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ABSTRACTS

TWENTIETH JOINT LEPROSY RESEARCH CONFERENCE

National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland, U.S.A.
12–13 August 1985

U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

Good morning, Ladies and Gentlemen:

It is again a great privilege for me to have the honor of welcoming you to a U.S.–Japan Leprosy Research Conference. It is a joy to welcome our Japanese friends and co-workers to the National Institutes of Health and to renew our friendships since we last met in Tokyo last year. This is a particular pleasure since it marks the twentieth anniversary of our association together as part of the U.S.–Japan Cooperative Medical Science Program. Few bilateral government programs have even lasted for a generation. Even fewer have been consistently productive. Virtually no other program has witnessed the revolution that has occurred in our understanding of leprosy as reflected in these conferences—a revolution due, in large part, to the encouragement, visibility, and support provided by the U.S.–Japan Cooperative Medical Science Program.

As we look back over these 20 years of incredible progress, it is appropriate that we remember our leaders and teachers who have so greatly influenced us over these many years. Tragically, two legendary men in leprosy research whom we were privileged to know as colleagues will no longer be joining us at these conferences.

Professor Mitsugu Nishiura expired on the 18th of January this year. For over 30 years at Kyoto University, Prof. Nishiura pioneered in the electron microscopy of leprosy. We will miss his meticulous research, his patient teaching, and his warmth as a personal colleague.

Exactly one month after the loss of Prof. Nishiura, on the 18th of February this year, Dr. Charles Shepard of the Centers for Disease Control in Atlanta died unexpectedly. Chairman of the U.S. Leprosy Panel from its inception in 1965 until 1977, Dr. Shepard was best known for his landmark discovery that *Mycobacterium leprae* are capable of multiplying in the foot pads of mice—a discovery which created the whole field of experimental leprosy. We will miss his painstaking research, his gentle guidance, and his quiet joy of life.

As both Prof. Nishiura and Dr. Shepard would have wanted, we are again privileged to have enjoyed a year of excellent progress in leprosy research on both sides of the Pacific, as judged by the outstanding group of papers which are to be presented here. We are delighted to have the opportunity of meeting in conjunction with our esteemed colleagues in tuberculosis to once more exchange ideas and share common problems in our understanding of the two major mycobacterial afflictions of mankind.

Finally, I would like to express our deep appreciation to Dr. Bill Jordon, Dr. Darrel Gwinn, and to the staff of the U.S.–Japan Cooperative Medical Science Program for yet another superbly organized U.S.–Japan Leprosy Research Conference this year. I look forward very much to the rest of the week.

Thank you very much.

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

PROGRAM
TWENTIETH JOINT LEPROSY CONFERENCE

12 August 1985, Monday

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

Session I

Co-Chairmen: Dr. Hiroko Nomaguchi
Dr. Zanvil Cohn

Nomaguchi, H., Kohsaka, K., Miyata, Y., Mori, T. and Ito, T. Induction of gamma interferon with various mitogens in mice pretreated with *M. lepraemurium* and the effect of *in vivo* interferon production on the growth of *M. lepraemurium* in mice

Modlin, R. L., Mehra, V., Fujimiya, Y., Wong, L., Horwitz, D., Rea, T. H. and Pattengale, P. Lepromin-induced suppressor activity in lymphocytes cultured from leprosy skin lesions

Kaplan, G., Weinstein, D. E., Steinman, R. M., Nathan, C. F., Levis, W. R. and Cohn, Z. A. The spectrum of *in vitro* T-cell responsiveness in lepromatous leprosy

Uyemura, K., Modlin, R. L. and Rea, T. H. *In vitro* cyclosporin A restoration of lepromin-induced suppressor activity in patients with erythema nodosum leprosum

Izaki, S., Hsu, P.-S., Tanji, O., Hibino, T., Tokairin, S., Ohkuma, M. and Izaki, M. Plasminogen activator associated with hypersensitivity granulomas in murine leprosy

Truman, R. W., Shannon, E. J. and Hastings, R. C. Host responses to the phenolic glycolipid-I antigen of *M. leprae*

Session II

Co-Chairmen: Dr. Tsuyoshi Fujiwara
Dr. Patrick Brennan

Jacobs, W. R., Clark-Curtiss, J. E., Docherty, M. A. and Curtiss, R., III. Identification of cloned *Mycobacterium leprae* genes in *Escherichia coli*

Cocito, C. Comparative properties of *Mycobacterium leprae* and leprosy-derived corynebacteria

Hunter, S. W., Gaylord, H., Stewart, C. and Brennan, P. J. Isolation and preliminary characterization of the major intracellular carbohydrate-containing antigens of *Mycobacterium leprae*

Miller, R. A., Harnisch, J. P., Gilliland, B. C., Lukehart, S. A. and Buchanan, T. M. The molecular basis of positive treponemal antibody, antinuclear antibody and immune complex tests among Hansen's disease patients

Tsutsumi, S. and Gidoh, M. On a role of antileprotic agents as the scavengers of active oxygen radicals

13 August 1985, Tuesday

Session III

Co-Chairmen: Dr. Seiichi Izaki
Dr. Thomas Buchanan

Abe, M., Miyaji, I., Minagawa, F. and Yoshino, Y. Salivary anti-*M. leprae* antibodies of household contacts and inhabitants in a leprosy-endemic area

Cho, S.-N., Chatterjee, D., Gelber, R. H., Rea, T. H. and Brennan, P. J. A diverse approach to the serodiagnosis of leprosy based on the phenolic glycolipid

Buchanan, T. M. Species-specific epitopes on protein molecules of *Mycobacterium leprae* or *M. tuberculosis*: elucidation with monoclonal antibodies

Mohagheghour, N., Cho, S.-N., Gelber, R. H., Foung, S. K. H. and Engleman, E. G. Production of human monoclonal antibodies to *Mycobacterium leprae*

Gillis, T. P. Affinity purification of a 65,000 dalton protein *Mycobacterium gordonae* using a monoclonal antibody which recognizes a shared epitope on the homologous protein of *Mycobacterium leprae*

Tung, K. S. K., Nelson, K., Rubin, L., Wagner, D., Umland, E., Schauf, V., Scollard, D., Vithayasai, P., Vithayasai, V. and Worobec, S. Serum soluble interleukin-2 receptors in leprosy patients

Session IV

Co-Chairmen: Dr. Kazunari Nakamura
Dr. Thomas Rea

Nakamura, K. and Yogi, Y. The athymic rodent as an experimental lepromatous leprosy model (continued): effect of genetic background of nude rats and age of ICR nude mice

Chehl, S. K., Shannon, E. J., Krahenbuhl, J. L., Job, C. K. and Hastings, R. C. Adoptive transfer of cell-mediated immunity in *M. leprae*-infected nude mice with *M. leprae*-immunized allogeneic leukocytes depleted of Thy-1.2-bearing cells and Lyt-2.2-bearing cells

Tung, K. S. K., Teuscher, C., Koster, F., Yanagahara, D., Umland, E. and Brennan, P. Strain variation in murine immune response to the *M. leprae* glycolipid antigen, phenolic glycolipid-I

Eustis-Turf, E., Benjamins, J. A. and Lefford, M. J. Neural auto-antibodies in leprosy

Job, C. K., Harris, E. B., Allen, J. L. and Hastings, R. C. A possible mode of transmission of armadillo leprosy in the wild and a simple method to conduct a random survey of its prevalence

Thomas, D. A., Mines, J. S., Mack, T. M., Thomas, D. C. and Rea, T. H. Armadillo exposure among Mexican-born patients with lepromatous leprosy

14 August 1985, Wednesday

Combined Leprosy and Tuberculosis Symposium
Section II. Immunology of Leprosy and Tuberculosis

Co-Chairmen: Dr. Masahide Abe
Dr. Robert Hastings

Cohn, Z. A., Kaplan, G., Steinman, R. M., Witmer, M. and Nath, I. The role of lymphokines in cell-mediated immunity

Goren, M. B., Bruyninckx, W. J., Leung, K.-P., Swendson, L. S., Heifetz, L. and Fiscus, J. Functionality of secondary lysosomes in murine resident peritoneal macrophages

Fujiwara, T., Izumi, S. and Brennan, P. J. The synthesis and activity of the sugar derivatives related to phenolic glycolipids of *Mycobacterium leprae* and preparation of sugar-protein conjugates

Mehra, V., Sweetser, D., Bloom, B. R. and Young, R. Recombinant DNA clones expressing *M. leprae* and *M. tuberculosis* antigens: potential new tools for immunodiagnosis and prophylaxis

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

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ABSTRACTS OF LEPROSY CONFERENCE

Nomaguchi, H., Kohsaka, K., Miyata, Y., Mori, T., Ito, T., Nagata, K., Okamura, H. and Shoji, K. Induction of gamma interferon with various mitogens in mice pretreated with *M. lepraemurium* and the effect of *in vivo* interferon production on the growth of *M. lepraemurium* in mice.

Interferon (IFN) is produced in antigen-sensitized mice after challenge with antigen

or mitogens. We tried to see the IFN production in mice sensitized with *Mycobacterium lepraemurium* (MLM) and the attenuated MLM after challenge with lipopolysaccharide (LPS) and staphylococcal enterotoxin A (SEA).

In vivo production of IFN was induced by the challenge with LPS in C57BL/6 mice pretreated 2 weeks previously with MLM *in vivo* (10 mg net weight i.p.). IFN activity appeared in the blood around 2 hr after challenge with LPS, and reached a maximum at about 5 hr. These IFN were characterized as to their molecular types by a neutralization test with anti-IFN-α,β antiserum. The IFN at 2 hr post-challenge was mostly α,β-type; those at 5 hr were predominately γ-type.

The enhanced production of IFN by SEA or LPS reached a maximum 2 weeks after treatment with MLM *in vivo*. At this time, a huge hepatosplenomegaly was observed. Spleen weight and liver weight reached a maximum about 2 weeks after injection of MLM *in vivo*. The enhanced production of IFN was observed not only by the pretreatment with MLM *in vivo* but also by MLM Ogawa 7th, 8th, 84th and heated MLM (56°C for 30 min) in C57BL/6 mice.

The induction of IFN was then tested in CBA mice. However, no production of IFN was observed by the same treatment in these mice, showing that C57BL/6 mice are high responders and CBA mice are low responders in the production of IFN.

The effect of *in vivo* production of IFN on the survival time of mice infected with MLM was also tested. C57BL/6 mice inoculated with MLM (10 mg net weight i.p.) died of heavy murine leprosy after 3–4 months. The C57BL/6 mice were challenged with SEA or LPS to induce lymphokine *in vivo,* including IFN. However, an effect of the SEA or LPS challenge was not observed on the survival time of mice infected with a large dose of MLM, although the survival time of a very few mice in the group challenged with LPS or SEA was prolonged from 1 month to 1 year.

IFN-alpha beta material was injected intravenously 4 times (12,000 IU each) into mice infected with a large dose of MLM. However, the survival time was not changed by the treatment of IFN α,β exogenously.

MLM Ogawa 84th isolated from smooth-growing colonies lost their ability to produce a large leproma in mice (attenuated bacilli). However, their ability to induce IFN after challenge with mitogens was retained well, as shown in this paper. The effect of the attenuated MLM (MLM Ogawa 84th) on the survival time of mice infected with MLM *in vivo* was tested. MLM *in vivo* and MLM Ogawa 84th were co-injected (i.p.) and the mice challenged with LPS. An effect of the attenuated MLM on the survival time of mice infected with MLM *in vivo* was not observed by this method.—[Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita-shi, Osaka; Hyogo College of Medicine, Mukogawa, Nishinomiya-shi, Hyogo, Japan]

Modlin, R. L., Mehra, V., Fujimiya, Y., Wong, L., Horwitz, D., Rea, T. H. and Pattengale, P. K. Lepromin-induced suppressor activity in lymphocytes cultured from leprosy skin lesions.

Infectious granulomas are unique aggregates of lymphocytes and macrophages cooperating in an immune response to invading microorganisms. The tuberculoid leprosy granuloma is successful in eliminating *Mycobacterium leprae,* whereas the lepromatous leprosy granuloma is not. The mechanism of unresponsiveness of the lepromatous granuloma has been inferred from results of peripheral blood studies and immunoperoxidase studies of tissue sections. Peripheral blood studies have been controversial in determining a role for suppressor lymphocytes in lepromatous leprosy. Although immunoperoxidase studies have revealed an excess of suppressor/cytotoxic phenotypes in lepromatous but not in tuberculoid granulomas, the actual functional properties of these cells are unknown.

We sought to study the functional properties of lymphocytes derived from leprosy granulomas. Because sufficient numbers of lymphocytes for functional study could not be obtained directly from skin lesions, we set up short-term cultures as follows: Skin biopsy specimens were obtained from 3 tuberculoid and 5 lepromatous patients. Cells were extracted through surgical mesh and sorted with monoclonal antibodies, T4 and T8 (Coulter Electronics, Hialeah, Florida, U.S.A.), using FACS. The percentages of

lesion-derived, T-lymphocyte phenotypes were more similar to those reported by immunoperoxidase methods than those obtained by simultaneous measurement of peripheral blood. Therefore, we were confident that the cells we collected were predominantly from tissue granulomas and not from within blood vessels. Ten to 1000 cells were seeded per well and cultured in the presence of interleukin-2 (Electonucleonics, Inc., Silver Spring, Maryland, U.S.A.) and irradiated allogeneic feeder lymphocytes. Using these techniques, we were able to obtain 30 million cells from each starting well. Cultured cells were washed free of feeders and the phenotype redetermined. In >90% of the cultures, the final T4 or T8 phenotype was that of the original seeded cells. A portion of cultured cells was assayed for lepromin-induced suppression. Suppression was determined by adding 5×10^4 cultured cells to 2×10^5 normal peripheral blood mononuclear cells. Dharmendra lepromin (1:10) and concanavalin A (ConA, 2.5 μg/ml) were added and ^{3}H-thymidine incorporation was measured at 3 days with suppression calculated as:

$$100 - \frac{\text{cpm, lepromin + ConA}}{\text{cpm, ConA}} \times 100.$$

The results obtained on some of these cultures are presented below.

Culture no.	% T4	% T8	% lepromin-induced suppression of ConA
L1-2[a]	4	99	42
L1-4	0	89	28
L5-7	1	99	39
L5-1	96	5	22
T1-1[b]	25	50	−132
T2-3	17	93	−47
T2-4	8	97	−57

[a] L = lepromatous.
[b] T = tuberculoid.

The results indicate that although suppressor/cytotoxic phenotypes are present in both tuberculoid and lepromatous granulomas, lepromin-induced suppressor cells were found only in cells derived from lepromatous granulomas. We hypothesize that the intimate association of these lepromin-induced suppressor cells with other lymphocytes and macrophages contributes to the inability of cells in the lepromatous granuloma to eliminate infection.—[Departments of Dermatology and Pathology, University of Southern California School of Medicine, Los Angeles, California, U.S.A.; Departments of Immunology and Microbiology, Albert Einstein College of Medicine, Bronx, New York, U.S.A.]

Acknowledgment. This work was supported by grants from the National Hansen's Disease Center, the National Institutes of Health, the World Health Organization (IMMLEP), The Heiser Program for Research in Leprosy, and a gift from the Military and Hospitaller Order of St. Lazarus of Jerusalem.

Kaplan, G., Weinstein, D. E., Steinman, R. M., Nathan, C. F., Levis, W. R. and Cohn, Z. A. The spectrum of *in vitro* T-cell responsiveness in lepromatous leprosy.

The lesions of lepromatous leprosy patients are characterized by extensive replication of *Mycobacterium leprae* within dermal macrophages. Blood monocytes from lepromatous patients appear to be activated normally by lymphokines. When peripheral blood mononuclear leukocytes from lepromatous patients are challenged with *M. leprae in vitro* proliferation and lymphokine production by T cells are impaired. The cause(s) of this anergy is as yet unclear.

In this study we have examined further the *in vitro* response of T cells to *M. leprae.* In addition, the effects of exogenous recombinant interleukin-2 (IL-2) and suppressor cell populations on the response to *M. leprae* were examined. We found that lepromatous patients with the same histopathological diagnosis were heterogenous in their response to *M. leprae.* While some patients were unable to respond, as assessed by T-cell proliferation and immune (gamma) interferon (IFN-γ) release, the T cells of other patients showed low but detectable responses, relative to tuberculoid controls. Attempts to restore responsiveness in cells from these patients have been unsuccessful in our hands. Exogenous IL-2 had no significant effect on the response of T cells from nonresponder patients. The T cells from low-responder patients showed en-

hanced responsiveness in the presence of IL-2. This enhancement was proportional to the magnitude of the original response to *M. leprae*. Similarly, deletion of monocytes and/or OKT8+ T cells with monoclonal antibodies and complement had no effect on the response to *M. leprae* by nonresponder patients. However, the proliferation in some low and high responders was enhanced when monocytes were depleted from the cultures. This enhancement is not *M. leprae* specific, since it is also observed in responses to BCG.

We conclude that the defect in lepromatous low- and nonresponders does not result from a simple lack of IL-2 production and that IL-2 cannot induce responsiveness in the majority of unresponsive lepromatous patients. Furthermore, neither OKT8+ T cell and/or monocyte mediated suppression is the principal cause(s) of the anergy to *M. leprae*. Possibly, there is a low level or lack of *M. leprae*-responsive T cells in the circulation of these patients.—[The Rockefeller University and The Irvington House Institute, New York, New York 10021, U.S.A.]

Acknowledgment. This work was supported by a grant from The Heiser Program for Research in Leprosy.

Uyemura, K., Modlin, R. L. and Rea, T. H. *In vitro* cyclosporin A restoration of lepromin-induced suppressor activity in patients with erythema nodosum leprosum.

Erythema nodosum leprosum (ENL), a major source of morbidity in patients with lepromatous leprosy (LL), is of uncertain pathogenesis. We have previously suggested a cell-mediated immune response in the pathogenesis of ENL based on immunoperoxidase studies showing a tissue helper-cell predominance in ENL versus a suppressor-cell predominance in LL. Also, we demonstrated an order of magnitude more interleukin-2 (IL-2) positive cells in ENL versus LL. Lepromin-induced suppressor cells are present in the peripheral blood of LL and absent in ENL (Mehra, *et al.*, unpublished data). These immunologic changes do not benefit the ENL patient so that a return to the quiescent LL state may be desirable. Current ENL therapy is problematic due to the teratogenicity of thalidomide and the serious side effects of long-term steroid therapy. Cyclosporin A (CsA) is an immunomodulator with few serious side effects when used in low doses. Its mechanism of action is thought to be related to inhibition of IL-2 production and sparing of suppressor-cell functions. Therefore, the immunologic efficacy of CsA may be suitable for the potential treatment of ENL patients. We evaluated the *in vitro* effect in CsA on lepromin-induced suppression.

Lepromin-induced suppression was measured strictly adhering to the protocol of Mehra, *et al.* (J. Immunol. 123:1813, 1979) in 5 normal, 4 LL, and 16 ENL patients. Peripheral blood mononuclear cells (PBMC) were incubated with CsA (50 ng/ml) or media control for 6 hr and then washed. PBMC (2×10^5/well) were then cultured in concanavalin A (ConA, 2.5 μg/ml) or Dharmendra lepromin (1:10) with ConA. Tritiated thymidine incorporation was determined after 3 days of culture. Percent suppression of ConA response was calculated as follows: 100 − (cpm, lepromin + ConA)/(cpm, ConA) × 100.

We confirmed that lepromin-induced suppression was present in LL but not in ENL. In addition, we found that CsA treatment of PBMC from ENL patients restored the lepromin-induced suppression to levels observed in LL patients. No change was observed in normal controls.

The mechanism of action of CsA may involve lymphocytes and/or adherent accessory cells. Investigation of the mechanism of CsA restoration of lepromin-induced suppression in ENL may serve as a useful tool to dissect the immunology of leprosy. These findings are also significant in that they provide the first evidence for the potential efficacy of CsA in the treatment of ENL.—[Department of Pathology and Dermatology, University of Southern California School of Medicine, Los Angeles, California, U.S.A.]

Izaki, S., Hsu, P.-S., Tanji, O., Hibino, T., Tokairin, S., Ohkuma, M. and Izaki, M. Plasminogen activator associated with hypersensitivity granulomas in murine leprosy.

Plasminogen activator activity was extracted from hypersensitivity-type murine

lepromas induced in C57BL/6N, resistant, mice but not in murine lepromas without hypersensitivity in CBA, susceptible, mice. The detection of the enzyme was correlated with immunohistochemical findings, such as fibrin deposition and expression of IA antigen by macrophages. Biochemical characteristics of the plasminogen activator associated with hypersensitivity granulomas (gPA) were investigated and compared with plasminogen activator secreted from cultured macrophages, as well as tissue-type and urokinase-type plasminogen activators. The data obtained suggest possible cellular origin of gPA.

Enzyme activity was measured in a Tris-saline soluble fraction of two polar types of murine lepromas in C57BL/6N and CBA mice with ^{125}I-fibrin microplates with and without purified plasminogen, and synthetic chromogenic peptide substrates, such as Ile-Pro-Arg-pNA (s-2288), Val-Leu-Lys, pNA (s-2251), and Glu-Gly-Arg-pNA (s-2444). The electrophoretic enzymography method was utilized to detect plasminogen activator activity after SDS-10% polyacrylamide slab gel electrophoresis. Thioglycollate stimulated mouse peritoneal macrophages were cultured with Dulbecco's modified MEM (DMEM) + 10% fetal calf serum (FCS) for 24 hr, followed by incubation with DMEM without FCS. Plasminogen activator was collected from DMEM without FCS.

Sephacryl S-200 gel chromatography of the plasminogen activator activity in the tissue extract of hypersensitivity-type murine lepromas demonstrated a major peak of enzyme activity at a molecular weight of 24,000 (gPA), and an additional peak with 45,000 (gPA′). gPA and gPA′ were separated by further chromatography with a DEAE anion exchanger. The separated gPA and gPA′ similarly showed trypsin-like serine proteinase properties; they were inhibited by DFP, PMSF, TLCK, aprotinin, and antipain, and hydrolyzed synthetic peptide substrates with Arg or Lys residues. gPA showed the highest hydrolytic activity for Ile-Pro-Arg-pNA (Km = 1.4×10^{-4} M), second highest for Val-Leu-Lys-pNA (Km = 5.2×10^{-4} M), and lowest for Glu-Gly-Arg-pNA (Km = 9.3×10^{-4} M). The enzymes showed the optimal pH between pH 8 and 9. The enzyme activity was lost at 56°C but was fairly stable at 37°C.

Cultured macrophages secreted approximately 0.2 CTA U/ml of plasminogen activator activity in the culture media. By the electrophoretic enzymography technique the condensed culture media demonstrated that macrophage plasminogen activator consists of enzyme molecules with 24,000 and 45,000 molecular weights, as well as some greater molecules. On the same slab gel, the gPA and gPA′ preparation showed identical mobility to 24,000 and 45,000 molecules, respectively, whereas they were distinguished from tissue-type and urokinase-type plasminogen activators. Furthermore specificity for the synthetic peptide substrates of both macrophage plasminogen activator and gPA and gPA′ were mostly identical, revealing highest affinity for Ile-Pro-Arg-pNA, and a fairly high affinity for Val-Leu-Lys-pNA, whereas they were distinguished from both tissue-type and urokinase-type plasminogen activators that did not hydrolyze Val-Leu-Lys-pNA effectively.

The tissue induction of plasminogen activator in hypersensitivity-type murine lepromas showed a clear correlationship with a) fibrin deposition in the inflammatory tissue, b) ultramicroscopic differentiation of macrophages, c) expression of IA antigen on macrophage cell membrane, and d) other tissue remodeling reactions associated with hypersensitivity and healing reactions. The plasminogen activator secreted from activated macrophages stimulated by granulomatous hypersensitivity is assumed to play roles during the development of murine lepromas in the resistant host animals.—[Iwate Medical College, Morioka-shi, Iwate, Japan]

Truman, R. W., Shannon, E. J. and Hastings, R. C. Host responses to the phenolic glycolipid-I antigen of *M. leprae.*

Enzyme-linked immunosorbent assays (ELISA) have been developed to detect antibodies to the phenolic glycolipid-I (PGL-I) antigen of *Mycobacterium leprae.* Statistical definitions for positive/negative interpretations in ELISA were derived by testing sera from 226 leprosy patients, 100 National Hansen's Disease Center (NHDC)

staff members, and 108 Pima Indians. Leprosy has not been diagnosed in the native American Indian, and Pimas reside in a section of the United States where contact with leprosy would be rare. False-positive reactions were not found in sera from Pima Indians. Therefore, Pimas probably represent "true negatives" for leprosy serology.

IgM, IgA, and IgG antibodies to PGL-I were detected in 46–62% of the leprosy patients tested. These antibodies were also detected in 12–14% of presumed healthy NHDC staff members. No significant correlation between ELISA absorbences and the bacterial indices of lepromatous patients was found. Coefficients of correlation did not exceed 0.22. It is presumed that PGL-I may persist in tissues to promote a humoral response even in the absence of detectable bacilli. Antimicrobial therapy with dapsone had no significant effect on the serologic profiles of lepromatous patients with respect to serum IgM, IgA, IgG, C3, and specific IgM, IgA, IgG antibodies to the PGL-I antigen.

Patients suffering erythema nodosum leprosum (ENL) had no discernable trends in antibodies to PGL-I. ENL was noted in patients both with and without detectable antibodies to PGL-I. Serological monitoring of patients to predict ENL does not seem feasible. The drug thalidomide had a significant effect on the levels of total serum IgM and specific IgM antibodies to PGL-I. Levels of other serum constituents or specific antibodies to PGL-I were not significantly affected. Further studies on the persisting nature of this antigen and its possibility to stimulate a chronic antibody response may yield insight into the character of immune regulation in leprosy.—[National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Acknowledgment. This work was supported by grants from the Hansen's Disease Foundation and the National Hansen's Disease Center, Carville, Louisiana.

Jacobs, W. R., Clark-Curtiss, J. E., Docherty, M. A. and Curtiss, R., III. Identification of cloned *Mycobacterium leprae* genes in *Escherichia coli.*

We have previously reported the construction of recombinant libraries of *Mycobacterium leprae* using both plasmid (pBR322) vectors and cosmid (pHC79) vectors. Although each of the pHC79::*M. leprae* libraries theoretically represents greater than 99.99% of the *M. leprae* genome, complementation of 7 different mutations in the *Escherichia coli* host strain had not been observed.

Additional genomic libraries of *M. leprae* DNA have been prepared using the plasmid expression vector pYA626. This vector is a derivative of pBR322 which possesses the promoter region of the β-aspartate semialdehyde dehydrogenase gene (*asd*) from *Streptococcus mutans,* which is very efficiently expressed in *E. coli.* Several of these clones were subsequently identified by their ability to complement separate mutations in the *E. coli* host strain: citrate synthase (*gltA*), dehydroquinate synthetase (*aroB*), and anthranilate synthase (*trpE*).

The polypeptide specified by the clones that complement the *gltA* mutation in *E. coli* minicells has been analyzed by SDS-PAGE. This polypeptide is a 46KD protein specified by a 2.6 kb *Pst*I digestion fragment. The *gltA* complementation activity is present when this fragment is in one orientation within pYA626 (pYA1036), but not when it is in the reverse orientation (pYA1041), which implies that the protein is being synthesized only when the gene is in transcriptional phase with the *asd* promoter. Minicell analysis of the proteins specified by recombinant molecules in each orientation demonstrated that the 46KD protein was only synthesized in minicells containing pYA1036.

When this 2.6 kb insert was subcloned into pHC79 (pYA1044) the 46KD protein was synthesized, but at lower levels so that this recombinant molecule was unable to complement the *gltA* mutation in the *E. coli* strain. We thus conclude that *M. leprae* genes can be expressed in *E. coli* although, in the clones analyzed thus far, expression appears to depend upon the presence of promoters recognized by the *E. coli* transcription system.—[Departments of Biology and Microbiology, Washington University, St. Louis, Missouri 63130, U.S.A.]

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Health Organization Program on Research in Tropical Diseases, IMMLEP, and THELEP Working Groups.

Cocito, C. Comparative properties of *Mycobacterium leprae* and leprosy-derived corynebacteria.

Two kinds of microorganisms can be recognized in tissues of leprosy patients: *Mycobacterium leprae* (ML), and leprosy-derived corynebacteria (LDC). ML from untreated patients are endowed with alcohol-acid-fastness, which can be lost upon treatment with antibiotics and in the course of a strong immune response (tuberculoid leprosy). Vulnerable ML thus produced can be reversibly de-stained by organic solvent; in tissue sections from tuberculoid and treated patients, more bacteria are revealed by the Wade-Fite method (which involves a special de-paraffination technique) than by the Ziehl-Neelsen procedure.

Organisms of genera *Corynebacterium* s.s., *Mycobacterium,* and *Nocardia* (CMN group) have DNA with percent guanine-plus-cytosine contents (%GC) of 50–60, 69–72, and 68–70, respectively. GC values of DNA from ML and LDC are close to 56%. DNA from different LDC strains display high homology among them, and low homology with reference corynebacteria.

CMN cell wall consists of interconnected peptidoglycan and polysaccharide-mycolate complex. Peptidoglycan of LDC (and of known CMN organisms) has the polysaccharide backbone linked to a tetrapeptide of L-ala, D-glu, m-DAP (meso-diaminopimelate), D-ala. In ML, L-ala is replaced by glycine. Mycobacterial wall polysaccharides (that of ML is unknown) are branched arabinogalactans with end arabinose linked to C70 and C90 mycolates. Peripheral polysaccharides of LDC are arabinogalactomannans with lateral strands of arabinose and mannose. Mycolic acids of LDC are of the corynomycolic type (C32, C34, and C36 with 1–4 double bonds), and those of ML are of the mycobacterial type.

Components of CMN wall and cytoplasm are immunologically active as antigens (polysaccharides, proteins), haptens (lipids), and adjuvants (peptidoglycans). Strong intragenera and weak intergenera crossreactions are observed among CMN bacteria; LDC preparations, however, crossreact strongly with ML and mycobacteria, and weekly with reference corynebacteria. LDC in leprosy tissues can, thus, be revealed by fluorescent anti-LDC antisera as well as by anti-ML antisera. The main crossreacting component is antigen Ml of LDC, which corresponds to antigens Ag 7 of ML and Ag 60 of BCG, the active components of lepromin and tuberculin, respectively. Antigen Ml has a polysaccharide moiety crossreacting with the wall polysaccharide of LDC. Immunological reactivity in leprosy apparently is directed toward the polysaccharide moiety during the tuberculoid phase, and the polypeptide moiety during the lepromatous phase.

Immunological kinship of LDC and ML suggests their possible cooperation in leprosy development. Infection of small numbers of LDC in one foot pad of mice challenged in both foot pads with ML produced a faster proliferation of ML suggestive of synergism. Leprosy can, thus, be considered a disease which is produced by ML, organisms of uncertain taxonomic position, possibly helped by LDC, a unique group of corynebacteria which are well characterized both chemically and immunologically.—[Microbiology and Genetics Unit, Institute of Cell Pathology, University of Louvain Medical School, Brussels 1200, Belgium]

Acknowledgments. Most of the work on LDC was carried out with LDC strains from the collections of J. Delville (University of Louvain) and L. Barksdale (New York University, New York, U.S.A.). Some experiments herewith related were carried out in collaboration with Prof. M. Harboe (University of Oslo, Oslo, Norway) and Prof. J. Delville. The following scientists have participated in the present research project: C. Abou-Zeid, M. Coene, P. Danhaive, F. Fontaine, C. Gaily, M. C. Gueur, E. Janczura, R. Laub, and F. Vanlinden.

Hunter, S. W., Gaylord, H., Stewart, C. and Brennan, P. J. Isolation and preliminary characterization of the major intracellular carbohydrate-containing antigens of *Mycobacterium leprae.*

We have isolated from *Mycobacterium leprae* a family of major carbohydrate-containing macromolecular antigens. All are

mannose- and arabinose-containing, the arrangement of which we do not yet know. They are highly lipidated, acylated exclusively by 10-methyloctadecoanoate. Hence, they are water-soluble lipopolysaccharides, which we call lipoarabinomannans. Lipoarabinomannan-B (LAM-B), a highly acidic polymer, has been purified to PAGE and chromatographic homogeneity. It is the dominant immunogen in *M. leprae,* is immunologically crossreactive with a related, but not identical, product from *M. tuberculosis,* and contains α-glycerolphosphate as itself or as part of some other phosphodiester. There is also present a sizeable quantity of nitrogeneous material, suggesting further novel immunochemical features. Some of the structural features of LAM-B evoke comparisons with lipoteichoic acid of Gram-positive bacteria with images of what this means in terms of physiology, immunogenicity, and pathogenicity. Lipoarabinomannan-A (LAM-A), chromatographically and electrophoretically distinct from LAM-B, unlike LAM-B, does not react with monoclonal antibodies or with antibodies from human lepromatous leprosy serum. It is also devoid of the majority of its acidic functions, suggesting that these are the major antigen determinants.

These lipoarabinomannans are undoubtedly the major so-called "common antigens" of the leprosy bacillus and should be further examined, since there is now a fair body of accumulated experimental evidence demonstrating the existence of cross-reactivity in acquired protection to various mycobacteria.—[Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.]

Miller, R. A., Harnisch, J. P., Gilliland, B. C., Lukehart, S. A. and Buchanan, T. M. The molecular basis of positive treponemal antibody, antinuclear antibody, and immune complex tests among Hansen's disease patients.

Numerous studies in the literature have commented on the high prevalence of autoantibodies and false-positive syphilis serologies in patients with Hansen's disease (HD). However, as we reported at the Carville meetings last summer, we have found little evidence of nonspecific antibody production among the patients followed in the Seattle HD clinic. Although we could detect circulating immune complexes in 48% (21/44) of our patients, none were positive for rheumatoid factor or had detectable cryoglobulinemia. Only 4% (2/45) had antibodies to SS-A (a cytoplasmic nucleic-acid antigen). We were able to confirm, though, the existence of a low prevalence of positive antinuclear antibody (ANA) and syphilis serology assays in our population.

Of the HD sera tested to date, 16% (7/45) had positive ANA results. However, of these 7 positive sera, none were positive for antibody to native DNA, to histones, to extractable nuclear antigens (ENA), or to centromeres. This pattern implies antibody activity directed against complexed nucleic acids and nucleoproteins, and would be typical of a weak, crossreactive antibody response. We cannot definitively dismiss the hypothesis that antimycobacterial lipid antibodies crossreact with the phosphate backbone of DNA, although the negative results on the native DNA binding assays make this unlikely. Sera from patients with systemic lupus erythematosus failed to react on nitrocellulose immunoblots to *Mycobacterium leprae* carbohydrates or proteins, or by ELISA against the phenolic glycolipid antigen of *M. leprae.*

Positive serologic tests for syphilis were detected in several HD patients; 16% (9/55) had a positive VDRL test (titer range 1:2–1:4), 9% (5/55) had positive results on a specific anti-treponemal test (FTA-ABS), and 7% (4/55) were positive by both assays. Eight sera from HD patients were tested by immunoblot for reactivity to proteins from *Treponema pallidum.* None of these 8 patients had a history of syphilis, yaws, or pinta. All of the sera, including the 3 sera from patients negative by both VDRL and FTA-ABS, reacted with several treponemal proteins, most prominently with antigens of 48,000 molecular weight (7/8) and 41,000 molecular weight (8/8). The pattern is typical of normal, nonimmune human serum. Previous work had shown two antigens of 12,000 and 14,000 molecular weights to be specific for pathogenic treponemes. Of the 4 sera from patients positive by both VDRL and FTA-ABS, 3/4 recognized the 14,000 antigen and 1 of those 3 identified both antigens. The 1 serum which reacted with nei-

ther antigen was from a patient with a 1:4 VDRL titer but only a 2+ FTA-ABS, which is often interpreted as borderline reactivity. The serum from a patient positive by FTA-ABS but negative by VDRL reacted strongly with the 12,000 antigen and weakly with the 14,000 molecule. None of the seronegative sera reacted with the 14,000 antigen, but 1/3 weakly recognized the 12,000 protein. Patients infected with pathogenic treponemes generally develop antibodies to both the 14,000 and 12,000 antigens. We believe that antibody to the 14,000 molecular weight antigen of *T. pallidum* which develops in a subset of HD patients is the most common cause of false-positive FTA-ABS tests in this population.—[Immunology Research Laboratory and Department of Medicine, Pacific Medical Center, Seattle, Washington 98144; Department of Medicine, Harborview Medical Center, Seattle, Washington; National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Acknowledgment. This research was supported by Contract No. 258-83-0063 from the National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.

Tsutsumi, S. and Gidoh, M. On a role of antileprotic agents as the scavengers of active oxygen radicals.

Niwa, *et al.* reported the scavenging activity of dapsone (DDS) on singlet oxygen, hydroxyl radical (OH·) and H_2O_2 and an additional effect of clofazimine on superoxide (O_2^-, SO) and OH·. Since we felt there was a contradiction between this probable antibacterial mechanism of clofazimine and its strong inhibitory action on adjuvant-induced rat arthritis, we examined the effects of antileprotic agents on active oxygen radicals (AO). The results found up to the present are as follows:

1) The order of scavenging activities of drugs on a fixed level of SO produced by hypoxanthine and xanthine oxidase was: RFP, RFPQ > enoxacin > 2-mercapto-3-hydrazinoquinoxaline (MHQ) > DDC > sulfadimethoxine (SDM), CCA (Lobenzarit) > clofazimine. In this experimental system, the activities of DDS, levamisole, dexamethasone (DX), ofloxacin, ATSO (a plant source polysaccharide) and therafectin (a mild immunomodulator) were trifling or nearly negative.

2) When SO was put in contact with a mixture of drug and SOD, clofazimine, RFPQ, DDC, and MHQ all strongly enhanced the scavenging of SO by SOD, while the enhancement of SDM or enoxacin became weak when compared with that in 1. On the contrary, ATSO strongly inhibited the scavenging by SOD.

3) When peritoneal adherent cells collected from normal Wistar rats were exposed to a drug for 96 hr, no drug except DDC inhibited the scavenging of SO by the adherent cells. DDC is known to be a substance which decreases endogenous SOD. In addition, we noticed a deformation/granulation of adherent cells after exposure to 20 μg/ml of DDC. The other drugs more or less enhanced the scavenging of SO by the adherent cells. The order of the enhancement was: cyclosporin-A (Mn-type > total SOD), ATSO (both) > MHQ (both) > DDS (both) > clofazimine (both), DX (total > Mn-type), SDM (both), ofloxacin (both), enoxacin (both), and levamisole (both). The scavenging activity of RFPQ disappeared in this cellular system.

4) Eight out of 11 drugs examined, including clofazimine, significantly decreased the counts of cellular chemiluminescence using luminol after contact incubation of adherent cells with these drugs for 96 hr. The order of the decrease was: MHQ > DX > levamisole, ATSO > clofazimine, cyclosporin-A > DDS > ofloxacin. The chemiluminescence of cells following contact with MHQ became nearly negative. RFPQ alone significantly increased the chemiluminescence, suggesting a contribution of this phenolic quinone to the aerobic respiration of adherent cells. The decrease by SDM or enoxacin was insignificant.

5) The significance of these scavenging activities of drugs is discussed, especially regarding the following points: a) a possible cause of results different between Niwa, *et al.* and us; b) a possible mutual influence between bacterial Mn-type SOD and intracellular SO, especially when the latter is stimulated; and c) a contradiction such as the dose of SOD to SOD-increased autoimmune diseases and the recommendation of immunostimulants to Mn-SOD-lowered

cancer patients, which are also AO stimulators.

6) Using a far larger number of cells, the influences of drugs on AO themselves are being examined. The results hitherto found are as follows:

Coincidental to the report by Berton, *et al.* who noticed the lowering in the ability of adhered cells to produce AO after *in vitro* incubation, we also noticed a serious lowering in the production of SO. Thus, we could not find clear enough results using a ferricytochrome C method. The results found are: a) The response of freshly adhered cells to phorbol-12-myristate-13-acetate (PMA) was superior to the stimulation of the cells by washed opsonized zymosan-A. b) Although PMA was used, the scavenging effect of DDS still could not be detected due to the low production of SO. c) Nevertheless, no increase in SO production was found with RFPQ-, clofazimine-, levamisole-, DX-, and cyclosporin-A-contacted cells.

Accordingly, the production level of OH· was, at most, 1/10 of those noted in earlier reports by other authors in which freshly isolated cells were used. However, in this experiment a significant GC peak decline could be found in 6 out of the 11 drugs examined. The declines were clear in clofazimine-, DX-, and MHQ-contacted cells.—[National Institute for Leprosy Research, Higashimurayama-shi, Tokyo, Japan]

Abe, M., Miyaji, I., Minagawa, F. and Yoshino, Y. Salivary anti-*M. leprae* antibodies of household contacts and inhabitats in a leprosy-endemic area.

A modified technic for the fluorescent leprosy antibody absorption (FLA-ABS) test was employed for detecting salivary anti-*Mycobacterium leprae* antibodies in household contacts and inhabitants in a leprosy-endemic area. The test was positive in 20 (58.8%) of 34 household contacts, 92 (39%) of 236 school children, and 36 (16.9%) of 213 adults. The percentage of positive reactions was significantly higher in the contacts of lepromatous patients than in those of tuberculoid patients, and also higher in school children with a suspicious neural symptom than in those without the symptom. The salivary and serum FLA-ABS tests were compared in 234 school children and 38 adults. Concordant positive reactions were seen in 58 (24.8%) children and in only 1 adult. A significant number of individuals showing a positive reaction with the saliva but a negative reaction with the serum suggested subclinical infection through the mucous membrane. The delayed-type hypersensitivity (DTH) responses of 228 school children were examined by the skin reaction to Dharmendra's antigen. The percentage of positive DTH responses was lowest in the salivary antibody-positive but circulating antibody-negative responders, while it was highest in the reverse responders.

The serological specificity of salivary anti-*M. leprae* antibodies was checked by cross-reactions and additional absorption tests. Only 7 out of 55 specimens of saliva showing a positive FLA-ABS test were cross-reactive with some species of mycobacteria. Positive reactions against *M. leprae* were not influenced at all by additional absorptions with crossreacting mycobacteria, but became negative after an additional absorption with *M. leprae*. Twenty-three specimens of saliva from patients with tuberculosis did not give positive reactions when the saliva was absorbed with double doses of BCG and *M. vaccae*. Therefore, a modified technic of the FLA-ABS test was found specific for *M. leprae* and useful for detecting subclinical leprosy infection. These findings are discussed from the immunological and epidemiological points of view.—[National Institute for Leprosy Research, Higashimurayama-shi, Tokyo, Japan]

Cho, S.-N., Chatterjee, D., Gelber, R. H., Rea, T. H. and Brennan, P. J. A diverse approach to the serodiagnosis of leprosy based on the phenolic glycolipid-I.

In previous studies, we described the synthesis of a series of neoglycoproteins containing the 3,6-di-*O*-methylglucopyranose epitope of phenolic glycolipid-I which had been conjugated to carrier protein via an 8-methylene (octyl) linker arm (Chatterjee, *et al.*, unpublished observations). All of those neoglycoproteins proved to be serologically highly reactive and showed good concor-

dance with the native phenolic glycolipid-I (PGL-I) in detecting anti-PGL-I antibodies. However, in more than 10% of serum specimens from leprosy patients, the reactivity against the semi-synthetic antigens was less than that against the glycolipid, suggesting that more of the native structure, beyond the terminal epitope, is required for optimal reaction with anti-PGL-I antibodies. Accordingly, in this present study, we synthesized more extensive neoglycoprotein antigens, those containing the partially unmethylated disaccharide, 3,6-di-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-rhamnopyranoside-octyl-BSA (disaccharide-octyl-BSA; D-O-BSA) and 3,6-di-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-methyl-α-L-rhamnopyranoside-octyl-BSA (natural disaccharide-octyl-BSA; ND-O-BSA). These semi-synthetic antigens were compared to PGL-I and the earlier neoglycoproteins in their ability to detect anti-PGL-I antibodies in serum specimens from leprosy patients and control groups, using ELISA. Of 94 patients' sera specimens tested, 66 were positive to ND-O-BSA and PGL-I and 62 to D-O-BSA and M-O-BGG, indicating a marginal advantage of ND-O-BSA in detecting anti-PGL-I antibodies. However, among 223 control sera examined, only 5 (2.2%) were positive to ND-O-BSA compared to from 9 to 15 against the other neoglycoproteins, demonstrating substantially greater specificity. Thus, ND-O-BSA showed the greatest sensitivity and specificity among neoglycoprotein antigens synthesized to date, and it would appear that the more of the native structure one can introduce into the synthetic product, the greater the efficacy in serodiagnosis. Accordingly, synthesis of the entire trisaccharide-octyl-BSA is now proceeding.

Chemical and immunological procedures for the quantitation of phenolic glycolipid-I itself have been developed. Serum samples (0.5–1 ml) were extracted with $CHCl_3$-CH_3OH mixtures and fractionated on short columns of silicic acid. Direct thin-layer chromatography with a sensitivity of about 0.5 μg allowed direct detection of the glycolipid in untreated lepromatous and borderline patients, and high-pressure liquid chromatography allowed quantitation; 0.8–3.7 μg per ml of serum from 4 patients. Enzyme-linked immunosorbent assay (ELISA)-inhibition using anti-glycolipid polyclonal antibodies, which displayed greater avidity for the glycolipid than monoclonal antibodies, corroborated these figures. Dot-ELISA on nitrocellulose using polyclonal and monoclonal IgG antibodies allowed for greater sensitivity (0.5 ng) and semi-quantitative evaluation. Small quantities of glycolipid (20–35 ng per ml) were observed in urine of lepromatous leprosy patients. Among patients at the lepromatous end of the disease spectrum, the levels of glycolipid and anti-glycolipid immunoglobulin M were generally in accord. Longitudinal studies demonstrated that in sera obtained from patients undergoing chemotherapy, the glycolipid was cleared sooner than anti-glycolipid antibody. The application of this experimental approach to diagnosis, bacillary quantification, and standardization of skin test reagents and vaccines, as well as to assessing the role of *M. leprae*-specific antigens in incubating leprosy, neuropathy, and reactional states, was also discussed.—[Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523; Seton Medical Center, Daly City, California 94015; LAC/USC Medical Center, Los Angeles, California 90033, U.S.A.]

Buchanan, T. M. Species-specific epitopes on protein molecules of *Mycobacterium leprae* or *M. tuberculosis*: elucidation with monoclonal antibodies.

The Immunology Research Laboratory, University of Washington, Pacific Medical Center, Seattle, Washington, U.S.A., recently participated in the analysis of 33 coded monoclonal antibodies to *Mycobacterium tuberculosis* as part of a workshop sponsored by the World Health Organization. Seven of the 33 antibodies within the group originated in our laboratory. The methods used for the analysis included dot blot immunoassay to determine species specificity, and Western blot and gel immunoradioassay (GIRA) to determine the molecule recognized. Eight monoclonal antibodies were specific for *M. tuberculosis*, and evidence was obtained which located these epitopes on probable protein molecules of *M. tuberculosis* of 14K, 23K, 38K, and 64.5K daltons. The 64.5K and 23K

molecules may also contain crossreactive epitopes, as defined by 4 other monoclonals within the coded set of antibodies. The crossreactive monoclonals to the 64.5K protein of *M. tuberculosis* recognized a protein of similar molecular weight in *M. leprae,* which is known to contain both crossreactive and at least 1 *M. leprae* species-specific epitope. The crossreactive monoclonals to the 24K protein of *M. tuberculosis* recognized a 28K protein of *M. leprae* which is known to contain at least 1 *M. leprae* species-specific epitope in addition to 2 crossreactive epitopes. No monoclonals with crossreactivity were found that reacted with the 14K or the 38K protein molecules of *M. tuberculosis.* Dr. Rick Young, Whitehead Institute, Cambridge, Massachusetts, U.S.A., isolated recombinant DNA clones that directed synthesis in *Escherichia coli* of species-specific epitopes of *M. leprae* for each of 5 different protein molecules previously characterized by monoclonals as bearing epitopes specific for the leprosy bacillus. The results of these studies with *M. tuberculosis* suggest that similar approaches may yield recombinant DNA clones capable of directing synthesis of protein epitopes specific for the tubercule bacillus. These data provide promise for eventual development of improved skin-test reagents and possibly a vaccine containing species-specific protein epitopes.—[Immunology Research Laboratory and University of Washington Pacific Medical Center, Seattle, Washington; National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

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Mohagheghpour, N., Cho, S.-N., Gelber, R. H., Foung, S. K. H. and Engleman, E. G. Production of human antibodies to *Mycobacterium leprae.*

The clinical classification of leprosy is partly based on a shifting balance between humoral and T cell-mediated reactions to *Mycobacterium leprae.* Further, some immunological reactions against the leprosy bacilli have adverse effects on the host. Not yet known is whether B cells and T cells recognize and respond to the same or different *M. leprae* antigens, and if patients with different forms of leprosy produce antibodies to different antigens and whether anti-*M. leprae* antibodies aid in recovery from disease or contribute to disease manifestations. To better understand B and T cell response in various forms of leprosy, a more precise characterization of antigenic determinants on the leprosy bacillus will be required. One approach has utilized the ability to produce murine monoclonal antibodies (Mab) against leprosy bacilli. However, immunization of rodents with *M. leprae* antigens may yield antibodies with antigenic specificities quite different from those generated in naturally infected humans. Because antibodies derived from patients' B cells will allow a more direct analysis of moieties immunogenic in man, we have elected to produce human Mab to *M. leprae* antigens. To generate such reagents, we have initiated an effort to produce Mab to *M. leprae.* Our initial findings are reported here.

Human hybrids secreting antibody against *M. leprae* were generated using peripheral blood B lymphocytes from 3 patients with lepromatous leprosy. Fresh or Epstein-Barr virus transformed B cells were fused with our human-mouse cell line, SBC-H20, and positive hybrids were cloned and subcloned at 0.5 cell/well. Eight clones generated from these fusions were found to exhibit long-term (6–16) months, continuous antibody secretion (3–20 μg/ml) in tissue culture. These Mab, all IgM, reacted with whole cell preparations from *M. leprae* and *M. bovis.* However, none reacted with arabinomannan, a complex cell-associated polysaccharide common to all mycobacterial species. These Mab reacted with phenolic glycolipid (PGL-I) purified from *M. leprae* and *M. bovis,* suggesting a shared epitope. Little or no reaction was detected to phthiocerol dimycocerosate, whole cell preparations from *M. avium* serovar 2 and *M. intracellulare* serovar 8 and C-mycoside, indicating that the phenol ring may be the crossreactive determinant. To further examine the serological specificity of these Mab, they were tested for reactivity against the PGL-I terminal sugar, mycoside A, and mycoside B. Supernatants from 3 clones (ML1, ML4, and

ML5) reacted strongly with the PGL-I terminal sugar. No reaction was detected to the mycoside A and the mycoside B, suggesting that the rhamnose-containing residue on the *M. leprae* phenolic glycolipid is a strong immunogen.—[Stanford Medical School Blood Center and Department of Pathology, Stanford University School of Medicine, Stanford, California 94305; Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523; Seton Medical Center, Daly City, California 94025, U.S.A.]

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Gillis, T. P. Affinity purification of a 65,000 dalton protein of *Mycobacterium gordonae* using a monoclonal antibody which recognizes a shared epitope on the homologous protein of *Mycobacterium leprae.*

Recent discoveries using monoclonal antibodies (MCAB) have established the fact that certain proteins of *Mycobacterium leprae* or epitopes on these proteins constitute species-specific antigens or antigenic determinants of *M. leprae.* In addition, some of these epitopes have been shown to be immunogenic in patients with Hansen's disease. One such protein is the cell wall-associated protein of *M. leprae* (M_R = 65,000) which carries 1 species-specific epitope and at least 3 crossreactive epitopes. We have established methods for the purification of the homologous protein from *M. gordonae* by affinity chromatography using MCAB IIC8 which binds a crossreactive epitope on the 65,000 dalton protein of *M. leprae.*

A soluble extract of *M. gordonae* (Mg100KS) produced by sonication was reacted for 18 hr at 4°C with an affinity matrix consisting of MCAB IIC8 covalently coupled to Sepharose 4B. The reaction mixture was poured into a column (9 cm × 1.8 cm) and elution of unbound components initiated with equilibration[1] buffer. Ten fractions (5 ml/fraction) were collected, and the absorbances of individual fractions were monitored at 280 nm. Subsequent elution conditions consisted of wash I[2] buffer (50 ml), wash II[3] buffer (50 ml), UBA-6[4] buffer (50 ml), UBA-4[5] buffer (50 ml), and UA-3[6] buffer (50 ml). Fractions representing the first peak from the column (equilibration) and the first wash (wash I) were pooled separately and concentrated to 15 ml and 10 ml, respectively, by pressure filtration. Fractions representing each subsequent elution condition were combined, dialyzed (distilled water), lyophilized, and reconstituted in 2 ml of sterile phosphate buffered saline, pH 7.0. Each fraction was analyzed by SDS-PAGE under reducing conditions for number and quantity of polypeptides and immunoblotting for immunoreactive components. Immunoreactivity with MCAB IIC8 was observed in Mg100KS starting material, UBA-4, and UA-3 fractions. Correlation of immunoreactivity and protein staining indicated that the major band of immunoreactivity was associated with a single protein (M_R = 65,000). Blots of UBA-4 showed some reactivity with lower molecular weight bands, also observed by Coomassie staining. In contrast UA-3 showed a single protein species (M_R = 65,000) both by immunoblotting and by staining with Coomassie R-250. The protein content of UBA-4 and UA-3 was 80 μg/ml and 20 μg/ml, respectively. Together these two fractions represent approximately 0.5% of the total protein present in the starting material applied to the affinity column. Studies are in progress to test the ability of the affinity purified material to induce the *in vitro* transformation of lymphocytes from various strains of mice sensitized with either *M. gordonae* or *M. leprae.*—[National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

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[1] Equilibration: NaCl (150 mM), Tris/HCl (50 mM, pH 8), NP-40 (0.05%).

[2] Wash I: NaCl (450 mM), Tris/HCl (50 mM, pH 8), NP-40 (0.05%).

[3] Wash II: NaCl (450 mM), Tris/HCl (50 mM, pH 8).

[4] UBA-6: Urea (2.4 M), barbital/acetate (30 mM, pH 6).

[5] UBA-4: Urea (2.4 M), barbital/acetate (30 mM, pH 4).

[6] UA-3: Urea (2.0 M), acetic acid (1.0 M, pH 3).

Tung, K. S. K., Nelson, K., Rubin, L., Wagner, D., Umland, E., Schauf, V., Scollard, D., Vithayasai, P., Vithayasai, V. and Worobec, S. Serum soluble interleukin-2 receptors in leprosy patients.

Rubin, *et al.* recently detected soluble receptors for interleukin-2 (IL-2R) in the culture media of lymphocytes responding to lectin or soluble antigen. Elevated levels of IL-2R have also been found in patients with T-cell leukemia. Since IL-2 and IL-2R are known regulators of the magnitude and specificity of T-cell responses, an elevated IL-2R in the serum and tissue fluids may represent a significant marker of profound T-cell activation. We have, therefore, quantitated IL-2R levels in the sera from a variety of leprosy patients and their household or non-household contacts.

Serum IL-2R was quantitated by a highly reproducible, solid-phase, enzyme-linked immunoassay utilizing two monoclonal antibodies, each recognizing a different epitope of IL-2R. Serum IL-2R levels were determined in the following subjects from Chiang Mai, Thailand: 22 untreated LL, 14 untreated BL, 3 untreated BB, 32 untreated BT, 8 untreated TT, 11 treated LL/BL, 13 treated BT/TT, 11 active ENL, 15 patients with active reversal reaction, 14 household contacts of BL/LL, 12 non-household contacts. On 6 patients with reversal reactions and 6 with ENL reactions, IL-2R were determined before treatment, and then weekly for 3–4 weeks on corticosteroid therapy. The diagnoses were based on clinical findings and skin histopathology. All serum samples were stored at −70°C before IL-2R study.

Nonreactive leprosy patients and contacts. a) The IL-2R levels of untreated LL, BL, and BB patients were comparable and, as a group, they did not differ significantly from those of household or non-household contacts (means = about 800 units/ml). b) Patients with TT and BT had significantly lower IL-2R levels than those of contacts; and patients with TT had significantly lower levels than patients with LL, BL, or BB. c) The IL-2R levels in LL/BL patients were comparable among treated and untreated groups. In contrast, the IL-2R levels of treated BT/TT patients were significantly higher than the untreated TT/BT patients, and they reached levels comparable to those of untreated LL/BL patients.

Leprosy patients with reactions. a) The IL-2R levels in patients with reversal reactions were greatly elevated (means = 2846 units/ml; SEM = 729), although the levels among patients were variable (range = 996–10,503 units/ml). b) In contrast to BL patients with reversal reactions, IL-2R levels of ENL patients were only slightly elevated. c) Patients with reversal reactions and patients with ENL responded differently to corticosteroid treatment. Thus, in 6 of 6 patients with reversal reactions, IL-2R levels fell precipitously during a 2–3 weeks' treatment period. In contrast, the IL-2R levels in 6 of 6 ENL patients did not change during corticosteroid therapy.

It is interesting that TT and BT patients, who should exhibit a positive delayed skin reaction (a T-cell reaction) to lepromin, actually had lower IL-2R levels than did LL and BL patients, who should be unresponsive to lepromin. It is tempting to speculate that the very elevated serum IL-2R levels in patients with reversal reactions represent a state of profound immunoregulatory disturbance that is associated with a heightened helper T-cell and/or reduced suppressor T-cell functions.

Since patients with reversal reactions and those with ENL differ in their basal IL-2R levels, and in the responses of the IL-2R levels to corticosteroid therapy, the two reaction states most likely have different pathogenetic mechanisms. It can be anticipated that as the meaning of elevated soluble IL-2R levels becomes better clarified, we will also gain insights into the pathogenesis of the reactive states of leprosy.—[Department of Pathology, University of New Mexico, Albuquerque, New Mexico 87131; Department of Preventive Medicine, University of Illinois, Chicago, Illinois 60612; Metabolic Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, U.S.A.; Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand]

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Nakamura, K. and Yogi, Y. The athymic rodent as an experimental lepromatous leprosy model (continued): effect of genetic background of nude rats and age of ICR nude mice.

We have successfully established as an animal model for experimental lepromatous leprosy the NFS/N nude mouse and SHR nude rat which gave excellent results with the development of a heavy lepromatoid formation. The formation of the lepromatoid lesion in athymic rodents was influenced by the genetic background gene of the animals as we previously reported. In this report, we compared the susceptibility of the SHR(N2) nude rat with that of a "resistant" F344(N4) nude rat, and tested the effect of aging in a "resistant" ICR nude mouse by determining whether or not a heavy lepromatoid lesion would occur.

SHR(N2) nude rats, established by a mating system (cross and intercross) with 2 F344(N4) male nude rats and 4 normal SHR female rats, and 2–4-week-old SHR(N2) and F344(N4) nude rats were used. ICR nude mice were also used: 2–7 days (neonates), 4–6 weeks, and 3–4 months (adult) after birth. The *Mycobacterium leprae* strain used was transferred in nude mice and nude rats, respectively, and nude mice and rats were maintained in vinyl isolators under SPF conditions.

Following subcutaneous inoculation into the right hind foot pad with 1.7×10^6 *M. leprae* into SHR(N2) and F344(N4) nude rats, a heavy swelling due to *M. leprae* growth was observed in the SHR nude rats. The bacillary counts in infected paws were approximately 9.8×10^9 and 3.5×10^{10} bacilli (with redness of the tissue) per gram in the SHR nude rats 225 days post-inoculation. The infection (foot, developing to lower leg) occurred with marked nodules 225 days after inoculation in all of the tested SHR nude rats. More advanced infection with heavy lesions was seen in the SHR nude rats than in the F344 nude rats; gross weights were from 7.4 to 14.5 grams in the SHR(N2) nude rats. We found that the high susceptibility of nude rats for *M. leprae* growth depended on a susceptible gene in the genetic background of the animals. This was easily shown with a mating system (cross, intercross) at the N2 generation using F344 male nude rats and normal SHR female nude rats. Indeed, a heavy lepromatoid lesion was produced similar to those in SHR nude rats at the N4 generation, as previously reported.

With infection in the right hind foot pads of ICR nude mice, the heavy lepromatoid lesions were enhanced in neonatal (2–7 days of age) animals. The neonatal ICR nude mice had larger lesions than those of adult animals (3–4 months of age).

In summary, we have established the SHR nude rat at the N2 generation as a new leprosy model, having previously reported on SHR nude rats at the N4 generation. With infection in some ICR neonatal nude mice there were heavier lesions than those in adult ICR nude mice. Thus, the development of a heavy lepromatoid formation in the nude mice may be influenced by aging effects in the host.—[National Institute for Leprosy Research, Tokyo, Japan]

Chehl, S. K., Shannon, E. J., Krahenbuhl, J. L., Job, C. K. and Hastings, R. C. Adoptive transfer of cell-mediated immunity in *M. leprae*-infected nude mice with *M. leprae*-immunized allogeneic leukocytes depleted of Thy-1.2-bearing cells and Lyt-2.2-bearing cells.

We have previously reported a dose-responsive induction of reversal reactions in *Mycobacterium leprae*-infected athymic nude mice with adoptive transfer of cell-mediated immunity (CMI) using unimmunized and *M. leprae*-immunized allogeneic splenic leukocyte infusions derived from heterozygote (nu/+) donor mice (Chehl, *et al.*, Int. J. Lepr. **51**:649, 1983). We further reported the efficacy of 3 vaccines: a mixture of BCG with heat-killed *M. leprae,* heat-killed *M. leprae* alone, and BCG alone. The effective dose 50% was lowest for the mixture, followed by *M. leprae* alone, followed by BCG. Quantitative analysis of resulting reversal reactions in the nu/nu recipient mice was evaluated by the degree of foot pad inflammation and swelling, decreased morphological indices of bacteria, and mononuclear cell infiltrations examined histopathologically (Hastings, *et al.*, Int. J. Lepr. **52** Suppl.:736, 1984). Having established the dose-responsive adoptive transfer of CMI in infected nude (nu/nu) mice by immunized allogeneic leukocyte in-

fusions, we have now investigated the role of leukocyte subsets in the production of reversal reactions in *M. leprae*-infected homozygous nude mice by infusing *M. leprae*-immunized allogeneic leukocytes depleted of Thy-1.2- and Lyt-2.2-bearing cells.

The splenic leukocyte suspension obtained from *M. leprae*-immunized heterozygous nu/+ mice was passed through Lympholyte density cell-separation medium and treated with anti-Thy-1.2 or anti-Lyt-2.2 monoclonal antibodies, followed by rabbit complement. Undepleted cells were treated with complement alone. Depletion of the T cells and suppressor T cells from appropriate suspensions was confirmed by using fluorescein-conjugated anti-Thy-1.2, anti-Lyt-2.2 and anti-Lyt-2.1 monoclonal antibodies. The suspension was then enumerated and diluted to contain 4×10^6, 4×10^5, and 4×10^4 viable splenocytes per 200 μl. The undepleted naive suspension and antibody-treated suspensions were adjusted to contain cell numbers equivalent to the doses of the enumerated undepleted sensitized cell suspension. *M. leprae*-infected homozygous nude mice, bearing approximately 3×10^8 acid-fast bacilli (AFB) per infected foot pad were injected intravenously with various doses of antibody-treated and untreated splenocytes. Control mice were given 200 μl of medium RPMI 1640. The animals were observed at regular intervals for gross indications of reversal reactions (foot pad erythema and edema), sacrificed 28 days after cell infusions, and the foot pads harvested for bacteriological and histopathological examination.

The Lyt-2.2 (T suppressor) depleted suspension of cells from immunized mice induced the most marked erythema and edema, followed by undepleted immune cells, Thy-1.2 (T cell) depleted immune cells and undepleted naive cells. No significant changes were observed in the number of AFB per foot pad. However, a dose-responsive reduction occurred in the morphological index (MI) in recipients of all cell suspensions. The effective dose 50% (ED50), i.e., the number of cells required to reduce the MI below 5% in 50% of the animals, was lowest for Lyt-2.2-depleted cells.

Histological assessment of reversal reactions was based on reductions in the number of AFB, granulation of AFB, lymphocyte infiltration, and the presence of epithelioid cells. Reversal reactions of varying degrees were observed in response to all cell suspensions at the 4×10^6 dose. The undepleted sensitized cell suspension was most effective in causing histologic reversal reactions, followed by Lyt-2.2 (T suppressor) depleted, naive undepleted, and Thy-1.2 (T cell) depleted cells.

These data suggest that adoptively transferred helper T cells are responsible for inducing reversal reactions in *M. leprae*-infected nude mice.—[Laboratory Research Branch, National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

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Tung, K. S. K., Teuscher, C., Koster, F., Yanagahara, D., Umland, E. and Brennan, P. J. Strain variation in murine immune response to the *M. leprae* glycolipid antigen, phenolic glycolipid-I.

Phenolic glycolipid-I (PGL-I) is a major antigenic component of *Mycobacterium leprae.* Antibodies to PGL-I are detectable in leprosy patients. PGL-I appears to preferentially stimulate T cells of the suppressor phenotype *in vitro.* Variations of the immune response to *M. leprae* antigens including PGL-I in the heterogenous population may determine the clinical stage of leprosy. In this study, we analyzed the variations of humoral and cellular immune responses to PGL-I among inbred and congenic mice following immunization with *M. leprae* organisms.

Humoral antibody response to PGL-I. Eighteen strains of inbred, congenic and F_1 female mice were immunized twice with PGL-I in incomplete adjuvant, on days 0 and 20. Sera from day −14 and day 30 were studied for anti-PGL-I antibodies with the solid phase radioimmunobinding assay. ^{125}I-labeled anti-mouse kappa and ^{125}I-labeled protein A were used to detect antibodies of all Ig classes and of IgG class, respectively. A paired *t* test was used to analyze the experimental results. a) A continuous distribution in antibody levels among mouse strains was found, and this indicates polygenic control of the antibody response. b)

There is influence of H-2 genes on antibody response as suggested by the findings that mice of H-$2^{q, b\&k}$ were high responders and mice of H-$2^{s\&d}$ were low responders and, among H-2 congenic BALB/c and C57BL/10 mice, only those of H-2^k genotype (BALB.K and B10.BR) were high responders. c) Igh allotype complex-linked genes also influence antibody response since among BALB/c with different Igh allotypes, some were high responders while others were low responders. d) Based on the study on F_1 progenies of 3 high × low responder combinations, low antibody response to PGL-I was uniformly inherited as an autosomal dominant trait.

Cellular immune responses to PGL-I. Thirteen inbred, congenic and F_1 mouse strains were immunized with *M. leprae* in incomplete Freund's adjuvant on days 0, 21, and 42. Skin test responses (foot pad swelling) to PGL-I in liposome (control = liposome alone) were determined on day 52, and lymphocyte proliferative responses to PGL-I on day 62. For the latter, lymphoid cells from thioglycollate-elicited peritoneal exudate, lymph nodes, and spleens were enriched for T cells by glass adherence and nylon wool deletion of non-T cells. Enriched T cells were cultured with or without PGL-I (0.1–10 μg per well), and stimulation indices calculated. a) Of the 13 mouse strains tested for delayed hypersensitivity reactions to PGL-I, only 4 responded and the magnitude was considerably below that of the response to *M. leprae.* b) Five of 13 mouse strains demonstrated an *in vitro* proliferative response to PGL-I, and mice with the highest responses were BALB.K and DBA/1. There was no obvious correlation between responsiveness and the H-2 haplotype. c) Among BALB/c congenic at the Igh allotype, some were responders while others were nonresponders. d) There was no correlation between mice that responded in the delayed hypersensitivity assay and the lymphocyte proliferation assay, and these did not correlate with the strain variations seen in the humoral antibody study.

The study demonstrated marked strain variations in the humoral and cellular immune responses to PGL-I in mice immunized with *M. leprae.* Antibody response to PGL-I was influenced by both H-2 and non-H-2 genes, including those of the Igh allotype-complex; the low-responder phenotype was the dominant genetic trait. While both responder and nonresponder mouse strains were identified with respect to cellular immune responses to PGL-I, there was no obvious relationship between these findings and the mouse genotypes. Thus, despite the earlier finding that the PGL-I epitope recognized by T lymphocytes exhibited exquisitely fine specificity, the immune response to PGL-I is under complex genetic control.—[Departments of Pathology and Medicine, University of New Mexico, Albuquerque, New Mexico 87131; Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.]

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Eustis-Turf, E. P., Benjamins, J. A. and Lefford, M. J. Neural auto-antibodies in leprosy.

The mechanisms by which leprosy neuropathy is produced have not been satisfactorily elucidated. This is particularly true in lepromatous leprosy in which nerve damage may occur in the absence of infiltrating inflammatory cells. In this respect, leprosy neuropathy is similar to the Guillain-Barré syndrome and the M-protein peripheral neuropathy. In the latter two diseases, demyelination and loss of nerve function is thought to be mediated by autoantibodies that bind to peripheral nerve myelin proteins. In light of these observations, we decided to explore the role of autoimmune mechanisms in the neuropathy of leprosy.

Sera from 43 leprosy patients were tested for anti-neural antibodies capable of binding to normal human nerves. The leprosy patients were from Chiang Mai, Thailand, and represented all Ridley-Jopling classifications of the disease. The sera were screened by an indirect immunofluorescent antibody technique on frozen sections of normal human sciatic nerve and spinal cord obtained at autopsy. Seventeen of the 43 sera (38%) produced fluorescent staining at a dilution of 1:20. The positive sera were distributed across the disease spectrum and were not predominant in any one type of leprosy. The peripheral nerve staining appeared as a ring

at the junction of the axon and the myelin sheath. The spinal cord showed fluorescent staining in the white matter, particularly in the dorsal and ventral horn regions. The antibody involved was of the IgG isotype exclusively.

Control sera were obtained from 10 healthy leprosy contacts living in Thailand, and 10 tuberculosis patients from the United States. These were all found to be negative. Sera from 10 healthy U.S. subjects were also tested and all but one of these was negative, the exception showing weak staining of a pattern similar to that seen with the positive leprosy sera.

It has been reported that lepromatous patients are often hypergammaglobulinemic and/or have a high prevalence of autoantibodies. Although many of the positive sera were drawn from nonlepromatous patients, it was necessary to preclude the possibility that nonspecific binding, due to either an increased amount of IgG in the serum, a generalized polyclonal activation, or binding of IgG to Fc receptors, was the cause of the fluorescent staining. Firstly, serum IgG levels were measured by radial immunodiffusion on samples from 10 patients with and 10 without anti-neural antibodies (ANeAB). Raised IgG levels were found in both populations but the mean values were not significantly different, indicating that an increase in serum IgG did not account for the positive staining pattern. Secondly, 10 ANeAB-positive and 10 ANeAB-negative serum samples were tested for autoantibodies against smooth muscle, cardiac muscle, mitochondria, nuclei, and parietal cells. No antibodies to parietal cells or mitochondria were found in either group, and the frequency of smooth muscle and cardiac muscle antibodies was similar in both groups. Anti-nuclear antibodies were found more often in the ANeAB-negative group. These results strongly suggest that the anti-neural staining was not due to a generalized B-cell activation. Thirdly, the binding of human IgG Fc fragments to peripheral nerve sections was examined. There was slight background staining when sections were incubated with Fc fragments, followed by anti-isotype fluorescent serum. But when sciatic nerve sections were first treated with Fc fragments, then with the patients' sera, and finally with a fluorescent anti-light chain serum, the characteristic axonal staining pattern was unimpaired, indicating that binding via Fc receptors was not the cause of the positive reactions.

To determine if the antibody involved was primarily directed against *Mycobacterium leprae* but able to crossreact with a peripheral nerve component, sera from ANeAB-positive and -negative patients were absorbed with irradiated *M. leprae*. This treatment did not eliminate the positive fluorescent staining of nerves.

Having established that the neural antibodies were not artifactual, attempts were then made to identify the antigen(s) against which the antibodies were directed. Absorption studies have been done with myelin and crude axolemma fractions of human peripheral nerves. These fractions were unable to absorb the autoantibody. Human myelin components were also separated by SDS-PAGE and transferred to nitrocellulose paper. These immunoblots were tested with patient sera and an enzyme-conjugated second antibody. These results were negative, confirming the lack of myelin staining in the frozen sections and the inability of myelin fractions to absorb the anti-neural antibody.

A neurofilament fraction of human peripheral nerve and an intermediate filament (IF) fraction from human spinal cord have been isolated. Using the spinal cord IF fraction immunoblots were prepared from SDS-PAGE gels, and were reacted with anti-neural antibody-positive and -negative serum samples. Subsequent staining showed the presence in the sera of positive patients of IgG antibodies that bind to a protein band migrating at approximately 50,000 daltons. A rabbit antiserum raised to a bovine brain IF preparation showed staining of a protein band of the same molecular weight, as well as bands corresponding to other intermediate filament proteins. Further characterization of the antigen(s) to which the human neural antibodies bind is in progress.—[Wayne State University, School of Medicine, Detroit, Michigan, U.S.A.]

Acknowledgment. This work was supported by U.S.P.H.S. grant no. AI-20198.

Job, C. K., Harris, E. B., Allen, J. L. and Hastings, R. C. A possible mode of transmission of armadillo leprosy in the wild

and a simple method to conduct a random survey of its prevalence.

When experimental armadillos develop disseminated disease, the ears are always found to be infiltrated by lepromatous granulomas. In a study of indigenous armadillo leprosy, 28 out of 34 ear specimens examined from infected animals showed evidence of disease. Ear biopsies are routinely used to screen experimentally infected animals to detect generalized disease.

It is a common sight on the roads of Louisiana to see armadillos hit and killed by automobiles, and these animals provide an opportunity to conduct a random survey of leprosy among armadillos. Both ears in their entirety are removed from the killed animals, fixed in 10% buffered Formalin, and brought to the laboratory by the staff of the Laboratory Research Branch. Two representative pieces from each ear are processed for paraffin section. Five μm sections are made and stained for acid-fast organisms according to the modified Fite's method. The slides are carefully examined for acid-fast bacilli (AFB), and the histopathological changes are studied. The exact geographical location of the animals is also recorded.

To date, of the 330 specimens studied 41 were autolyzed and were discarded. Of the remaining 289, 9 showed macrophage granulomas containing AFB. The presence of these organisms inside the nerves in all of the AFB-positive specimens confirms that they are *Mycobacterium leprae.* The prevalence rate of the disease in this study was, therefore, 3.1% compared to the 6.8% and 4.6% rates in previous studies done in Louisiana.

An analysis of the distribution of the disease in the parishes of Louisiana from which most of the specimens are collected showed evidence of clustering.

Most of the ear specimens showed nonspecific inflammation, but a prominent feature was the presence of thorns in 25.3% of them. Thorns were seen in the epidermis and dermis and there was a well-marked, foreign body reaction around most of them, followed by disintegration. In some there was no reaction at all.

In 27 animals, the tip of the nose was excised along with the ears and examined histopathologically. Thorns were seen in 25.5% of them. Diffuse infiltration by macrophages containing AFB were seen in 2 animals which also had lepromatous disease of the ears. In 1 animal with no evidence of ear involvement, sections of thorns were seen at four separate areas in the nose. Localized to the tissue immediately surrounding 1 of the 4 thorns, scattered macrophages containing AFB were present. Also AFB were seen inside a nerve bundle, confirming that they are, indeed, *M. leprae.* The absence of AFB in other areas of the nose and in the ears of this animal in all probability rules out generalized disease. Therefore, it is reasonable to suggest that AFB entered the tissue along with the thorn, and that wild armadillo leprosy is transmitted through infected thorns.—[Laboratory Research Branch, National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Thomas, D. A., Mines, J. S., Mack, T. M., Thomas, D. C. and Rea, T. H. Armadillo exposure among Mexican-born patients with lepromatous leprosy.

The findings of a leprosy-like illness in feral armadillos (*Dasypus novemcinctus*) in Louisiana, Texas, and most recently in Mexico, a disease identical to that produced in captive armadillos inoculated with human-derived *Mycobacterium leprae,* has raised questions concerning a possible relationship between leprosy in man and leprosy in armadillos. The initial study addressing this issue, conducted in Louisiana, found no evidence connecting leprosy in the two species. However, a report from Texas of leprosy in man associated with exposure to armadillos does not permit the question to rest.

Of the conceivable relationships, two kinds appear to be amenable to testing by epidemiological studies. In one, humans may be at risk to develop leprosy by virtue of contact with armadillos or armadillo products, i.e., direct exposure. This possibility would be of importance for people living in areas inhabited by nine-banded armadillos, but the problem would be a regional one, confined to North America.

In the other possibility, humans and armadillos share an ecological niche in common, making both species vulnerable to the development of leprosy, i.e., indirect ex-

posure. This possibility is of general importance, even where armadillos do not exist, because of its clear implication that *M. leprae* may be a soil organism and that propagation of leprosy may not depend upon person-to-person transmission. In this possibility the armadillo is envisioned as a mobile combination of Petri dish, culture media, and incubator, uniquely qualified to identify *M. leprae.* These two possibilities are not mutually exclusive, i.e., humans could be at risk to develop leprosy by armadillo contact directly and both species could be at risk to develop leprosy because of a shared ecologic factor.

Because both possibilities are of importance, we have conducted a small, case-control study designed to find or to exclude an association between armadillo exposure and leprosy in Mexican residents of Los Angeles, California, U.S.A. Eighty-nine patients were classified as follows: 76 LL, 8 BL, 2 BB, and 3 BT. Eighty controls, chosen from patients attending two Los Angeles County comprehensive health care clinics, were frequency matched for age and sex. Criteria for selection of participants included residence in Mexico and the east side of Los Angeles County, California. Two kinds of exposure were sought and distinguished from one another: direct contact, i.e., occupational, dietary or reactional; and indirect contact, i.e., lived in an area inhabited by armadillos with or without direct exposure.

Direct contact with armadillos was more frequent among male patients, 32 of 56, than among male controls, 9 of 41, but was similar in women, 9 of 33 patients and 6 of 39 controls. Indirect armadillo exposure was more common in patients than in controls in both sexes: male patients, 44 of 56, and male controls, 18 of 41; female patients, 22 of 33, and female controls, 16 of 39. Also, indirect exposure was similar before and after age 10. However, cases and controls differed on a number of potentially confounding factors, notably the region of Mexico and the size of town from which they had come. Male cases also reported more frequent direct iguana and deer exposure; although not significant, the difference suggested a possible reporting bias. Armadillo effects were therefore adjusted for age, size of town, region, and exposure to iguana or deer using an unconditional multiple logistic analysis, treating exposure both as binary and as continuous.

The adjusted odds ratios for indirect and direct exposure, relative to no relationship between exposure and outcome being assigned a numerical value of 1.0, are summarized. In men, the adjusted odds ratio for indirect exposure was 4.2 and for direct exposure, 6.8 ($p < 0.001$ in both). In women, the adjusted odds ratio for indirect exposure was 3.7 and for direct exposure, 2.6 ($p < 0.001$ and $p < 0.01$, respectively).

Therefore, in men and women both direct and indirect armadillo exposure appear to be significant risk factors in the development of leprosy. Because of the skewed patient population, these conclusions pertain only to lepromatous leprosy.—[University of Southern California School of Medicine, Los Angeles, California 90033, U.S.A.]

Acknowledgment. This work was supported by a gift from the Military and Hospitaller Order of St. Lazarus of Jerusalem.

Cohn, Z. A., Kaplan, G., Steinman, R. M., Witmer, M. and Nath, I. The role of lymphokines in cell-mediated immunity.

In the presence of an appropriate accessory cell, helper T cells and antigen, T cells are stimulated to undergo blastogenesis, replication and secrete a number of potent cytokines into their environment. These include both interleukin-2 (IL-2) and gamma-interferon (γ-IFN). One of the important defects in patients with lepromatous leprosy is their inability to respond to *Mycobacterium leprae* antigens. This is expressed both as an inability of their T cells to incorporate ^{3}H-Tdr and to release both γ-IFN and IL-2. We currently believe γ-IFN is the most potent macrophage-activating factor now known. Human monocytes exposed to γ-IFN will, in the subsequent 48 hr, demonstrate a markedly enhanced production of toxic oxygen intermediates and the ability to kill intracellular parasites. Monocytes from patients with lepromatous leprosy respond normally to active T-cell supernatants and to recombinant γ-IFN. However supernatants from T cells of lepromatous patients exposed to *M. leprae* fail to activate normal monocytes, whereas those from tuberculoid patients do activate monocytes for

an intracellular kill. This suggests that monocytes of lepromatous patients are responsive, but that the defect is in lymphokine formation.

Gamma-interferon has other effects which may also influence cell-mediated immunity. It enhances the expression of Ia determinants and Fc receptors on a variety of cell types. In addition, A. Luster in our laboratory has discovered the rapid induction of a novel protein in macrophages, endothelium, and fibroblasts. This polypeptide has a strong homology to platelet factor 4 and is a potent (10^{-9} M) chemotactic factor. A synthetic peptide based upon the derived structure is also strongly chemotactic. These data suggest a more global influence of γ-IFN on cells other than "professional immunocytes" but cells which are also in the milieu of the delayed reaction.

Ongoing studies on leprosy patients in New Delhi, India (in collaboration with I. Nath) are evaluating the dermal responses to a delayed-type tuberculin reaction. Preliminary results indicate that some lepromatous patients can respond positively with the influx of large numbers of helper T cells and monocytes. In addition, there appears to be a dramatic reduction in the overlying Langerhans' cells of the epidermis and their presence within the reaction of the upper dermis. These cells have recently been shown to be potent accessory cells in T-cell responses (Schuler and Steinman). Finally, very dramatic modifications of the overlying epidermal keratinocytes take place. These include proliferative response, cell enlargement, thickening of the epidermis, and the expression of Ia on the surface of the new keratinocytes. This suggests the production of potent epidermal growth factor(s) by the cells accumulating in the delayed reaction. The induction of this type of response by purified lymphokines is discussed.

Studies at the electron microscope level are evaluating the influence of newly emigrating T cells and monocytes on the pre-existing foamy macrophages and intracellular *M. leprae.*—[The Rockefeller University and The Irvington House Institute, New York, New York 10021, U.S.A.; All India Institute of Medical Sciences, New Delhi, India]

Goren, M. B., Bruyninckx, W. J., Leung, K.-P., Swendson, L. S., Heifetz, L. and Fiscus, J. Functionality of secondary lysosomes in murine resident peritoneal macrophages.

During the last decade, a variety of lysosomotropic agents have been recognized that allow manipulation of lysosomal activities in mouse peritoneal macrophages. Polyanionic agents (which embrace the sulfatides of *Mycobacterium tuberculosis* because of their micellar form in aqueous environment and include dextran sulfate, poly-*D*-glutamic acid, etc.) accumulate in secondary lysosomes and inhibit their ability to fuse with phagosomes. Some weak bases also perturb lysosomal behavior (probably both primary and secondary lysosomes). Ammonium ion was described as inhibiting fusion, while chloroquine is alleged to promote fusion. Both raise the intralysosomal pH and therefore antagonize the activity of the acid hydrolases.

We studied the effects of polyanions on several functions of mouse resident peritoneal macrophages in order to assess the consequences of the antagonism of secondary lysosomal fusion function, and further examined the behavior of resident cells after they had endocytosed granules obtained from freshly isolated human neutrophils and thereby acquired myeloperoxidase (MPO).

We originally established, by electron microscopy, that polyanion-treated cells labeled with Thorotrast were inhibited in delivering the lysosomal marker to yeast-containing phagosomes. When the target for phagocytosis was heat-killed (boiled) yeasts, the inhibition was maintained as fully as with viable yeasts. This contradicts the earlier conclusions drawn from observations on cells labeled with acridine orange, and illustrates one of the artifactual consequences of using this fluorescent marker.

We first studied intracellular digestion of radiolabeled particulate substrates as affected by polyanion accumulation in secondary lysosomes. The time course of digestion was followed by measuring the release of TCA-soluble label. Our results showed that digestion of ^{125}I-KLH (keyhole limpet hemocyanin)-anti KLH, of ^{32}P-labeled viable or heat-killed *Saccharomyces cerevi-*

siae, of ^{125}I-labeled zymosan, and of ^{125}I-labeled heat-killed yeasts was identical in control and in "polyanion" cells. We suggest that this is probably a consequence of primary lysosomal activity—and that it may represent the state of affairs in both normal and polyanion macrophages, i.e., secondary lysosomes may play only a minor role in digestion.

Polyanion and control cells also exhibited essentially identical microbicidal activity for the targets we examined: *Escherichia coli* and *Streptococcus faecalis.* This confirms the conclusions of other investigators as well. Therefore, lysosomal accumulation of polyanions seems to have essentially no effect on two principal functions of mouse resident peritoneal macrophages.

As a third study, we examined the functionality of neutrophil granule enzymes after they had been incorporated into macrophage secondary lysosomes by endocytosis. We had earlier found that the endocytosed neutrophil myeloperoxidase (MPO) was functional in Klebanoff-type iodination elicited by a phagocytic pulse of zymosan. In current studies, electron microscopic cytochemistry confirmed that the macrophages accumulated significant MPO—presumably in secondary lysosomes—and that they delivered this in abundance to phagosomes enclosing targets of yeast or *S. faecalis,* for example. Despite this delivery of enzymatically active MPO, these and control cells (no MPO) showed identical killing activity for *E. coli, S. faecalis* and *M. intracellulare.* Killing of the mycobacteria was enormously enhanced after the macrophages endocytosed glucose oxidase conjugated to zymosan, but the effect was identical with control and with "MPO" cells.

In toto, these data suggest that in resident murine peritoneal macrophages, secondary lysosomes do not play a significant role in digestion or in intracellular killing, and that the resident cells are incapable of utilizing MPO for its bactericidal potential once it is incorporated into the secondary lysosomal system.—[National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206, U.S.A.]

Fujiwara, T., Izumi, S. and Brennan, P. J. The synthesis and activity of the sugar derivatives related to phenolic glycolipids of *Mycobacterium leprae* and preparation of sugar-protein conjugates.

The synthesis of the nonreducing end disaccharide of phenolic glycolipid-I (PGL-I), 3,6-di-*O*-Me-β-D-Glc*p*(1 → 4)2,3-di-*O*-Me-α-L-Rha*p*, was much more improved by using methylation with diazomethane-boron trifluoride. This synthetic method was simple and effective enough for the mass production of the disaccharide.

The disaccharides with aglycones were synthesized and their activities were tested by ELISA. α-Linked stearyl disaccharide and α-linked lauryl disaccharide showed high activities. Stearyl disaccharide with the 2′-*O*-acetylated glucose residue showed very high activity but specificity was decreased.

Natural disaccharide was coupled with bovine serum albumin (BSA) by reductive amination to give a highly active sugar-BSA conjugate. In this case, specificity was also decreased. The disaccharides with methyl 3-(*p*-hydroxyphenyl)-propionate were coupled with BSA by the acyl azide method to give a highly active sugar-BSA conjugate. The conjugates had 20–30 moles of disaccharides/mole-BSA which were very good incorporation rates. The 3,6-di-*O*-Me-β-D-Glc*p*(1 → 4)2,3-di-*O*-Me-α-L-Rha*p*-BSA conjugate showed very good concordance with the natural PGL-I in ELISA on its specificity and activity. These results strongly suggest the possibility of the practical use of the conjugate for the serodiagnosis of leprosy.

The chemical synthesis of the trisaccharide with the linker arm, methyl 3-(*p*-hydroxyphenyl)-propionate, is also reported.—[Institute for Natural Science, Nara University, Nara, Japan; Leprosy Research Laboratory, Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan; Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.]

Mehra, V., Sweetser, D., Bloom, B. R. and Young, R. Recombinant DNA clones expressing *M. leprae* and *M. tuberculosis* antigens: potential new tools for immunodiagnosis and prophylaxis.

We have used a recombinant DNA strat-

egy to isolate genes from *Mycobacterium leprae* and *M. tuberculosis* that encode immunologically relevant proteins in order to generate new tools potentially useful for basic biological studies and development of more effective immunodiagnostic studies and vaccines. Large recombinant DNA expression libraries containing *M. leprae* and *M. tuberculosis* genomic DNA fragments were co: structed in the bacteriophage vector lambda gt11. These libraries were screened with monoclonal antibodies directed against many of the major proteins of both bacilli. Recombinant DNA clones which produced antigens that reacted with these antibodies were isolated and further characterized. All clones were subjected to restriction endonuclease mapping. Particular attention has been focused on the 65KD antigen gene of *M. leprae,* which is being sequenced. This strategy offers an opportunity a) to examine the precise location of each epitope, b) to determine the relevance of each antigen to delayed-type hypersensitivity, and c) to assess the usefulness of these antigens for immunodiagnosis and vaccination purposes.—[Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10561; Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02141; Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, U.S.A.]

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CLOSING REMARKS

Dr. Fauci, Dr. Goren, Dr. Tokunaga, Dr. Hastings, Ladies and Gentlemen:

On behalf of the Japanese Leprosy Panel and participants, I would like to express our deepest appreciation to Dr. Hastings, to the members of the U.S. Leprosy Panel, to Dr. Goren, to the members of the U.S. Tuberculosis Panel, to Dr. Fauci, and to the staff of the National Institute of Allergy and Infectious Diseases for their many acts of kindness and for splendidly organizing the Twentieth U.S.–Japan Leprosy and Tuberculosis conferences sponsored by the U.S.–Japan Cooperative Medical Science Program. Since 1983, the leprosy and the tuberculosis panels have held a joint symposium, and today we are meeting again with our colleagues in tuberculosis. Although we have finished the Twentieth U.S.–Japan Research Conference over the past two days and are just closing this symposium, we look forward to attending the Twentieth U.S.–Japan Tuberculosis Research Conference over the next two days.

It has been a great pleasure to have had the opportunity of participating in these meetings and to have learned much new knowledge on leprosy, especially the remarkable progress in the immunology of leprosy. We have, moreover, been encouraged and inspired by the presentations on the immunology of leprosy, tuberculosis and other diseases at this symposium. We strongly stress the importance of immunology for leprosy and tuberculosis research. However, our final goal—the eradication of the disease—is so broad and so distant that we must make every effort to organize more powerful cooperation among different approaches toward this ultimate goal.

At this opportunity, I would like to express our deepest condolences on the deaths of Dr. Charles Shepard and Dr. Mitsugu Nishiura who both contributed greatly to the development of the U.S. and the Japanese Leprosy Panels. Their sudden deaths will be an irrevocable loss for our activities. Therefore, it is our sincere desire that this loss will soon be supplemented by renewing our old friendships, by beginning new friendships, and by sharing new knowledge, and that a more effective and powerful approach toward our final goal will be achieved by this form of international cooperation. I look forward to welcoming you to our country next year for the Twenty-first U.S.–Japan Leprosy Research Conference.

Thank you very much.

—Masahide Abe, *Chairman*
Japanese Leprosy Panel