

ABSTRACTS

FIRST U.S. HANSEN'S DISEASE RESEARCH CONFERENCE

National Hansen's Disease Center
Carville, Louisiana, U.S.A.
13–15 August 1984

OPENING REMARKS

First let me extend my heartiest welcome to Carville. We are delighted that so many of you have been able to attend this, the First U.S. Hansen's Disease Research Conference.

The pace of research in Hansen's disease is increasing and research findings are accumulating more and more rapidly. At the first meeting of the HD Research Advisory Committee at Carville last year, there was felt to be a need for U.S. investigators in HD to have an annual forum to present and discuss their findings. The traditional opportunities for these presentations have come at the annual U.S.–Japan Leprosy Research Conferences sponsored by the U.S.–Japan Cooperative Medical Science Program and the National Institute of Allergy and Infectious Diseases of NIH. As a practical matter, when the U.S.–Japan Leprosy Research Conferences are held in a country, either the U.S. or Japan, there are only a limited number of participants from the other country who are able to attend. In an effort to provide more rapid communication of a broader amount of research findings and on the unanimous advice of the

HD Research Advisory Committee, the National Hansen's Disease Center decided to sponsor a U.S. HD Research Conference here at Carville this year—a year when the U.S.–Japan Leprosy Research Conference will be held in Tokyo. Next year we look forward to seeing you at the U.S.–Japan Leprosy Research Conference to be held in this country. We hope to again sponsor a U.S. HD Research Conference in 1986, when the U.S.–Japan Conference will again be held in Japan.

We are honored to have with us, as chairmen of the scientific sessions, the extramural members of the HD Research Advisory Committee. We are extremely fortunate in having an excellent group of scientific papers. If there is anything we can do to make your stay more comfortable or more enjoyable, please let us know.

Thank you all very much for coming.

—John R. Trautman, M.D.
*Assistant Surgeon General
Director, National Hansen's
Disease Center*

FIRST U.S. HANSEN'S DISEASE RESEARCH CONFERENCE

PROGRAM

13 August 1984, Monday

Welcome and Announcements: Dr. John R. Trautman, Assistant Surgeon General;
Director, National Hansen's Disease Center

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Jacobson, Robert R. Clinical aspects

Williams, K., Bahlinger, V., Heroman, B., Madrigal, D. and Brantley, P. The relationship between perceived social support and illness reports in Hansen's disease patients

Schauf, V., Ryan, S., Nelson, K., Brown A., Scollard, D. and Smith, T. Tuberculoid leprosy in Thailand is associated with HLA-DR2

Ching, C. Y., Pollack, M., Reichert, E., Hokama, Y., Fujikawa, R., Loui, W., Sato, D., Wong, C. and Ching, N. Analysis of immunologic and genetic factors in multicase families with Hansen's disease in Hawaii

Cynamon, M. H., Palmer, G. and Sorg, T. B. *In vitro* activity of ampicillin compared to ampicillin and sulbactam against *Mycobacterium tuberculosis*

Harris, E. B. and Prabhakaran, K. Malonyl-CoA decarboxylase activity in *Mycobacterium leprae*

Shannon, E. J., Truman, R. W., Christy, S. A., Vadiie, R. and Hastings, R. C. Effect of thalidomide on induction of antibody synthesis in mice to a T-independent antigen—DNP Ficoll

Morales, M. J., Piper, L., Yoder, L., Job, C. K. and Hastings, R. C. The pharmacology of intermittent clofazimine therapy in Hansen's disease

Session II. *Chairman: W. M. Meyers*

Dhople, A. M., Kazda, J. and Storrs, E. E. Presence of "difficult-to-grow" mycobacteria in armadillos

Cehl, S. K. and Hastings, R. C. An anomalous response of *M. leprae* to dapsone chemotherapy in nude mice

Skinsnes, O. K., Chang, P. H. C., Kuba, B. and Gaines, S. Numerical taxonomic and immunologic comparison of 36 leprosy-derived mycobacterial strains and 17 members of the MAIS complex

Prabhakaran, K. and Harris, E. B. Temperature effects on viability, diphenoloxidase, and permeability of *Mycobacterium leprae*

Martin, L. N., Gormus, B. J., Wolf, R. H., Baskin, G. B., Meyers, W. M., Walsh,

G. P. and Binford, C. H. Relationship between depressed lymphocyte responsiveness to mitogens and the percentage of OKT8⁺ lymphocytes in experimental leprosy in the mangabey (*Cercocebus atys*)

Kvach, J. T., Neubert, T. A. and Heine, H. S. The adenosine triphosphate content and death rate of *Mycobacterium leprae* isolated from armadillo tissue by Percoll buoyant density centrifugation

Jacobs, W. R., Docherty, M. A., Clark-Curtiss, J. E. and Curtiss, R., III. Expression of cloned *Mycobacterium leprae* DNA in *Escherichia coli* K-12

Imaeda, T. and Portaels, F. DNA relatedness among *Mycobacterium leprae*, leprosy-derived bacteria, and established mycobacterial species

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Session III. Chairman: C. C. Shepard

- Gelber, R. H.** Studies on antimicrobial activity against *M. leprae* infections of the mouse foot pad
- Gelber, R. H., Humphres, R. C. and Fieldsteel, A. H.** A comparative study of four rodent systems to monitor initial therapy of lepromatous leprosy: in search of a more sensitive system to assess bacterial viability and optimal bactericidal therapy
- Navalkar, R. G. and Ibegbu, C.** Immunologic assessment of *Mycobacterium leprae* antigens using isoelectric focusing and chromatofocusing separation
- Lefford, M. J.** *Mycobacterium lepraemurium* infection of T cell-depleted mice: implications concerning the immune response in intact mice
- Humphres, R. C. and Winters, M. A.** Effects of *Mycobacterium leprae* vaccination on cell-mediated immune function of Lewis rats: specific sensitization vs tolerance
- Collins, F. M. and Orme, I. M.** Immunocompetence of the heavily infected host
- Truman, R. W., Shannon, E. J., Hugh-Jones, M. E., Hagstad, H. V. and Hastings, R. C.** Detection of IgM class antibodies in armadillos to the phenolic glycolipid-I antigen of *M. leprae*
- Baskin, G. B., Wolf, R. H., Gormus, B. J., Martin, L. N., Walsh, G. P., Binford, C. H. and Meyers, W. M.** Experimental leprosy in the mangabey (*Cercopithecus atys*): necropsy findings
- Malaty, R., Meyers, W. M., Walsh, G. P., Binford, C. H., Zimmerman, L. E., Baskin, G. B., Wolf, R. H., Gormus, B. J., Martin, L. N. and Gerone, P. J.** Histopathological changes in the eye of a mangabey monkey with lepromatous leprosy

Session IV. Chairman: G. Stoner

- Long, E. G., Smith, J. S., Head, E. C., Brennan, P. J. and Smith, J. H.** A specific glucose oxidase immunoenzyme stain for *Mycobacterium leprae*
- Krotoski, W. A. and McCormick, G. T.** Immunofluorescence studies on *Mycobacterium leprae* in fixed tissues
- Young, D. B., Khanolkar, S. R. and Buchanan, T. M.** Multiple strategies for the identification of antigen and species specificity of monoclonal antibodies reactive with *Mycobacterium leprae*
- Mohagheghpour, N., Fong, S. K. H., Gelber, R. H., Brennan, P. J. and Engleman, E. G.** Production of human monoclonal antibodies to *Mycobacterium leprae*
- Gillis, T. P., Miller, R. A. and Buchanan, T. M.** Characterization of a competitive-antibody-binding assay with a radiolabeled monoclonal antibody specific for *Mycobacterium leprae*
- Brennan, P. J.** Diagnosis of leprosy based on the 3,6-di-O-methyl- β -D-glycopyranosyl epitope; assay of antibodies with natural and synthetic probes; assay of antigen by chemical and immunological means
- Job, C. K., Sanchez, R. S. and Hastings, R. C.** Phenolic glycolipid-I: an immunogen for a Mitsuda response
- Mehra, V., Brennan, P. J. and Bloom, B. R.** Mechanism of unresponsiveness in lepromatous leprosy

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Session V. *Chairman: D. Gwin*

- Douglas, J. T., Brennan, J. P. and Gelber, R. H.** ELISA evaluation of phenolic glycolipid-I (PG-I) and whole *M. leprae* with sera collected from leprosy patients during the course of chemotherapy
- Rea, T. H., Modlin, R. L. and Taylor, C. R.** Anti-interleukin-2 positive and anti-Tac positive cells in erythema nodosum leprosum, lepromin reactions, and tuberculin reactions
- Modlin, R. L., Tapia, F. J., Bloom, B. R., Convit, J. and Rea, T. H.** *In situ* characterization of the cellular immune response in leprosy and leishmaniasis
- Mohaghehpour, N., Gelber, R. H., Sasaki, D. T., Brennan, P. J. and Engleman, E. G.** Effects of recombinant IL2 on the *Mycobacterium leprae*-induced T cell response in lepromatous leprosy
- Miller, R. A., Harnisch, J. P., Gilliland, B. C., Lukehart, S. A. and Buchanan, T. M.** Low prevalence of serologic evidence of polyclonal B cell activation among Hansen's disease patients in Seattle
- Schuller-Levis, G., Cutler, E., Davis, R., Harris, D. and Levis, W.** Monocyte chemotaxis in leprosy
- Levis, W. R., Meeker, H. C., Sersen, G. and Schwerer, B.** IgM and IgG antibodies against phenolic glycolipid-I from *Mycobacterium leprae* in leprosy sera: relationship to bacterial index and erythema nodosum leprosum
- Tausk, F., Schreiber, R., Lopez, D. A. and Gigli, I.** Reduced expression of C3b receptors on erythrocytes from patients with lepromatous leprosy
- Campbell, P. B., Tolson, T. A., Yoder, L., Loesch, J. and Krahenbuhl, J. L.** Defective regulation of monocyte leukotaxis in leprosy

PARTICIPANTS

- Agrawal, Krishna**, Pharmacology Department, Tulane University School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112
- Almodovar, Pablo I.**, 617 Dr. Pavic St., Santurce, Puerto Rico 00909
- Atkinson, Maureen**, 1326 NE-68, Seattle, WA 98115
- Bahlinger, Vernon**, Social Services Department, National Hansen's Disease Center, Carville, LA 70721
- Baskin, Gary**, Delta Regional Primate Center, Three Rivers Road, Covington, LA 70433
- Beach, Robert**, Rehabilitation Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Bell, Judith**, Rehabilitation Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Binford, Chapman**, Microbiology Department, Armed Forces Institute of Pathology, 6825 16th St. N.W., Washington, DC 20306-6000
- Bizorik, Dorothy**, Hansen's Disease Contract Health Services Program, County of Los Angeles, 313 North Figueroa St., Los Angeles, CA 90012
- Brandsma, William**, Rehabilitation Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Branon, Dana**, P.O. Box 3911, La Feria, TX 78559
- Brennan, Patrick**, Associate Professor, Department of Microbiology, Colorado State University, Fort Collins, CO 80523
- Brown, Linda**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Bruhjill, Helene**, Maricopa County Health

- Department, P.O. Box 2111, ATTN: TB Control, Phoenix, AZ 85001
- Buchanan, Thomas**, Professor of Medicine and Pathobiology, Immunology Research Laboratory, Pacific Medical Center, 1200 112th Ave. South, Seattle, WA 98144
- Buford, William**, Rehabilitation Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Campbell, Peter**, Department of Medicine, East Carolina University School of Medicine, Greenville, NC 27834
- Cariappa, A.**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Chehl, Sumir**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Ching, Clara**, Department of Medicine, University of Hawaii Medical School, St. Francis Hospital, Sullivan #476, 2230 Liliha St., Honolulu, HI 96817
- Christy, Sterling**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Clark-Curtiss, Josephine E.**, Department of Biology, Washington University, Campus Box 1137, St. Louis, MO 63130
- Clements, Bruce**, Deputy Chief, Clinical Branch, National Hansen's Disease Center, Carville, LA 70721
- Collins, Frank**, Medical Research Laboratories, Trudeau Institute, Inc., Saranac Lake, NY 12983
- Courrege, Mary Lou**, 937 Kerlerec St., New Orleans, LA 70116
- Dhople, Arvind**, Florida Institute of Technology, 7725 W. New Haven Ave., Melbourne, FL 32901
- Diggs, Cheryl**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Douglas, Jim**, Department of Microbiology, University of Hawaii, 2538 The Mall, Honolulu, HI 96822
- Douglas, Martin A.**, Washington University, St. Louis, MO 63120
- Dube, S. K.**, Department of Pharmacology, Yale University Medical School, P.O. Box 3333, New Haven, CT 06510
- Ellis, Irma**, National Hansen's Disease Center, Carville, LA 70721
- Elwood, Julia**, National Hansen's Disease Center, Carville, LA 70721
- Figueson, Judith**, Department of Dermatology, University of Puerto Rico School of Medicine, GPO Box 5067, San Juan, Puerto Rico 00936
- Foy, Kathleen**, Hawaii State Department of Health, 3650 Maunalei Ave., Honolulu, HI 96816
- Gelber, Robert**, Seton Medical Center, 1900 Sullivan Ave., Daly City, CA 94015
- Gigli, Irma**, Professor of Medicine, Chief, Division of Dermatology, University of California Medical Center, San Diego, 225 Dickinson St., San Diego, CA 92103
- Gillen, Janet**, 387½ Jersey St., San Francisco, CA 94114
- Gillis, Thomas P.**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Gooch, John**, Hansen's Disease Program, Hawaii Department of Health, P.O. Box 3378, Honolulu, HI 96801
- Goodman, Dee**, Assistant Editor, International Journal of Leprosy, National Hansen's Disease Center, Carville, LA 70721
- Gormus, Robert**, Delta Regional Primate Center, Three Rivers Road, Covington, LA 70433
- Grosset, Jacques**, Bacteriologie et Virologie, Faculte de Medecine Pitié-Salpêtrière, 91 Boulevard de l'Hopital, 75634 Paris Cedex 13, France
- Gwinn, Darrel D.**, Leprosy Program Officer, Bacteriology and Virology Branch, Microbiology and Infectious Diseases Program, National Institute of Allergy and Infectious Diseases, 738 Westwood Building, Bethesda, MD 20014
- Hagstead, Harry**, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803-8416
- Harnisch, James**, Division of Dermatology, Room 14, Department of Medicine, University of Washington, Seattle, WA 98144
- Harris, Eugene**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Hastings, Robert C.**, Chief, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Heine, Henry**, Leonard Wood Memorial, Department of Immunology, Johns Hopkins University, 615 N. Wolfe St., Baltimore, MD 21205

- Heroman, Bunny**, Social Services Department, National Hansen's Disease Center, Carville, LA 70721
- Humphres, Robert**, Immunology Section, Stanford Research International, 333 Ravenswood Ave., Menlo Park, CA 94025
- Imaeda, Tamotsu**, Professor, Department of Microbiology, University of Medicine and Dentistry, 100 Bergen St., Newark, NJ 07103
- Jacobs, William**, Department of Biology, Washington University, Campus Box 1137, St. Louis, MO 63130
- Jacobson, Robert R.**, Chief, Clinical Branch, National Hansen's Disease Center, Carville, LA 70721
- Job, C. K.**, Pathology Research Department, National Hansen's Disease Center, Carville, LA 70721
- Juokins, Carol**, San Diego Hansen's Disease Clinic, North San Diego Health Center, 2440 Grand Ave., San Diego, CA 92109
- Kahkonen, Martti**, Pathology Research Department, National Hansen's Disease Center, Carville, LA 70721
- Kanatani, Frank**, National Hansen's Disease Center, Carville, LA 70721
- King, Barry**, Tulane University, 1430 Tulane Ave., New Orleans, LA 70112
- Knapp, Merrill**, Stanford Research International, 333 Ravenswood Ave., Menlo Park, CA 94025
- Krahenbuhl, James L.**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Krotoski, W. A.**, Deputy Chief, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Kuechler, Carolyn**, Department of Dermatology, M/C 624, University of Illinois College of Medicine at Chicago, P.O. Box 6998, Chicago, IL 60680
- Kvach, James**, Leonard Wood Memorial, Department of Immunology and Infectious Diseases, Johns Hopkins University, 615 N. Wolfe St., Baltimore, MD 21205
- LaChine, Alleine**, Department of Dermatology, M/C 624, University of Illinois College of Medicine at Chicago, P.O. Box 6998, Chicago, IL 60680
- Langa, Andy**, 2357 Channing Ave., Scotch Plains, NJ 07090
- Lefford, Maurice J.**, Department of Immunology and Microbiology, Wayne State University School of Medicine, 540 E. Canfield Ave., Detroit, MI 48201
- Levis, William**, Chief, Dermatology Department, Bayley-Seton Hospital, Bay St. and Vanderbilt Ave., Staten Island, NY 10304
- Li, Su-Chen**, Biochemistry Department, Tulane University School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112
- Locniskar, Mary**, New England Medical Center, 171 Harrison Ave., Boston, MA 02111
- Loesch, Julie**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Long, Earl**, Department of Pathology, The University of Texas Medical Branch at Galveston, Galveston, TX 77550
- Longley, Jack**, Columbia Presbyterian Medical Center, 630 West 168th St., New York, NY 10032
- Lopez, D. A.**, Clinical Director, Hansen's Disease Clinic, North San Diego Health Center, 2440 Grand Ave., San Diego, CA 92109
- Madrigal, Dan**, Social Services Department, National Hansen's Disease Center, Carville, LA 70721
- Malaty, Raga**, Francis I. Proctor Foundation for Research in Ophthalmology, University of California-San Francisco, San Francisco, CA 94143
- Martin, Louis**, Delta Regional Primate Center, Three Rivers Road, Covington, LA 70433
- Maxwell, Barbara**, National Hansen's Disease Center, Carville, LA 70721
- McCormick, Gregory**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Meeker, Cliff**, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314
- Mehra, Vijay**, Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461
- Meyers, Wayne**, Walter Reed Army Medical Center, Department of Infectious and Parasitic Diseases, Division of Microbi-

- ology, Armed Forces Institute of Pathology, Room 4111, Washington, DC 20306
- Miller, Richard**, Acting Assistant Professor of Medicine, Division of Infectious Diseases, Pacific Medical Center, 1200 12th Ave. South, Seattle, WA 98144
- Millikan, Larry**, Dermatology Department, Rm. 3551, Tulane University Medical Center, 1430 Tulane Ave., New Orleans, LA 70112
- Modlin, Robert**, Department of Dermatology, LAC-USC Medical Center, 1200 N. State St., Los Angeles, CA 90033
- Mohaghehpour, Nahid**, Stanford Medical School Blood Center, 800 Welch Road, Palo Alto, CA 94304
- Morales, Melvyn**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Moretta, Jane**, Director of Nursing, National Hansen's Disease Center, Carville, LA 70721
- Moschella, Samuel**, Department of Dermatology, Lahey Medical Center, 41 Mall Road, Burlington, MA 01803
- Mroczkowski, Tom**, Dermatology Department, Rm. 3551, Tulane University Medical Center, 1430 Tulane Ave., New Orleans, LA 70112
- Nakaj, Betty F.**, Hawaii State Department of Health, 3650 Maunalei Ave., Honolulu, HI 96816
- Navalkar, Ram G.**, Professor and Chairman, Department of Microbiology and Immunology, Morehouse School of Medicine, 830 Westview Dr., Atlanta, GA 30314
- Neel, Bobbie**, Rehabilitation Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Nelson, Kenrad**, University of Illinois Medical Center, P.O. Box 6998, Chicago, IL 60680
- Neubert, Tom**, Leonard Wood Memorial, Department of Immunology and Infectious Diseases, Johns Hopkins University, 615 N. Wolfe St., Baltimore, MD 21205
- Pasqua, J. P.**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Pepper, Philip P.**, Executive Officer, National Hansen's Disease Center, Carville, LA 70721
- Prabhakaran, K.**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Ramos-Caro, Francisco**, Department of Dermatology, University of Miami School of Medicine (R-250), P.O. Box 016250, Miami, FL 33101
- Randhawa, Baljit**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Rea, Thomas**, Professor of Medicine (Dermatology), University of Southern California School of Medicine, 2025 Zonal Ave., Los Angeles, CA 90033
- Richard, Vernon**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Ross, Ian**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Sanchez, Rita**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Schauf, Victoria**, University of Illinois College of Medicine at Chicago, P.O. Box 6998, Chicago, IL 60680
- Schnorr, Ken**, Department of Veterinary Microbiology, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803-8416
- Schuller-Levis, Georgia**, Bayley-Seton Hospital, Bay St. and Vanderbilt Ave., Staten Island, NY 10304
- Shannon, E. J.**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Shepard, Charles C.**, Leprosy and Rickettsial Unit, Bureau of Lab., Bldg. 7, Rm. B5, Centers for Disease Control, Atlanta, GA 30333
- Skinsnes, Olaf K.**, John A. Burnes School of Medicine, University of Hawaii at Manoa, Leahi Hospital, 3675 Kilauea Ave., Honolulu, HI 96816
- Smith, Cleve**, Strong Hospital, P.O. Box 295, Rochester, NY 14642
- Sorg, Timothy**, Veterans' Administration Medical Center, 800 Irving Ave., Rm. 1118, Syracuse, NY 13210
- Spears, Laura**, 11033 Budwood Lane, Corpus Christi, TX 78410
- Stephens, Joe**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721

- Stewart, Maggie**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Stoner, Gerald**, Department of Health and Human Services, Public Health Service, National Institutes of Health, Building 36, Room 43-17, Bethesda, MD 20205
- Theriot, Stacy**, Rehabilitation Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Todd, Bill**, Department of Microbiology and Parasitology, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803-8416
- Trautman, John R.**, Assistant Surgeon General, Director, National Hansen's Disease Center, Carville, LA 70721
- Truman, Richard W.**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Vadiee, Reza**, Laboratory Research Branch, National Hansen's Disease Center, Carville, LA 70721
- Vitek, Isabel**, Texas State Department of Health, 1100 W. 49th St., Austin, TX 78756
- Walsh, Gerald**, Armed Forces Institute of Pathology, Washington, DC 20306
- Winters, Mark A.**, Stanford Research International, 333 Ravenswood Ave., Menlo Park, CA 94025
- Worobee, Sophie**, Department of Dermatology, M/C 624, University of Illinois College of Medicine at Chicago, P.O. Box 6998, Chicago, IL 60680
- Yoder, Leo**, Clinical Branch, National Hansen's Disease Center, Carville, LA 70721
- Young, Douglas**, Immunology Research Laboratory, Pacific Medical Center, 1200 12th Ave. South, Seattle, WA 98144
- Young, Saroj**, Immunology Research Laboratory, Pacific Medical Center, 1200 12th Ave. South, Seattle, WA 98144

ABSTRACTS OF FIRST U.S. HD RESEARCH CONFERENCE

Jacobson, R. R. Clinical aspects.

Although the prevalence of the disease has changed very little in the last two decades, leprosy remains a major public health problem in many Third World countries. The Ridley-Jopling classification is widely used to classify the disease although the simplified method proposed by the WHO Study Group on the Chemotherapy of Leprosy for Control Programs is increasingly popular. This divides all patients into paucibacillary and multibacillary types. Reactions continue to be a major complication of therapy occurring in up to 50% of the cases with varying degrees of severity. Management of the disease is complicated both by reactions and by the prolonged period of treatment required. It is now widely accepted that combination drug therapy should be used for the management of all cases, but poor compliance and primary and secondary resistance to dapsone have, until now, limited the success of attempts to control the disease with chemotherapy. Widespread acceptance and implementation of the WHO Study Group regimens proposed in 1982 may improve compliance and diminish the incidence of sulfone resistance. Current research efforts are focused on such things as the development of new drugs, serologic tests for the early diagnosis of the disease, and an antileprosy vaccine, and further elucidation of the exact nature of the immune defect in patients which allows them to develop the disease.—[National Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Williams, K., Bahlinger, V., Heroman, B., Madrigal, D. and Brantley, P. The relationship between perceived social support and illness reports in Hansen's disease patients.

The concept of social support has emerged as a central issue in psychosocial research involving chronic illness. Although empirical tests regarding the importance of social support in illness are few, a recent review of the literature suggests that adaptive social support is associated with recovery and cop-

ing with serious illness or injury. Only a limited group of studies would indicate possible negative effects of social support. Studies which address the role of social support in Hansen's disease (HD) patients are noticeably absent from the psychosocial literature. The purpose of the present study, therefore, was to examine the relationship of social support to patients' reports of physical symptoms in HD patients.

Subjects were randomly selected from the inpatient population of HD patients at the National Hansen's Disease Center in Carville, Louisiana. Participation was limited to patients who possessed a satisfactory proficiency with the English language. Following the signing of an informed consent, patients were interviewed according to the format in the "Interview Schedule for Social Interaction" (ISSI) developed by Henderson, Duncan-Jones, Byrne and Scott (1980). The ISSI is a standard interview consisting of 52 questions which explore various types of social relationships and provisions afforded by relationships in terms of availability and perceived adequacy. The interview time averaged approximately one hour, and all interviews were conducted by the same social service staff member. The ISSI yielded four separate measures of social support which include: 1) the availability of attachment; 2) the perceived adequacy of attachment; 3) the availability of social integration; and 4) the adequacy of social integration. Following completion of this interview, patients were also requested to complete the Wahler Physical Symptoms Inventory, an instrument designed to assess the intensity of physical complaining behavior.

Correlational analyses were conducted holding age and sex of the subjects as a constant. Of the four measures of social support, the one most highly correlated with physical symptom reports of HD patients was the patient's perception of the adequacy of social integration. Results indicated that perceptions regarding the quality of social relationships was significantly related to the number of physical symptoms they endorsed of the physical symptom checklist

($r = -0.36$, $p < 0.01$). The actual number of social contacts appeared far less important as a determinant of illness reports. Such findings are discussed in terms of their relevance for the psychosocial rehabilitation of HD patients.—[National Hansen's Disease Center, Carville, Louisiana, 70721, U.S.A.]

Schauf, V., Ryan, S., Nelson, K., Brown, A., Scollard, D. and Smith, T. Tuberculoid leprosy in Thailand is associated with HLA-DR2.

HLA-DR associations have been demonstrated for several familial diseases involving aberrant immune responses including leprosy, multiple sclerosis, diabetes, and systemic lupus erythematosus. Because of several previous reports associating tuberculoid leprosy and HLA-DR, we compared the frequency of HLA-DR2 in Thai tuberculoid leprosy patients to that in healthy Thai controls. We performed HLA-DR typing in unrelated Thai patients with borderline tuberculoid (BT) or polar tuberculoid leprosy (TT) and in control subjects who are male blood bank donors. HLA-DR types were determined for B lymphocytes separated from peripheral blood monocytes on a nylon wool column using a microcytotoxicity assay with a well-characterized serum panel. HLA-DR2 was present in over 90% (29/32) of the tuberculoid (BT and TT) leprosy patients. In contrast, HLA-DR2 was found in only 64% (14/22) of the control subjects. The frequency of HLA-DR2 was significantly increased in tuberculoid leprosy ($\chi^2_c = 4.31$, $p < 0.05$). The odds ratio for tuberculoid leprosy was 5:52 among DR2-positive individuals. Preliminary studies do not indicate an HLA-DR2 association in unrelated Thai patients with lepromatous leprosy. These findings, together with the high frequency of DR2 in Thai controls (64%), suggest that DR2 or DR-linked genes may be advantageous in spite of increasing the risk of tuberculoid leprosy. Our data in the Thai population support previous observations in other populations that HLA-DR2 is associated with tuberculoid leprosy.—[University of Illinois College of Medicine, Chicago, Illinois, and McKean Rehabilitation Institute, Chiang Mai, Thailand]

Ching, C. Y., Pollack, M., Reichert, E., Hokama, Y., Fujikawa, R., Loui, W., Sato, D., Wong, C. and Ching, N. Analysis of immunologic and genetic factors in multicausal families with Hansen's disease in Hawaii.

A prospective study of immunologic and genetic factors in family groups with Hansen's disease has been initiated. Two families with more than one index case of Hansen's disease were analyzed for antigens of the major histocompatibility complex, the histocompatibility leukocyte antigen (HLA) linked immune response (IR) genes; for associations between the Gm allotype-linked IR genes; and the immune responses to certain antigens. Familial clustering of Hansen's disease in Hawaii suggests the involvement of genetic as well as immunological factors in the pathogenesis of this disease.

Studies on the HLA-A, -B, -C, and -DR (D-related) antigens have so far been inconclusive. The segregation of the HLA-DR2 antigen with the tuberculoid form of Hansen's disease has been reported (van Eden, *et al.*, 1980; de Vries, *et al.*, 1981). In our preliminary studies, the DR2 antigen was observed in all affected members of both families with either the lepromatous or tuberculoid forms of Hansen's disease.

In family No. 1, all family members carried the DR2 MB1 antigen. The father was homozygous at the DR2 locus and the two affected offspring also shared the paternal haplotype, Aw24 B40(w6) DR2(MB1), while the normal sibling had the A25 B62(w6) DR2(MB1) haplotype. Interestingly, the unaffected sibling, with no evidence of Hansen's disease (NEHD), responded in a lymphoproliferative assay to armadillo-lepromin antigen as measured by ^3H -thymidine uptake. However, an even more interesting finding was a very rare Gm haplotype 1,3:21 which segregated with the father and two siblings affected with lepromatous leprosy. This Gm haplotype 1,3:21 is very rare, even in the Filipino ethnic group (Pandey, personal communication). Mother and sibling, both with NEHD, did not carry this Gm 1,3:21 haplotype. The affected LL individuals did not respond to armadillo lepromin *in vitro*.

In family No. 2, Gm typing was uninformative since the same Gm phenotype was

shared by all members of the family. The DR2 antigens were present in family members with the lepromatous as well as the tuberculoid form of the disease. All family members had lower than normal responses to armadillo lepromin in the *in vitro* lymphoproliferative test.

In conclusion, the finding of DR2 associated susceptibility in family No. 1 and a rare Gm haplotype 1,3:21 associated with the LL form of Hansen's disease suggests that further investigations may determine if HLA and/or Gm linked immune response genes may be associated with increased susceptibility to Hansen's disease. Such a study may define individuals in a family at risk of developing the more serious lepromatous form of the clinical disease and may aid in the control, prevention, and treatment of the more problematic manifestation of the disease for clinicians and public health epidemiologists.

NK function in leprosy patients. The heterogeneity of the NK cell population and cytotoxicity to a herpes-virus infected fibroblast, NK(HSV-1), and a chronic myeloid leukemia cell line, NK(K562), was examined in patients with a wide spectrum of the clinical form of leprosy. NK functions were summarized to NK(HSV-1) and NK(K562) in normal donors [NK(HSV-1) 45% \pm 14, N = 50, (mean \pm S.D.); NK(K562) 53% \pm 14, N = 45], the polar leprosy groups TT and LL(ENL-); LL(ENL+); and leprosy patients with cancer.

NK activity (mean \pm S.D.) for the 15 TT patients was NK(HSV-1) 51% \pm 20, NK(K562) 51% \pm 17 at an effector:target ratio of 50:1; in 24 LL(ENL-) patients: NK(HSV-1) 46% \pm 16; NK(K562) 43% \pm 15. As a group, no significant differences in NK function were observed between patients with TT and LL(ENL-) ($p > 0.05$) and normal donors ($p > 0.05$).

NK cytotoxicity was more likely to be observed as deficient in patients with reactional episodes, the lepromatous leprosy patient with erythema nodosum leprosum, LL(ENL+), and in the immunolabile group with borderline leprosy (BT, BL). NK activity for the 10 LL(ENL+) patients as compared to the LL(ENL-) group was slightly lower than normal, NK(HSV-1) 28% \pm 18, NK(K562) 36% \pm 23.

In a prospective analysis, NK function was evaluated in a 29-year-old Hawaiian male lepromatous leprosy patient with erythema nodosum leprosum LL(ENL+). This patient had severe and necrotic ENL reaction, lymphadenopathy, panniculitis and a temperature of 104°. NK cytotoxicity was low during the acute ENL reaction: NK(HSV-1) 3%; NK(K562) 6%. At 21 days, with the ENL subsiding, NK(HSV-1) 5%; NK(K562) 20%. Treatment of PBL with IFN α *in vitro* augmented NK to both targets, NK(HSV-1) 19%; NK(K562) 55%. At 53 days, during which no ENL lesions were observed, NK activity returned to normal levels: NK(HSV-1) 37%; NK(K562) 41%. Exogenous IFN α augmented NK(HSV-1) 55%; NK(K562) 68%. The removal of a suppressor adherent cell or macrophage by Sephadex G-10 filtration of PBL appeared to increase NK activity.

Two of 14 borderline lepromatous (BL) patients studied had low NK(HSV-1) 17%, 20%; NK(K562) 19%, was deficient in 1 of 13 cases [NK(HSV-1), 45% \pm 21, N = 14 BL; NK(K562), 58% \pm 18, N = 13 BL]. In borderline tuberculoid (BT) patients, 4 of 9 patients had low NK(HSV-1) 19%, 14%, 10%, 10%. NK(K562) 18%, was low in 1 of these 4 BT patients with low NK(HSV-1) [NK(HSV-1) 27% \pm 14; NK(K562) 48% \pm 21, N = 9 BT].

Finally, leprosy patients with impaired cell-mediated immunity have not been reported to have a significantly increased incidence of cancer. NK function was studied in leprosy patients with cancer. A 65-year-old Japanese female studied for three years after resection of adenocarcinoma of the sigmoid colon with no evidence of recurrence had NK(HSV-1) 30%, NK(K562) 33% values within the lower normal range. A 63-year-old Melanesian male with undifferentiated large cell carcinoma of the lung had NK(HSV-1) 1%, NK(K562) 19%. At the time of metastasis, 1 year and 4 months later, NK(HSV-1) 18%, NK(K562) 9%. His PML were not augmented to normal levels following treatment with exogenous IFN α , NK(HSV-1) 13%, NK(K562) 19%.

Thus, low NK function has been observed in select patients with the wide spectrum of the clinical forms of the leprosy disease. Correlations between NK functions and HLA antigens in multicase families may

provide valuable information on the immunogenetics of the NK-IFN system and in defining individuals at risk of developing cancer or microbial diseases.—[University of Hawaii Medical School, Departments of Medicine, Pathology and Surgery; Hawaii State Department of Health, Hansen's Disease Prevention Program, Honolulu, Hawaii and Sloan-Kettering Memorial Cancer Center, New York, New York]

Acknowledgments. This work was supported in part by grants from the National Institutes of Health (NIHAI-17403), the Lani Booth Foundation of Hawaii, and the Associated Chinese University Women.

Cynamon, M. H., Palmer, G. and Sorg, T. B.

In vitro activity of ampicillin compared to ampicillin and sulbactam against *Mycobacterium tuberculosis*.

The *in vitro* activity of ampicillin alone and in combination with sulbactam against 14 isolates of *Mycobacterium tuberculosis* was evaluated by broth dilution susceptibility testing in 7H10 broth. Bactericidal activity was determined by plating in duplicate on 7H10 agar, and was defined as a 99% reduction in colony counts. The initial inoculum was about 5×10^4 CFU/ml. The concentrations of ampicillin tested were serial twofold dilutions from 32 to 1 μ g/ml, and the ampicillin/sulbactam combinations were tested in 1:1 ratios by weight.

The minimal concentration required to inhibit 50% (MIC₅₀) and 90% (MIC₉₀), as well as the bactericidal concentrations, are listed below (in μ g/ml for each agent):

	MIC ₅₀	MIC ₉₀	MIC _{range}	MBC ₅₀	MBC ₉₀
Amp.	32	>32	16–>32	>32	>32
Amp./Sul.	4	8	1–8	8	16

The addition of sulbactam to ampicillin increases the *in vitro* activity against *M. tuberculosis* by fourfold.—[Veterans' Administration Medical Center and State University of New York, Upstate Medical Center, Syracuse, New York]

Harris, E. B. and Prabhakaran, K. Malonyl-CoA decarboxylase activity in *Mycobacterium leprae*.

Multi-branched fatty acids occur in many biological systems, including mycobacteria.

The unusual features of these acids probably help the bacteria to resist the degradative enzymes of the host tissue.

Recent studies suggest that the enzyme malonyl-CoA decarboxylase may be involved in the regulation and biosynthesis of branched-chain fatty acids present in mycobacteria. The precursors of long-chain fatty acids are acetyl-CoA and malonyl-CoA. Malonyl-CoA is formed from CO₂ and acetyl-CoA in a reaction that requires biotin and is catalyzed by the enzyme acetyl-CoA carboxylase. It was proposed that by specifically decarboxylating malonyl-CoA, methyl malonyl-CoA would be the only chain elongating substrate available, therefore bringing about the production of methyl-branched acids.

Using ¹⁴C-labeled malonyl-CoA, we have detected malonyl-CoA decarboxylase activity in *Mycobacterium leprae* separated from the tissues of experimentally infected armadillos. The decarboxylation appears to be biotin dependent as shown by its sensitivity to avidin. This inhibition could be partially reversed by the addition of excess biotin to the reaction mixture. The malonyl-CoA decarboxylase activity was nearly completely inhibited by coenzyme A, but was unaffected by the SH reagent iodoacetamide.

These results suggest that malonyl-CoA decarboxylation in *M. leprae* may not be the result of a specific malonyl-CoA decarboxylase but, rather, could be the reverse reaction of an avidin-sensitive acetyl-CoA carboxylase.—[National Hansen's Disease Center, Carville, Louisiana 70721]

Shannon, E. J., Truman, R. W., Christy, S. A., Vadiie, R. and Hastings, R. C. Effect of thalidomide on induction of antibody synthesis in mice to a T-independent antigen—DNP Ficoll.

Elucidation of the mechanism of action of thalidomide in ENL may be beneficial in understanding the pathogenesis of this syndrome. On an immunological basis, we have shown thalidomide to: 1) inhibit IgM and not affect IgG antibody formation when fed to mice 5 or 7 days before immunization with sheep erythrocytes; 2) exert a selective decrease of serum IgM among leprosy patients being treated with thalidomide for

ENL, and 3) significantly decrease the absolute numbers of T-helper/inducer cells in peripheral blood of healthy males. Since ENL has been hypothesized to be an immune-complex-mediated disease, thalidomide may inhibit a cell or interaction among regulator cells (helper and suppressor T lymphocytes and macrophages) and B lymphocytes required for IgM immunoglobulin production.

To exclude T cell interaction in an inductive phase of IgM antibody synthesis, we determined the effect of thalidomide on the ability of mice to respond to DNP-Ficoll. The B cell response to this polysaccharide is IgM, not dependent on T cell help, and requires a strict accessory macrophage cell interaction.

Having first determined a median effective dose (ED 50) for a four-day plaque-forming cell (PFC) response to DNP-Ficoll, mice were fed for 5 days a 0.03% w/w concentration of thalidomide in powdered rodent chow. This procedure had been shown in our lab to achieve a mean blood level of thalidomide in mice of 0.84 $\mu\text{g/ml}$, which is equivalent to that of 0.9 $\mu\text{g/ml}$ acquired in humans following a 100 mg oral dose of thalidomide. Control mice were fed powdered rodent chow only. After 5 days both groups of mice were injected with $10^{3.5}$ ng of DNP-Ficoll and their spleens assayed for PFC to TNP-sensitized sheep erythrocytes. The thalidomide-fed group of 8 mice had a mean PFC response of 1284 ± 410 , and the control group of 8 mice had a mean PFC response of 968 ± 238 . Thalidomide did not inhibit, it augmented ($p < 0.05$), the ability of mice to respond to DNP-Ficoll. Thalidomide does not interfere with *de novo* IgM antibody synthesis by B cells nor does it interfere with macrophage collaboration in response to T-independent antigens.—[National Hansen's Disease Center, Carville, Louisiana 70721]

Morales, M. J., Piper, L., Yoder, L., Job, C. K. and Hastings, R. C. The pharmacology of intermittent clofazimine therapy in Hansen's disease.

Clofazimine (B663, Lamprene) was administered orally in a once-monthly dosage to patients with Hansen's disease who were previously untreated with clofazimine but

were on other concomitant chemotherapy. One group of patients received 600 mg of clofazimine and another group received 1200 mg of clofazimine in a once-monthly dosage for a period of 6 months. Blood, urine, feces, and punch skin biopsies were obtained at various intervals in order to assess the absorption, distribution, and measurement of clofazimine during this time period. Analysis of clofazimine content in the various tissues was accomplished using high-performance liquid chromatography.—[National Hansen's Disease Center, Carville, Louisiana 70721]

Dhople, A. M., Kazda, J. and Storrs, E. E. Presence of "difficult-to-grow" mycobacteria in armadillos.

For the first time in the 12 years since the discovery of armadillos as a model of human lepromatous leprosy, considerable concern has developed among investigators making use of this model because of published reports on the isolation of unknown, difficult-to-grow mycobacteria from the liver and spleen of the nine-banded armadillo. Because the reports were based on a very small number of animals (4 armadillos) and because of possible implications of these reports on current and future leprosy research, this study was undertaken to find the answers to several questions arising from the reports.

The livers and spleens from these armadillos, non-infected as well as infected earlier with *M. leprae*, were harvested aseptically. One piece of each tissue was processed in Florida, and the other piece of the same tissue was sent to Borstel on dry ice with coded numbers. All of the procedures used for processing the tissues, including decontamination, were identical in both laboratories. Four types of media were used: 2 conventional mycobacterial media and 2 on which the growth of "difficult-to-grow" mycobacteria has been reported. The inoculum on each medium was very heavy (suspension containing approximately 0.4 g tissue). Cultures on each of the 4 media were incubated at 32°C, 34°C, and 37°C, and observed once a week for up to 6 months.

So far, we have included 70 samples in the study, and the findings on 28 of these

have been analyzed. The results from both laboratories agree well. Among the 28 samples, only one sample (#4) showed colonies on original Ogawa egg-yolk medium, while another sample (#19) showed colonies only on modified Ogawa medium (containing mycobactin, sodium chloride and malachite green). Colonies from both of these samples were pale yellow, contained numerous acid-fast bacilli (AFB), and initial growth was observed only after 8–10 weeks at 32°C. However, on subsequent subcultures the AFB from sample #4 grew rapidly on original Ogawa medium as well as on Löwenstein-Jensen and Middlebrook 7H11. Various taxonomic studies revealed the AFB from this sample to belong to the MAIS complex. On the other hand, subcultures from sample #19 grew relatively slowly and only on modified Ogawa medium compared to those from sample #4 and, on the basis of various tests done so far, these organisms could not be placed in any one particular group of known mycobacteria. However, neither of the samples exhibited any growth on any of the media when normal inoculum size (10 ml) was used.

The extent of contamination of these samples with difficult-to-grow mycobacteria and the results of the remaining 42 samples are discussed. Analyses of the results from such a large number of samples should indicate whether such difficult-to-grow mycobacteria were present in armadillos in the wild or introduced during captivity or during laboratory harvests. Significance of such mycobacteria on current and future leprosy research is discussed.—[Medical Research Institute, Florida Institute of Technology, Melbourne, Florida 32901 and Borstel Research Institute, Borstel, Federal Republic of Germany]

Acknowledgments. This work was supported by the Germany Leprosy Relief Association and the National Institute of Allergy and Infectious Diseases, NIH NO1AI 92637.

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

See abstract in *Int. J. Lepr.* **52** (1984) 608–609.

Skinsnes, O. K., Chang, P. H. C., Kuba, B. and Gaines, S.* Numerical taxonomic and immunologic comparison of 36 leprosy-derived mycobacterial strains and 17 members of the MAIS complex.

A total of 51 cultivated mycobacterial strains are involved in this evaluation: 34 cultivated from lepromatous patients and armadillos infected with *Mycobacterium leprae*, 9 strains of *M. scrofulaceum*, 4 of *M. avium*, and 4 of *M. intracellulare*. The leprosy cultures were isolated on LA-3 culture medium (Skinsnes, *et al.*, *Int. J. Lepr.* **43**:193–203, 1975). The members of the *M. avium-intracellulare-scrofulaceum* (MAIS) complex were predominantly obtained from the Denver Mycobacterial Culture Collection, and two uncultivated strains used in the immunologic studies came from a biopsy of a lepromatous patient in Hong Kong and from tissues of Carville armadillo 225 obtained from the National Institutes of Health. Patients from whom cultures were derived involve a broad geographic area, including Thailand, China, Hong Kong, India, Brazil, Dakar, and Hawaii.

All cultures were characterized by the following determinations: growth at 25°C, 37°C, 42°C; pigment formation; catalase production; catalase stability at 68°C; niacin secretion; reduction of nitrate; hydrolysis of Tween 80; tellurite reduction; 5% NaCl tolerance; arylsulfatase hydrolysis; growth on MacConkey agar; urease production; iron uptake; hydrolysis of β -galactoside; and surface versus bottom growth on LA-3 medium. Additionally, drug sensitivity determinations were made to: INH, DDS, rifampin, cephaloridin, thiacetazone, B663, saccharic acid, kanamycin, p-nitrobenzoic acid, hydroxylamine, and thiophen-carboxylic acid hydrazide. Computer analysis provided taxonomic matching of all cultivated strains on the basis of these characteristics.

As a second line of taxonomic approach, all of these mycobacterial strains were subjected to reaction with peroxidase-labeled human lepromatous serum purified to IgG (Skinsnes, *et al.*, *Int. J. Lepr.* **46**:394–413, 1978) and adsorbed against bacterial powders from *M. tuberculosis*, *M. avium*, *M.*

* Deceased, May 1982.

scrofulaceum, *M. vaccae*, and BCG. Rabbit antibodies were raised against *M. avium*, *M. scrofulaceum*, *M. intracellulare*, *M. leprae* derived from tissues of Carville armadillo 225, and against two cultured mycobacterial strains from lepromatous patients (HI-75 and HK-79). These antibodies were also purified to IgG, variously adsorbed against mycobacteria, and labeled with peroxidase. All of the antibodies were applied to all of the cultivated strains and to tissue-derived bacilli from the armadillo and from the lepromatous patient as long as the human-derived bacilli were available. The cultures were all tested at 7–13 days, this being the timeframe previously determined when the cultures showed the greatest antigenic response, except for a few slow-growing MAIS cultures. The latter were harvested as soon as there was adequate growth to form the necessary pellets for electron microscopy (EM). All cultures were grown on Wallenstein medium. Five pellets were made by centrifugation for each culture determination and fixed with OsO_4 . The pellets were embedded in Spurr medium, and ultrathin sections were cut at 600 Å on a LKB Ultratome. Visualization was at 28,000 and 84,000 magnification. All determinations were made on a Zeiss EM 9 S2 electron microscope.

The results of the EM evaluations were graded according to the intensity of the peroxidase reaction and the proportion of bacilli reacting. The various mycobacterial strains were matched by computer on the basis of the results.

Finally, the results of the numerical taxonomy determinations and the immunoperoxidase determinations were combined in computer matching.

The results of the determinations are such as to support the conclusions of David, *et al.* [Ann. Microbiol. (Paris) 134:367–377, 1978], who included six of the strains in our group of cultivated bacilli from lepromatous patients and armadillos in their taxon I as being of nonclassified mycobacteria. The results of the immunoperoxidase studies tend to show the same affinities as do *M. leprae* derived from human lepromatous patients and armadillos. These affinities differ from those of the MAIS complex.

The realities of the leprosy-derived cul-

tures are more remarkable than their hypothetically derived characteristics.—[Department of Pathology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii 96816]

Prabhakaran, K. and Harris, E. B. Temperature effects on viability, diphenoloxidase, and permeability of *Mycobacterium leprae*.

Many of the biological properties of *Mycobacterium leprae* remain obscure, and it is not known why the organism does not grow in any culture media. Evidence for the presence of enzymes of glycolysis and citric acid cycle in *M. leprae* is inconclusive. Our studies as well as those of others have established that, among mycobacteria, the enzyme diphenoloxidase is a specific metabolic property of the Hansen bacillus. The antileprosy drug dapsone (DDS), which inhibits diphenoloxidase, does not readily penetrate intact *M. leprae*. DDS inhibited the enzyme when the bacilli were disrupted, which indicated a permeability barrier of the cell membranes of the organisms to the drug. Dapsone penetrated the bacteria prepared from fresh tissues of experimentally infected armadillos when the bacteria were exposed to warmer temperatures, or washed with NaOH but not with trypsin. The bacilli obtained from infected tissues stored at 0°C for about a week or kept frozen at –80°C did not show any permeability barrier to the drug. Diphenoloxidase of freshly prepared *M. leprae* was stimulated when the bacteria were exposed to 50°C for 10 min prior to the assay; at 60°C the activity decreased, and at 100°C the enzyme was completely inactivated. Exposure to 50°C for 10 min increased the diphenoloxidase of the bacilli washed with trypsin or NaOH. When the assay was done at temperatures below 37°C, the activity was considerably lower, suggesting that *M. leprae* may not be a psychrophilic organism in this respect. Suspensions of the bacteria purified from fresh tissues retained their viability when frozen at –80°C. The frozen bacilli prepared from tissues stored at 0°C for about a week did not multiply in mouse foot pads, suggesting disruption of cell membranes and consequent damage to cytoplasmic components

by ice crystals. NaOH or trypsin treatment did not impair the viability of the bacteria. The inhibition of diphenoloxidase of *M. leprae* by dapsone could serve as a model system to test the integrity of the cell membranes of the organisms.—[National Hansen's Disease Center, Carville, Louisiana 70721]

Martin, L. N., Gormus, B. J., Wolf, R. H., and Baskin, G. B., Meyers, W. M., Walsh, G. P. and Binford, C. H. Relationship between depressed lymphocyte responsiveness to mitogens and the percentage of OKT8⁺ lymphocytes in experimental leprosy in the mangabey (*Cercocebus atys*).

We have previously reported on a naturally acquired case of leprosy in a mangabey monkey and on the experimental transmission of leprosy to normal mangabeys. The experimentally induced disease has been characterized as borderline-lepromatous to lepromatous in nature. Immunological alterations developed during the course of disease in two mangabeys with untreated experimental leprosy. These animals have been studied immunologically since the outset of the project in 1980. Lymphocyte blastogenic responses to the mitogens concanavalin A (ConA), phytohemagglutinin (PHA), and pokeweed mitogen (PWM) became severely depressed as the experimental disease became progressively disseminated in the third and fourth years post-inoculation. This depression in lymphocyte blastogenic responses to mitogens was associated with high percentages of OKT8⁺ blood lymphocytes in infected monkeys compared to uninoculated controls.

Studies of normal mangabeys have demonstrated cyclic annual alterations of lymphocyte blastogenic responsiveness to mitogen. Increased responsiveness occurred in July to August and decreased responsiveness occurred in March to April over two successive years. The percentage of OKT8⁺ blood lymphocytes also varied annually, with lower percentages in August to October and higher percentages in March to April. Values obtained from the normal mangabeys demonstrated a highly significant negative correlation between the percentages of

OKT8⁺ lymphocytes and the blastogenic responses to mitogens. Lymphocytes obtained in July to August had lower percentages of OKT8⁺ cells and higher mitogen responsiveness than lymphocytes obtained in March to April. The association of higher percentages of OKT8⁺ lymphocytes with lower mitogen responsiveness suggested that the OKT8⁺ lymphocyte subset may contain suppressor activity in mangabeys as has been demonstrated in man and rhesus monkeys.

Experimentally infected mangabeys with disseminated lepromatous leprosy consistently had a high percentage of OKT8⁺ lymphocytes and depressed responsiveness to mitogens. The negative association between the lymphocyte blastogenic responses to mitogens and the percentage of OKT8⁺ lymphocytes in normal and lepromatous mangabeys strongly suggests that the depressed mitogen responsiveness of lymphocytes from mangabeys with disseminated disease may result from a leprosy-induced increase in OKT8⁺ cells.—[Delta Regional Primate Research Center, Tulane University, Covington, Louisiana 70433 and Armed Forces Institute of Pathology, Washington, D.C. 20306]

Kvach, J. T., Neubert, T. A. and Heine, H. S. The adenosine triphosphate content and death rate of *Mycobacterium leprae* isolated from armadillo tissue by Percoll buoyant density centrifugation.

Mycobacterium leprae's ATP content and death rate have been determined using cells isolated from armadillo liver by Percoll buoyant density centrifugation. The newly developed isolation procedure requires approximately 2½ hr and effectively separates the bacteria from armadillo ATP and tissue debris. The purified *M. leprae* possessed an ATP level similar to cultivable bacteria and died exponentially, as monitored by ATP, under defined conditions. The conditions consisted of 0.1 M potassium phosphate buffer, pH 7.0, inoculated with 10⁷ bacteria per ml incubated at 25°C in test tubes filled to 50% of capacity.

Establishment of a reproducible death rate under the conditions provided an internal control mechanism for evaluating the relative merit of changes to *M. leprae*'s envi-

ronment and, thereby, a means of optimizing conditions for, first, the maintenance and then the *in vitro* growth of the organism. Freeze-thawing *M. leprae* in armadillo tissue or as purified suspensions damaged the organisms, resulting in an accelerated loss of ATP when the bacteria were incubated under the defined conditions. Treatment with clofazimine and incubation at temperatures between 20–35°C accelerated the rate of ATP decay which was directly proportional to drug concentration and temperature. Demonstration of clofazimine activity *in vitro* represents the first step toward the development of a means for screening for clofazimine resistance in leprosy patients.—[The Johns Hopkins University, Baltimore, Maryland and The George Washington University, Washington, D.C.]

Jacobs, W. R., Docherty, M. A., Clark-Curtiss, J. E. and Curtiss, R., III. Expression of cloned *Mycobacterium leprae* DNA in *Escherichia coli* K-12.

Recombinant DNA technology is a powerful tool for studying the genetics and physiology of organisms for which genetic systems are not well developed or for organisms which are difficult to cultivate in the laboratory, such as *Mycobacterium leprae*. High molecular weight DNA isolated from *M. leprae* has been digested with the restriction endonuclease PstI and the 30 to 40 kilobase fragments produced were cloned into the cosmid vector pH79 to generate libraries of recombinant *M. leprae* DNA which theoretically represent greater than 99.99% of the *M. leprae* genome. The pH79::*M. leprae* recombinant molecules were again digested with PstI and the fragments of *M. leprae* DNA were subcloned into pYA626, a plasmic vector derived from pBR322 which also possesses the very strong promoter from the *Streptococcus mutans* aspartic acid semialdehyde dehydrogenase (*asd*) gene. The pYA626::*M. leprae* recombinant molecules were transformed into a minicell-producing strain of *Escherichia coli* K-12. Minicells were purified from these recombinant plasmid-containing strains, the polypeptides specified by the plasmids were labeled with [³⁵S]-methionine, and the labeled polypeptides were analyzed by sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS-PAGE). At least 5 different pYA626::*M. leprae* recombinant molecules produced 1 (and with 3 molecules, 2 or 3) unique polypeptide(s).

We are currently analyzing polypeptides produced by both pH79::*M. leprae* recombinant molecules and pYA626::*M. leprae* molecules in minicells by immunological screening with anti-*M. leprae* sera from leprosy patients (a gift of R. Gelber), from armadillos (a gift of C. Shepard), and with monoclonal antibodies to the 68 kd protein (a gift of T. Gillis) to identify *M. leprae*-specific antigenic determinants.—[Department of Biology, Washington University, St. Louis, Missouri 63130]

Imaeda, T. and Portaels, F. DNA relatedness among *Mycobacterium leprae*, leprosy-derived bacteria, and established mycobacterial species.

The present study was undertaken to clarify DNA relatedness between *Mycobacterium leprae* grown experimentally in armadillos and other mycobacterial and corynebacterial strains isolated from leprosy patients or animals infected with *M. leprae*. Phylogenetic study was also carried out to establish the position of *M. leprae* within the genus *Mycobacterium*. Dot blot hybridization, kinetic analysis of DNA-DNA hybridization in free solution, and restriction enzyme cleavage analysis were employed.

DNAs of *M. leprae* and uncultivable acid-fast organisms isolated from a mangabey monkey and wild armadillos with leprosy-like disease were 100% homologous, indicating that they are the same organism.

In order to estimate the genomic relationship between *M. leprae* and other bacteria, we have designed a practical method which involves a dot blot hybridization technique using biotin-labeled DNA probes of test organisms and a standard set of nitrocellulose filters dotted with single-stranded DNAs of representative 16 to 24 strains of mycobacterial species, including *M. leprae*. This method revealed that *M. haemophilum* is the closest species to *M. leprae* among mycobacterial species tested, although their DNA homology does not exceed 40% in the kinetic analysis.

DNAs of various mycobacterial species were digested with restriction endonucleases, such as Eco RI, Eco RV, Kpn I, Bcl I and Bgl II, electrophoresed in agarose gel, Southern-transferred to nitrocellulose filters, and finally hybridized with a biotin-labeled DNA probe of *M. leprae*. Several restriction fragments were homologous to *M. leprae* DNA, indicating that common sequences between *M. leprae* and other mycobacteria are most likely clustered in these mycobacterial DNAs.

The dot blot hybridization method also showed that DNAs of leprosy-derived mycobacteria (LDM) contain sequences which are relatively similar to those of *M. avium*, *M. intracellulare*, and *M. scrofulaceum*, as observed in classical taxonomic analysis. However, in the kinetic analysis which provides more accurate information on DNA relatedness, none of these species showed more than 50% homology to LDMs. Restriction enzyme cleavage analysis also showed different patterns in both location and distribution of restriction sites in these strains. These results suggest that LDMs belong to a new cluster within the genus *Mycobacterium*.

Leprosy-derived corynebacteria (LDC) contain DNAs with a very low degree of homology to that of *M. leprae*. A mixture of LDC and LDM strains showed a low degree of DNA homology to *M. leprae* grown in armadillos. This suggests that the presence of LDCs and LDMs in leprosy tissues may not be directly related to the disease. — [Department of Microbiology, New Jersey Medical School, Newark, New Jersey 07103 and Institut de Medecine Tropicale "Prince Leopold," Antwerpen, Belgium]

Gelber, R. H. Studies on antimicrobial activity against *M. leprae* infections of the mouse foot pad.

The proportional bactericidal test (Lepr. Rev. 49:7, 1978) provides an incontrovertible method of assessing antimicrobial bactericide and comparing degrees of bacterial killing. By this technique, Colston established that dapsone 0.01% in mouse chow is 78% bactericidal and that rifampin 0.003% and 0.01% in mouse chow are 99.99% and 100% bactericidal, respectively (Lepr. Rev. 49:7, 1978). In order to assess the relative

killing of *Mycobacterium leprae* by graded levels in mouse chow of dapsone (0.01%, 0.001%, 0.0001%, and 0.00001%) and rifampin (0.01%, 0.005%, 0.003%, 0.001%, and 0.0003%), we utilized the proportional bactericide technique. By this method dapsone was respectively 68%, 68%, 0%, and 0% bactericidal, while rifampin was respectively 99.9%, 90%, 52%, 37%, and 20% bactericidal.

Previously, Shepard (Proc. Soc. Exp. Biol. Med. 109:636, 1962) found that multiplication of *M. leprae* in mice continuously fed diet containing 0.5% cycloserine was only delayed and partially suppressed. In order to assess the activity against *M. leprae* of some newer hydroxamic acid derivatives, beta alanyl and glycyl and cycloserine (as a control) were incorporated into mouse chow at 0.0025%, 0.025%, 0.1%, 0.5%, and 2% each, and groups of mice infected with 5×10^3 *M. leprae* were so treated from 60 to 150 days following infection (kinetic technique). All concentrations of beta alanyl hydroxamic acid and glycyl hydroxamic acid were inactive. Lower concentrations of cycloserine were also inactive, but 0.5% and 2% cycloserine resulted in a growth delay of *M. leprae* of greater than 1 year. These active concentrations of cycloserine were retested in mice by the kinetic technique, and a growth delay of over 1.5 years was observed.

The activity of a number of other antimicrobial agents with promise for efficacy in mycobacterial disease were also studied against *M. leprae* infection of the mouse foot pad by the kinetic technique. The activity against *M. leprae* of a new and promising dihydrofolate reductase inhibitor, brodimoprim (0.03%) (Chemotherapy 29:249, 1983) and trimethoprim (0.1%) were studied alone and in combination with dapsone (0.0001%). Only the combination of brodimoprim and dapsone resulted in a significant delay in the growth of *M. leprae*, 120 days. Minocycline (0.025%) but not doxycycline (0.01%) or erythromycin (0.04%) resulted in growth delay of *M. leprae*. Cephadrine (0.5%) was found purely bacteriostatic for *M. leprae* while cefoxitin, cefamandole, cefotaxime, moxalactam, and cephradine 250 mg/kg administered intraperitoneally 5 times weekly from day 60 to 150 following mouse foot pad infection were inactive. The

beta lactamase inhibitor, clavulanic acid, when combined with amoxicillin (5 times weekly by gavage) resulted in significant delay of growth in *M. leprae* in this study.— [Seton Medical Center, Daly City, California 94015]

Acknowledgments. These studies were supported by grants from the National Hansen's Disease Center and the United States Public Health Service's Central Clinical Investigations Committee.

Gelber, R. H., Humphres, R. C. and Fieldsteel, A. H. A comparative study of four rodent systems to monitor initial therapy of lepromatous leprosy: in search of a more sensitive system to assess bacterial viability and optimal bactericidal therapy.

The testing of new antileprosy regimens is handicapped by the lack of a highly susceptible animal that can detect small numbers of viable *Mycobacterium leprae* in the presence of large numbers of dead *M. leprae*, as occurs in patient tissue following chemotherapy. Shepard, *et al.* (Am. J. Trop. Med. Hyg. 21:446, 1972 and 23:1120, 1974) demonstrated previously that the standard 5×10^3 inoculum of *M. leprae* from skin biopsies of previously untreated lepromatous patients taken as little as 3 days after the initiation of rifampin 600 mg daily or a single 1500 mg dose regularly do not multiply in the feet of mice. Similarly, Kohsaka, *et al.* (abstract in Int. J. Lepr. 52 Suppl:751, 1984) presented the finding that large inocula of bacilli ($>10^6$) obtained from skin biopsies of human lepromatous leprosy patients treated for only 2 days with 450 mg rifampin daily did not multiply in nude mice. However, all therapeutic regimens used thus far, including those utilizing rifampin, are not sterilizing and, despite years of treatment, patients continue to harbor drug-sensitive, viable bacilli, "persisters" (Br. Med. J. 1:133, 1978). In order to define optimal chemotherapy of lepromatous leprosy, which might safely permit discontinuation of therapy, a more sensitive monitoring system to assess the relative initial killing of *M. leprae* by candidate regimens is required.

To this end, we have compared the sen-

sitivity of the usual foot pad inoculum into normal mice (5×10^3), large inocula [$\sim 10^6$ in neonatally thymectomized Lewis rats (NTLR)], nude rats (congenitally athymic), and normal mice. For this purpose 15 previously untreated lepromatous leprosy patients were randomly assigned to treatment regimens of either a single initial 1500 mg dose of rifampin plus daily 100 mg dapsone or weekly 900 mg rifampin plus daily 100 mg dapsone. Skin biopsies taken a few days, 1 week, 2 weeks, and 4 weeks after the initiation of therapy were used as the source of *M. leprae* inocula in these studies. Only two of 58 (4%) skin biopsies grew in mouse foot pads inoculated with 5×10^3 bacilli. In contrast, 22 of 59 skin biopsies grew *M. leprae* unequivocally directly in NTLR, while 10 biopsies showing equivocal growth in NTLR were confirmed to contain viable *M. leprae* upon subsequent mouse passage. Thus, in all, 32 of 59 (54%) biopsies grew *M. leprae* in NTLR directly or in subsequent mouse passage. Larger inocula were most commonly associated with the demonstration of viable bacilli. Of the 15 instances in which NTLR or their passage mice confirmed viability, when both $<10^6$ and $>10^6$ bacilli were inoculated, there was only 1 instance when the $<10^6$ inoculum grew but the $>10^6$ did not, 6 instances where both the $>10^6$ and the $<10^6$ inocula grew, and 8 instances in which the $>10^6$ inoculum grew but the $<10^6$ did not. Although NTLR detected viable *M. leprae* most commonly at the earlier biopsy times, even at 4 weeks, 40% of the biopsies contained viable bacilli as detected by the NTLR. The percentage of patients exhibiting viable bacilli by NTLR inoculation was higher for the patients receiving the multidose rifampin regimen at all 4 time periods.

Only 2 of 27 biopsies grew *M. leprae* unequivocally in nude rats, and 2 more were substantiated by subsequent mouse passage. Thus, only 14% of biopsies tested grew in nude rats. In none of the 27 instances did nude rats demonstrate *M. leprae* when parallel inocula in NTLR did not, but 11 instances when viable bacilli were detected in NTLR but not in nude rats.

Nine biopsies were inoculated in parallel into mice utilizing small inocula (5×10^3) mice utilizing large inocula ($\sim 10^6$), and

NTLR utilizing large inocula. Small inocula in no instances demonstrated viable bacilli in mice, while 5 biopsies demonstrated viable bacilli in mice given large inocula following subsequent mouse passage. The NTLR demonstrated viable *M. leprae* in those 5 instances and in an additional 3 instances.

A colony of neonatally thymectomized Lewis rats (NTLR) has been successfully established and maintained at the Seton Medical Center vivarium. A collaborative study involving untreated lepromatous patients from the San Francisco, Los Angeles, San Diego, and Seattle contract care sites has been initiated, wherein patients are randomly assigned to a therapy with either regimen A (dapsons 100 mg daily + rifampin 600 mg daily) or regimen B (dapsons 100 mg daily, rifampin 600 mg daily + clofazimine 100 mg daily). These regimens were selected because they have the potential of being more potent than those used in the above-described trial and correspond to the usual therapy utilized in the United States (regimen A) or contain the three-drug combination (although not dosage) advocated by the World Health Organization (regimen B). For purposes of this study *M. leprae* from skin biopsies obtained at 3–4 days, 1 week, 2 weeks, and 4 weeks after the initiation of therapy are being inoculated in parallel into BALB/c mice ($\sim 10^3$ and 10^6 bacilli/foot pad) and NTLR ($\sim 10^6$ bacilli per foot pad).—[Seton Medical Center, Daly City, California 94015 and Stanford Research Institute, Stanford, California]

Navalkar, R. G. and Ibegbu, C. Immunologic assessment of *Mycobacterium leprae* antigens using isoelectric focusing and chromatofocusing separation.

Isoelectric focusing (IEF) represents a major advance in the field of high resolution separation of proteins and other amphoteric macromolecules. It is an equilibrium method in which proteins are segregated according to their isoelectric points (pI) in pH gradients.

This technique has been employed to determine various protein antigens of *Mycobacterium leprae* and several other clinically relevant mycobacteria. Various prepara-

tions of *M. leprae*, such as autoclaved and untreated, showed significant differences in their protein content as determined by the number of bands stained. Such differences were also noted between *M. leprae* and other mycobacteria, not only in the number of bands but also their location within a given pH range. Bands of all mycobacteria, including *M. leprae*, located themselves in acidic pH range when polyacrylamide gel (PAG) plates at pH 3.5 to 9.0 were used. Subsequently, when PAG plates with pH 4.5 to 6.0 were used, other mycobacterial bands were seen between pH 4 and 5.0. However, only *M. leprae* bands extended to pH 6.5.

This property of *M. leprae* now permits identification of the organism from all mycobacteria including *in vitro*-grown *M. leprae*. In addition, it also permits recognition of immunogenic components of the organism because of the differences seen between each of the two *M. leprae* preparations, untreated and autoclaved ones.

In addition, we have also used a chromatofocusing technique in which a pH gradient can be produced on an ion exchanger by taking advantage of the buffering action of the charged group of the ion exchanger. When this method is used to elute proteins bound to the ion exchanger, they elute in order of their pI, resulting in a band sharpening, sample concentration and very high resolution.

These studies have yielded several fractions which have been screened for detecting delayed hypersensitivity to *M. leprae* and other mycobacteria in guinea pigs and have resulted in the recognition of specific and crossreactive antigens of *M. leprae*.

Having confirmed the elicitation capabilities of each of the *M. leprae* antigens, studies are now underway to evaluate their ability to induce delayed hypersensitivity. An appropriate number of BALB/c mice have been sensitized through foot pad inoculation and are scheduled to be sacrificed at predetermined intervals for an evaluation of foot pad swelling and lymphocyte transformation. Data obtained in these studies are presented together with the information already obtained.—[Department of Microbiology and Immunology, Morehouse School of Medicine, Atlanta, Georgia 30310]

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

See abstract in *Int. J. Lepr.* **52** (1984) 611–612.

Humphres, R. C. and Winters, M. A. Effects of *Mycobacterium leprae* vaccination on cell-mediated immune functions of Lewis rats: specific sensitization vs tolerance.

Although clinical trials employing a killed *Mycobacterium leprae* vaccine are scheduled to start in the near future, its use as an effective method for the prevention of leprosy in susceptible individuals remains controversial. The controversy involves two major issues. On one hand, it is argued that a certain segment of the population is genetically predisposed to develop the lepromatous form of leprosy and will not benefit from vaccination due to an inherent defect in their immune response preventing development of protective immunity. Alternatively, it is argued that the impairment in cell-mediated immunity (CMI) exhibited by lepromatous patients is due to an active suppressive mechanism which develops during the course of the disease, and that vaccination prior to the onset of multibacillary leprosy may prevent the development of this immunosuppression and induce protective immunity. In this regard, a better understanding of the cellular mechanisms that occur following vaccination with *M. leprae* may be of value for evaluation of its efficacy as a vaccine.

Splenic and lymph node cells from Lewis rats receiving intradermal administration of killed armadillo-derived *M. leprae* are being evaluated for their ability to respond *in vitro* in the presence of *M. leprae* antigen. Immune responsiveness is being determined at the cellular level following co-culture of lymphoid cells with specific *M. leprae* antigen by measuring the level of lymphocyte blastogenesis, the release of migration inhibitory factor (MIF), the level of spontaneous cellular cytotoxicity against Yac-1 tumor target cells, and the level of interleukin 2 (IL2) released into culture supernatants. The above immune parameters are also being evaluated in Lewis rats that have been

treated by intravenous or intraperitoneal administration of large numbers of *M. leprae*, prior to intradermal sensitization, as a means of inducing specific immune tolerance to *M. leprae* antigen.

In the present study, we have observed a significant enhancement in the lymphocyte blastogenic response of splenic (Spl) and inguinal lymph node (LN) cells obtained from Lewis rats receiving intradermal (ID) administration of killed *M. leprae* whole cell preparations (WCP) when co-cultured *in vitro* with *M. leprae*-derived antigen preparations. Kinetic studies have shown that enhanced lymphocyte activity can be detected from 14 to 53 days post ID immunization with peak responses occurring at approximately 35 days. Similar levels of lymphocyte activation were achieved when *M. leprae* concentrations of 2×10^7 and 1×10^7 per animal were used for sensitization, but a concentration of 5×10^6 *M. leprae* per animal failed to induce a detectable *in vitro* response. The development of immunological memory following *M. leprae* immunization was evaluated by administering a secondary (2°) immunogenic dose of *M. leprae* 120 days post primary (1°) immunization. Measurement of lymphocyte activation 14 days post 2° immunization showed significant enhancement in the lymphocyte blastogenic response following *in vitro* co-culture with *M. leprae* antigen as compared to animals receiving 1° immunization only.

In addition to the *in vitro* lymphocyte blastogenesis assay as a measure of *in vivo* activation of *M. leprae*-specific lymphocytes, we have employed another *in vitro* measure of CMI, spontaneous cellular cytotoxicity against Yac-1 tumor target cells. The measurement of natural killer (NK) cell activity was chosen as an additional parameter of elevated CMI since this cell type may be involved in immunoregulation of other lymphoid cells by augmenting or suppressing effector cell function. Parallel studies of lymphocyte blastogenesis and NK activity of inguinal lymphoid cells from ID immunized animals showed a close correlation between enhanced lymphocyte blastogenic activity and enhanced NK activity. In contrast, splenic cells from the same animals did not show a similar augmentation in NK activity even though lymphocyte blastogenesis was dramatically enhanced.

Further evidence for T cell activation following ID immunization of Lewis rats with killed *M. leprae* was assessed by using assays for determination of MIF and IL2 activity in the presence of specific antigen. Significant levels of both lymphokines were detected in culture supernatants following *in vitro* co-culture of sensitized splenic lymphocytes and *M. leprae* antigen. A kinetic study of IL2 release following addition of *M. leprae* antigen to sensitized splenic cells showed significant levels after 24 hr of culture, peak production by 48 hr, and gradual diminution of IL2 activity during the next 3 days of *in vitro* culture.

Tolerance to *M. leprae* antigens is being examined using the method of Shepard, *et al.* for its induction. A significant depression of *in vitro* lymphocyte blastogenesis to *M. leprae* antigen was observed if Lewis rats were first treated with a high dose of *M. leprae* (1×10^8 organisms intravenously or intraperitoneally on days -7 or -14) prior to intradermal immunization with heat-killed *M. leprae*. In contrast, lymphoid cells from tolerant animals did not show a significant depression in their *in vitro* response to the T cell mitogen, concanavalin A, or to a crossreactive mycobacterial antigen derived from *M. marinum*. Preliminary results using spleen cells from tolerant animals showed a significant reduction in their ability to release IL2 when co-cultured *in vitro* with *M. leprae* antigen. The ability of splenic cells from tolerized animals to secrete MIF during *in vitro* culture is presently under investigation.—[Immunobiology Section, Life Sciences Division, SRI International, Menlo Park, California 94025]

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Collins, F. M. and Orme, I. M. Immuno-competence of the heavily infected host.

See abstract in *Int. J. Lepr.* **52** (1984) 610-611.

Truman, R. W., Shannon, E. J., Hugh-Jones, M. E., Hagstad, H. V. and Hastings, R. C. Detection of IgM class anti-

bodies in armadillos to the phenolic glycolipid-I antigen of *M. leprae*.

An enzyme linked immunosorbent assay (ELISA) using the phenolic glycolipid-I (PGI) antigen of *Mycobacterium leprae* and crossreactive antisera specific for human IgM μ chains was developed to detect IgM class antibodies to *M. leprae* in armadillos. Testing sera from recently captured and experimentally inoculated armadillos showed the assay to have high sensitivity and specificity. A retrospective serological survey for *M. leprae* antibodies in armadillos was performed. A total of 182 serum samples taken from feral armadillos between the years 1960 and 1964 were examined. IgM class antibodies to PGI were detected in 9 of the sera tested. Reactivity to PGI in these 9 samples was abolished when sera were absorbed with whole *M. leprae*. Absorption with *M. scrofulaceum*, *M. terrae*, *M. rhodesiae*, and *M. diernhoferi* failed to abolish or significantly alter reactivity to PGI in these samples. Possible implications for the transmission of leprosy are discussed.—[Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana 70803 and National Hansen's Disease Center, Carville, Louisiana 70721]

Baskin, G. B., Wolf, R. H., Gormus, B. J., Martin, L. N. and Walsh, G. P., Binford, C. H. and Meyers, W. M. Experimental leprosy in the mangabey (*Cercocebus atys atys*): necropsy findings.

Naturally occurring and experimentally induced leprosy have recently been described in the mangabey monkey (*Cercocebus atys atys*). We recently had the opportunity to examine at necropsy an adult male mangabey which had been experimentally infected with *Mycobacterium leprae* 46 months prior to death. The animal had been inoculated intradermally and intravenously with *M. leprae* derived from a naturally infected mangabey. The monkey subsequently developed progressive generalized nodular and diffuse thickenings of the skin of the face, ears, scrotum, and distal extremities. Biopsies revealed a disease histologically similar to human subpolar lepromatous leprosy.

The mangabey died unexpectedly 46 months after infection and was examined

at necropsy. Grossly apparent changes were observed in the skin, nasal mucosa, lymph nodes, peripheral nerves, and testicles. Histologically, lesions similar to human lepromatous leprosy were seen in the skin, nasal mucosa, lymph nodes, peripheral nerves, and testicles. These consisted of severe infiltration by histiocytes which contained numerous acid-fast bacilli (AFB) by Fite-Faraco acid-fast stain. The other internal organs were only minimally involved with either a few microgranulomas being present or rare AFB being noted in capillary endothelial cells.

The necropsy findings further define the mangabey as an animal model for leprosy and are very encouraging due to the close similarity between what is seen in humans and in the mangabey.—[Delta Regional Primate Research Center, Covington, Louisiana 70433 and Armed Forces Institute of Pathology, Washington, D.C. 20306]

Malaty, R., Meyers, W. M., Walsh, G. P., Binford, C. H., Zimmerman, L. E. and Baskin, G. B., Wolf, R. H., Gormus, B. J., Martin, L. N. and Gerone, P. J. Histopathological changes in the eye of a mangabey monkey with lepromatous leprosy.

Ocular complications are seen in 6–9% of all leprosy patients, and it is estimated from hospital-based studies that up to 1 million are blinded by this chronic ocular infection. To date little is known about the pathogenesis of leprosy of the eye since histopathological studies have been limited to eyes removed surgically or at autopsy. Microscopic examination of the eyes of nine-banded armadillos with experimental leprosy has shown extensive invasion of the tissues of the anterior segment of the eye, including massive infiltration of the uveal tract. In man these are also the later, more obvious manifestations of ocular involvement, observed clinically as well as pathologically.

We have recently had the opportunity to examine histopathologically the eyes of a sooty mangabey monkey (*Cercocebus atys*) that died with disseminated leprosy 46 months after inoculation with *Mycobacterium leprae* from a mangabey monkey that had naturally acquired leprosy (Meyers, *et*

al., Int. J. Lepr. 48:495–496, 1980). This provided the first opportunity to observe the early changes in the pathogenesis of ocular leprosy in a primate. Histopathological study showed vascularization of the corneoscleral area in the upper temporal quadrant of the eye. The superficial stromal tissue was invaded by histiocytes laden with acid-fast bacilli. Blood vessels at the corneal limbus contained lepra bacilli in the endothelial cells, and the corneal nerves were also invaded by acid-fast bacilli.

This is the first report of ocular manifestations in a primate in which the duration of the infection is known. The only previous report of leprosy in a subhuman primate was that of a naturally occurring infection in a chimpanzee, the duration of whose disease was unknown. Further study of experimentally infected primates is warranted to clarify the pathogenesis and help in the management of the ocular complications of this disease.—[Armed Forces Institute of Pathology, Washington, D.C. 20306 and Delta Regional Primate Research Center, Covington, Louisiana 70433]

Long, E. G., Smith, J. S., Head, E. C., Brennan, P. J. and Smith, J. H. A specific glucose oxidase immunoenzyme stain for *Mycobacterium leprae*.

The isolation of a specific phenolic glycolipid (PGI) from *Mycobacterium leprae*-infected armadillo livers has resulted in the development and application of a sensitive and specific enzyme-linked immunosorbent assay for the detection of anti-*M. leprae* antibodies in infected patients (Cho, *et al.*, Infect. Immun. 41:1077–1083, 1983). As a corollary of these advances, we have attempted to use a rabbit anti-PGI antiserum in an immunoenzyme stain for *M. leprae* in tissue.

An indirect peroxidase method using rabbit anti-PGI as the primary antiserum and a commercial peroxidase conjugate was unsuccessful with deparaffinized skin sections because of high background staining.

The experiment was repeated using a goat anti-rabbit IgG conjugated to glucose-oxidase (Cappel, Malvern, Pennsylvania) and a disclosing reagent composed of α -D-glucose as enzyme substrate, phenazine methosulfate as the electron-transport agent, and

a tetrazolium salt 2-(2'-benzothiazolyl)-5-styryl-3-(4'-phthalhydrazidyl) tetrazolium chloride as an electron receptor (Rathlev, *et al.*, Clin. Chem. 27:1513-1515, 1981). Thirty-two mounted but unstained biopsy specimens were obtained from the Department of Dermatology at the University of Texas Medical Branch at Galveston, Texas. Previous diagnosis of infection based on clinical history and acid-fast stain was unknown to the investigators who did the immunoenzyme stains.

Sections were prepared for staining by deparaffinization in xylene, passage through increasing dilutions of ethanol and rehydration in phosphate-buffered saline (PBS), pH 7.4. Hydrated specimens were covered with a small volume of a 1:100 dilution of anti-PGI antiserum and incubated in a humid chamber at room temperature for 30 min. Slides were washed in 3 changes of PBS and covered with a 1:200 dilution of the glucose-oxidase conjugate and incubated for 30 min at room temperature. After another wash, the sections were covered with the disclosing reagent and incubated for 45 min at 37°C in a moist chamber. The disclosing reagent was rinsed off with PBS and the slides were washed briefly in distilled water, dehydrated in alcohol, cleared in xylene, and mounted in Permout. Soon after the initial trials, it was found necessary to use a light-green counterstain since the glucose-oxidase method caused negligible background staining and some sections were almost invisible.

Positive specimens were identifiable by a dark-red stain of bacilli in globi and in surrounding tissue. Indeed, most positive specimens could be detected upon gross examination. The glucose-oxidase stain correctly identified all 12 cases of lepromatous leprosy but was positive for a specimen from a marine turtle with unidentified acid-fast bacilli. The strain was negative for 5 specimens from tuberculoid leprosy patients, 3 specimens of lung tissue with *M. tuberculosis*, 1 specimen with *M. kansasii*, 1 with *Coccidioides immitis*, and 1 with *Sporotrix schenckii*. The remaining 9 negative specimens came from skin disorders of a non-infectious etiology.

Smears of 15 species of mycobacteria from stock cultures were stained to probe the specificity of the stain. There was some background staining, but the organisms did

not stain and could be seen as "ghosts" against a pale-pink background.

Extensive testing of the glucose-oxidase immunoenzyme method was precluded by access to a limited number of specimens. Our results indicate that the method may be useful in differentiating *M. leprae* from other mycobacteria in skin biopsies as well as in noncutaneous tissues.—[Department of Pathology, The University of Texas Medical Branch at Galveston, Galveston, Texas 77550; Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523]

Krotoski, W. A. and McCormick, G. T. Immunofluorescence studies on *Mycobacterium leprae* in fixed tissues.

Employing immunofluorescence conditions similar to those developed by one of us specifically for investigations on the tissue stages of malaria parasites (Am. J. Trop. Med. Hyg. 22:159-162, 1973 and 31:24-35, 211-225, 1291-1293, 1983), we have undertaken a study of *Mycobacterium leprae* *in situ* in armadillo tissues.

Specimens of liver and skin from a heavily infected armadillo were divided and fixed in either Carnoy's solution or in buffered formalin, and were then dehydrated and embedded in paraffin. Microtome sections on glass slides were deparaffinized in xylene and split into two portions to permit controlled comparisons of different sera. An indirect immunofluorescence (IFA) system employing lepromatous patients' sera and rabbit anti-human IgG conjugated to fluorescein (FITC) was used for these initial studies. A cytoplasmic counterstain was employed to decrease autofluorescence and to permit exact localization of specifically immunofluorescent sites.

As in the malaria system, standard formalin fixation proved to be inferior to Carnoy's fixative, in that tissue fixed in the former showed far more nonspecific, diffuse fluorescence than the latter, particularly in regard to normal skin (collagen) components. Although less permanent than formalin, Carnoy's fixative permitted a clear-cut immune localization of active mycobacterial antigens; individual bacteria could be readily identified in Carnoy's-fixed

tissue (but not in formalin-fixed), although only a small proportion of the organisms present in large clusters in the skin were fluorescent.

In the liver, a specific amorphous fluorescence was present within the Kupffer cells in tissue fixed by either method, but was far brighter in Carnoy's-fixed material; this amorphous fluorescence was distinctly not present in control sections treated with sera from leprosy-naïve patients. Individual mycobacterial cells could not be distinguished within the Kupffer cells in formalin-fixed material, but were brightly fluorescent after Carnoy's fixation.

Post-IFA restaining of sections by the standard Fite-Faraco method, followed by relocation of specifically fluorescent objects, permitted an exact determination of their correspondence with AFB (or lack of it in control sections). Of interest is the observation that, in skin, only a small proportion of the organisms present in large clusters were fluorescent, although greater numbers were distinguishable in individual globi by this specific immune method.

By comparison of fluorescence characteristics obtained with different sera, it appears that different patients "see" the same things differently. Thus, in the same (split) armadillo liver section reacted with different sera, an equal degree of fluorescence of the mycobacterial bodies per se can be accompanied by widely disparate degrees of Kupffer cell fluorescence.—[National Hansen's Disease Center, Carville, Louisiana 70721]

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

See abstract in Int. J. Lepr. **52** (1984) 621–622.

Mohaghehpour, N., Fong, S. K. H., Gelber, R. H., Brennan, P. J. and Engleman, E. G. Production of human monoclonal antibodies to *Mycobacterium leprae*.

Progress in understanding the immunologic defect in leprosy has been hindered by a paucity of reagents that define *Mycobacterium leprae* antigens. Such reagents could be used to detect *M. leprae*-specific epitopes

in infected tissues, to identify epitopes that either induce or suppress specific immunity and, ultimately, to facilitate the development of specific vaccines. To generate such reagents, we have initiated an effort to produce human monoclonal antibodies to *M. leprae*. Our initial findings are reported below.

B lymphocytes were isolated from the peripheral blood of a patient with lepromatous leprosy by a standard sheep erythrocyte rosetting technique and transformed into lymphoblastoid cell lines with Epstein-Barr virus (EBV) containing supernatants from the marmoset B-958 line. To identify EBV transformed B cells that secrete antibody to *M. leprae*, supernatants were screened by ELISA using *M. leprae* antigen. Cells from positive wells were plated at 10 cells/well in liquid medium over irradiated human red blood cells as feeders. Expanded polyclones were fused with our human-mouse cell line, SBC-H20, yielding multiple hybridomas secreting anti-*M. leprae* antibodies. Positive hybrids were cloned and subcloned at 0.5 cell/well. Supernatants from two different hybrid subclones showed significant reactivity, even at a 1:64 dilution, when tested against *M. leprae* sonicate: 1.1D11 = 0.435; 1.1G7 = 0.424; control medium alone = 0.004.

Both subclones have been maintained in continuous culture for 3 months and secrete IgM antibody to 10 µg/48 hr/10⁶ cells. These results indicate that potentially useful human monoclonal antibodies can be produced to *M. leprae* antigens.—[Stanford University, Stanford, California; Seton Medical Center, Daly City, California and Colorado State University, Fort Collins, Colorado]

Gillis, T. P., Miller, R. A. and Buchanan, T. M. Characterization of a competitive-antibody-binding assay with a radiolabeled monoclonal antibody specific for *Mycobacterium leprae*.

Murine monoclonal antibody IVD8 has been shown to bind an epitope of a 68,000 dalton protein of *Mycobacterium leprae*. This epitope (68Kd^Λ) appears to be unique to *M. leprae* as defined by ELISA, FLA-ABS, and immunoblotting. Accordingly, antibody to this epitope in the sera of an

individual would indicate current or previous infection with *M. leprae*. We have designed a competitive-antibody-binding assay (CABA) with radiolabeled monoclonal antibody IVD8 and cell wall antigen from *M. leprae*. Serial tenfold dilutions of serum samples from normal individuals and tuberculosis or leprosy patients were tested in the standardized assay. At serum dilutions of 10^{-3} and 10^{-2} , 8 of 8 normals were unable to inhibit the binding of IVD8 in the CABA. At a dilution of 10^{-1} only 1 normal serum gave a 5% level of inhibition. In contrast, 7 of 8 leprosy patients showed levels of inhibition from 2–49% at dilutions of 10^{-3} and 10^{-2} . At 10^{-1} all 8 leprosy sera showed levels of inhibition from 9–66%. Only 3 tuberculosis patients have been tested thus far and none of them showed levels of inhibition above 20% at any dilution tested. Therefore, preliminary data suggest that the 68Kd protein of *M. leprae* is immunogenic in leprosy infections in man. In addition, we report the development and characterization of a competitive-antibody-binding assay which may be used to detect serum antibody to the unique epitope 68Kd^A of *M. leprae*.—[National Hansen's Disease Center, Carville, Louisiana 70721 and Immunology Research Laboratory, Pacific Medical Center, Seattle, Washington 98144]

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

See abstract in *Int. J. Lepr.* **52** (1984) 618–620.

Job, C. K., Sanchez, R. S. and Hastings, R. C. Phenolic glycolipid-I: an immunogen for a Mitsuda response.

Recent antigenic analysis of *Mycobacterium leprae* has yielded a phenolic glycolipid-I (PGI) specific to the organism and at least its serological uniqueness is attributed to its trisaccharide component. Several studies are underway to detect antibodies to this specific antigen in leprosy patients and their contacts. The hydrophobic property of this compound has limited its use in the available *in vitro* systems to study cell-

mediated immune reactions. Since the granulomatous response obtained in the Mitsuda test is considered a correlate of a satisfactory cell-mediated immune reaction to antigens of *M. leprae*, an *in vivo* study using PGI was undertaken.

Purified PGI was obtained from Dr. Brennan's laboratory and a suspension in 0.85% w/v saline was prepared by sonicating 1.9 mg of PGI in 1.9 ml of saline for 30 sec using a Branson Sonifier cell disruptor at 100 watts power setting. The suspension was then diluted with saline so that 0.1 ml contained 100 μ g of PGI.

Two groups of armadillos were used in the study. One group of 3 was previously infected intravenously with 10^8 *M. leprae*, was found to be resistant to the disease, and was Mitsuda positive. The other group of 3 contained 2 armadillos previously infected intravenously with 10^8 *M. leprae* which were found to be resistant to the disease but were Mitsuda negative, and 1 uninfected Mitsuda-negative armadillo.

All 6 armadillos were injected intradermally in the abdomen with 100 μ g of PGI suspended in 0.1 ml of saline, and a tattoo mark was placed around the injected site. At 21 days the skin reactions were measured and biopsied.

The skin reactions in the lepromin-positive armadillos were 3 mm, 3 mm, and 7 mm. All of the lepromin-negative armadillos had a negative response. The histopathological appearance of the positive response showed a characteristic granulomatous reaction composed of epithelioid cells, macrophages, and lymphocytes. In one animal there was an area of necrosis surrounded by epithelioid cells. The negative reaction was similar in all animals and showed small scattered collections of macrophages with abundant foamy cytoplasm. Electron microscopic studies confirmed the histological findings. The lepromin-negative animals showed macrophages with abundant cytoplasm which had numerous large vacuoles of various sizes. Most of these phagosomes contained granular material. There was hardly any evidence of activation in the cells. Endoplasmic reticulum and mitochondria were few. In the lepromin-positive animals the macrophages showed all signs of activation. There were numerous mitochondria and marked proliferation of the rough en-

doplasmic reticulum. The cell surface had numerous pseudopodia and microvilli.

A positive, delayed hypersensitivity granuloma strongly suggestive of a cell-mediated immune reaction to PGI in lepromin-positive animals and a negative response in lepromin-negative animals is established in this study. It strongly suggests that PGI is an immunogen that elicits cell-mediated immune responses in animals sensitized to *M. leprae*.—[National Hansen's Disease Center, Carville, Louisiana 70721]

Mehra, V., Brennan, P. J. and Bloom, B. R. Mechanism of unresponsiveness in lepromatous leprosy.

Although serological comparisons of the antigens of *Mycobacterium leprae* indicate that all known proteins or glycoproteins are identical or crossreactive with those of *M. tuberculosis*, lepromatous patients who are anergic to antigens of *M. leprae* frequently show positive cell-mediated immunity to antigens of the tubercle bacillus. In view of the serologic crossreactivity between the protein antigens of *M. leprae* and other mycobacteria, the specific and selective immunological unresponsiveness of lepromatous patients remains problematic.

The hypothesis which we have suggested to explain the selective unresponsiveness in lepromatous leprosy is that there must be one or a small number of unique antigenic determinants of *M. leprae* capable of inducing specific suppressor cells which then inhibit the reactivity of helper T cells to other specific or crossreactive determinants.

In order to examine this hypothesis we have developed a simple *in vitro* assay for measuring the ability of *M. leprae* to induce suppression of mitogenic response of peripheral blood lymphocytes to ConA. We have shown previously that: a) Dharmendra lepromin induced *in vitro* suppression of the ConA response of lepromatous and borderline patients, but not tuberculoid patients or normals; b) two cell populations contribute to the suppression, monocytes and T cells; c) all the lepromin-induced Ts activity was associated with a 20–30% subpopulation of T cells defined by the Th₂, monoclonal OKT5 or OKT8 antibodies; d) a high percentage of the Ts subset expressed acti-

vation markers, Ia and Fc receptors; e) no significant suppression *in vitro* was found with lymphocytes from 60 lepromatous patients after immunotherapy with BCG + killed *M. leprae*, and the number of Ia⁺ OKT8 cells returned to normal levels.

Since the only unique antigen demonstrated on *M. leprae* is a phenolic glycolipid (PGI), we have explored the possibility that this glycolipid might activate the suppressor cells in lepromatous patients. The results indicate that PGI is as effective as lepromin in inducing suppression of lymphocyte proliferation to ConA, suggesting that it may be one of the major determinants responsible for activation of suppressor T cells in lepromatous leprosy. The specificity of recognition of this antigen was elucidated by measuring the ability of modified *M. leprae* glycolipid and analogous glycolipids from *M. bovis* and *M. kansasii* to induce suppression.—[Albert Einstein College of Medicine, Bronx, New York 10461 and Colorado State University, Fort Collins, Colorado 80523]

Douglas, J. T., Brennan, P. J. and Gelber, R. H. ELISA evaluation of phenolic glycolipid-I (PGI) and whole *M. leprae* with sera collected from leprosy patients during the course of chemotherapy.

IgM antibody levels to phenolic glycolipid-I (PGI) in the same 53 leprosy sera obtained from the San Francisco United States Public Health Service contract care site were studied by somewhat different ELISA methodologies at the University of Hawaii and Colorado State University. Excellent agreement in 47 of 53 instances was found between the two laboratories. IgM antibody to whole *Mycobacterium leprae* in these same sera was studied by ELISA at the University of Hawaii. The optical density (OD) response to whole *M. leprae*, except in one instance, was as much as, and frequently more than, that to PGI. Lepromatous sera more commonly demonstrated antibody and at higher titer levels to both PGI and whole *M. leprae* than did tuberculoid sera. Tuberculoid sera contained antibody more commonly to whole *M. leprae* (79%) than to PGI (50%). The effect of 2 years of therapy on the generally falling level of IgM antibodies to PGI and whole *M.*

leprae in 20 previously untreated lepromatous and 10 tuberculoid patients are presented.—[University of Hawaii, Honolulu, Hawaii; Colorado State University, Fort Collins, Colorado; Seton Medical Center, Daly City, California]

Rea, T. H., Modlin, R. L. and Taylor, C. R. Anti-interleukin-2 positive and anti-Tac positive cells in erythema nodosum leprosum, lepromin reactions, and tuberculin reactions.

See abstract in *Int. J. Lepr.* **52** (1984) 612–614.

Modlin, R. L., Tapia, F. J., Bloom, B. R., Convit, J. and Rea, T. H. *In situ* characterization of the cellular immune response in leprosy and leishmaniasis.

Leprosy and American cutaneous leishmaniasis have similar clinical, pathologic, and immunologic spectrums. Tuberculoid leprosy is similar to localized cutaneous leishmaniasis (LCL) in its presentation with few skin lesions and rare organisms observed. Lepromatous leprosy is similar to diffuse cutaneous leishmaniasis (DCL) in its presentation of multiple skin lesions with numerous organisms parasitizing macrophages. Organism-specific, delayed hypersensitivity reactions are present in tuberculoid leprosy and LCL but absent in lepromatous leprosy and DCL. In fact, both lepromatous leprosy and DCL patients have been shown to have organism-specific suppressor cell activity. The present study was undertaken to compare immunopathologic aspects of these diseases and examine the *in situ* cellular immune response.

For the present study, 10 tuberculoid (BT) and 10 lepromatous (LL) patients were classified according to the criteria of Ridley and studied at the Los Angeles County/University of Southern California Medical Center. Eight patients with LCL and 2 with DCL were studied; 9 of 10 were studied at the Instituto Nacional de Dermatologia in Caracas, Venezuela. The diagnosis of DCL was made in the presence of multiple skin lesions and numerous organisms parasitizing macrophages. Frozen sections were evaluated with monoclonal antibodies and immunoperoxidase techniques. The specifici-

ties sought included the pan T-cell marker, Leu 4; the helper/inducer phenotype, Leu 3a; the suppressor/cytotoxic phenotype, Leu 2a; interleukin-2 and interleukin-2 receptors (Tac). Previous studies have shown that the anti-interleukin-2 antibody identifies interleukin-2 producer cells.

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

Percent of cells in granulomas by phenotype

	Tubercu- loid	Lepromatous	LCL	DCL
IL-2	0.46±0.28	0.028±0.016	0.22±0.10	0.031±0.011
Tac	2.1±0.7	1.5±0.5	2.1±1.1	3.4±0.2
Leu 4	54±13	33±9	58±13	43±4
Leu 2	21±6	27±9	36±11	23±4
Leu 3	41±12	13±6	26±4	18±4
Leu 3a/2a	2.1±0.5	0.5±0.3	0.8±0.2	0.8±0.3

In tuberculoid granulomas, interleukin-2-positive cells and suppressor/cytotoxic cells were distributed in the mantle surrounding the granuloma, while helper/inducer cells and interleukin-2 receptor cells were distributed throughout the granuloma. In lepromatous leprosy, LCL, and DCL, all cells were distributed throughout the granuloma. The helper:suppressor ratio was >1 in tuberculoid leprosy and <1 in lepromatous leprosy, DCL, and LCL. Interleukin-2 cells were rare in lepromatous leprosy and DCL but an order of magnitude greater in tuberculoid leprosy and LCL. Interleukin-2 receptor cells were present in all 4 disorders.

Several conclusions are of particular importance for leprosy. Because the presence of the interleukin-2 receptor cell means that antigen has been presented and that interleukin-1 has been seen and because the numbers of interleukin-2 receptor cells are similar in lepromatous and tuberculoid patients, the evident failure of *Mycobacterium leprae*-specific CMI in lepromatous leprosy is not a failure of antigen presentation nor an event preemptive of antigen recognition. Rather, because interleukin-2-producing cells are significantly reduced in lepromatous as compared with tuberculoid patients, we think it is likely that the *M. leprae*-specific failure of CMI in lepromatous patients

is secondary to reduced, probably inhibited, interleukin-2 production, in accord with the *in vitro* studies of Haregewoin, *et al.* and Nogueira, *et al.*

Furthermore, the similar nature of the immunopathologic infiltrates in lepromatous leprosy and DCL suggests a common immunologic mechanism for the CMI unresponsiveness in these two disorders. Both LCL and tuberculoid leprosy infiltrates contained similar numbers of interleukin-2-producer cells; however, the ratio and microanatomic location of T cell subpopulations in LCL was more similar to DCL and lepromatous leprosy than tuberculoid leprosy. This suggests that functional attributes of tissue infiltrating cells, such as cytokine production, are more closely related to the effectiveness of the cell-mediated immune response than numbers or locations of T cell phenotypes.—[LAC-USC Medical Center, Los Angeles, California 90033; Albert Einstein College of Medicine, Bronx, New York; Instituto Nacional de Dermatologia, Caracas, Venezuela]

Mohagheghpour, N., Gelber, R. H., Sasaki, D. T., Brennan, P. J. and Engleman, E. G. Effects of recombinant IL-2 on the *Mycobacterium leprae*-induced T cell response in lepromatous leprosy.

See abstract in *Int. J. Lepr.* **52** (1984) 617–618.

Miller, R. A., Harnisch, J. P., Gilliland, B. C., Lukehart, S. A. and Buchanan, T. M. Low prevalence of serologic evidence of polyclonal B cell activation among Hansen's disease patients in Seattle.

Several studies have reported evidence of polyclonal B cell activation in leprosy patients, particularly in patients with lepromatous and borderline lepromatous disease, or in patients with ENL. False-positive serologic tests for syphilis and antinuclear antibodies have been the most extensively studied markers, but other autoantibodies have also been reported. The vast majority of these studies were performed on patients in tropical developing countries. Little or no information was provided concerning other intercurrent illnesses, parasitic infections, or chronic neurotrophic ulcers in these patient populations. The current study at-

tempts to address the issue of autoantibody production in a well-characterized population of patients in the United States.

Forty-four patients have been enrolled in the study to date. Distribution by disease class is: LL, 3; BL, 9; BB, 5; BT, 7; TT, 4; I, 2. Definitive classification is pending on the remainder. Duration of specific therapy ranged from 0 to 15 years, with the majority of patients falling in the 1–3 year group. The majority of our patients were begun on therapy before major nerve loss had occurred, and the general level of personal hygiene is quite high. As a result, only 1 patient has chronic cutaneous ulcerations. None of the patients has evidence of amyloidosis or renal dysfunction, with the exception of 1 unusual patient who has an active urine sediment (see below). The population is composed largely of three ethnic groups: Hispanics, Samoans, and Southeast Asian refugees. Although they have resided in the United States for variable lengths of time, their overall health status is very good. Seven patients have experienced ENL reactions, and 3 have had reversal reactions.

Serologic tests for syphilis have been performed on 30 patients. Three had positive VDRL tests (all low titer), and 7 had equivocal FTA-ABS examinations. None had clearly positive results on the FTA-ABS test. Two of the patients with weakly positive VDRL tests had borderline FTA-ABS results.

Screening for antinuclear antibodies is ongoing. Only 1 positive has been detected among the first 25 sera tested. Tests for antibodies to extractable nuclear antigens, histones, SS-A(Ro), and others are pending. Circulating cryoglobulins have been detected in only 1 of 42 patients tested—the same patient who had antinuclear antibodies—and 1 of the 2 patients with positive VDRL and borderline FTA tests. This patient is a 16-year-old Samoan female with lepromatous leprosy. Subsequent to the diagnosis of leprosy, she developed a clinical syndrome consistent with systemic lupus erythematosus, including fevers, arthritis, pleuritis, and nephritis. Her serum is positive for anti-DS DNA antibodies. Her cryoglobulin has been analyzed and was a mixed IgG-IgM cryoglobulin. No monoclonal spike was found on protein electrophoresis. No mycobacterial antigens could be detected in the

cryoglobulin by Western Blot analysis with pooled human leprosy sera, and the antigenic specificities of the IgG in the cryoglobulin was analogous to that of her serum IgG. The cryoglobulin has been consistently present in serial specimens over a six-month period.

Erythrocyte sedimentation rates (ESR) have been determined in most patients, and they tend to be higher in patients with disease toward the lepromatous end of the spectrum. Moderate elevation of the ESR was sometimes observed during acute ENL reactions, but values over 100 mm/hr were not seen. A curious observation was that all patients tested during acute reversal reactions had extremely low ESRs, usually in the 0–2 mm/hr range. The explanation for this phenomenon is unclear, but it may prove to be of some benefit in the diagnosis of reversal reaction in certain situations.

Although this study is still in progress, we have thus far failed to reproduce the findings of previous investigators who observed frequent evidence of autoantibody production and false-positive tests for syphilis in Hansen's disease patients. Part of this difference may be explained by differences in the ethnic make-up of the study populations, the relatively small number of lepromatous cases, and the comparatively early treatment of our cases. It may also reflect a component of B cell activation secondary to chronic antigenic stimulation by non-*Mycobacterium leprae* antigens, e.g., chronic skin infection, which was absent in our population.—[Immunology Research Laboratory, and Department of Medicine, Pacific Medical Center; University of Washington, Seattle, Washington 98144 and National Hansen's Disease Center, Carville, Louisiana 70721]

Acknowledgment. This research was supported by Contract No. 258-83-0063 from the National Hansen's Disease Center.

Schuller-Levis, G., Cutler, E., Davis, R., Harris, D. and Levis, W. Monocyte chemotaxis in leprosy.

Lepromatous leprosy (LL) is characterized by a large number of tissue macrophages laden with *Mycobacterium leprae*. Because of these large numbers of cutaneous macrophages, as well as variations in sub-

populations of lymphocytes, a study of macrophage migration and production of lymphocyte-derived chemotactic factor (LDCF) is important. Two previous papers have documented, in multibacillary leprosy patients with active disease, a deficit in neutrophil chemotaxis, secondary to a serum chemotaxis inhibitor.

In our initial studies, chemotaxis was performed (Snyderman, *et al.* In: *Leukocyte Chemotaxis*. New York: Raven Press, 1978, 73–78) using blind well chambers and 5.0 μ m pore polycarbonate filters. The number of migrated cells, tested in triplicate, was determined microscopically by examining 20 oil immersion fields. To prepare LDCF, human mononuclear cells were separated by Ficoll-Hypaque density gradients, washed 3 times, and adjusted to 2×10^6 cells/ml. The cells were incubated with ConA and 0.5% autologous serum at 37°C in 5% CO₂. After 48 hr, cell-free supernates were stored at –70°C until use, as a standard chemotactic factor.

The table shows a significant difference in the ability of monocytes from normal controls and active LL patients to migrate toward a concentration gradient. In contrast, there was no significant difference between inactive LL and normal controls.

Monocyte chemotaxis to LDCF		
Subjects	Chemotaxis* (mean \pm S.D.)	Significance ^b
Normal controls (n = 14)	29.6 \pm 10	
Active LL ^c (n = 8)	14.6 \pm 8	p < 0.01
Inactive LL (n = 5)	27.7 \pm 7	Not significant

* Mean number of monocytes per oil immersion field. Negative control (culture media) was always < 10 cells per oil immersion field.

^b Normal values compared to active LL and inactive LL.

^c Bacterial Index ranged from 3+ to 6+.

We are currently using a new 48-well chemotaxis chamber which has greatly minimized manipulation time compared to the blind well chamber. Combined with the use of an image analyzer to count the migrated cells, the method is suitable for clinical research on the functional state of monocytes in large groups of patients.

We investigated the production of lymphocyte-derived chemotactic factor (LDCF) for mononuclear phagocytes from LL peripheral blood lymphocytes. Briefly, normal

and LL peripheral blood mononuclear cells were isolated by Ficoll-Hypaque separation and stimulated with ConA. Supernatants collected at 4, 24, 48, 72, and 96 hr were tested for production of LDCF using normal human monocytes in a modified Boyden chamber. Optimal conditions with respect to cell density, lectin concentration, and culture time have been established with LL patients and normal controls. Of the 2 LL patients studied, both have decreased production of LDCF (4–96 hr) when compared to normal controls.

The use of the newly designed, 48-well chemotaxis chamber with an image analyzer offers the advantages of decreasing the cell number and chemotactic factor needed, decreasing the working and counting time, as well as increasing the accuracy of the assay. This study should provide meaningful data for comparison of monocyte chemotaxis and LDCF production to Bacterial Index, therapy, as well as correlation (or lack of correlation) with phenolic glycolipid antibodies.—[New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314]

Levis, W. R., Meeker, H. C., Sersen, G. and Schwerer, B. IgM and IgG antibodies against phenolic glycolipid-I from *Mycobacterium leprae* in leprosy sera: relationship to bacterial index and erythema nodosum leprosum.

See abstract in *Int. J. Lepr.* **52** (1984) 614–615.

Tausk, F., Schreiber, R., Lopez, D. A. and Gigli, I. Reduced expression of C3b receptors on erythrocytes from patients with lepromatous leprosy.

The receptor for the C3b fragment of the third component of the complement system was first reported by Nelson as the immune adherence receptor on primate erythrocytes (E) and nonprimate platelets. Subsequently this receptor has been shown to be present on membranes of a number of other blood and tissue cells. This receptor participates in a number of immunologic functions, including processing of immune complexes, regulation of complement activation, and host cell-mediated cytotoxic responses. Certain human immune complex diseases such

as systemic lupus erythematosus and rheumatoid arthritis are associated with reduced levels of erythrocyte CR1, providing clinical evidence to support the concept that this defect can result in an impairment to process circulating immune complexes effectively.

We have studied the expression of CR1 on erythrocytes of patients with Hansen's disease. We were motivated by the spectrum that spans from lepromatous leprosy, with high levels of circulating immune complexes, to the tuberculoid pole, featuring the absence of immune complexes. To measure the CR1 on erythrocytes, we developed a radioligand assay using Fab fragments of a monoclonal IgG raised in mice against the purified receptor. We have shown that this monoclonal antibody is directed toward an epitope different from the C3b binding site and, therefore, capable of measuring the total number of receptors on the cell surface.

With this technique we measured 47 patients (19 LL, 5 BL, 4 BB, 5 BT, and 14 TT) and a normal, age-matched population consisting of 31 subjects. The E from the normal controls displayed a mean of 645 CR1 per cell, with a range from 319 to 1320. The 19 patients identified as LL expressed a significant reduced level of E-associated CR1—361 per cell, with a range from 125 to 687 ($p < 0.001$). In contrast, the 14 TT patients had normal CR1 receptor levels, a mean of 649 with a range from 387 to 1313 ($p = 0.3$). No significant results were obtained in the other subgroups due to limited population size.

These findings could bear importance in the ability of the host to inactivate and clear immune complexes from the circulation in lepromatous leprosy since it has been shown that immune complexes bind to erythrocytes and are delivered to the liver where they are apparently removed by local macrophages. Whether the defect of CR1 expression on erythrocytes is genetically determined or acquired is presently under investigation.—[Division of Dermatology, University of California Medical Center, San Diego, San Diego, California 92103]

Campbell, P. B., Tolson, T. A., Yoder, L., Loesch, J. and Krahenbuhl, J. L. Defective regulation of monocyte leukotaxis in leprosy.

Our laboratory in Greenville has maintained an interest in the regulation of monocyte function in patients with granulomatous disorders. We have previously shown that monocytes from patients with active tuberculosis (TB) and sarcoidosis display defective monocyte leukotaxis *in vitro*. This defect is effected by an increase in activity of a cell-directed inhibitor of monocyte leukotaxis (CDI-MLx), a 230,000 dalton constituent plasma protein. CDI-MLx activity in these patients returned to normal within days of initiating therapy in the form of rifampin or prednisone. CDI-MLx is a lymphocyte product, clearly distinct from migration inhibitory factor, leukotactic lymphokines, and immunoglobulins. The elaboration of CDI-MLx *in vitro* is augmented by soluble and cell-associated antigen and certain mitogens. Peripheral blood mononuclear cells from patients with TB and those with sarcoidosis and culture supernatants from cutaneous sarcoid granulomata spontaneously elaborate CDI-MLx *in vitro* which likely accounts for the increased plasma activity of this immunoregulator in these disorders.

Since leukotaxis appears requisite for both the early and continuing immigration of phagocytes into foci of microbial invasion, we have sought to evaluate those mechanisms regulating peripheral blood monocyte locomotion in patients infected with *Mycobacterium leprae*. Plasma leukotactic inhibitory activity was assayed by comparing the effects on normal human monocytes of preincubation in either undiluted test plasma or in TC 199 with 2% BSA. After a 30 min preincubation, each aliquot of cells was resuspended in 2% BSA and leukotactic responsiveness to 10% zymosan-activated normal human serum assessed in a double-filter modification of Boyden's technique. For studies of the *in vitro* production of leukotactic inhibitors, peripheral blood mononuclear cells were isolated by Ficoll-Hypaque sedimentation and resuspended at 1 million per ml of RPMI-1640 with 10% heat-inactivated fetal calf serum with or without 100 μ g/ml concanavalin A. After 72 hr, the supernatants were harvested and assayed for inhibitory activity as above.

Plasma was obtained from 8 normal subjects and 30 patients with active leprosy.

The inhibitory activity of the plasma from the normal subjects did not differ from controls (mean \pm S.D. = $7.2 \pm 8.2\%$). However, significant ($>15\%$) leukotactic inhibitory activity was detected in the plasma of 4 of 6 patients with BT disease ($23.5 \pm 10.2\%$, range 12.1 to 30.9%); of 5 of 7 with BL disease ($24.0 \pm 15.6\%$, 0.2 to 43.1%); and of 12 of 17 with LL ($24.3 \pm 15.1\%$, 3.7 to 45.5%). There was no significant difference in the frequency or magnitude of leukotactic inhibitory activity among BT, BL, or LL patients, nor did there appear to be correlations between inhibition and disease duration, type of antileprosy or anti-inflammatory chemotherapy, occurrence of ENL, or bacillary index.

Lymphocyte culture supernatants from 3 patients with BL and 9 with LL were also assayed. Unstimulated culture supernatants inhibited the leukotactic responsiveness of normal monocytes by $3.8 \pm 5.4\%$, which was not different from normal supernatants ($5.4 \pm 7.6\%$). Similarly, inhibitory activity in ConA stimulated supernatants of leprosy and normal lymphocytes were equivalent ($40.0 \pm 4.7\%$ vs $45.6 \pm 10.2\%$). No correlation between plasma leukotactic inhibitory activity and the activity in either unstimulated or mitogen-stimulated culture supernatants was observed.

These data indicate that plasma from a majority of patients with borderline or lepromatous leprosy contains substances that inhibit the locomotion of monocytes. The current focus of our work is to determine the identity of this inhibitory substance(s). Our inability to demonstrate production of enhanced inhibitory activity by stimulated peripheral blood mononuclear cells suggests that leukotactic inhibition in leprosy may be effected by mechanisms fundamentally different from those in other mycobacterial diseases. Whether this plasma inhibitory activity contributes to the pathogenesis of this disease is not known. The identity of these inhibitors is also not clear, and the possibility that they are produced locally in the *M. leprae*-infected tissues remains to be determined.—[Department of Medicine, East Carolina University, Greenville, North Carolina; Clinical and Laboratory Research Branches, National Hansen's Disease Center, Carville, Louisiana 70721]