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ABSTRACTS

TWENTY-SECOND JOINT LEPROSY RESEARCH CONFERENCE

National Institutes of Health
Bethesda, Maryland, U.S.A.
20 and 22 July 1987

U.S.–Japan Cooperative Medical Science Program

OPENING REMARKS

Ladies and gentlemen; panel members and participating scientists of the Japanese component of the U.S.–Japan Cooperative Leprosy Program; fellow researchers from the United Kingdom, Ethiopia, Belgium, The Netherlands, India, the People's Republic of China; friends and benefactors from the Leonard Wood Foundation and the American Leprosy Missions; fellow U.S. scientists: on behalf of the U.S. panel of the U.S.–Japan Cooperative Leprosy Program and on behalf of Dr. Darrel Gwinn, Leprosy Program Officer of the National Institutes of Health; Dr. Earl Beck, Coordinator of the U.S.–Japan Cooperative Medical Science Program; and on behalf of our hosts, the National Institutes of Health, I would like to welcome you to the Twenty-Second U.S.–Japan Leprosy Research Conference.

This, our twenty-second meeting, is an historic one in that we have survived the years of infancy and teenhood and have now acquired full maturity. This is also a crucial meeting in that, in the great tradition of self examination inherent to this Program, we will evaluate our past performance and future prospects over the next few days.

The past few years have been a splendid time for leprosy research and for participants in this and other international programs. Among some extraordinary accomplishments too numerous to mention, we, as researchers in leprosy, have been responsible for the differentiation of the major protein antigens of the leprosy bacillus through the use of an extraordinary collection of monoclonal antibodies and an unprecedented degree of international cooperative research. The technological development of recombinant DNA cloning and T-cell cloning have been responsible for the mapping of many of the T-cell and B-cell epitopes on those protein antigens. Several of these antigens have been produced in unprecedented quantities through recombinant DNA technology, and many individual peptide segments have been synthesized, such that experiments to define the immunogenicity and protective efficacy of individual proteins and their peptides are now underway.

Phenolic glycolipid, the definition of which must be a major accomplishment of the Program, saw its complete fruition with chemical synthesis of the specific triglycosyl entity and its incorporation into highly specific and facile seroepidemiological tools. The lipopolysaccharide antigens of *Mycobacterium leprae* and much of its cell wall have been structurally defined in recent times, and an examination of their roles in the immunopathogenesis of leprosy has begun. Spectacular advances have been made in immunobiology, with the delineation of T-cell subsets, lymphokines, MHC-genes and the overall role of the macrophage, all of which have contributed to a better comprehension of the immunopathogenesis of the disease. Substantial progress has also been made in the therapeutic application of these findings, through the clinical appli-

cation of lymphokines or antigens, alone or in combination. Intensive work on several animal models was responsible for the recognition of new drug combinations and the requirements of future subunit vaccines.

Many of the unprecedented accomplishments of the past few years were made possible only through close cooperation between participants in this and other international programs. Leprosy remains a major health problem for the poorest sections of humanity, and much of it is distributed in endemic areas adjacent to Japan. There, our Japanese colleagues, through their own resources, scientific and financial, are now implementing, in close concert with U.S. colleagues, the fruits of this program; a good example of such cooperative endeavors is found in the implementation of leprosy serodiagnosis in the Western Pacific and S.E. Asia regions. Soon we will see the emergence of a second-generation candidate vaccine, through molecular cloning or further fractionation of the bacillus. Diagnostic tests for paucibacillary leprosy to complement those in existence for multibacillary leprosy will also emerge, probably from immunological assessment of recombinant proteins. The avenues are open through this and other programs for the immediate implementation in leprosy-endemic areas of these needed developments.

Although the past five years have witnessed extraordinary progress in leprosy research, much has yet to be done. The global objectives of developing improved methods for assessing specific cellular and humoral immune responses to the antigens of *M. leprae*; of defining the immunopathological mechanism underlying tissue damage in leprosy and of reversal of specific immunological unresponsiveness; and of developing antileprosy vaccines and new chemotherapeutic combinations; all of these await our resolution. Only through commitment to the ideals of the U.S.–Japan Cooperative Program and other international programs can U.S.-based workers complement the acquisition of fundamental knowledge with its practical implementation. Thus, the primary challenge for us Americans in the future years is innovative, brave, new research, which must always be our priority, and then the imparting of this knowledge in forums such as this, followed by its humanitarian applications, again, through the medium of the U.S.–Japan Cooperative Program or our Indo–U.S. programs or the WHO programs. It is only through the existence of such programs that we, primarily basic researchers, can contribute to the ultimate eradication of leprosy.

Again, on behalf of fellow panel members, Drs. Clark-Curtiss, Cohn, Rea, and Krahenbuhl, I welcome you and wish for you a fulfilling meeting.

—Patrick J. Brennan, *Chairman*
U.S. Leprosy Panel

PROGRAM
TWENTY-SECOND JOINT LEPROSY CONFERENCES

20 July, Monday

Opening Remarks: Dr. Patrick Brennan, Chairman, U.S. Leprosy Panel

Session I

Co-Chairmen: Dr. Tonetaro Ita
Dr. Thomas Rea

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Douglas, J. T., Steven, L. M., Cellona, R. V., Fajardo, T., Abalos, R. M. and Madarang, M. G. Serological reactivity among contacts of lepromatous patients in Cebu, The Philippines

Levis, W. R., Meeker, H. C., Schuller-Levis, G., Sersen, E., Brennan, P. J. and Fried, P. Mycobacterial carbohydrate antigens for serologic testing of leprosy

Gelber, R. H., Tsang, M., Murray, L. P. and Siu, P. Chemotherapy trials in the neonatally thymectomized Lewis rat: a model for the study of the therapy of lepromatous leprosy

Ito, T., Kohsaka, K. and Miyata, Y. Effect of ofloxacin on experimental leprosy

Dhople, A. M., Osborne, L. J., Seydel, J. K. and Schonenberger, H. Development of new chemotherapeutic agents in leprosy

Session II

Co-Chairmen: Dr. Tatsuo Mori
Dr. Zanvil Cohn

Converse, P. J., Ottenhoff, T. H. M., Ehrenberg, J. P. and Kiessling, R. The repertoire of mycobacterial antigens recognized by peripheral blood cells and sera of healthy leprosy patient contacts

Ottenhoff, T. H. M. and de Vries, R. R. P. An HLA-DR3 immune response gene for *Mycobacterium leprae* predisposes to tuberculoid leprosy

Mshana, R. N., Krahenbuhl, J. L. and Hastings, R. C. Interferon-gamma induces the expression of major histocompatibility complex antigens by *in vitro* cultured murine Schwann cells

Draper, P. Mycobacterial wall lipopolysaccharide: aspects of organization and structure

Brennan, P. J., Hunter, S. W., McNeil, M., Stewart, C., Bloom, B. R., Melancon-Kaplan, J., Modlin, R. L. and Rea, T. H. The cell wall of *M. leprae*; isolation, composition, and immunogenicity

22 July, Wednesday

Session III

Co-Chairmen: Dr. Shinzo Izumi
Dr. James Krahenbuhl

Krahenbuhl, J. L., Sibley, L. D. and Chae, G. T. Defective macrophage effector function in lepromatous leprosy

Sibley, L. D., Ramasesh, N., Franzblau, S. G. and Krahenbuhl, J. L. Functional responses of normal and activated macrophages infected with *Mycobacterium leprae in vitro*

Holzer, T. J., Arnold, J. J., Vachula, M. and Andersen, B. R. Phenolic glycolipid-I of *Mycobacterium leprae* induces altered monocyte oxidative responses *in vitro*

Cohn, Z. A. and Kaplan G. The role of T6+ Langerhans' cells in the cutaneous immune response in leprosy

Kaplan, G. and Cohn, Z. A. The role of IFN-γ in the regulation of DTH in human skin

Rea, T. H. and Modlin, R. L. Characterization of CD4-positive T-lymphocyte subsets in leprosy granulomas

Session IV

Co-Chairmen: Dr. Kazunari Nakamura
Dr. J. Clark-Curtiss

Mori, T. Acid-fast bacilli detected in umbilical cord and skin specimens

Nakamura, K. and Yogi, Y. *M. leprae* susceptibility of NOD hybrid nude mice

Portaels, F., Walsh, G. P., DeRidder, K., Malaty, R., Silva, M. T., Binford, C. H. and Meyers, W. M. Cultivable mycobacteria isolated from 32 newly captured armadillos (*Dasypus novemcinctus*) from Louisiana

Clark-Curtiss, J. E., Walsh, G. P. and Portaels, F. Use of recombinant DNA molecules in epidemiological studies of leprosy

JOINT U.S.–JAPAN TUBERCULOSIS AND LEPROSY SYMPOSIUM
National Institutes of Health
Bethesda, Maryland, U.S.A.

21 July, Tuesday

Session II—Immunology of Leprosy and Tuberculosis

Co-Chairmen: Dr. Masahide Abe
Dr. Patrick Brennan

Sathish, M., Esser, R. E., Curtiss, R., III, and Clark-Curtiss, J. E. Detection and characterization of antigens of *Mycobacterium leprae* reacting with sera from leprosy patients

Izumi, S., Fujiwara, T., Ikeda, M., Nishimura, Y. and Sugiyama, K. Gelatin-particle agglutination test for serodiagnosis of leprosy—a new simple test useful for a large-scale field study

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ABSTRACTS

Abe, M. Some strategies for developing serodiagnosis of leprosy.

Some strategies for developing a new serological test as well as for promoting practical use of the known tests are discussed from the immunological aspects of leprosy. Since the chemical structures of *Mycobacterium leprae*-specific carbohydrate and peptide epitopes have been clarified recently, synthesized compounds corresponding to each epitope have been or will be used as the antigen for the serodiagnosis of leprosy. Humoral immune responses against these epitopes may not always be parallel during the clinical or subclinical course of leprosy. This was shown by a comparison of serological tests using different antigen preparations. It is, therefore, suggested that a nonantigenic carrier substance or particles conjugated with carbohydrate or peptide or both may be useful as an ideal antigenic substitute for *M. leprae* for the different purposes of serological testing in leprosy. The comparison also showed that tests with high sensitivity tend to have low specificity and vice versa. Since the prevalence rate of leprosy is very low and the frequency of subclinical infection is relatively high, criteria for serological testing should be set according to the purpose of their use. For example, high specificity is required to avoid overdiagnosis; whereas high sensitivity is favorable for screening subclinical infection.

In general, serodiagnosis is useful for: a) early detection of the disease, b) epidemiological study, c) evaluation of the effect of treatment, d) prognosis of the disease, and e) identification of the immunoglobulin class of antibody. Their usefulness in leprosy is discussed based on recent findings. A simple rapid test is required for a large-scale survey in the field. Passive agglutination of particles coated with *M. leprae*-specific carbohydrate or peptide epitope conjugate is suitable for this purpose, provided that the reliability of such a test is kept constant, even under field conditions.

Finally, a strategy for establishing the test system both in the field and in the central laboratory is discussed. Pretreatment of test materials and the correct methods of transportation are needed in the field, while microtitration and automation are necessary for multipurpose tests in a central laboratory.—[National Institute for Leprosy Research, Tokyo, Japan]

Douglas, J. T., Steven, L. M., Cellona, R. V., Fajardo, T., Abalos, R. M. and Madarang, M. G. Serological reactivity among contacts of lepromatous patients in Cebu, The Philippines.

See page 718 of the Correspondence section of this issue.

Levis, W. R., Meeker, H. C., Schuller-Levis, G., Sersen, E., Brennan, P. J. and Fried, P. Mycobacterial carbohydrate antigens for serologic testing of leprosy.

Antibodies to the phenolic glycolipid-I (PGL-I) antigen of *Mycobacterium leprae* have been shown to be potentially useful in the serodiagnosis of leprosy and the monitoring of leprosy patients. However, many nonlepromatous patients do not develop antibodies to PGL-I and, accordingly, additional adjunctive tests would be helpful in establishing a serological profile for the diagnosis of leprosy throughout the entire disease spectrum. Recently, a highly immunogenic lipoarabinomannan (LAM) has been isolated from both *M. leprae* and *M. tuberculosis* and partially characterized. We have field tested these antigens in the New York City Regional Hansen's Disease Program in order to determine if antibodies to LAM might prove useful adjuncts to PGL-I in the detection and monitoring of leprosy.

PGL-I was incorporated into liposomes with sphingomyelin, cholesterol, and dicetyl phosphate. Control liposomes were made without PGL-I. After coating (2.5 μg PGL-I/ml), the plates were washed, then blocked with phosphate buffered saline plus 3% bovine serum albumin (PBS-BSA). Sera, diluted 1:20 in PBS-BSA, were added in duplicate to wells containing PGL-I liposomes and control liposomes. The plates were then washed and goat anti-human IgM peroxidase conjugate was added. The plates were again washed and substrate solution was added [1.8 mM 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) plus 0.1 mM H_2O_2 in phosphate buffer]. The reaction was stopped and read at 405 nm. Results were expressed as ΔE = EPGL-I-liposome coat) − E(control liposome coat).

LAM was dissolved in carbonate-bicarbonate buffer (2 μg/ml). The plates were coated, one half with the LAM solution and one half with coating buffer alone. After coating, the plates were washed and blocked with PBS-BSA. Sera diluted 1:100 and 1:200 (for detection of IgM and IgG, respectively) were added in duplicate to LAM-coated and uncoated wells. The plates were washed and goat anti-human IgG (or IgM) peroxidase conjugate was added. The plates were again washed and substrate solution (1.8 mM ABTS plus 0.1 mM H_2O_2 in citrate phosphate buffer) was added. Results were expressed as ΔE = E(LAM-coated wells) − E(uncoated wells).

When the data were analyzed by the nonparametric Kruskal-Wallis test, LL, BL, and BB patients were significantly higher for anti-PGL-I IgM, anti-LAM IgM and IgG than controls (The Table). BT patients were significantly higher than controls for anti-PGL-I IgM and anti-LAM IgG, while TT patients were significantly higher for anti-LAM IgG than controls. Sera from five tuberculosis patients were significantly higher than controls for IgG to *M. tuberculosis* LAM. All five were positive for IgG to LAM (range, ΔE = 0.4 to 1.13). Over 85% (53 of 62) of the multibacillary leprosy patients tested positive for at least one of the three antibodies assayed (anti-PGL-I IgM, anti-LAM IgM and IgG). This was significantly higher than the number of controls positive for at least one antibody ($\chi^2 = 48.89$, $p < 0.001$). The 14.5% of multibacillary patients who were negative for all three antibodies were mostly long-standing cases (mean length of disease 16.1 years ± 11.4, range 1–30 years) inactive by biopsy (mean BI = 0.22 ± 0.44). Additionally, 71% of paucibacillary patients had at least one positive antibody reading ($\chi^2 = 23.31$, $p < 0.001$ when compared with controls). Significantly elevated seropositivity was seen among household contacts, but to a lesser extent (31.8% positive for at least one antibody, $\chi^2 = 5.02$, $p. < 0.05$). A symptomatic contact (wife of a LL patient) with a neuropathy of the right posterior tibial nerve was seen to be initially positive for IgM to both PGL-I and LAM and IgG to LAM (ΔE = 0.33, 0.55, and 1.88, respectively, for anti-PGL-I IgM, anti-LAM IgM, and anti-LAM IgG; electrodiagnostic nerve conduction study measured distal latency at 7.0 msec and amplitude of response at 2 mV).

THE TABLE. *Detection of antibodies to LAM and PGL-I in leprosy patients, contacts, and controls.*

Ridley-Jopling classification	Anti-PGL-I-LAM		Anti-LAM IgM		Anti-LAM IgG	
	No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.
LL	51	0.59 ± 1.35[a]	55	0.41 ± 0.43[a]	55	0.60 ± 0.57[a]
BL	10	0.66 ± 0.43[a]	12	0.31 ± 0.35[b]	12	0.76 ± 0.49[a]
BB	5	0.23 ± 0.17[b]	6	0.16 ± 0.08[c]	6	0.87 ± 0.41[b]
BT	24	0.13 ± 0.24[b]	25	0.11 ± 0.06	25	0.32 ± 0.34[a]
TT	3	0.03 ± 0.03	3	0.05 ± 0.02	3	0.45 ± 0.30[c]
TB[d]	3	0.02 ± 0.04	5	0.14 ± 0.13	5	0.85 ± 0.35[b]
NC	5	0.01 ± 0.02	5	0.08 ± 0.05	5	0.05 ± 0.05
HC	66	0.03 ± 0.07	78	0.10 ± 0.07	78	0.11 ± 0.24
Controls	33	0.02 ± 0.08	35	0.08 ± 0.03	35	0.07 ± 0.10

[a] Significantly higher than mean control values by Kruskal-Wallis test ($p < 0.001$).
[b] Significantly higher than mean control values by Kruskal-Wallis test ($p < 0.001$).
[c] Significantly higher than mean control values by Kruskal-Wallis test ($p < 0.01$).
[d] TB = tuberculosis.

After 14 months of dapsone therapy, an improvement in the neuropathy was noted (distal latency = 4.4 msec, amplitude = 10 mV), and a concurrent drop in antibody level was seen ($\Delta E = -0.07$, 0.17, and 0.04 for anti-PGL-I IgM, anti-LAM IgM, and anti-LAM IgG, respectively).

A positive linear correlation was observed between BI and both IgM ($r = 0.4147$, $p < 0.001$) and IgG ($r = 0.3366$, $p < 0.001$) antibody to *M. tuberculosis* LAM. This finding suggests that the level of antibody to LAM is reflective of the total bacillary load and, as such, a good indicator of disease activity. When patients with ENL were analyzed separately, no statistically significant correlation was seen between either BI and anti-LAM IgM or BI and anti-LAM IgG.

A group of 28 patients, 10 contacts, and 3 controls was assayed for antibodies to both *M. tuberculosis* and *M. leprae* LAM. Overall, excellent correlation was found between antibody levels to *M. tuberculosis* LAM and *M. leprae* LAM ($r = 0.8350$, $p < 0.001$ and $r = 0.9637$, $p < 0.001$ for IgG and IgM, respectively), indicating considerable antigenic similarity between the two LAM preparations. One patient was highly elevated for IgG to *M. leprae* LAM but negative to *M. tuberculosis* LAM (ΔE IgG = 1.06 vs ΔE IgG = 0.13). This may reflect a response to a *M. leprae*-LAM determinant not shared with *M. tuberculosis*.

Determination of antibody levels to LAM should prove to be a useful adjunct to anti-PGL-I IgM in the serodiagnosis and monitoring of leprosy.—[New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314; Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.]

Gelber, R. H., Tsang, M., Murray, L. P. and Siu, P. Chemotherapy trials in the neonatally thymectomized Lewis rat: a model for the study of the therapy of lepromatous leprosy.

Although the normal mouse has proved enormously useful in establishing a scientific basis for the chemotherapy of leprosy, particularly for screening drugs and evaluating their bactericidal potential, it is an insensitive monitor of clinical trials utilizing bactericidal agents such as rifampin and wanting as a model of lepromatous leprosy amenable to direct evaluation of therapeutic regimens. Previously, we (Int. J. Lepr. **54**:273–283, 1986) established that utilization of the neonatally thymectomized Lewis rat (NTLR) provides a superior and more sensitive monitoring system than the BALB/c mouse for detecting viable *Mycobacterium leprae* in skin biopsies of patients undergoing initial therapy. Because the NTLR is seriously impaired immunologically and develops large numbers of *M. leprae*, it is also a potentially useful model of lepromatous leprosy suitable for direct studies of chemotherapy regimens. In the first studies of chemotherapy in the NTLR, Fieldsteel (Am. J. Trop. Med. Hyg. **25**:854–857, 1976) found that 0.00005% dapsone in rat chow, a concentration of drug which when fed to NTLR continuously from the

time of infection prevented *M. leprae* multiplication, did not eliminate viable *M. leprae* from established heavily infected NTLR. Furthermore, 100 times that concentration of dapsone, 0.005% alone, up to 10 doses of rifampin, 10 mg/kg, alone or combined with 0.00005% dapsone, and 0.005% dapsone with a single dose of rifampin, 10 mg/kg, were also unable to sterilize established and heavily infected NTLR (Int. J. Lepr. **47**:106–110, 1979 and **48**:267–276, 1980). Thus, established infection of the NTLR, as in human lepromatous leprosy, allows persisting *M. leprae* despite potent antimicrobial therapy.

In our current chemotherapy studies, NTLR were infected in both hind foot pads with 5×10^3 *M. leprae*. At 1 year, 16 foot pads (8 NTLR) were harvested and found to be uniformly heavily infected, average 2×10^8 *M. leprae*/foot pad (range 4×10^7–8×10^8). One year following infection, groups of these NTLR were treated for 4 months with the following six regimens:

1. Dapsone (DDS) 0.005% + 10 doses of rifampin (RMP) 10 mg/kg by gavage
2. Dapsone 0.005% + rifampin 0.01%
3. Rifampin 0.01%
4. Clofazimine (CLO) 0.01% + rifampin 0.01%
5. Dapsone 0.005% + ethionamide (ETH) 0.2%
6. Rifampin 0.01% + ethionamide 0.2%

Regimens 1–3 were studied because they provided more intensive schedules of rifampin alone and combined with dapsone than those studied previously by Fieldsteel. Regimens 4 and 5 were selected because Shepard (Int. J. Lepr. **40**:33–39, 1972) had found these two combinations to be the only ones of the four clinically utilized drugs (dapsone, rifampin, clofazimine, and ethionamide) that were demonstrated to be consistently additive in their antimicrobial activity against *M. leprae* in infected mice. Regimens 4 and 6 were evaluated because these combinations (rifampin plus ethionamide and rifampin plus clofazimine) appear to be the most bactericidal in mice (Lepr. Rev. **47**:7–15, 1978). NTLR foot pads were harvested and *M. leprae* enumerated during therapy and at intervals up to 4 months after discontinuation of therapy in order to determine by the most sensitive methods available to us whether regrowth of any survivors could be prevented. For these purposes, viability of *M. leprae* from treated NTLR was determined by passage to mice (5×10^3 *M. leprae* per foot pad) and to NTLR (the maximal number of bacilli, generally $>10^6$ *M. leprae* per foot pad). When passage NTLR demonstrated less than a fourfold increase in *M. leprae* but $>10^5$ bacilli, viability was finally determined by further mouse subpassage. The results of these studies are summarized in the table below.

It is noteworthy that while the three regimens which included dapsone commonly resulted in the detection of viable *M. leprae* both during therapy, 6 of 16 NTLR (38%), and after therapy was discontinued, 11 of 23 NTLR (48%), the three regimens that did not utilize dapsone resulted uniformly in the detection of no viable *M. leprae* during therapy, 0 of 11 NTLR (0%), and rarely, only 2 of 25 NTLR (8%), even after therapy was discontinued. This suggests that the utilization of a fundamentally bacteriostatic agent, dapsone, reduces the bactericidal activity of bactericidal agents such as RMP, ETH, and CLO in this system. Furthermore, both during therapy and especially after therapy had been discontinued, the two regimens of DDS and RMP were less effective in eliminating viable *M. leprae* than RMP alone. If confirmed by larger numbers of observations, this would imply antimicrobial antagonism in the NTLR for the combination of DDS and RMP.

Of the combinations studied, CLO + RMP and RMP + ETH appear superior,

Survival of M. leprae *in NTLR**

	Regimen					
	A DDS/RMP	B DDS/RMP	C RMP	D CLO/RMP	E DDS/ETH	F ETH/RMP
During Rx	2/7 (29%)	3/6 (50%)	0/4 (0%)	0/3 (0%)	1/3 (33%)	0/4 (0%)
After Rx	4/5 (80%)	4/9 (44%)	2/7 (29%)	0/9 (0%)	3/9 (33%)	0/9 (0%)

* NTLR with viable *M. leprae*/total NTLR studied (% NTLR with viable *M. leprae*).

entirely eliminating viable *M. leprae* both during therapy and preventing the growth of survivors up to 4 months following discontinuation of therapy. Thus, these regimens appear the best candidates to permit safe discontinuation of therapy in lepromatous patients. The key to effective short-course therapy in experimental tuberculosis in the mouse and in human disease appears to depend on the sterilizing activity of two bactericidal agents. Our studies suggest that such an approach in the therapy of leprosy offers similar promise.—[Seton Medical Center, Daly City, California 94015; GWL Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Ito, T., Kohsaka, K. and Miyata, Y. Effect of ofloxacin on experimental leprosy.

Ofloxacin, a synthetic quinolone and antibiotic with a broad antimicrobial spectrum, was examined for anti-*Mycobacterium leprae* activity in experimental leprosy using normal and nude mice.

In nude mice, ofloxacin treatment with a drug-containing diet at a concentration of a 0.025% for 100 days was slightly effective; a 0.05% or 0.075% drug-containing diet for 100 days showed significant suppression of the growth of *M. leprae*. In normal mice, however, a concentration of 0.075% drug-containing diet showed no effects. On the other hand, treatment of normal mice with 1 mg of ofloxacin 6 times a week for 100 days (equivalent to 0.025% in diet) showed significant suppression of *M. leprae* growth.—[Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan]

Dhople, A. M., Osborne, L. J., Seydel, J. K. and Schonenberger, H. Development of new chemotherapeutic agents in leprosy.

There is an urgent need for new chemotherapeutic agents in leprosy because a) multidrug-resistant strains of *Mycobacterium leprae* are now a real possibility, b) none of the existing drugs clear the "persistent" organisms, and c) the cost of first-line drugs such as rifampin and clofazimine is still too high for large-scale use in most of the endemic countries.

Drugs are usually screened for antimicrobial activity by exposing the target organisms to the drugs in a cell-free culture. Recently, we have succeeded in obtaining limited but definite *in vitro* multiplication of *M. leprae* in a cell-free medium using biochemical parameters (ATP, DNA and ^{3}H-thymidine) to measure growth. This system enables us to evaluate the action of drugs on *M. leprae*. Using the system, the minimal inhibitory concentration (MIC) of dapsone (DDS) was found to be 20–25 ng/ml and that of rifampin, 250–300 ng/ml.

In an effort to develop newer antileprosy agents, three classes of compounds were screened using the above system. The first one was dapsone derivatives. Replacing hydrogen from various positions in the benzene ring with other substituents did not yield any active compound. Only four derivatives gave 20%–40% inhibition in the growth of *M. leprae* when used at 100–200 ng/ml.

The second class of compounds was trimethoprim derivatives. Trimethoprim is a known powerful inhibitor of dihydrofolate reductase, but its activity is restricted to gram-negative bacteria only. Therefore, various derivatives of trimethoprim were synthesized in an effort to design a compound with inhibitory activity against dihydrofolate reductase of mycobacteria. Among the nine derivatives screened, only one (K-130) was as effective as dapsone against *M. leprae* with a MIC of 30 ng/ml; two other derivatives had a MIC of 100 ng/ml, while another two derivatives gave only 50% inhibition with 100 ng/ml. The activity of K-130 was equally good against a dapsone-resistant strain of *M. leprae*. Synergism was observed between dapsone and K-130; growth of *M. leprae* was totally inhibited with a combination of 5 ng/ml DDS and 20 ng/ml K-130. On the other hand, the other two derivatives with MICs of 100 ng/ml did not show any synergism with dapsone. K-130 was also shown to inhibit the growth of *M. leprae* in the foot pads of mice fed with the drug at a dose of 0.03%; in mice it proved to be bactericidal, since 9 months after terminating the treatment no growth of *M. leprae* was observed in the foot pads. Another trimethoprim derivative tested extensively was brodimoprim. In *in vitro* tests,

its MIC against *M. leprae* was 15 μg/ml, but in combination with 10 ng/ml DDS, 1 μg/ml of brodimoprim completely stopped the growth of *M. leprae*. In mouse foot pad experiments, 0.1% brodimoprim alone or 0.05% brodimoprim in combination with 0.0001% DDS completely inhibited the growth of *M. leprae*.

The third class of compounds screened was N-alkylbenzylamines. This is a class of compounds with a specific action against mycobacteria, particularly *M. tuberculosis*. Among the two compounds tested so far in this series, only one (#93) gave 70% inhibition of growth of *M. leprae* in an *in vitro* system at 10 μg/ml, while in the mouse foot pad system 50% inhibition was obtained when used at 0.1%. Further studies on these compounds are in progress.—[Medical Research Institute, Florida Institute of Technology, Melbourne, Florida 32901, U.S.A.; Borstel Research Institute, Borstel, Federal Republic of Germany; University of Regensberg, Regensberg, Federal Republic of Germany]

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Converse, P. J., Ottenhoff, T. H. M., Ehrenberg, J. P. and Kiessling, R. The repertoire of mycobacterial antigens recognized by peripheral blood cells and sera of healthy leprosy patient contacts.

Protective immunity against mycobacteria is dependent on antigen-specific T cells. Antibodies against mycobacteria are induced upon immunization but have little or no role in protecting the host against infection. The rational design of mycobacterial vaccines and skin-test reagents depends on the identification of antigens that are capable of inducing T-cell responsiveness. Serological techniques, using monoclonal or polyclonal antibodies to screen crude or purified mycobacterial preparations and recombinant DNA libraries have detected some antigens that are also recognized by human T cells but may fail to recognize others. Potentially, there may be differences in the epitopes seen by the T-cell and B-cell anti-mycobacterial antigen repertoires.

We have adopted a new screening technique by which the different components of sonicated *Mycobacterium leprae* or BCG were separated according to their molecular weight (mw) by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then electroblotted on nitrocellulose paper. Blots were cut into horizontal sections, sterilized, cut further into 4 × 4 mm squares, and tested directly in proliferation assays. Our results indicate that peripheral T cells of healthy leprosy patient contacts respond preferentially to the lower mw (<70 kD) and not to the higher mw fractions of BCG and *M. leprae*, in contrast to the humoral response of these same individuals. The most important fractions in inducing a lymphoproliferative response were in the regions of 11–16 kD of both BCG and *M. leprae* and to the 22–26 kD region of *M. leprae*. No antibodies could be detected in these components of BCG in these contacts. These fractions appeared to represent mw regions that were, in some instances, clearly distinct from previously defined antigens. The data also supported earlier studies on the relevance of an antigen in the 18 kD region in responses by individuals immunized with *M. leprae*. It was further shown that lymphoproliferation in response to mycobacterial fractions correlated with the production of interferon, a lymphokine required for macrophage activation and elimination of mycobacteria.

These studies allow the direct assessment of antigens involved in protective T-cell immunity, and should be helpful in selecting relevant antigens for the design of skin-test reagents as well as vaccines for the prevention of mycobacterial diseases.—[Armauer Hansen Research Institute, Addis Ababa, Ethiopia]

Ottenhoff, T. H. M. and de Vries, R. R. P. An HLA-DR3 immune response gene for *Mycobacterium leprae* predisposes to tuberculoid leprosy.

Antigen-specific immune responses are controlled by polymorphic immune response (Ir) genes. In experimental animals, the majority of these Ir genes has been

mapped to the major histocompatibility complex (MHC). MHC class II Ir genes have been found to control immune reactivity against T-cell-dependent foreign antigens. The products of these MHC class II Ir genes are the MHC class II molecules. These molecules have been found to restrict and to regulate T-cell activation.

It has been recognized that the HLA class II region contains similar, polymorphic antigen-specific Ir and Is (immune suppression) genes and products. It is generally assumed that at least several of the known associations between HLA class II alleles and certain diseases may be a consequence of such HLA class II Ir/Is genes. The polymorphism of these Ir/Is genes and, consequently, their products would lead to genetically controlled interindividual variability in immune responsiveness, and thus in susceptibility or resistance to disease. The unraveling of this chain of events not only is important for the theoretical understanding of the mechanisms of HLA-disease associations, but may also be practically relevant and lead to preventive and therapeutical applications.

One important example of HLA class II Ir genes is provided by leprosy, the chronic infectious disease caused by *Mycobacterium leprae*. HLA class II-linked Ir genes most probably control the type of leprosy which develops upon infection as well as the type of cell-mediated immune reactivity against *M. leprae* and other mycobacteria in skin tests. Since both leprosy type and skin test responsiveness strongly correlate with helper T-cell responsiveness against *M. leprae*, it is likely that the HLA class II-linked Ir genes actually control these antigen-specific T-cell responses. Since HLA class II molecules as mentioned above restrict and regulate antigen presentation to helper T cells, one would expect that these HLA class II Ir genes code for class II molecules which are involved in the differential regulation of *M. leprae*-specific helper T-cell responsiveness.

In this paper, we present our studies carried out in a Surinam population where HLA-DR3 is associated with the TT but not the BL-LL type of leprosy. Thus, a DR3-associated immune response gene may predispose to TT leprosy by regulating Th-cell activity against *M. leprae*. We report here a differential role for DR3 versus other DR (non3) products in Th-cell responsiveness against *M. leprae* and related antigens, both in TT patients and in healthy individuals. The results show that: a) DR3 tended to protect against nonresponsiveness against mycobacterial antigens in healthy individuals (N = 99) as measured by lymphocyte transformation tests ($p = 0.05$); b) Th-cell responses of *M. leprae*-induced Th-cell lines (TCL_{lep}) of healthy controls as well as TT patients were mainly HLA-DR restricted [studied in respectively 148 ($p = 3 \times 10^{-4}$) and 197 ($p = 10^{-6}$) allogeneic Th cell-APC (antigen-presenting cell) combinations]; c) DR3 was associated with high Th-cell responsiveness when in DR3/non3 heterozygous healthy controls (N = 8) the DR3-restricted responses of TCL_{lep} were compared with the DRnon3-restricted ones in, respectively, 54 and 33 allogeneic Th cell-APC combinations ($p = 6 \times 10^{-3}$). In contrast, DR3 was associated with low Th-cell responsiveness in DR3/non3 heterozygous TT patients (N = 7) when similar (respectively, 51 and 40) Th cell-APC combinations were studied ($p = 10^{-5}$); d) DR3 was associated with high Th-cell responsiveness against *M. tuberculosis* (PPD) in the same TT patients (N = 3) as well as in healthy controls (N = 3), studied in, respectively, 26 and 36 combinations (respectively, $p = 0.015$ and $p = 0.020$); e) the DR3-associated low TCL_{lep} responsiveness in TT patients is induced by *M. leprae*-specific antigens but, once induced, is directed against common mycobacterial antigens. The DR3-associated high responsiveness of TCL_{lep} from patients and of TCL_{lep} from healthy individuals similarly is directed against common antigens on mycobacteria; f) DR3-restricted *M. leprae*-reactive cloned T cells from TT patients were found to recognize other antigenic determinants on the bacillus and seemed to be suppressed more often and more strongly by autologous T cells than the DRnon3-restricted clones. Such a haplotype-related suppression might account for the DR3-associated low responsiveness of TCL_{lep} in TT leprosy.

This study has enabled us to identify DR3 as the product of a *M. leprae*-specific DR3-coded Ir gene, and to study the role of DR3 in the regulation of the immune response

against *M. leprae*. Thus, this study may provide a model for the unraveling of the mechanism of other HLA class II-disease associations.—[Department of Immunohematology and Bloodbank, University Hospital, Leiden, The Netherlands]

Mshana, R. N., Krahenbuhl, J. L. and Hastings, R. C. Interferon-gamma induces the expression of major histocompatibility complex antigens by *in vitro* cultured murine Schwann cells.

Immunopathological mechanisms involved in the pathogenesis of peripheral neuropathies are as yet unknown. In leprosy, peripheral neuropathy is the most important consequence of the disease. Cell-mediated immune reactions to intraneurally located *Mycobacterium leprae* antigens are thought to be pathogenetically related to the neuropathy seen in borderline leprosy patients; whereas the pathogenesis of lepromatous leprosy neuropathy is unclear.

In our previous studies, we reported that in the nerve, leprosy neuropathy is associated with expression of major histocompatibility complex (MHC) antigens on cells morphologically indistinguishable from Schwann cells. Similar findings have recently been reported in nerve biopsies from patients with chronic demyelinating polyradiculoneuropathy. These observations suggest that Schwann cells could be recruited, by expressing MHC antigens, to directly participate in an immune reaction rather than merely acting as innocent bystander cells. The present study was designed to further evaluate induction of Ia antigen expression on *in vitro*-cultured Schwann cells.

Dorsal root ganglia were dissected from 1–2-day-old mice, digested with trypsin, and the cells cultured in a medium containing 0.69% glucose for 24 hr. The medium was then replaced with fresh medium containing 10 M cytosine arabinocide-C for 72 hr. Cells were typsinized and treated with monoclonal IgM anti-Thy 1.1 and normal rabbit serum to complement lyse fibroblasts. The viable cells were collected and cultured in the presence of 10–50 ng cholera toxin. Seventy-two hr before induction experiments, the cells were washed and the medium replaced with one without cholera toxin. Astrocytes and peritoneal cells were obtained and cultured using conventional methods.

Cultured cells were treated with various concentrations of either crude lymphokine (derived from rat spleen cells stimulated with ConA) or recombinant interferon-γ. MHC antigen expression was assessed by indirect immunofluorescence after incubation with appropriate monoclonal antibodies and development with fluoescinated goat anti-mouse IgG.

Our results show that interferon-γ can induce MHC class I antigens on virtually all astrocytes and Schwann cells after an incubation period of at least 48 hr. MHC class II (Ia) antigens were induced on only 10%–15% of the Schwann cells or astrocytes, while almost all peritoneal cells were able to express Ia antigens under similar conditions. Cells were judged to be Schwann cells by morphological appearance, lack of Fc receptor, lack of fibronectin, and the presence of S-100 protein. Astrocytes were identified by glial fibrillary acidic protein (GFAP) staining. The proportion of Ia-expressing Schwann cells did not increase with prolonged incubation or incubation with higher concentrations of interferon-γ.

The role of Ia-expressing Schwann cells in the pathogenesis of peripheral neuropathies is unclear at the moment, but our studies offer a way of probing mechanisms involved in intraneural antigen presentation. Current studies are focused on further characterizing the Ia-expressing Schwann cells and their capacity to present *M. leprae* antigens to T lymphocytes or to act as targets for cell-mediated cytotoxicity. Because of the limited numbers of primary Schwann cells, the possibility of using Schwannoma cell lines is under investigation.—[Laboratory Research Branch, GWL Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Draper, P. Mycobacterial wall lipopolysaccharide: aspects of organization and structure.

The detailed structure of the wall lipopolysaccharide (mycoloyl arabinogalactan) of mycobacteria was worked out two decades ago by French and Japanese scientists. More recently, Belgian workers compared

the wall polysaccharides of several mycobacteria, nocardias and corynebacteria and confirmed the sequence of the mycobacterial arabinogalactan. However, some important information about this otherwise well-recognized structure remains to be obtained. Little is known of: a) its biosynthesis, b) its physical arrangement in living mycobacteria, or c) whether there is a specialized "linker group" attaching the polysaccharide to the peptidoglycan of the wall (as is common in other bacteria). Some ideas and experiments bearing on points b) and c) are discussed. Electron microscopic evidence for an extended (probably hydrated) configuration of the lipopolysaccharide in the living mycobacterium is presented.—[National Institute for Medical Research, London, England]

Brennan, P. J., Hunter, S. W., McNeil, M., Stewart, C., Bloom, B. R., Melancon-Kaplan, J., Modlin, R. L. and Rea, T. H. The cell wall of *M. leprae*; isolation, composition, and immunogenicity.

Anatomically distinct cell walls of *Mycobacterium leprae* or large fragments of cell walls were isolated by a variety of procedures, involving disruption, differential centrifugation and sucrose gradient centrifugation, or differential solvent extraction. Examination of these showed the expected presence of mycolates, arabinogalactan and phosphate, all covalently bound to peptidoglycan. However, the unexpected presence of large quantities of proteinaceous amino acids proved to be of the most significance. Only a small proportion of the cell-wall proteins could be extracted from the basic cell wall using a variety of procedures; the majority were tenaciously associated with the cell-wall core. Sequential removal of mycolates and arabinogalactan did not dislodge this protein from peptidoglycan. The resulting cell-wall protein-peptidoglycan complex (CW-PPC) contained significant immunological reactivity. For instance, lymphocytes from tuberculoid patients and contacts responded as well to CW-PPC as they did to the intact bacillus. Also, guinea pigs previously sensitized to intact *M. leprae*, when later tested with CW-PPC, developed a distinct delayed-type hypersensitivity reaction. Throughout its fractionation, the cell-wall protein-containing fraction displayed highly unusual physico-chemical properties in that it was consistently of large molecular weight and insoluble in most aqueous buffers. Clearly, the cell wall of *M. leprae* and other mycobacteria represents an important source of highly immunoreactive proteins.—[Colorado State University, Fort Collins, Colorado 80523; Albert Einstein College of Medicine, Bronx, New York 10461; University of Southern California School of Medicine, Los Angeles, California 90033, U.S.A.]

Krahenbuhl, J. L., Sibley, L. D. and Chae, G. T. Defective macrophage effector function in lepromatous leprosy.

In the nu/nu mouse foot pad, growth of *Mycobacterium leprae* is virtually unchecked, yielding enormous numbers of organisms and producing granulomas resembling those of lepromatous leprosy. To address the hypothesis that the intracellular presence of live *M. leprae* or *M. leprae* constituents compromises the functional capacity of the involved (parasitized) macrophages, we have previously characterized heavily infected foot pad macrophages from nu/nu mice and tested them to determine if they were capable of being activated by lymphokines. Lepromatous foot pad granulomas were excised from *M. leprae*-infected nu/nu mice, dispersed with enzymes, and the cells characterized. Cells gorged with *M. leprae* predominated after centrifugation through Nycodenz and appeared to be macrophages by virtue of their capacity for adherence and phagocytosis, positive staining for esterase, and possession of Fc and C3bi receptors. Upon phagocytosis of *Candida*, these *M. leprae*-burdened cells appeared to reduce NBT and, like peritoneal macrophages, they also supported the intracellular growth of *Toxoplasma gondii*. However, doses of macrophage-activating factor or murine recombinant interferon-gamma (Mu rIFN-γ) which readily activated peritoneal macrophages from nu/+ or nu/nu mice to kill or inhibit the obligate intracellular protozoan *Toxoplasma gondii* failed to activate *M. leprae*-burdened foot pad macrophages.

Further studies have been carried out to explore defective effector function of gran-

uloma macrophages from nu/nu mice. Foot pad macrophages were also refractory to rIFN-γ induced activation as measured by cytotoxicity for EL-4 tumor target cells. Thus, both the microbicidal and cytotoxic effector functions are compromised in the lepromatous macrophages. Although our previous characterization of these foot pad granuloma cells included evidence that they were capable of producing superoxide as measured by reduction of NBT to formazan granules around phagocytized *Candida*, precise quantitation of superoxide generation by measurement of cytochrome C reduction in response to phorbol myristate acetate (PMA) revealed no base-line production of superoxide anion. Moreover, even after prolonged high doses of rIFM-γ, we observed no enhanced generation of this oxidative metabolite. Defects in cytotoxicity and oxidative metabolism appeared to be restricted to the local foot pad since we did not observe defective response to rIFM-γ in peritoneal macrophages from these same nu/nu mice. Similarly, although lepromatous foot pad granuloma macrophages produced large amounts of the immunoregulating arachidonic acid metabolite PGE2, nu/nu peritoneal macrophages did not. Incorporation of indomethacin into the media for as long as 72 hr did not reverse the failure of these cells to become activated by rIFN-γ as measured by anti-*Toxoplasma* activity, cytotoxic activity for tumor target cells, or production of superoxide anion production. Thus, production of PGE2 may not be the sole mechanism underlying defective effector function by lepromatous macrophages.

In an attempt to determine whether other cytokines might be effective, *M. leprae*-infected foot pad macrophages were treated with various doses of recombinant tumor necrosis factor (TNF) alone or in combination with IFN. No evidence of activation was observed regardless of the lymphokine/cytokine combination.

Preliminary studies have been carried out to determine the effects of rifampin chemotherapy of infected nu/nu mice on the responsiveness of foot pad granuloma macrophages to rIFN-γ. Our intention was to determine whether foot pad macrophages could respond to lymphokines when gorged with killed bacilli or constituents of *M. leprae* that remain after clearance has begun. Initial results suggest that 14 weeks after initiation of treatment, the foot pad macrophages are at least partially responsive to IFN as measured by challenge with *Toxoplasma*. Studies are continuing in this model to monitor the response to lymphokines of foot pad macrophages and the recruitment and turnover (or local multiplication) of macrophages in the foot pads of *M. leprae*-infected nu/nu mice treated with rifampin or Mu rIFN-γ. Results of these latest studies are pending.—[Immunology Department, Laboratory Research Branch, GWL Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Acknowledgment. This work was supported by grant no. AI 22442 from the National Institute of Allergy and Infectious Diseases.

Sibley, L. D., Ramasesh, N., Franzblau, S. G. and Krahenbuhl, J. L. Functional responses of normal and activated macrophages infected with *Mycobacterium leprae in vitro*.

Activated macrophages demonstrate nonspecific microbicidal capacity for a number of phylogenetically different microorganisms. To explore the hypothesis that activated macrophages may inhibit or kill *Mycobacterium leprae*, we have used both *in vivo*-activated macrophages from mice given *Corynebacterium parvum* or infected with *Toxoplasma* and macrophages activated *in vitro* using recombinant murine gamma interferon (IFN-γ). When pre-activated, mouse macrophages showed considerable capacity to inhibit the synthesis of phenolic glycolipid-I and greatly reduced the content of ATP recovered from intracellular *M. leprae*. Additionally, activated macrophages showed considerable enhancement of lysosome fusion and capacity to digest viable suspensions of *M. leprae* which resisted digestion in normal macrophages.

In contrast, *M. leprae*-burdened macrophages obtained from *nu/nu* mouse granulomas lack the capacity to respond to macrophage-activating signals such as IFN-γ. This defection in macrophage activation is evident as a reduced capacity for toxoplasmacidal activity, tumoricidal activity, O_2^- production, and Ia expression following

treatments of IFN-γ that readily activate normal peritoneal macrophages. To further explore the effector functions of *M. leprae*-induced macrophages, we have examined the early development of reduced activation potential in mouse peritoneal macrophages infected *in vitro*. Defective macrophage activation was dependent on viability of the *M. leprae* inoculum, the burden of intracellular bacilli, and duration of *in vitro* culture. Thus, if IFN-γ activation was initiated prior to or simultaneously with *M. leprae* infection, macrophages were fully activated. The development of defective activation was closely paralleled by macrophage production of prostaglandin E_2 (PGE_2) that reached a peak level 2–3 days after infection with *M. leprae*. Defective IFN-γ activation was reversible by indomethacin and a similar block of IFN-γ activation was produced in normal macrophages exposed to exogenous PGE_2. Thus, infection with *M. leprae* appears to partially restrict macrophage activation by early induction of PGE_2. Elevated production of PGE_2 is also characteristic of granuloma macrophages from *M. leprae*-infected nu/nu mice, and may be related to their lack of IFN-γ responsiveness. Additionally, we have measured high levels of PGE_2 produced *in vitro* by biopsies from lepromatous leprosy patients, suggesting that a similar suppression of IFN-γ activity may occur in humans. The inability of macrophages to dispose of *M. leprae* is a conspicuous feature of lepromatous leprosy, and may be related to reduced macrophage responsiveness to immune mediators, including IFN-γ. These studies suggest that recruitment of pre-activated macrophages to the tissue lesions may ultimately be necessary for the killing and clearance of *M. leprae*.—[Immunology Research Department, GWL Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.]

Holzer, T. J., Arnold, J. J., Vachula, M. and Andersen, B. R. Phenolic glycolipid-I of *Mycobacterium leprae* induces altered monocyte oxidative responses *in vitro*.

Phthiocerol-containing lipids produced by *Mycobacterium leprae* are found in infected tissues in quantities approaching twice the weight of the organism itself. These lipids appear to exist as both constituents of the bacterial capsule and as extracellular free "droplets." It is clear that the phenolic glycolipid-I (PGL-I) and the corresponding diacylphthiocerol are present in large quantities in human lepromas and are detectable in patient sera. Several recent studies have suggested a possible role for PGL-I in the suppression of the concanavalin-A-induced mitogenic responses of lymphocytes from leprosy patients. The mononuclear phagocyte in lepromatous disease is known to ingest *M. leprae* but fails to kill the organism and, in fact, *M. leprae* proliferates within these cells. Whether PGL-I plays a role in protecting *M. leprae* from macrophage bactericidal mechanisms has not been addressed to our knowledge.

Studies were performed to determine if normal, human peripheral blood monocytes could incorporate PGL-I and if exposure to the lipid would alter oxidative responses to particulate and soluble stimuli. PGL-I was prepared as lipid micelles by drying stock solutions of PGL-I in $CHCl_3$:CH_3OH (2:1) under N_2, adding Hanks' balanced saline solution (HBSS) and sonicating the preparation. Peripheral blood monocytes were enriched from Ficoll-Hypaque isolated mononuclear cells by adherence to plastic or by elutriation centrifugation. Suspensions of PGL-I micelles (100 μg) were added to 10^6 monocytes with either HBSS, 30% normal or lepromatous patient sera in HBSS, or monoclonal anti-PGL-I antibody. The mixtures were gently mixed at 37°C for 3 hr in teflon vials. The cells were extensively washed by centrifugation, and the supernatants and cells lyophilized overnight. The dried samples were extracted with $CHCl_3$:CH_3OH, and processed for immunodot quantitation by the method of Cho (J. Infect. Dis. **153**:560, 1986). In six experiments, PGL-I incorporation ranged from 1%–20% of the 100 μg. Maximal incorporation occurred in the presence of either 30% normal or patient sera. Cell damage as determined by trypan blue uptake did not appear to differ from the non-lipid-exposed control cells.

To determine if PGL-I incorporation influenced monocyte killing mechanisms, we measured the superoxide anion (O_2^-) generating capacity of lipid-treated monocytes

stimulated with zymosan (Z; 50 particles/cell) or phorbol myristate acetate (PMA; 1 μg/ml). O_2^- was quantitated using cytochrome c reduction measured in an automated ELISA reader. In experiments using 8 different monocyte donors, 7 of the 8 experiments demonstrated increased PMA-stimulated O_2^- release from the PGL-I-treated cells as compared to nonlipid-treated control cells. When serum-treated zymosan was used as a particulate stimuli, cells from five donors exhibited decreased O_2^- release, while cells from three donors released slightly more O_2^- than did nonlipid exposed cells. The PMA-stimulated O_2^- release differed moderately, but consistently, from the control cells, while the zymosan-stimulated O_2^- release was more heterogeneous and differed only slightly from the control cells. It appears the PGL-I, when incubated with pheripheral blood monocytes, is incorporated at significant levels into some or all of the cells and that the oxidative responses of PGL-I-treated monocytes are altered. Studies are in progress to determine if PGL-I is incorporated into all or some of the monocytes exposed to the micelles, and if PGL-I and the related diacylphthiocerol also influence other mononuclear phagocyte functions.

These studies demonstrate that extracellular *M. leprae*-derived PGL-I can be spontaneously incorporated into phagocytic cells and that these lipid-treated cells exhibit altered oxidative responses to both soluble and particulate stimuli. This may be an important factor in protecting *M. leprae* from macrophage bactericidal mechanisms and may play a role in altering macrophage/lymphocyte collaboration.—[University of Illinois at Chicago, Department of Medicine, and Westside VA Medical Center, Chicago, Illinois 60612, U.S.A.]

Cohn, Z. A. and Kaplan, G. The role of T6+ Langerhans' cells in the cutaneous immune response in leprosy.

See Abstract in Int. J. Lepr. **55** (1987) 748.

Kaplan, G. and Cohn, Z. A. The role of IFN-γ in the regulation of DTH in human skin.

The aim of our studies is to examine the role of gamma interferon (IFN-γ) in the regulation of cell-mediated immunity to *Mycobacterium leprae* and *M. leprae* destruction in the lesions of leprosy patients. For this purpose we have administered IFN-γ into the skin of patients with lepromatous leprosy and evaluated the local response 24 hr to 3 weeks after IFN-γ administration. In many ways the response to this recombinant lymphokine (rIFN-γ) resembles that seen with a delayed-type hypersensitivity (DTH) reaction to PPD. This includes the proliferation of epidermal keratinocytes, their expression of IA antigen, thickening of the epidermis, and upward migration of T6+ epidermal Langerhans' cells. In addition, mononuclear leukocytes migrate into the injection site. In contrast to the PPD reaction, IFN-γ injection (up to three injections of 1–20 μg) fails to result in the accumulation of T6+ Langerhans' cells in the dermis in association with helper T cells. The absence of these potent T-lymphocyte-directed accessory cells may indicate lack of T-lymphocyte activation in the dermis.

In a recent study carried out in this laboratory, IFN-γ was shown to rapidly induce the expression of a new group of genes in macrophages, fibroblasts, and endothelial cells. One of these genes has been isolated and its DNA sequenced. The polypeptide deduced from the nucleic acid sequence (γIP-10) has been synthesized and an antiserum raised against this peptide. We have used this antiserum to demonstrate the expression of the γIP-10 peptide in the recombinant IFN-γ-injected lesions. By 18 hr after the injection of rIFN-γ into the dermis of lepromatous patients, cells in the basal layer of the epidermis showed clear immunohistological staining for the induced peptide. Identification of IFN-γ-induced peptides in inflammatory lesions provides us with a direct way of demonstrating the local production and release of IFN-γ.

We have shown that the epidermal and inflammatory cells of tuberculoid but not lepromatous lesions express the IFN-γ-induced peptide. This observation is compatible with our hypothesis that IFN is released locally in tuberculoid leprosy lesions but not in lepromatous leprosy lesions. When PPD responses were examined, we

observed that the IFN-γ-induced peptide was expressed by 24 hr in some of the basal cells of the epidermis. At 48 hr, all of the basal cells of the epidermis and many of the mononuclear leukocytes and endothelial cells in the dermal accumulation were positive for γIP-10. γIP-10 expression was observed for up to 2 weeks in these lesions. The local production of IFN-γ in the site of DTH reaction to PPD in the lesions of lepromatous leprosy patients corresponds to the local destruction of foamy bacilli-laden macrophages.—[The Rockefeller University, New York, New York 10021, U.S.A.]

Rea, T. H. and Modlin, R. L. Characterization of CD4-positive T-lymphocyte subsets in leprosy granulomas.

To further investigate immunoregulation in leprosy granulomas, we have utilized immunoperoxidase techniques and double-staining with monoclonal antibodies 2H4 and Leu3 (CD4) to identify the T-suppressor/inducer (CD4+ 2H4+) (Morimoto, J. Immunol. **137**:3247, 1986) and T-helper/inducer (CD4+ 2H4−) subsets in 10 lepromatous (LL and BL) and 8 tuberculoid (BT) lesions.

Five percent of the cells in both BT and LL granulomas double-stained as CD4+ suppressor/inducer cells, but the percentage of CD4+ 2H4− helper/inducer cells was four times greater in tuberculoid than in lepromatous lesions. The helper/inducer: suppressor/inducer ratio was 14:1 in BT and 1.1:1 in LL lesions, while the peripheral blood ratio was 1.2:1 and 1.9:1, respectively. In distribution, the suppressor/inducers were admixed with the macrophages in LL, but were restricted to the lymphocytic mantle in BT granulomas.

The specific cell-mediated immune unresponsiveness in LL may result in part from the interaction of the T-suppressor/inducer cells with the T-suppressor/cytotoxic (CD8+) cells. The higher, 14:1, helper/inducer: suppressor/inducer ratio in BT lesions, and the lower, 1.2:1, in blood indicate some selective entry, selective retention or proliferation of the CD4+ T-cell subsets in tissues. The restriction of both suppressor/inducers and suppressor/cytotoxic phenotypes to the lymphocytic mantle is further evidence of precisely regulated, immunologically active, micro-environments in tuberculoid granulomas.—[Department of Dermatology, LAC/USC Medical Center, Los Angeles, California 90033, U.S.A.]

Mori, T. Acid-fast bacilli detected in umbilical cord and skin specimens.

Twenty years ago, I detected many acid-fast bacilli (AFB) in the abdominal skin of a uterine carcinoma patient, but the bacilli did not grow on the whole egg 3% Ogawa medium. Many AFB were detected in this specimen without any cell inflammation. A bacterial suspension antigen was made from the isolated AFB. The Mitsuda reaction was compared to Dharmendra antigen in lepromatous and tuberculoid leprosy patients and in healthy controls. The antigen of the isolated bacilli was negative in the tuberculoid leprosy patients despite strong positive reaction to Dharmendra antigen. Since I speculated that this unknown bacillus might introduce a kind of tolerance, it was pursued as a pseudo-leprosy bacillus for 20 years.

Method. All glassware used for acid-fast staining was cleaned with potassium perchromate : sulfuric acid and washed in (millipore) filtered distilled water. Surgical skin and umbilical cord specimens from Caesarian operations were cut into small pieces, ground in a mortar, and dried completely; the drying process is important for the mucous umbilical cord. The dried material was emulsified with a small quantity of distilled water and then filtered through sterile absorbent cotton. The filtrate was centrifuged for 30 min at 10,000 rpm. The pellet was homogenized with a small volume of distilled water. Tissue fragments were removed by filtering through sterile absorbent cotton. The filtrate was neutralized with sterile M/10 phosphate buffer, pH 6.6, and centrifuged for 30 min at 10,000 rpm. The pellet was used for the detection of AFB.

Results. AFB were detected in the smear preparations of 13 (27%) out of 49 skin specimens. Three specimens were smear-positive; cultivation was negative. AFB were detected in the smear preparations of 13 (15%) out of 86 umbilical cord specimens;

cultivation was possible in 8 (9%) out of 86 cases. Five specimens were smear-positive with negative cultivation. Of the AFB isolated, 4 were *Mycobacterium scrofulaceum*, 1 was *M. intracellulare*, and 15 were unidentified but had the following characteristics: strongly positive niacin reaction, growth at 45°C, and urease positive. In one case, 8×10^5 AFB were detected in 10 g of umbilical cord; the baby was born having 2.8×10^8 AFB.

Discussion. Clearly, there has been transplacental transmission of AFB; the mechanism of such transmission, however, is not clear. The recipient, who carries the AFB throughout life, may have low cell-mediated immunity, predisposing to leprosy and atypical mycobacteriosis.—[National Leprosarium Tama Zensho-en, Tokyo, Japan]

Nakamura, K. and Yogi, Y. *M. leprae* susceptibility of NOD hybrid nude mice.

We have successfully established an animal model for experimental lepromatous leprosy by using NFS/N nude mice which gave excellent results with the development of marked lepromatoid lesions. The formation of the lepromatoid lesion in nude [NFS/N and N:NIH(s)] mice was influenced by the genetic background, resembling the case of nude (SHR) rats previously reported by us.

In this report, we compared the susceptibility of NOD hybrid nude mice with that of "resistant" CD-1 nude mice.

Non-obese diabetic (NOD) mice were selected from Jc1:ICR mice obtained from CLEA, Tokyo. We have established the NOD × CD-1 (ICR) hybrid nude mice (N1) by mating the CD-1 male nude mice and NOD female mice. Thirteen NOD hybrid nude mice and 15 CD-1 nude mice 6–9 weeks old were used. The inoculum size was 6×10^5 bacilli derived from foot passage of nude mice. The site of injection was the right dorsal aspect of the hind foot. Mice were maintained in a vinyl isolator under specific pathogen-free (SPF) conditions.

At 389 days after infection, there were marked nodular lesions in the infected foot, developing to the lower leg and toes, of NOD hybrid nude mice. In comparison, the CD-1 nude mice showed lesions with a slight swelling at the infected site. Severe lepromatoid lesions with ulceration in the infected site of NOD hybrid nude mice were observed at 425 days after infection. At that time, bacillary counts were over 10^{11} bacilli per g with ulcerating tissues. The foot (including swollen lower leg and toes) weight of the NOD hybrid nude mice was 1.5–2.0 g. In contrast, the foot weight of the CD-1 nude mice was 0.25–0.35 g.

We have confirmed that the CD-1 nude mice were "resistant" compared with the NOD hybrid nude mouse. The acid-fast bacilli (AFB) obtained from swollen, abscess- and ulcer-forming tissue have bacteriologically been distinguished from other mycobacteria: reinoculation test in normal (ICR) mice confirmed persistent infection at the site of injection only, loss of acid-fastness by pyridine extraction, and lack of growth when incubated on modified Nemoto's egg-yolk medium and 1% Ogawa's medium at 33°C and 37°C for 3 months. In addition, a section of the infected foot of a NOD hybrid nude mouse without insulitis, when stained with Fite-Faraco and hematoxylin and eosin, indicated a severe lepromatoid lesion and a large number of lepra cells, respectively. A section of bone marrow from the infected foot contained a large number of bacilli and globi. Thus, the AFB obtained from the lesions could not be distinguished from *Mycobacterium leprae*. Therefore, we have established the NOD hybrid nude mouse as a new model for experimental lepromatous leprosy as well as the NFS/N and N:NIH(s) nude mice, resembling the previously reported cases of Swiss mice.

NOD mice originated from Swiss-Webster mice (Jc1:ICR outbred strain) and NFS/N and N:NIH(s) mice originated from Swiss mice. Moreover, the genetic variance of the laboratory outbred Swiss mice was reported. The original colony of outbred Swiss mice, brought from Lausanne, Switzerland, in 1926 by Clara Lynch, was dispersed as Swiss and Swiss-Webster mice to researchers and commercial dealers. Litter sizes of NOD hybrid nude mice are characterized as, and similar to, ICR and CD-1 nude mice without diabetes. The development of a heavy lepromatoid formation in the NOD hybrid nude mice (N1) may be

produced by the genetic background of the Swiss and Swiss-Webster colony (except for ICR and CD-1 nude mice). The same may be true for SHR hybrid nude rats originating from the Wistar colony which differ from "resistant" WKY and WM nude rats in experimental lepromatous leprosy. Therefore, pathogenesis of severe lepromatoid lesions may be influenced by a combination of genes, such as those associated with insulin-dependent diabetes and hypertension.

In summary, we have established the NOD hybrid nude mouse (NOD × CD-1 nude mice) at the N1 generation as a new model for experimental lepromatous leprosy.—[National Institute for Leprosy Research, Tokyo, Japan]

Portaels, F., Walsh, G. P., DeRidder, K., Malaty, R., Silva, M. T., Binford, C. H. and Meyers, W. M. Cultivable mycobacteria isolated from 32 newly captured armadillos (*Dasypus novemcinctus*) from Louisiana.

The isolation of several cultivable mycobacterial strains (MAIS, *Mycobacterium gordonae*, ADM) from armadillos experimentally infected with *M. leprae* was recently reported. To determine the origin and significance of cultivable mycobacteria in these animals, we examined the tissues of 32 armadillos captured from three locations in southwest Louisiana. Three of the 32 animals had naturally acquired leprosy as confirmed by histopathology and multiplication in the mouse foot pad. Mycobacteria were cultivated from all tissues studied (liver, spleen, lymph nodes) from leprosy-infected animals and from the inguinal lymph nodes of all nonleprous animals. Ninety-six percent of the mesenteric lymph nodes, 4% of the livers, and 31% of the spleens from nonleprous armadillos contained cultivable mycobacteria. Tissues from leprosy-infected armadillos always contained greater numbers of cultivable mycobacteria than tissues from nonleprosy-infected armadillos.

The most frequently isolated species from both leprosy-infected and nonleprosy-infected animals belonged to the MAIS complex. *M. gordonae* and *M. simiae* were frequently isolated from both leprosy- and nonleprosy-infected armadillos. Some species (*M. terrae, M. fortuitum, M. malmoense, M. chelonei, M. marinum*) were isolated only from tissues from nonleprous armadillos. Most of these mycobacterial species are present in the environment, and do not appear to be responsible for established infections in wild armadillos. Interestingly, the MAIS complex was isolated from all of the tissues in the three animals with leprosy, suggesting that, in addition to infection with *M. leprae*, these animals were probably infected with MAIS strains. Mycobacteria belonging to new groups of armadillo-derived mycobacteria (ADM) were isolated from five nonleprous samples and four samples from leprous armadillos. This confirms that ADM strains similar to those isolated from experimentally infected armadillos were also present in wild armadillos. Environmental samples from Florida and Louisiana, collected in the natural biotope of armadillos, have produced more than 600 mycobacterial strains but no ADM strains. This does not prove that these ADM are not in the environment; these species, like *M. simiae* or *M. malmoense*, may be present in nature in very low numbers.

The isolation of more mycobacteria from *M. leprae*-infected tissues than from noninfected tissues suggests that infection by *M. leprae* favors the multiplication of some cultivable mycobacteria. An alternate explanation is that a defect in host defenses related to susceptibility to leprosy also favors colonization by other mycobacteria. The wide variety of mycobacteria demonstrated in armadillo tissues indicates that appropriate measures must be taken to detect cultivable mycobacteria in tissues of armadillos experimentally infected with *M. leprae*. Specific chemical markers, monoclonal antibodies, and other techniques should be used in conjunction with cultivation to ensure that *M. leprae* preparations are free of other mycobacterial species.—[Institute of Tropical Medicine, Antwerp, Belgium; Armed Forces Institute of Pathology, Washington, D.C. and Louisiana State University Eye Center, New Orleans, Louisiana, U.S.A.]

Clark-Curtiss, J. E., Walsh, G. P. and Portaels, F. Use of recombinant DNA mol-

ecules in epidemiological studies of leprosy.

Inability to grow *Mycobacterium leprae* on conventional bacteriological media has greatly hampered our understanding of the physiological capabilities of this organism. Classification of an organism as *M. leprae* is usually based on isolation of the organism from characteristic lesions of patients, the capacity of the bacterium to be acid-fast stained, and the inability of the organism to be cultivated on conventional mycobacterial media. Moreover, there is presently no means by which isolates from leprosy patients in different parts of the world can be differentiated.

We have used the technique of restriction fragment length polymorphism (RFLP) analysis to establish specific patterns for chromosomal DNA of *M. leprae* from two different sources of human biopsy material, from a naturally infected armadillo and from a naturally infected mangabey monkey. Chromosomal DNA from eight cultivable mycobacteria, including *M. bovis* BCG, "*M. lufu*," *M. tuberculosis* H37Rv, and *M. vaccae*, were also tested. The probes used in these experiments were nine recombinant molecules from an *M. leprae* genomic library chosen at random, a recombinant molecule that specifies citrate synthase and another that specifies an enzyme involved in purine metabolism. A fragment containing the rRNA operon from *M. smegmatis* was also used as a probe.

Eight of the probes hybridized only to *M. leprae* DNA; the citrate synthase-containing fragment, the rRNA operon probe and one random probe were slightly homologous to chromosomal DNA fragments of the cultivable mycobacteria, but in all cases, the probes hybridized to different sized fragments of the cultivable mycobacterial chromosomes than they did to *M. leprae* DNA fragments. No polymorphisms were detected among the chromosomes of *M. leprae* from human leprosy patients, from the naturally infected armadillo, or from the naturally infected mangabey monkey. One probe, pYA1065, hybridized to multiple fragments of chromosomal DNA from *M. leprae*. Since this probe did not hybridize to any DNA except *M. leprae*, it appears to be a candidate for development as a diagnostic reagent.—[Washington University, St. Louis, Missouri; Armed Forces Institute of Pathology, Washington, D.C., U.S.A.; Institute of Tropical Medicine, Antwerp, Belgium]

Acknowledgments. This work was supported by U.S. Public Health Services grant AI23470 from the National Institutes of Health and funds from the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases (IMMLEP and THELEP).

Sathish, M., Esser, R. E., Curtiss, R., III, and Clark-Curtiss, J. E. Detection and characterization of antigens of *Mycobacterium leprae* reacting with sera from leprosy patients.

The construction of recombinant libraries of *Mycobacterium leprae* in plasmid, cosmid, and phage vectors has recently been reported. Of these, the λgt11 phage expression vector system has been used to isolate clones which encode polypeptide antigens recognized by mouse monoclonal antibodies. The immunological relevance of these antigens is as yet unclear. In an attempt to isolate antigens which may be relevant to the pathogenesis of the disease, we screened the λgt11 library with sera from leprosy patients.

Dr. Thomas H. Rea provided the sera from 21 lepromatous leprosy patients which were pooled. The activity of the sera against *Escherichia coli* and *M. leprae* was monitored by ELISA and the anti-*E. coli* reactivity was removed by passing the sera through sepharose beads coupled to an *E. coli* sonic extract. These pooled, adsorbed sera were used to probe the λgt11 library. Positive colonies were identified by blotting the plaques onto nitrocellulose membranes and reacting with the pooled sera followed by alkaline phosphatase-conjugated anti-human antibodies. Clones showing positive reactivity were plaque purified. Twenty clones which react with the serum pool have been identified so far. Different clones showed varying degrees of reactivity.

The reactivity of the clones was confirmed by Western blot analysis using the

same pooled sera. All of the clones except two appear to be producing proteins fused to β-galactosidase and showed bands of high molecular weight. Reactivity of the clones to monoclonal antibodies has also been analyzed by the technique of Western blotting. Three of the clones reacted strongly with MC 0401, a monoclonal antibody against the 65 kD antigen. None of the clones tested showed any specific reactivity with antibodies 5205, 8908, 4220, 8026, and 5828.

Three of the clones have been subcloned, and the expression of mycobacterial protein is being evaluated. Studies on further molecular and immunological characterization of these clones are in progress.—[Department of Biology, Washington University in St. Louis, St. Louis, Missouri 63130, U.S.A.]

Acknowledgment. This research was supported by fellowships from the Heiser Foundation (M.S. and R.E.) and by U.S.P.H.S. grant DE06669.

Izumi, S., Fujiwara, T., Ikeda, M., Nishimura, Y. and Sugiyama, K. Gelatin-particle agglutination test for serodiagnosis of leprosy—a new simple test useful for large-scale field study.

The chemically synthesized trisaccharide moiety of the phenolic glycolipid-I (PGL-I) antigen of the leprosy bacillus was conjugated with bovine serum albumin (BSA) via parahydroxy phenyl propionate. This semisynthetic antigen (NT-P-BSA) has the same antigenicity as natural PGL-I. Recently, we produced a new agglutination reagent for the serodiagnosis of leprosy by coating gelatin particles with NT-P-BSA. The antibody levels in 926 sera from various sources were tested by both particle agglutination (PA) and indirect ELISA.

The results clearly demonstrated good correlation between the two serological methods. Although it is a semiquantitative method, PA is a simple and inexpensive serological test suitable for large-scale epidemiological studies of leprosy in developing countries in which leprosy is endemic.—[Leprosy Research Laboratory, Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto; Department of Natural Science, Nara University, Nara; Central Research Laboratories, Fujirebio Inc., Hachioji, Japan]

CLOSING REMARKS

Distinguished Guests, Ladies and Gentlemen:

On behalf of the Japanese Leprosy Panel and participants, I express our deepest appreciation to Dr. Brennan, to the members of the U.S. Leprosy Panel, to Dr. Collins, to the members of the U.S. Tuberculosis Panel, and to the staff of the National Institutes of Health for their many acts of kindness and for admirably organizing the Twenty-second U.S.–Japan Leprosy and Tuberculosis Conferences sponsored by the U.S.–Japan Cooperative Medical Science Program. It has always been a great pleasure to have the opportunity to participate in this symposium and to learn of the remarkable progress in the immunology of leprosy, tuberculosis, and other related diseases. We are also looking forward to the Joint U.S.–Japan Tuberculosis Symposium and the Twenty-third Joint Committee Meeting to learn of their progress. It seems too early for closing remarks now, but this may be my last opportunity as Chairman of the Japanese Leprosy Panel.

I express my deep gratitude to all of the friends of both the U.S. Leprosy and Tuberculosis panels who have encouraged and inspired me since the beginning of this program. How much progress has been achieved during these two decades? We can answer promptly with the following: successful cultivation of murine leprosy bacilli, development of the mouse foot pad technique, experimental leprosy in the armadillo and the athymic nude mouse, effective therapeutic regimens using drug combinations instead of monotherapy, development of the serodiagnosis of leprosy, immunological understanding of the unresponsiveness in lepromatous leprosy, and so on.

Although my contribution to the progress

in these research areas would be like a drop of water in the Pacific Ocean, I do not regret being unable to complete my work, because I believe that my dreams of yesteryear will soon be realized by younger scientists who are supported and encouraged by the program. Needless to say, the permanent friendship and mutual understanding between the United States and Japanese scientists are a potential power for promoting the activities of the program toward the final goal—eradication of leprosy from the world.

We look forward to welcoming you to our country next year for the Twenty-third U.S.–Japan Leprosy Research Conference.

Thank you very much.

—Masahide Abe, *Chairman*
Japanese Leprosy Panel

In order not to delay publication of this issue, the Board of Directors of the JOURNAL has given its permission for the Index to Volume 55 to be published in the March 1988 issue of the JOURNAL. We hope this will not duly inconvenience readers who wish to bind their volumes promptly.—RCH