains were more sensitive to these antibiotics (especially rifampin) and most produced opaque or partially opaque colonies. These data suggest that rifampin resistance (and possibly kanamycin and streptomycin resistance) may be plasmid-mediated in some strains of *M. avium-intracellulare* and support the concept of a permeability barrier. We are currently attempting to confirm this relationship via plasmid-curing and plasmid-transfer experiments.—[Department of Microbiology, Kurume University School of Medicine, Kurume 830, Japan]

Matsuoka, M., Kawaguchi, K. and Kawatsu, K. Multiplication of *M. leprae* in nude mice after infection through different routes and suitable site for the growth.

BALB/c-nu/nu mice were infected with *Mycobacterium leprae* intravenously, subcutaneously, and intracutaneously. Intravenously infected nude mice with 2.5×10^6 bacilli revealed disseminated infection at the late stage of the infection. Larger number per unit weight of each organ was recorded in hind foot pads, exceeding 10^6/mg, and the total bacillary number in this site was more than 10^8. Considerably large numbers were also shown in the fore foot pad, ear, and lip. On the other hand, no bacilli were detected from lung or kidney throughout the observation period, and much less bacilli were seen in the liver and spleen than in the ear and lip. The results indicate that there are pronounced differences in multiplication of the bacilli among these organs even in nude mice. Different bacillary multiplication among visceral organs suggests that the multiplication of *M. leprae* is controlled

by not only body temperature but also by other biological characteristics.

After subcutaneous infection with 1.0×10^7 bacilli in the back, the bacilli decreased in number at 1 week but thereafter the organisms increased steadily in number in the inoculated site of BALB/c-nu/nu mice up to 50 weeks. No bacillary multiplication was shown in BALB/c mice. The development of the subcutaneous infection was very slow, and the rate of increase was low. However, dissemination into sites of predilection was also shown after this route of inoculation.

Intracutaneous infection with 1.5×10^6 bacilli was made in the back of BALB/c-nu/nu mice. Although the bacilli were recovered from infected and uninfected sites at 50 and 70 weeks, the bacillary number varied greatly among the mice. Further studies are required to conclude on bacillary multiplication after intracutaneous infection.—[National Institute for Leprosy Research, Tokyo, Japan]

Nakamura, K. and Yogi, Y. The athymic rodent as an experimental lepromatous leprosy model (continued): The SHR nude rat as a new model and effect of host in the formation of the lepromatoid lesion in nude mice.

We compared the susceptibilities of various strains of the nude rat by using SHR-rnu, ACI-rnu, LOU/N-rnu, WM-rnu, LEW-rnu, and rhr-rnu. The SHR nude rat gave excellent results with the development of a heavy lepromatoid formation. Thus, the development of a heavy lepromatoid formation in nude rats was influenced by the genetic background of the rats similar to the case with nude mice which we previously reported. Blood pressure among the various nude rats corresponded to those strains with hereditary hypertension, except for the WM-rnu rat.

In this report, in addition to comparing C57BL/6-nu, and C57BL/6N-nu, C57BL/6-hr-nu, C57BL/6J-bg-nu, NC-nu, NZB-nu, and AKR/J-nu mice, we also compared the susceptibilities among NFS/N-nu, N:NIH-(s)-nu, Crj:CD-1-nu, and ICR-nu mice. The NFS/N nude mouse was again confirmed as a powerful tool as an experimental lepromatous model. In some of the C3H/HeN+MTV (having murine tumor virus) mice, there appeared the loss of the infected hind foot due to heavy ulceration, with a necrosis of the ears and in uninoculated fore feet. With dorso-lumbar site infections, heavy ulceration occurred in comparison to NFS/N nude mice. In addition, we found sex differences in that some of the female C3H/HeN-MTV nude mice had no heavier lesions than those of the male animals. With combined infection in nude mice there were heavier lesions with shorter lifespans than following single infections. C57BL/6-hr-nu mice showed enhanced infections in comparison to C57BL/6N-nu mice with heavy lepromatoid lesions, with lesions in the kidney. In C57BL/6J-bg-nu mice, there was a slightly enhanced upper lip infection. In addition, in comparing BABL/c-LASAT(Dh/+nu), ICR-LASAT, and their athymic (+/+nu) mouse counterparts, some of the infections in LASAT mice were noted to be stronger than those of the control BALB/c-nu and ICR-nu mice. The lack of a spleen may be allowing enhanced growth of *Mycobacterium leprae* at an early stage of the infection. With long-term observation, unfortunately a high incidence of tumors occurred in BABL/c-LASAT mice with marked enlargement of lymph nodes, such as the mesenteric nodes. However, in ICR-LASAT and their athymic counterparts tumors were never seen, these animals having excellent swelling in comparison with Crj:CD-1-nu and ICR-nu mice.

Thus, the formation of lepromatoid lesions in the nude mouse may be influenced by a combination of genes. Secondary complications such as tumor-bearing in nude mice may influence the formation of the lepromatoid lesion, inhibiting it at an early stage of the infection. Later on, moderate occurrence of tumor-bearing in nude mice may influence having a moderately enhanced or inhibited formation of lepromatoid lesions. Other complications, such as ulceration due to other factors, erythroderma, exfoliative erythroderma and, in some of the nude mice, diarrhea due to transfer into a conventional animal room, were associated with the disappearance of infected foot swelling. After receiving *M. leprae*-infected, nine-banded armadillo tissues, such as liver, spleen, skin, ovary, and adrenal gland, the infected hind foot swelling in ICR-nu mice was inhibited except

after receiving inocula prepared from infected armadillo skin. Therefore the formation of lepromatoid lesions in nude mice may be influenced by various unknown factors in the endogenous and/or exogenous environment as well as the genetic background of the animals. Additionally, various genetic combinations and combined infections of the extremities (e.g., lower legs and feet), upper lips, dorso-lumbar sites, i.p., perianal (base of tail to external genitalia) skin, and both testicular inoculation methods influence the formation of lepromatoid lesions.—[National Institute for Leprosy Research, Tokyo, Japan]

Gormus, B. J., Martin, L. N., Wolf, R. H., Baskin, G. B., Meyers, W. M., Walsh, G. P. and Binford, C. H. Experimental leprosy in monkeys.

Two sooty mangabey monkeys (*Cercocebus atys*) which developed lepromatous leprosy after inoculation 48 months ago with *Mycobacterium leprae* isolated from a mangabey with naturally acquired LL type leprosy have continued to develop progressively more severe disease during the last 12 months. Both animals advanced to a similar severe stage of LL type leprosy with neurologic impairment (early "clawing" of hands) developing in one animal (AO22) and possible early neurologic impairment in the hands of the other (AO23). Ulcerating lesions became very numerous at multiple inoculated and uninoculated sites.

Mangabey AO22 died with marked disseminated LL type leprosy in February 1984. Its tissues have been processed for an exhaustive histologic study. Touch impressions of the liver, kidney, and spleen sections showed minimal leprosy involvement, if any, in these groups. Hematoxylin and eosin (H&E) stains of the liver, lung, and kidney confirmed that observation and showed the presence of amyloid in the kidney glomeruli. H&E sections of nasal septum showed diffuse granulomatous infiltration of nasal mucous membranes. Acid-fast stains of sections revealed numerous acid-fast bacilli (AFB). Sections of testicle revealed severe inflammatory infiltration and numerous AFB with secondary tubular atrophy. These early results indicate a disease similar to human lepromatous leprosy with extensive involvement of the skin, nerves, nasal mucous membranes, and testicles, but little involvement of other internal organs. The second recipient of mangabey-derived *M. leprae* is presently responding positively to treatment with rifampin and dapsone.

Two mangabeys that were inoculated with armadillo-passed human *M. leprae* 39 months ago continue to show LL type leprosy but, other than satellite lesions near inoculation sites, no extensive distant dissemination has yet occurred. Both animals have extensive involvement at and near some inoculation sites. AFB first became demonstrable in nasal secretions from one of these mangabeys at 25 months and in the other at 40 months postinoculation. The presence of *M. leprae* in nasal secretions may indicate an early stage of dissemination. In May 1983, a normal mangabey was inoculated with *M. leprae* from either of these two mangabeys to determine whether human *M. leprae* may become adapted for more rapid infection by serial passage in mangabeys.

The mangabey with naturally acquired leprosy initially responded to treatment with rifampin for 30 days followed by maintenance on dapsone. Approximately 18 months later signs of infection reappeared, indicating a resistance to dapsone. The mangabey's condition worsened until rifampin therapy was reinstituted. Subsequently, this animal has shown dramatic improvement. Neurologic damage ("clawing" of hands and feet) in both arms and one hind leg is advanced, but all lesions have now healed, nasal secretions no longer contain *M. leprae,* and the animal's general health and vigor appear near normal.

Our early data suggested that no significant change in the numbers of OKT4+ or OKT8+ T cell subsets occurred in advanced LL type leprosy in the mangabey. However, in the past year additional, new control mangabeys have been studied and several additional observations of the infected animals have been obtained. Also, sufficient observations on the circannual rhythm involving immune parameters in mangabeys have now been obtained to permit us to sort out changes due to leprosy from cyclic fluctuations. Decreased mitogen responses in advanced LL type leprosy now

appear to be correlated with increased percentages of OKT8+ (suppressor) cells. Correlations between decreased numbers of pokeweed mitogen-induced, immunoglobulin plaque-forming cells and increased numbers of OKT8+ cells in advanced disease still have not reached statistical significance, but may yet prove to be at least partially related to changes in OKT4+ or OKT8+ subsets. The data suggest that at least some decrease in the immune function in advanced leprosy may be due to increased percentages of OKT8+ cells.

In July 1983, eight new mangabeys obtained from the Yerkes Regional Primate Research Center were inoculated with titrated doses of mangabey-derived *M. leprae*. Five of these mangabeys are now showing significant lesion formation at one or several inoculation sites with a gradation of lesion development depending on the dose of the inoculum. Histopathologic results from the only two of these animals biopsied show leprosy near the LL region of the spectrum.

In February 1984, 11 mangabeys were inoculated with mangabey-derived *M. leprae* from donor A022. In this experiment three mangabeys (two had been previously skin tested with standard lepromin) were injected with the highest dose by both intravenous (i.v.) and intracutaneous (i.c.) routes. The eight remaining mangabeys received fewer total *M. leprae* by either the combined i.v.-i.c. route or by an i.v. or i.c. route alone in such a way as to titrate total doses and to test the effects of the route of administration. The skin-tested animals should provide evidence of whether lepromin skin testing can alter the course of experimental leprosy infection, e.g., by acting as a vaccination offering protection to the animal. The 11 mangabeys in this group have not yet developed lesions, but it is still too soon after inoculation to expect to see signs of the disease, based on our previous observations.

In addition, two mangabeys were inoculated via aerosol exposure using mangabey-derived *M. leprae* in September 1983, and a third one was given combined i.v.-i.c. organisms together with a "nose-drop" inoculation. The latter animal was a mother that was housed in a cage with her uninoculated baby for six months thereafter. A total of 29 mangabeys, including the index case, are presently being studied after inoculation with or exposure to viable *M. leprae*. Four control mangabeys are also being studied. The outcome of the described inoculations via various routes using titrated doses of *M. leprae* should provide important information regarding natural modes of leprosy transmission.

One of two rhesus monkeys (*Macaca mulatta*), a male, inoculated with mangabey-derived *M. leprae* 39 months ago continues to have advanced, disseminated, LL type leprosy; the second rhesus, a female, inoculated at the same time and 12 paired male and female rhesus monkeys inoculated 21 months ago with armadillo-passed human *M. leprae* have not yet given any indication of the disease. Four additional rhesus monkeys were inoculated 16 months ago with mangabey-derived *M. leprae*. Two of these animals (males) now have *M. leprae* in nasal smears and are showing signs of probable lesion development. Biopsies have been taken; isolates are to be inoculated into mouse foot pads and armadillos. The other two rhesus monkeys, both females, that received mangabey-derived *M. leprae* are being watched closely but have not yet shown signs of leprosy.

Three African green monkeys (*Cercopithecus aethiops*) inoculated with mangabey-derived *M. leprae* 39 months ago developed lesions with histopathologic characteristics of leprosy two years after inoculation. One of these monkeys currently has extensively disseminated lepromatous leprosy, the second is unclassified but active, and the third has shown a tendency to regress. Ten African green monkeys inoculated 31 months ago with armadillo-passed human *M. leprae* remain free of the signs of leprosy. Two squirrel monkeys (*Saimiri sciureus*) inoculated 39 months ago with mangabey-derived *M. leprae* also show no signs of leprosy.

The data continue to indicate that the sooty mangabey is a good model for the transmission and study of leprosy. Both African green and rhesus monkeys show strong promise of becoming alternative, more available species that may be susceptible to the experimental transmission of leprosy. Additional studies are necessary to more completely evaluate the extent of suscepti-

bility of these latter two species to leprosy. Squirrel monkeys appear to be very resistant to infection with *M. leprae*.—[Delta Regional Primate Research Center, Tulane University, Covington, Louisiana 70433 and the Armed Forces Institute of Pathology, Washington, D.C. 20306, U.S.A.]

Kohsaka, K., Miyata, Y., Mori, T. and Ito, T. Reversal reaction by thymus transplantation in experimental leprosy of the nude mouse.

In order to investigate the induction of reversal reactions in the *Mycobacterium leprae*-infected nude mouse by the transplantation of the thymus, we have studied the effect of thymus transplantation on the growth of *M. leprae* in nude mice.

Two experiments were carried out. One was a prophylactic experiment and the other was a therapeutic experiment in experimental leprosy. The *M. leprae* used in the experiments originated from a previously untreated lepromatous leprosy patient and were passaged 6 times in nude mice.

In the prophylactic experiment, 35 nude mice were inoculated with 2.5×10^7/0.05 ml of *M. leprae* into both hind foot pads, and they were divided into two groups. One group of 20 mice was the untreated control, and the other group of 15 mice were thymus transplanted. Each mouse was transplanted a thymus from a mouse subcutaneously into the back or intraperitoneally monthly for 12 months beginning on the 8th day after inoculation. In control mice, the number of bacilli gradually increased, and the bacilli were harvested as follows: 1.5×10^9 at 7.5 months, 6.2×10^9 at 13 months, 2.6×10^{10} at 15 months, and 3.5×10^{10} at 18.5 months after inoculation. In contrast, nearly the same number as the inoculum of 10^7 organisms were recovered from both of the groups receiving thymus transplants subcutaneously or intraperitoneally. Thus the growth of the bacilli was strongly suppressed in the thymus transplanted nude mice.

In the therapeutic experiment, 16 infected mice among the above control group which were inoculated with *M. leprae* 7.5 months earlier were used as recipients of immunotherapy. At that time, the number of organisms had reached 1.5×10^9 in the foot pad. They were also divided into two groups, and the mice of the treated group were transplanted with thymus intraperitoneally 7 times for seven months. In the untreated control group, the number of organisms gradually increased to 3.5×10^{10} at 18.5 months after the inoculation similar to the former experiment. In the treated group, however, the bacillary population was significantly reduced compared with the untreated control group. The results suggest that thymus transplantation is effective as immunotherapy of experimental leprosy in nude mice.

The possibility of immunotherapy is suggested by the results of thymus transplantation in that there is both a prophylactic effect on the growth of *M. leprae* and a therapeutic effect on established experimental leprosy in nude mice. The histopathological findings also revealed the induction of reversal reaction in experimental leprosy in nude mice. A similar experiment by one-shot transplantation of the thymus is being conducted.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Hastings, R. C. and Chehl, S. R. An anomalous response of *Mycobacterium leprae* to dapsone chemotherapy in nude mice.

A skin biopsy (SI-20) from a patient treated with a daily dose of 100 mg dapsone (DDS) for approximately ten years contained 4.27×10^8 AFB/g with a morphological index of 5. The biopsy was processed to prepare an acid fast-bacilli (AFB) suspension for mouse foot pad inoculation. In addition to the routine *M. leprae* drug sensitivity study in BALB/c mouse foot pads in a dose of 5×10^3 per foot pad, the suspension was inoculated into the left hind foot pads of ten nude (nu/nu) mice in a dose of 1.85×10^5 per foot pad. The nude mice were immediately provided and maintained on a diet of 0.1% DDS mixed in autoclavable Rodent Chow.

The inoculated foot pad of the DDS-treated nude mice showed a growth of *M. leprae* to 1.39×10^7 per foot pad, with a morphological index of 6, eight months after inoculation. On the other hand, the organisms were found to be completely sensitive to DDS in BALB/c mice at six months post-

inoculation. Similar observations showing the ineffectiveness of DDS in preventing the growth of *M. leprae* in nude mice were reported by Kohsaka, *et al.* (Abstracts in Int. J. Lepr. 47:107–108 and 673–674, 1979).

Drug sensitivity patterns in the AFB harvested from the DDS-treated nude mice were determined by passage into BALB/c mice. The bacilli showed full resistance to DDS on passage. It would seem, therefore, that this isolate of *M. leprae* contained mutants fully resistant to DDS at a frequency of at least 1 viable bacillus per 1.8×10^5 total bacilli, or an estimated 9.25×10^3 (morphological index of 5, 1.85×10^5 inoculum) viable bacilli. On the other hand, the frequency of even partial DDS-resistant mutants was less than 1 viable bacillus per 5×10^3 total bacilli, or an estimated 250 (morphological index of 5, 5×10^3 inoculum) viable bacilli.

These data suggest that there may be a substantial sampling error in the routine mouse foot pad drug sensitivity monitoring of *M. leprae* from a lepromatous leprosy patient. This patient, who may be harboring up to 10^{10} viable *M. leprae,* has fully DDS-sensitive bacilli by routine mouse foot pad testing but the data suggest that as many as 10^6 of these bacilli are actually fully resistant to DDS.—[National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Ito, T., Kohsaka, K. and Miyata, Y. Effect of INAH on experimental leprosy of the nude mouse.

The effect of isoniazid (INAH) monotherapy on leprosy was examined by means of experimental leprosy of the nude mouse. BALB/c-nu/nu mice were infected with 1.5×10^6 *Mycobacterium leprae* in the right hind foot pads. Mouse diet containing 0.008% w/w INAH was prepared, and this diet could suppress the growth of BCG in the foot pads of nude mice. The diet was given to the *M. leprae*-infected mice for 6 months from 1 month to 7 months after infection.

No suppressive effect of INAH on the growth of *M. leprae* in nude mice was seen in this experiment. A significant antileprotic effect cannot be expected with INAH monotherapy.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Gidoh, M. and Tsutsumi, S. Analysis of hydrophilic conjugates of DDS by high performance liquid chromatography.

Following the preceding paper in which a simultaneous analysis of Promizole, DDS 2-sulfonamide (s-DDS, internal standard (IS) for sulfones), DDS, MADDS, RFP and its main metabolites, phenylthiohydantoin(e-phenylthiocarbamyl)-lysine (PEPL, IS for RFPs and B 663) and clofazimine was reported by using μBondapak C18 (Waters Assoc.) and by switching mobile phases appropriative for sulfones or for the others, we examined the analysis of hydrophilic DDS metabolites by high performance liquid chromatography (HPLC). A column (Waters Z module Radial-Pak Cartridge μBondapak NH_2) enabled the separation of DDS mono-N-glucuronide (DDSG) and DDS-mono-N-sulfamate (DDSS) when developed with methanol–0.001 M Na_2HPO_4 (30:70). Nevertheless, we could not find an adequate IS other than Na-sulfanilate (NaSA), and an unknown peak derived from guinea pig serum (PGP), the retention time (Tr) was similar to that of tryptophan, co-eluted with NaSA. Thus we changed the column to Radial-Pak Cartridge μBondapak phenyl. The development with 0.01 M Na_2HPO_4 enabled the individual quantification of all the hydrophilic metabolites. The order of eluted peaks on chromatogram was free DDS/MADDSS, 4-aminobenzene sulfonamide (IS), PGP, DDSG, DDSS, MADDSG and MADDSS. Although the sensitivities to the latter two were lower than the conjugates of DDS itself, by using this method, we tried the analysis of all the metabolites of DDS in each of urine, plasma and saliva of man orally dosed with 300 mg of capsulated DDS powder and $NaHCO_3$.

Results found were: 1) The urinary metabolites were DDSG $\gg$ MADDSG > DDS > MADDSS $\gg$ DDSS. The detected peaks at which Tr was identical to that of DDSS were very small. 2) Those in plasma

were DDS > MADDS ≫ DDSG > MADDSG. The content of MADDSG seemed to be increasing until 6.5 hours after intake. The peak identical with DDSS or MADDSS could not be found. 3) Those in saliva were DDS ≫ MADDS. The content of MADDS seemed to be preservable during 1.5–6.5 hours and that of DDS increased until 6.5 hours. The detected hydrophilic conjugate was only DDSG at 6.5 hours. The constant ratio could not be found clearly in both salivary DDS/plasma DDSG and salivary MADDS/plasma MADDSG. The ratios, salivary DDS/plasma DDS and salivary MADDS/plasma MADDS, were lower than those found by Peters, *et al.* 4) The intricacy of base lines on the chromatograms of plasma and salivary hydrophilic conjugates could not be improved by the filtration of plasma and diluted saliva through Sep-Pak prior to the extraction of these humors.—[National Institute for Leprosy Research, Higashimurayama-shi, Tokyo, Japan]

Tsutsumi, S. and Gidoh, M. Possible role of immunomodulators in the control of ENL.

Based on the distinctive criteria covering pharmacodynamy, chemoimmunology, and the influence on intracellular NADH oxidase activity examined by a NBT assay using guinea pig peritoneal macrophages and Baker's yeast, the distinction between immunostimulators (IS), immunopotentiators (IP), and immunomodulators (IM) is discussed.

In addition to the tendency that the order of activity to induce NK cells and IFN is IS > IP > IM, IS induce adjuvant-induced arthritis; whereas IM can inhibit and IP are ineffective or promotive. The appearance of the IgM component named LB in murine serum (Mizuno, *et al.*) following the dose of immunological activator was also IS > IP, and it was negative in the case of IM. The inverse immunodepression due to the excessive dose of an immunological activator does occur most frequently and sensitively in the case of IS. Since this undesirable immunodepression was supposed to be caused by IS-induced cytotoxicity such as PGEs or an excessive formation of intracellular active oxygen radicals (AOR), we started a series of experiments by NBT assay in order to discover a substance moderately producing AOR.

DDS tended to decrease phagocytized cells; whereas sulfadimethoxine significantly increased NBT formazan (NBTF) positive macrophages in all of the concentrations, 0.01–10 μg/ml. Clofazimine also significantly increased NBTF-positive cells. In spite of the similarity of CCA to clofazimine, a typical mild immunomodulator CCA contrarily decreased NBTF-positive cells. Platonin, a photosensitizer and a strong IM, could not increase NBTF-positive macrophages. DDC (Prabhakaran) reduced NBT to NBTF without incubation with macrophages. Oyanagui reported the inhibition of superoxide dismutase by DDC. Nevertheless, the pharmacodynamic and immunological spectra of DDC were enough to indicate the behavior of this substance to be an IM. Among indomethacin (10 nM and 1 nM), dexamethasone (same), and ATSO (200 μg/ml and 20 μg/ml), which is a purified PSK (polysaccharides from *Basidiomycetes*), dexamethasone alone significantly decreased both NBTF-positive macrophages and those phagocytized yeast cells at 10 nM.

Despite these findings, the intracellular antibacterial mechanism of DDS again remained a problem to be elucidated.

Supplementing these findings, although putatively, the ideal characteristics of a substance to be a candidate for antileprosy chemotherapy are proposed and discussed.—[National Institute for Leprosy Research, Tokyo, Japan]

Collins, F. M. and Orme, I. M. Immunocompetence of the heavily infected host.

Specific pathogen-free B6D2 F_1 hybrid mice were infected intravenously, subcutaneously or aerogenically with increasing numbers of *Mycobacterium avium* (mouse virulent) or *M. simiae* (mouse avirulent), and their growth within the lung and the spleen was determined over a six-month period. The blastogenic responsiveness of spleen cells harvested from heavily infected, anergic donors (5×10^7 CFU i.v.) was substantially reduced compared with those taken from control animals infected with 10^6 CFU. Despite this reduced cellular responsiveness *in vitro,* the mycobacterial infec-

tion continued with little or no sign of a decline in viable counts for the spleen or lung analogous to that normally observed in *M. tuberculosis* or BCG-infected mice. Furthermore, heavily infected anergic mice could be sensitized *de novo* by an appropriate inoculum of sheep red blood cells (SRBC). The optimum dosage for normal uninfected mice was 10^5 SRBC i.v., but when this sensitizing dose was used in the anergic mice, the foot pad response to 10^8 SRBC injected into a hind foot pad five days later was almost completely absent. This ablative effect was thought to be due to excessive antigenic destruction by the greatly enlarged population of activated macrophages within the heavily infected spleen. It was hypothesized that this resulted in suboptimal sensitization of the T cells and a drop in foot pad reactivity. This response could be restored by increasing the dosage of SRBC 100- to 1000-fold. Under such circumstances, substantial numbers of sensitized T cells could again be detected within the spleen by means of an appropriate adoptive transfer assay.

Mice heavily infected with BCG Pasteur also become anergic to tuberculin, and their spleen cells were unresponsive to PHA and ConA stimulation *in vitro*. Blastogenic responsiveness by these cells could be restored by supplementing the culture medium with the T-cell growth factor, interleukin 2 (IL2). Spleen cells harvested from anergic donors at the time of their minimum T-cell responsiveness *in vitro* (days 15–30) showed an enhanced capacity to remove IL2 from HT-2 cultures, and this depletion would limit the amount of cell proliferation (^{3}H-TdR uptake) shown by the T cells cultured *in vitro*. Mice which have been heavily infected with mycobacteria rapidly acquire an IL2-dependent T cell population, apparently in response to the systemic infection. Such cells undergo blastogenic transformation only in the continued presence of IL2. If this growth factor is removed or absorbed by actively replicating cells present in the spleen, a sharp drop in ^{3}H-TdR uptake might be interpreted to indicate a state of immunosuppression within the heavily infected cell donor. The significance of this type of response with respect to the interpretation of *in vitro* blastogenic responsiveness reported for T cells harvested from lepromatous leprosy patients is discussed.—[Trudeau Institute, Inc., Saranac Lake, New York 12983, U.S.A.]

Lefford, M. J. *Mycobacterium lepraemurium* infection of T cell-depleted mice: Implications concerning the immune response in intact mice.

The efficacy of the cell-mediated immunity in normal, intact mice can be inferred by comparing the progress of an infection in mice of the same strain that have been depleted of T lymphocytes. This approach has been applied to *Mycobacterium lepraemurium* (MLM) infection of mice.

In the first series of experiments, nude (nu/nu) and nu/+ mice derived from the BALB/c strain were infected with two doses of MLM, 10^8 or 10^6, by the intravenous (i.v.) and foot pad (FP) routes. Three measures of the host response were monitored: FP swelling at the site of infection; the number of MLM in the FP or spleen following FP and i.v. infection, respectively; and FP or i.v. resistance to challenge with *Listeria monocytogenes* (LM). After FP infection, it was found that localized FP swelling occurred earlier in nu/+ mice than in nude mice, signifying hypersensitivity granuloma formation in the former. However, localized resistance to LM was not apparent in either strain of mouse. Similarly, neither strain developed nonspecific resistance to LM after i.v. infection with MLM. Most importantly, the growth of MLM in the nude and the nu/+ mice was indistinguishably similar. It was inferred that the protective immune response to MLM was completely ineffective in nu/+ mice, an observation that was attributed to the fact that mice with a BALB/c genetic background are susceptible to MLM.

In order to test this hypothesis, the experiments were repeated in mice that had been depleted of T lymphocytes by adult thymectomy, 900 rads ot total body gamma radiation and bone-marrow reconstitution (TXB mice). Two strains of mice were tested: $CB6F_1$ and $B6D2F_1$, strains that are susceptible and resistant to MLM, respectively.

After FP infection the intact mice of each strain developed FP swelling more rapidly than the corresponding TXB mice. Unlike the experience with nu/+ mice, however,

the onset of FP swelling in intact mice was associated with local resistance to LM. Surprisingly, the TXB mice also developed FP resistance to LM, although at a later stage of infection. Counts of MLM in the FP of CB6 mice showed no material difference between the intact and the TXB groups. Thus, once again, immunity was not protective despite being accompanied by macrophage activation. In the more resistant B6D2 mice, the FP of TXB mice harvested 20 weeks after infection did contain significantly more MLM than their corresponding controls. Normal and TXB mice of both strains developed nonspecific resistance to LM after i.v. infection with MLM, but the growth of MLM in the spleens of normal mice was closely similar to that in TXB mice of the same strain. However, MLM grew more rapidly in the FP and spleen of CB6 mice than in B6D2 mice.

It was deduced that even though normal mice respond to MLM infection by development of hypersensitivity granuloma formation and macrophage activation, the latter cells do not restrain the replication of MLM much more effectively than the macrophages of T-cell-depleted mice. It appears that the virulence of MLM to mice may depend on the insusceptibility of these bacteria to macrophage microbicidal mechanisms.—[Wayne State University School of Medicine, Detroit, Michigan 48201, U.S.A.]

Ohkawa, S. Activation of human monocytes.

We report the normal time course and modification by lymphokine of hydrogen peroxide production, superoxide production, and glucose consumption in monocytes.

Glucose consumption spontaneously increased and lymphokine enhanced the consumption rate.

Superoxide production increased spontaneously and from the 4th day decreased. Lymphokine added on the 4th day suppressed the decrease of superoxide production.

Hydrogen peroxide production increased until the 3rd day of culture. Twenty-four-hour incubation with lymphokine from 0 time to the 1st day had no effect on hydrogen peroxide production, while incubation from the 2nd to the 3rd day enhanced hydrogen peroxide production.

The supernatant of lymphocytes incubated with *Mycobacterium leprae* was prepared from tuberculoid and lepromatous patients. Supernatant from tuberculoid patients enhanced superoxide production and glucose consumption. Supernatant from lepromatous patients had no effect on glucose consumption and superoxide production.

From these data we suggest that monocytes cultured *in vitro* are not a homogenous cell population but they differentiate spontaneously during culture. When we use human monocytes as a model of macrophage activation, the time course of the culture would be an important factor.

Lepromatous patients have some defects in their immune system against *M. leprae.* In this work we report that lymphocytes of lepromatous patients did not secrete lymphokine in response to *M. leprae.* The main defect is not at the effector cell level but is at the stage of lymphokine production.—[Leprosy Research Laboratory, Kyoto University School of Medicine, Kyoto, Japan]

Rea, T. H., Modlin, R. L., Fullmer, M. A., Dugan, F. and Taylor, C. R. Anti-interleukin 2 positive and anti-Tac positive cells in leprosy lesions, lepromin reactions, and tuberculin reactions.

Monoclonal antibodies have been shown to be powerful tools in the study of the immunopathology of the skin lesions of leprosy. To date, most investigations have used antibodies which identify cell phenotypes, for example, T suppressor-cytotoxic cells or T helper-inducer cells. Such phenotypic antibodies are limited in that they allow an inference of function but do not permit proof of function. This limitation is obviated in part by the recent availability of monoclonal antibodies against interleukin 2 (IL2) and its receptor, Tac, molecules with understood functions. The present study is a report of our investigation of the number and distribution of anti-IL2 and anti-Tac positive cells in BT, LL, and ENL tissues. In addition, data on tuberculin and lepromin skin tests are presented.

Six mm punch biopsy specimens were placed in ornithine carbonyl transferase (OCT), snap frozen in liquid nitrogen, and

stored at −70°C until sectioned. Studies utilized a two-step immunoperoxidase procedure or the avidin-biotin-complex (ABC) method with aminoethyl carbazole (AEC) as the chromogenic substrate.

Leprosy tissues were classified according to the criteria of Ridley. Tuberculin reactions (5 TU) were obtained at 24 hr and 48 hr or at 48 hr and 96 hr from patients with active, culture-proven, pulmonary tuberculosis. Lepromin reactions were obtained from tuberculoid (TT/BT or BT) patients or kindred of lepromatous patients at 21 or 28 days.

The specificities sought and monoclonal antibodies used included pan T cells (Leu 4, 1/60), T helper/inducer cells (Leu 3a, 1/60), T suppressor/cytotoxic cells (Leu 2a, 1/60), Langerhans' cells (OKT6, 1/50), HLA-DR or Ia (H4, 1/1000, Dr. Ron Billing), IL2 (anti-IL2, 1/10, Dr. Stephen Gillis), and the IL2 receptor or Tac (anti-Tac, 1/5000, Dr. Thomas Valdmann).

If greater than 5% of the total infiltrate, cells staining positively for a particular antibody were estimated by two observers as the percent of the infiltrate staining positively. If less than 5% of the infiltrate, positive cells and all cells of the infiltrate were counted.

The numerical results in the dermal infiltrates of leprosy lesions, summarized below, are expressed as the mean percent of cells staining positively for each antibody:

	BT (10)	LL (10)	ENL (8)
Leu 4	54 ± 14	33 ± 9	47 ± 15
Leu 2a	21 ± 8	27 ± 9	16 ± 4[b]
Leu 3a	41 ± 13[a]	13 ± 5	35 ± 10[a]
Anti-IL2	0.46 ± 27[a]	0.03 ± 0.017	0.34 ± 13[a]
Anti-Tac	2.1 ± 8	1.5 ± 0.5	1.6 ± 0.8

[a] $p < 0.001$ as compared with LL.
[b] $p < 0.02$ as compared with LL.

The distribution of cells staining positively for phenotypic antibodies was similar to those previously reported. IL2 positive cells in BT lesions were distributed in the lymphocytic mantle surrounding the epithelioid tubercle, similar to that of cells bearing the Leu 2a phenotype. In LL and ENL lesions, IL2 positive cells were admixed with macrophages. Anti-Tac positive cells were distributed among the macrophages in all three types of lesions.

On double staining, the IL2 positive cells were Leu 4 and Leu 3a positive but were negative for Leu 2a, Tac, and OKT6. Intimate apposition of IL2 and OKT6 phenotypes was seen not infrequently.

In the dermis of tuberculin and lepromin reactions, the numbers of Leu 4, Leu 2a, Leu 3a, OKT6, and IL2 and anti-Tac positive cells were present in proportions similar to those found in BT lesions. However, in lepromin responses a granulomatous architecture was present, and the Leu 2a positive cells were restricted to the lymphocytic mantle about the epithelioid tubercles.

A triad of epidermal and hair follicle changes, Langerhans' cell hyperplasia, lymphocytic infiltration, and Ia expression on keratinocytes was found in tuberculoid leprosy lesions, tuberculin tests, and lepromin tests. None of these changes was observed in lepromatous lesions, and only Langerhans' cell hyperplasia was well developed in ENL. In tuberculin reactions, keratinocyte expression of Ia was found only in 96-hr specimens.

Several conclusions are of particular importance for leprosy. Because the presence of a Tac receptor means that the antigen has been presented and that IL1 has been seen, and because the numbers of Tac positive cells are similar in LL and BT patients, the evident failure of *Mycobacterium lepraе*-specific cell-mediated immunity (CMI) in tissues of LL is not a failure of antigen presentation nor an event preemptive of antigen recognition. Rather, because IL2-producing cells are significantly reduced in lepromatous as compared with BT patients, we think it likely that the *M. leprae*-specific failure of CMI in lepromatous patients is secondary to reduced, probably inhibited, IL2 production, in accord with the *in vitro* studies of Haregewoin, Godal, and their coworkers, and in the *in vitro* studies of Nogueira, *et al.* Furthermore, in ENL there is an evident de-inhibition of IL2 production. We interpret this de-inhibition of IL2 production as further evidence that ENL is a cell-mediated immune response, and suggest that the de-inhibition might be pathogenic.

Other, more general, conclusions are offered concerning the immunopathology of delayed-type hypersensitivity (DTH) response. In the dermis, both IL2 and Tac

positive cells appear to be present in DTH reactions. The triad of Langerhans' cell hyperplasia, lymphocytic infiltration, and Ia expression on keratocytes appears to be the signature of DTH upon epithelial structures.—[Departments of Dermatology and Pathology, Los Angeles County/University of Southern California Medical Center, Los Angeles, California 90033, U.S.A.]

Minauchi, Y., Tokunaga, H., Goto, M. and Suzuki, M. Immunocytochemical study of leprous neuritis—a trial of whether an immunological mechanism exists or not in progressive sensory leprous neuritis.

In general, sera from patients with lepromatous leprosy seemed to have some antibodies to the peripheral nervous system, although the titers were very weak. The antibodies were in the IgG fraction. On the contrary, sera from patients with tuberculoid leprosy and those from the normal controls had no such antibodies. There seemed to be no antibody to myelin sheaths.

The sera from patients with lepromatous leprosy seem to have antibodies to axons and Schwann cell nuclei. Antibody to axons reacted mainly with motor fibers, and antibodies to Schwann cell nuclei reacted with sensory fibers. Most of the sera had both antibodies.

The existence of these antibodies seems not to be connected with the results of seroimmunological data nor clinical and/or disease duration. The reason why such antibodies are produced in patients with lepromatous leprosy is unclear.—[National Leprosarium, Hosizuka Keiai-en, Kagoshima, Japan]

Matsuo, E., Yamada, K., Sasaki, N. and Skinsnes, O. K. Possible significance of β-glucuronidase of *M. leprae* on its permeation from blood stream to peripheral nerve. A histopathologic study of leprous neuropathy and an experimental one to support that utilizing perfusion of *E. coli* to rat iliac artery.

Leprosy is caused by the infection of a mycobacterium like tuberculosis. However, one of the major differences of leprosy from the other mycobacterioses is neuropathy caused by the neural invasion of *Mycobacterium leprae* (ML). This seems to be the result of hematogenous spreads of ML, therefore, the components of vascular walls which make up the blood-peripheral nerve barrier and which might be destroyed by something like enzymes of ML should be studied to understand the pathology of leprous neuropathy. On the other hand, ML have β-glucuronidase (B-Gase), as we reported years ago utilizing histochemistry. In this study, therefore, we wished to know 1) the presence of B-Gase labile substance (BLS) in unremarkable human peripheral nerves, 2) whether or not this BLS disappears from the vasculatures in leprous neuropathy, and 3) whether or not B-Gase carrying *Escherichia coli* can penetrate into rat peripheral nerves through blood vessels in the *in vitro* perfusion system of the rat leg modified from the isolated perfused rat kidneys of Orrenius, *et al.* In other words, the studies were conducted to determine whether B-Gase of ML plays a role in its penetration from the blood stream to endoneurium through the walls of blood vessels (VW) in peripheral nerves.

The following results were obtained: 1) In control nerves of humans and rats, the presence of BLS was observed by the positive stainings of the VW by colloidal iron stain (CI). These substances seemed to be removed markedly by treatment, either by B-Gase or hyaluronidase. 2) In leprous neuropathy, the stainings of the VW by CI seemed to be markedly diminished in all cases studied, associated with the presence of leprous lesions. 3) In rat nerves perfused with *E. coli,* the bacilli seemed to have invaded into the endoneurium.

All of the above, therefore, might suggest the importance of B-Gase of ML in the penetration from the blood stream to the endoneurium to initiate leprous neuropathy, through VW, destroying BLS which is a part of the blood-nerve barrier.—[Departments of Pathology, Kyorin University School of Medicine and National Institute for Leprosy Research, Tokyo, Japan; University of Hawaii School of Medicine, Honolulu, Hawaii, U.S.A.]

Levis, W. R., Meeker, H. C. and Schwerer, B. Serum IgM antibodies to *Mycobacte-*

rium leprae-derived phenolic glycolipid: Relationships to bacterial index and erythema nodosum leprosum.

A major problem in following patients with leprosy infections is assessing the effect of antibiotic therapy. The most widely used current method for following multibacillary patients is measuring the bacterial index (BI). Several groups have reported the ability to detect serum antibodies of the IgM class against *Mycobacterium leprae*-derived phenolic glycolipid I (PG) in leprosy patients. Cho, *et al.* (Infect. Immun. **41**: 1077–1083, 1983) showed a significant lowering of anti-PG IgM in nine patients treated for over two years with dapsone as compared to 24 newly treated patients. However, 10 of the 24 newly treated patients also showed very low anti-PG IgM antibodies. In contrast, Brett, *et al.* (Clin. Exp. Immunol. **52**: 271–279, 1983) showed relatively high anti-PG IgM in seven long-term treated LL patients. In the current study, we have measured serum IgM antibodies against phenolic glycolipid I in 104 leprosy patients and assessed the degree of correlation to the BI.

ELISA screening of sera for anti-PG IgM was performed using PG incorporated into liposomes (PG : sphingomyelin : cholesterol : dicetylphosphate, 0.1:2.0:1.5:0.2, molar ratios) for coating microtiter plates. Control liposomes were made as above but without PG. Optimal antibody binding was obtained at the very low coat concentration of 2.5 μg PG/ml. Sera were tested in duplicate at 1/20 and 1/80 dilution. Bound antibody was determined using goat anti-human IgM-peroxidase conjugate (Cappel; 1/1000 dilution) and ABTS as a substrate. Leprosy patients, clinically and histologically classified according to the Ridley-Jopling scale, consisted of 62 patients with lepromatous leprosy (LL), 15 borderline lepromatous leprosy (BL), 2 mid-borderline leprosy (BB), 21 borderline tuberculoid (BT), and 3 tuberculoid leprosy (TT). Furthermore, 14 household contacts (HC), 10 nosocomial contacts (NC), and 15 normal controls were screened. The bacterial load was measured on a semilogarithmic scale as 0, trace, 1–6+ (BI).

Serum levels of IgM antibodies to PG showed a characteristic pattern by the Ridley-Jopling classification as shown below:

	ΔE[a] (mean)	+S.D. (N[b])
LL	0.31	±0.33 (62)
BL	0.47	±0.40 (15)
BB	0.32	±0.35 (2)
BT	0.08	±0.13 (21)
TT	0.02	±0.03 (4)
HC	0.01	±0.03 (14)
NC	0	±0.02 (11)
Controls	0.03	±0.13 (15)

[a] ΔE = Extinction (E) (PG liposome coat) − E (Control liposome coat).

[b] N = number of sera tested.

In addition, our results on all patients showed a clear correlation between PG-antibodies and the bacterial index: Antibody levels were significantly higher in patients with a BI between trace and 6+ (mean ΔE = 0.43 ± 0.37; 53 sera tested) as compared to inactive (BI = 0) patients and household contacts (mean ΔE = 0.11 ± 0.16; 57 sera tested). Furthermore, the presence of erythema nodosum leprosum (ENL) in patients with a BI of 3+ or greater was found to be associated with significantly lower anti-PG IgM levels (mean ΔE = 0.31 ± 0.30; 17 patients) than in patients with comparable BI without ENL reaction (mean ΔE = 0.76 pm 0.29; 18 patients). Thus, the correlation between the anti-PG IgM and the BI was stronger when only non-ENL patients were taken into account.

We conclude: 1) Anti-PG IgM increases with a rising bacterial index. 2) Patients with erythema nodosum leprosum have lower anti-PG IgM levels than do patients without ENL and a comparable BI. This suggests anti-PG IgM is involved in the pathogenesis of ENL. 3) Further monitoring of bacillary negative patients with elevated anti-PG IgM is indicated to determine to what extent the anti-PG IgM is a reflection of bacillary persistence.—[Dermatology Department, Bayley-Seton Hospital, Staten Island, New York, U.S.A.]

Izumi, S., Sugiyama, K., Fujiwara, T. and Brennan, P. J. A novel qualitative complement fixation test for detection of anti-phenolic glycolipid-I antibodies.

We have established a new qualitative complement fixation test to detect anti-phenolic glycolipid antibody suitable for clinical and epidemiological practice. The an-

tigen for this test is a mixture of phenolic glycolipid, lecithin, and cholesterol (1:4:10); 40 times to 640 times diluted antigen was mixed with inactivated test serum and 2 units of complement, then incubated at 37°C for 60 min. Sensitized sheep red blood cells were then added to the mixture and incubated at 37°C for another 30 min. The grade of hemolysis was read with the naked eye.

By this technique, 86% of lepromatous, 38% of tuberculoid patients and 5% of occupational contacts and tuberculosis patients showed positive results. The positivity in noncontacts was about 6%.

To evaluate the reliability of the complement fixation test, the antibody titers in the same sera were tested by ELISA; 82% of lepromatous, 36% of tuberculoid, 11% of occupational contacts, and 26% of tuberculosis patients showed positive reactions. The positive rate in noncontacts was 5%.

The concordance rate of the ELISA and complement fixation test was 84.4%. These results suggest that the new complement fixation test has comparable reliability and sensitivity to the ELISA test. From the practical point of view, the complement fixation test is better than the ELISA.—[Leprosy Research Laboratory, Kyoto University School of Medicine, Kyoto; Institute for Natural Science, Nara University, Nara, Japan; Department of Microbiology, Colorado State University, Fort Collins, Colorado, U.S.A.]

Abe, M., Minagawa, F., Yoshino, Y., Miyaji, I., Ozawa, T. and Sanada, K. Anti-*M. leprae* antibody response induced by lepromin testing.

For elucidating the effect of lepromin testing on the production of anti-*Mycobacterium leprae* antibodies, the fluorescent leprosy antibody absorption (FLA-ABS) test was performed before and after skin testing with Mitsuda's type of lepromin in a total of 152 immigrants coming from leprosy endemic areas. The percentages of positive FLA-ABS tests before and after the skin test were 42.1 and 82.2, respectively, the difference being statistically significant. No case with overt or suspicious symptoms of leprosy was found, and no history of contact with leprosy patients during the lepromin test was recorded. Therefore, it is obvious that the increase of reactivity in the FLA-ABS test after the lepromin test is due to the production of anti-*M. leprae* antibodies induced by the lepromin testing and that 4 million heat-killed bacilli in the lepromin are sufficient for the production of antibody.

The Mitsuda's reaction was positive in every person of this group. The grades of the reaction, divided into a) 1+ and b) 2+ or more, did not correlate with the results of the primary FLA-ABS test nor with those of the secondary test. The grades of Mitsuda's reaction also did not correlate with the age distributions which were most frequent between 20 and 29, with a range from 8 to 62 years. A correlation between the Fernandez' and Mitsuda's reactions was not significant. However, the former was stronger in females than in males, while the latter was stronger in males than in females. On the other hand, the FLA-ABS test showed no significant differences according to age or sex in this group. These findings are compared with previous findings obtained by surveys in Okinawa and Thailand, and are discussed from an immuno-epidemiological point of view.—[National Institute for Leprosy Research, Tokyo, Japan]

Hirata, T., Shimizu, K., Ohi, S., Sanada, K., Ramasoota, T., Sampoonachote, P., Kongoebchart, K., Sampatavanich, S., Ochasanond, P. and Rungruang, S. A short preliminary report of the teichoic acid antibody in the sera of leprosy patients.

To check up and/or determine the usefulness of the teichoic acid antibody test in leprosy, we detected and measured the teichoic acid antibody by the gel-diffusion method in 319 leprosy patients and 107 contacts of leprosy patients. By the gel-diffusion assay, 52 of 152 lepromatous leprosy patients in Japan had teichoic acid precipitins in their sera. In the case of Thailanders, 0 of 1 indeterminate, 0 of 31 tuberculoid, 5 of 31 borderline and 48 of 104 lepromatous leprosy patients, and also 10 of 107 contacts had the precipitins. The precipitin line in the present test was observed only in high-titered level.—[National Institute for Leprosy Research; Tokyo Women's Medical College; National Leprosarium Tama Zensho-en, Tokyo, Japan; Leprosy Division, Bangkok, Thailand]

Kikuchi, I., Ozawa, T., Sanada, K., Koseki, M. and Sasazuki, T. Immunogenetic analysis of leprosy in the Japanese (second report).

The genetic control of the susceptibility to and the clinical manifestations of leprosy was investigated using 66 unrelated patients with leprosy and 128 (89 affected and 39 nonaffected) members of 28 multiple-case families. In 66 unrelated patients with leprosy, HLA-DR2 and MT1 were significantly increased (relative risk = 3.76, χ^2 = 16.60 for DR2; relative risk = 4.09, χ^2 = 11.10 for MT1) compared to healthy controls. HLA-DR2 and MT1 were significantly increased (relative risk = 3.95, χ^2 = 11.10; relative risk = 18.62, χ^2 = 12.64, respectively) in 34 tuberculoid patients. In 32 lepromatous patients, HLA-DR2 was significantly increased (relative risk = 3.57, χ^2 = 8.97). Out of 28 sibpairs affected with leprosy, 9 sibpairs shared two HLA haplotypes identical by descent, 17 sibpairs shared one HLA haplotype identical by descent, and 2 sibpairs were HLA nonidentical. These haplotype distributions shared by affected sibpairs differed from the random distribution (χ^2 = 4.78, D.F. = 2, $0.09 < p < 0.10$). It was therefore suggested that the susceptibility to leprosy was controlled by HLA-linked gene(s). Out of 19 sibpairs affected with lepromatous leprosy, 8 sibpairs shared two HLA haplotypes identical by descent and 11 sibpairs shared one HLA haplotype identical by descent. These haplotype distributions differed from the random distribution (χ^2 = 7.20, D.F. = 2, $p < 0.03$), suggesting the existence of an HLA-linked major gene for lepromatous leprosy. This method, however, could not distinguish between the recessive model with gene frequency of 0.48 and the dominant model with gene frequency of 0.00 for the HLA-linked disease susceptibility gene for lepromatous leprosy.

Tuberculoid patients showed a vigorous immune response to *Mycobacterium leprae* antigen *in vitro*; whereas lepromatous patients did not show any response to this antigen at all. The nonresponsiveness of lepromatous patients was antigen specific since even the patients with lepromatous leprosy showed a strong response to streptococcal cell-wall antigen or PPD. The antigen-specific nonresponsiveness to *M. leprae* antigen was controlled by T lymphocytes but not monocytes because T lymphocytes from tuberculoid patients showed a strong response to this antigen in the presence of allogeneic monocytes from HLA-DR identical or haploidentical lepromatous patients; whereas T lymphocytes from lepromatous patients failed to respond to this antigen even in the presence of allogeneic monocytes from HLA-DR identical or haploidentical tuberculoid patients. Furthermore, it was demonstrated that the antigen-specific suppressor T lymphocyte ($Leu2^+3^-$) was present in relapsed lepromatous patients. These observations suggest that an HLA-linked gene controlled the clinical manifestation of leprosy through immune regulation by T lymphocytes.—[Department of Human Genetics, Medical Research Institute, Tokyo Medical and Dental University; National Institute for Leprosy Research; National Leprosarium Tama-Zensho-en, Tokyo, Japan]

Mohagheghpour, N., Sasaki, D. T., Brennan, P. J., Engleman, E. G. and Gelber, R. H. Effects of recombinant IL2 on the *Mycobacterium leprae*-induced T cell: Response in lepromatous leprosy.

It is generally conceded that in patients with lepromatous leprosy anergy to *Mycobacterium leprae* antigens, as judged by *in vivo* skin test and *in vitro* lymphocyte proliferation, is specific and persistent. Supporting the specificity of unresponsiveness is the observation that many lepromatous patients exhibit positive cell-mediated responses to antigens from other mycobacterial species, most of which are crossreactive with those of *M. leprae*. Although several explanations, including defective macrophage functions, absence of *M. leprae*-reactive T cells from the circulation, and active suppression, have been offered to explain the selective unresponsiveness of lepromatous patients, the mechanisms underlying the depression of cell-mediated responses have yet to be elucidated.

In recent years, evidence has accumulated that T lymphocytes once activated by antigen stimulate the adherent cells to secrete interleukin 1 (IL1). In turn, IL1 and antigen trigger a T cell (which may or may not be

the same T cell) to secrete interleukin 2 (IL2) into their immediate environment, thus expanding the appropriate clone of T cells. Preparations enriched in the lymphokine IL2 have been shown to significantly augment *in vivo* immune responses following administration in animals and enhance some deficient immune responses in patients with acquired immunodeficiency syndrome (AIDS). Moreover, supernatants of mitogen-stimulated lymphocytes, known to contain IL2, have been shown to restore the proliferative response of lymphocytes from a number of lepromatous patients to leprosy bacilli. Although these findings are provocative and suggest that the specific unresponsiveness of lepromatous patients is not due to the absence of circulating antigen-reactive T cells, it is not known whether the effects were due to IL2 or to other substances present in the conditioned medium. We have studied the effects of pure human recombinant interleukin 2 (rIL2) produced by *Escherichia coli* containing the cloned human gene, provided by Cetus Corporation, Emeryville, California, U.S.A., on the *in vitro* proliferation of lymphocytes of lepromatous patients.

Thirty-three patients (21 BL and LL, and 12 BT and TT) were studied. Among the lepromatous patients, six showed an inversion of helper : suppressor (Th:Ts) ratio. In addition, the percentage of circulating cells expressing the natural killer (NK) cell associated Leu 7 determinants, as well as HLA-DR antigens, was elevated in leprosy patients as compared to healthy controls. Since leprosy patients do not have increased numbers of B cells, these results suggest that they may have an increased number of circulating HLA-DR + T cells. Characteristic anergy to *M. leprae* antigens was present in lepromatous patients (mean stimulation index: lepromatous = 1.5 ± 0.3 S.D.; tuberculoid = 6.0 ± 3.5 S.D.). Recombinant IL2 augmented the proliferative activity of peripheral blood lymphocytes (PBL) from all patients regardless of the presence or absence of antigenic stimuli (whole *M. leprae* or its sonicate, PPD, or tetanus toxoid). Treatment of PBL from lepromatous patients or healthy controls with various doses of rIL2 (1–100 μ/ml) increased the uptake of ^{3}H-thymidine significantly and to an equivalent level. Thus, while rIL2 augments the response nonspecifically, it does not specifically restore the response of most patients with lepromatous leprosy to *M. leprae* antigens.—[Stanford University, Stanford, California; Seton Medical Center, Daly City, California, and National Hansen's Disease Center, Carville, Louisiana; Colorado State University, Fort Collins, Colorado, U.S.A.]

Brennan, P. J. Diagnosis of leprosy based on the 3,6-di-*O*-methyl-β-D-glucopyranosyl epitope; assay of antibodies with natural and synthetic probes; assay of antigen by chemical and immunological means.

The unique trisaccharide, 3,6-di-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-methyl-α-L-rhamnopyranosyl-(1 → 2)-3-*O*-methyl-α-L-rhamnopyranose is the *M. leprae*-specific segment of a phenolic phthiocerol-containing glycolipid (phenolic glycolipid I) ([5]) which is highly selective for the serodiagnosis of leprosy ([1, 2, 8]). We have recently described the synthesis and serological activity of the trisaccharide and its inherent terminal disaccharide ([4]). Both were equally active in inhibiting binding between the phenolic glycolipid and antiglycolipid IgM from human leprosy sera, whereas analogous oligosaccharides in which the terminal sugar was 6-*O*-methyl-β-D-glucopyranose or β-D-glucopyranose, were much less active or entirely inactive, respectively ([4]). This and other evidence involving the use of phenolic glycolipid II and III and partially deglycosylated phenolic glycolipid I, demonstrated that the 3,6-di-*O*-methyl-β-D-glucopyranose is the hapten determinant of the species-specific glycolipid ([4]). Work by others has shown that a monoclonal IgM and IgG to phenolic glycolipid I react primarily with the distal 3,6-di-*O*-methyl-β-D-glucopyranose ([7,9]). In more recent work the ploy of reductive amination of the synthetic disaccharides was used to conjugate the intact 3,6-di-*O*-methyl-β-D-glucopyranose epitope, or 6-*O*-methyl-β-D-glucopyranose, or β-D-glucopyranose, to protein, which glycoconjugates were compared with one another and with the native glycolipid in their applicability to the serodiagnosis of leprosy ([3]). An alternative synthetic strategy in which only the 3,6-di-*O*-methyl-β-D-glucopyranosyl epitope was used is summarized as follows:

Commercially available 3-*O*-methylglucose was converted to its allyl-glycoside, tritylated at the primary hydroxyl position and benzylated to give allyl 2,4-di-*O*-benzyl-3-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside. Detritylation followed by methylation at the -6- position gave the 3,6-di-*O*-methyl derivative. Removal of the protecting groups and purification gave crystalline 3,6-di-*O*-methyl glucose in overall 60% yield. Condensation of 8-methoxyoctanol (6) with acetobromo-3,6-di-*O*-methylglucose gave 8-methoxycarbonyloctyl-2,4-di-*O*-methyl-β-D-glucopyranoside in 65% yield. The deacylated product was converted to the crystalline hydrazide and coupled to bovine gamma globulin (BGG) or poly-D-lysine (PDL) via intermediate acylazide formation. The products were highly sensitive in ELISA and showed good concordance with the native glycolipid in the analysis of sera from 185 patients throughout the granulomatous spectrum of leprosy; the correlation coefficients between the monosaccharide-BGG and monosaccharide-PDL and the glycolipid were 0.761 and 0.818, respectively. These results indicate that highly sensitive and specific antigen probes for serodiagnosis of leprosy can be constructed based on the single sugar epitope only.

Both the phenolic glycolipid and the related diacylphthiocerol are present in large quantities on the surface of *M. leprae,* in infected tissues, and in serum and biopsy specimens from leprosy patients. Untreated and relapsed lepromatous leprosy is associated with high level constant bacteremia. It has been hypothesized that at some early stage, perhaps following a pneumonitis, a bacteremia occurs. The timing and duration of this bacteremia (antigenemia) is yet to be determined, but since the incubation period of leprosy is a minimum of two years and can at times be 15 or more years, bacteremia likely occurs long before clinical signs of disease. Because individuals residing in leprosy-endemic areas frequently develop signs of delayed-type hypersensitivity, lymphocyte transformation to *M. leprae,* and rising antibody titers, but do not develop clinical leprosy, it may be that bacteremia and its associated antigenemia occur in individuals who do not develop clinical leprosy. Defining and quantitating the duration and timing of circulating antigenemia, and precisely circulating phthiocerol-containing compounds, when correlated with humoral antibody may together best define the pathobiology of incubating leprosy.

We have now developed chemical and immunological procedures for the quantitation of phenolic glycolipid I in patient sera. Sera (0.5–1 ml) were extracted with $CHCl_3$/CH_3OH and fractionated on short columns of silicic acid. Direct TLC with a sensitivity of *ca.* 0.5 μg allowed detection of phenolic glycolipid I in untreated lepromatous and borderline patients, and HPLC allowed quantitation; 0.8–3.7 μg/ml serum from 4 patients. An ELISA inhibition assay using anti-glycolipid polyclonal antibodies corroborated these figures. Dot-ELISA on nitrocellulose using both polyclonal and monoclonal anti-glycolipid antibodies allowed for greater sensitivity (0.5 ng) and semi-quantitative evaluation. Among patients throughout the disease spectrum, the levels of glycolipid and anti-glycolipid IgM were generally in accordance. In sera obtained serially from a patient undergoing chemotherapy, the glycolipid peaked earlier than antibody and was cleared sooner. Thus, data on both antigen and antibody may present a broader picture of the status of *M. leprae* and its products throughout the course of leprosy.—[Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.]

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Fujiwara, T., Izumi, S. and Brennan, P. J. A correlation between the structure of phenolic glycolipid and its antigenic activity and synthesis of the derivatives for development of serodiagnosis of leprosy.

The disaccharides of phenolic glycolipid I, 3,6-OMe_2-β-D-Glc*p*(1-4)2,3-OMe_2-α-L-Rha*p*, with various kinds of linker arm (benzyl alcohol, methyl 3-(*p*-hydroxyphenyl)propionate, lauryl alcohol and stearyl alcohol) and the disaccharides with modified glucose residue were chemically synthesized and their sero-reactivities were tested by ELISA inhibition assay.

Disaccharides with benzyl alcohol and methyl 3-(*p*-hydroxyphenyl)propionate had almost equal activities as free natural disaccharide. Disaccharides with 3-phenylpropanol and stearyl alcohol showed relatively high activities and disaccharide with lauryl alcohol showed high activity. From the results of ELISA inhibition assay, the possibility that there were active structures other than the natural one was suggested.

Disaccharides with lauryl alcohol, benzyl alcohol, methyl 3-(*p*-hydroxyphenyl)propionate and stearyl alcohol were active in direct ELISA and disaccharide with β-linked lauryl alcohol had high activity.—[Institute for Natural Science, Nara University, Nara, Japan; Leprosy Research Laboratory, Kyoto University School of Medicine, Kyoto, Japan; Department of Microbiology, Colorado State University, Fort Collins, Colorado, U.S.A.]

Douglas, J. T. and Steven, L. M. Comparison of natural and synthetic antigens for early detection of leprosy.

We have compared the reactivity of *Mycobacterium leprae,* phenolic glycolipid I (PGI) antigens, and a synthetic antigen, representing the terminal sugar of PGI which is a monosaccharide conjugated to bovine gamma globulin (mBGG), with 145 samples of eluates from blood-soaked disks collected in the Ponape district of the Federated States of Micronesia. These samples consist of the periodic collection of finger-prick blood since 1980, and constitute 43 cases of clinical leprosy which have developed since the initial bleeding. We also have examined samples collected from individuals who subsequently became suspects (biopsies pending). In addition, the antigens were examined for reactivity with our 12 standard sera and pooled lepromatous sera from our Hawaiian sera bank. Finally, we have compared these antigens for sensitivity in a biotin-avidin amplified ELISA (AE = amplified ELISA).

This preparation of monosaccharide BGG (mBGG) contained a protein-to-carbohydrate ratio of 1:48 M. It was prepared at a protein concentration of 3.1 mg/ml, and we determined that a 1:4500 dilution (or 0.69 μg/ml) produced reactivity comparable to 4 μg/ml of PGI. Thus, mBGG is approximately sixfold more reactive than PGI (on a weight basis) when applied to microtiter plates in our volatile antigen-coating buffer (0.01 M ammonium acetate/carbonate, pH 8.2). And, the mBGG antigen was found to be more reactive than the whole *M. leprae* antigen in relation to positive control sera, and less reactive with our negative sera controls. The mBGG antigen also tolerates Tween 20 (which lowers the background), while the use of Tween 20 is not possible with PGI (it is washed off the plastic plate). The AE system was found to be at least four times more sensitive for the three antigens than the unamplified ELISA. From our results with Ponape samples, it appears that the synthetic mBGG antigen coupled with AE will be useful in detecting clinical leprosy and should provide a valuable placement for PGI. The natural and synthetic antigens were kindly provided by Dr. P. J. Brennan, Colorado State University.—

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Young, D. B., Khanolkar, S. R. and Buchanan, T. M. Multiple strategies for the identification of antigen and species specificity of monoclonal antibodies reactive with *Mycobacterium leprae.*

Monoclonal antibodies are powerful tools for analysis of the antigens of *Mycobacterium leprae.* Antibodies recognizing epitopes specific to leprosy bacilli can be used for specific detection of *M. leprae* antigens in lesions and may also be useful in differentiating these bacilli from other related mycobacteria. Immunoaffinity chromatography with monoclonal antibodies may be useful in preparation of purified *M. leprae* antigens which can then be tested for activity in assays of cell-mediated immunity and possibly incorporated into (or removed from) future vaccine preparations.

Our laboratory has generated and characterized 100 monoclonal antibodies to *M. leprae* during the past two years. In addition, we have participated in characterization of the WHO IMMLEP monoclonal bank. Several strategies have emerged from this experience which are illustrated below.

Screening of antibodies. Antibodies to the surface of *M. leprae* can be detected by binding whole organisms to microtiter plates for ELISA tests or by using microscope slides with immunofluorescent or immunoperoxidase detection. Most of the monoclonal antibodies available at present are not directed to surface-exposed antigens. Radioiodination experiments suggest that the surface of *M. leprae* is coated with large amounts of lipids which may mask potential protein antigens which are exposed on the surface of other mycobacteria.

In order to expose internal antigens of *M. leprae,* it is convenient to disrupt the bacilli by ultrasonication followed by centrifugation to prepare "soluble" and "insoluble" fractions. Antigen can then be coated either to plastic or nitrocellulose supports to screen for antibody interaction in ELISA or radioimmunoassays. Plastic supports have conveniently low background binding properties but nitrocellulose has a higher binding capacity which can be useful in the case of epitopes present at low concentrations in the heterogeneous antigen mixture. Coating conditions (pH, ionic strength) can affect the amount of absorption of particular antigens to plastic but binding to nitrocellulose is relatively independent of such factors.

Species specificity. Species specificity can be tested by coating antigen from different mycobacteria under the same conditions as those described above. A particularly useful screening procedure is to carry out a "dot-blot" test with antigen from different mycobacteria spotted onto nitrocellulose strips. Strips are incubated with monoclonal antibody and then with labeled secondary antibody. An autoradiograph reveals a series of spots for crossreactive antibodies but only a single spot for *M. leprae*-specific antibodies.

Identity of antigen. Antibodies directed to protein antigens can be recognized by susceptibility of the antigen to protease digestion prior to the assay. A useful criterion for distinguishing between different protein antigens is to determine the subunit molecular weight by polyacrylamide gel electrophoresis in the presence of SDS. Two methods are available for this: a) gel-immunoradioassay (GIRA) involves slicing the gel into 50 micron sections which are sufficiently thin to allow direct interaction of the antibody with antigen, and b) Western Blot involves electrophoretic transfer of antigens onto a nitrocellulose support for antibody interaction. In our experience, GIRA is more convenient for preliminary screening of monoclonal antibodies but Western Blot is useful as a second test—particularly for low molecular weight antigens. In some cases, antigenic determinants are destroyed by the detergent treatment required for these tests. A convenient method for separation of non-denatured antigens is the Beckman Microzonal system. This involves a rapid electrophoresis on cellulose acetate support and antigens can subsequently be detected without the necessity for transfer to nitrocellulose. A drawback with the technique is that it gives no molecular weight data.

Monoclonal antibodies to lipid antigens can be tested on lipid samples separated by thin-layer chromatography, and this technique has been used to characterize antibodies to the major phenolic glycolipid of *M. leprae.*

Antibodies available to *M. leprae.*

Screening of monoclonal antibodies from our laboratory and those supplied by other laboratories indicates the availability of monoclonals to at least 8 different molecules of the leprosy bacillus. IgM and IgG monoclonal antibodies have been prepared to the major phenolic glycolipid. These antibodies are specific for *M. leprae* and are the only monoclonal antibodies currently available which are both specific and react with the surface of the organisms. A number of antibodies appear to react with carbohydrate, lipid or glycolipid cell wall-associated antigens. These antibodies show a characteristic pattern of broad crossreactivity with other mycobacteria, and at least some recognize determinants on mycobacterial arabinomannan. Several antibodies react with a peptide with molecular weight 65–68K protein. Antibodies are also available which react with peptides with molecular weights 36K, 28K, 18K, and 12K. In the case of the 65–85K group and the 28K peptide, a number of antibodies are available which recognize separate specific and crossreactive epitopes present on the same polypeptide chain. For the 36K, 18K, and 12K peptides, only antibodies to *M. leprae*-specific epitopes are currently available.

These monoclonal antibodies are likely to prove useful for dissection of the human cell-mediated immune response to the leprosy bacillus, as well as for recombinant DNA approaches to the production of *M. leprae* antigens.—[Immunology Research Laboratory, Pacific Medical Center, Seattle, Washington 98144, U.S.A.]

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Dr. Masahide Abe, Chairman, Japanese Leprosy Panel, and Dr. Robert C. Hastings, Chairman, U.S. Leprosy Panel.

CLOSING REMARKS

Dr. Tokunaga, Dr. Goren, Dr. Abe, Ladies and Gentlemen:

On behalf of the U.S. Leprosy Panel and participants, I would like to express our deepest appreciation to Dr. Abe, to the members of the Japanese Leprosy Panel, and to the staff of the National Institute for Leprosy Research here in Tokyo, for their many acts of kindness and for again superbly organizing a U.S.–Japan Leprosy Research Conference. This year, as in the United States last year, we are again meeting with our colleagues in tuberculosis. Today we have had the opportunity to share information on the immunology of the two diseases and we look forward, for the first time, to being able to attend the remainder of the Joint Research Conference on Tuberculosis over the next two days.

It has been a great pleasure to have had the opportunity of participating in this, the Nineteenth Joint Conference on Leprosy Research of the U.S.–Japan Cooperative Medical Science Program. In keeping with the tradition of the U.S.–Japan Leprosy Research Conferences, we have, once again, been brought to the forefront of knowledge about this ancient disease. Steady progress is evident on both sides of the Pacific in the microbiology of *M. leprae,* in experimental leprosy and its therapy, in drugs and immune responses, in tissue damage and antibodies, and in the immunology of leprosy. Through presentations, formal questioning, and informal discussions, we have again learned a great deal from each other over the past three days.

We have enjoyed the opportunity of renewing many old and deep friendships, and we have enjoyed the opportunity of beginning new friendships. Such relationships and such sharing of knowledge form the basis for international cooperation in research. Clearly leprosy is an international disease. Its victims deserve our very best international efforts to provide new knowledge on how to better control, better cure, better prevent, and to eventually eradicate this disease.

It has been a great privilege to see and to be a part of this effort over the past three days. On behalf of the U.S. participants, I would like to thank our Japanese colleagues for the knowledge you have shared with us, for your gracious hospitality, and for the renewed inspiration you have given us by your example. I look forward to welcoming you to our country next year for the Twentieth U.S.–Japan Leprosy Research Conference.

Thank you very much.

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