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ABSTRACTS*

Franzblau, S. G., Adams, L. B., White, K. E. and Krahenbuhl, J. L. Modeling human pharmacokinetics of single-dose combination regimens in macrophage cultures of *M. leprae.*

Using macrophage cover-slip cultures infected with *Mycobacterium leprae*, changes in drug concentrations over time in humans easily can be simulated by changing culture media. The viability of *M. leprae* can then be assessed radiorespiro-metrically at selected times post-treatment following lysis of host macrophages. In this manner, the relative activity of various regimens can be assessed. In addition to this model, we use the Alamar blue reagent, an oxidation-reduction indicator, to assess antimicrobial activity of drugs against *M. leprae* in axenic medium in 96-well plates.

Preliminary results suggested that after a total of 35 hr exposure (eight changes of media and drug concentrations), dapsone or clofazimine alone (modeled as daily doses) failed to effect a significant reduction in *M. leprae* viability while the combination of the two drugs was active. The addition of a single dose of rifampin increased activity but was not greater than rifampin or clarithromycin alone.

Minocycline, ofloxacin and sparfloxacin alone showed significant activity but less so than rifampin or clarithromycin. Sparfloxacin + minocycline, sparfloxacin + clarithromycin and ofloxacin + minocycline all showed less activity than either clarithromycin or rifampin alone or the combination of dapsone + clofazimine + rifampin. Rifampin + ofloxacin, rifampin + sparfloxacin and clarithromycin + ofloxacin all displayed activity roughly equivalent to rifampin or clarithromycin alone. The most active combinations were clarithromycin + minocycline and rifampin + minocycline; both with greater activity than rifampin or clarithromycin alone.

This model should allow the rational selection of one or two once-monthly regimens to be assessed in human trials. Additional experiments will model three-drug combinations and the effect of alternating doses of the two most active drugs, clarithromycin and rifampin, a combination which cannot be administered simultaneously due to the induction of clarithromycin metabolism by rifampin. In addition, synergistic and antagonistic interactions can be assessed. It may also be possible to assess the post-antibiotic effect by comparing radiorespirometric activity immediately after drug levels fall below effective doses and that measured after an additional incubation period in macrophages in drug-free media.

After 2 weeks of incubation with drugs, fluorometric measurement of the reduced form of the Alamar blue dye indicated marked dose responses to rifampin and clarithromycin, both displaying significant activity at 3.9 ng/ml. Dapsone and sparfloxacin showed significant activity at 31 ng/ml while minocycline and ofloxacin showed activity at 63–125 ng/ml and 250 ng/ml, respectively. Activities were somewhat lower when measured after only a 1-week incubation in most drugs. Clofazimine was inactive but did show activity at concentrations above 5 μg/ml in a separate experiment.

Alamar blue is an inexpensive, nontoxic, oxidation-reduction dye which can be measured either fluorometrically or colorimetrically or, with sufficient inoculum and/or incubation time, visually. This assay thus has great potential in countries where radiorespirometric assays are either too expensive or too difficult due to regulatory restrictions.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Fukutomi, Y., McCormick, G., Pasqua, J. P., Krahenbuhl, J. L., Matsuoka, M. and Minagawa, F. Elongation and septal formation of *M. leprae* in macrophages cultured *in vitro.*

Mycobacterium leprae, the causative agent of leprosy, is an obligate intracellular pathogen that prefers the mononuclear

*Some of these abstracts were not provided by the authors but are a synopsis prepared by the U.S. Co-Chair Dr. Brennan.

phagocyte as its host cell. Numerous efforts have been devoted to the *in vitro* cultivation of *M. leprae* since its discovery, yet the leprosy bacillus remains uncultivable and researchers are dependent upon experimental animals [conventional and athymic (nude) mice and armadillos] to grow *M. leprae* in host cells, including macrophages infected *in vitro*. Claims for success in *in vitro* cultivation remain unconfirmed.

The difficulty in assessing the viability of *M. leprae* has also impeded leprosy research. The availability in our laboratories of a constant supply of a large number of highly viable *M. leprae* from nude mice allowed us to adapt radiorespirometry as a measure of the metabolic activity of *M. leprae*, affording a rapid (2 week) quantitative method to assess the viability of the leprosy bacillus *in vitro*.

Previously, we reported that highly viable *M. leprae* from infected nude mice retained metabolic activity as assessed by radiorespirometry. Radiorespirometry data over 2 weeks showed *M. leprae* in macrophages at 31°C to be more metabolically active than at higher temperatures, such as 37°C. Moreover, the addition of interleukin 10 (IL-10), a well-known cytokine which suppresses macrophage activation, to the cultures clearly sustained *M. leprae* metabolism in macrophages for 8 weeks. The apparent increase in the number of *M. leprae*/macrophages was likely an artifact of loss and lysis of macrophages and rephagocytosis of released *M. leprae*. Noteworthy, however, was the elongation of individual bacilli after 4 weeks of culture in macrophages maintained in media with IL-10.

The present study explored further the long-term, *in vitro* cultivation of *M. leprae* in mouse peritoneal macrophages and armadillo blood monocyte-derived macrophages. During the course of 1–2 months in culture, the bacilli in these infected macrophages were studied by radiorespirometric assay and observed under light- and transmission electron microscopy (TEM). TEM confirmed the elongation of *M. leprae* in mouse macrophages sectioned after 4 weeks under the ideal culture conditions described above. In addition, armadillo macrophages cultured *in vitro* also supported metabolism as well as elongation.—[National Institute for Leprosy Research, Tokyo, Japan; Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Gidoh, M. and Saito, H. *In vitro* and *in vivo* activities of a novel new quinolone, DU-6859a, against *M. leprae.*

We compared the antimicrobial activity of a newly developed quinolone, DU-6859a, with that of sparfloxacin (SPFX) in *Mycobacterium leprae*-infected mice given at doses of 1.0, 5.0 and 10 mg/kg once daily, 6 times per week by gavage. It was found that DU-6859a was partially bactericidal at 10 mg/kg, while SPFX was bactericidal at the same dosage. The *in vitro* susceptibility of *M. leprae* to some new quinolones, such as ofloxacin (OFLX), SPFX, DU-6895a, and AM-1155, was studied using the Buddemeyer system, by which the rate of $^{14}CO_2$ production from ^{14}C-labeled palmitic acid by acid-fast bacilli is measured. The antileprosy activity was in the order of rifampin > DU-6895a > SPFX > AM-1155 > OFLX.—[National Institute for Leprosy Research, Tokyo, Japan]

Gomez, J., DeMaio, J., Ko, C. and Bishai, W. Sporulation regulatory gene homologs in *M. tuberculosis* and *M. leprae.*

The *sigF* and *whiB* genes are sporulation regulatory genes in *Bacillus subtilis* and *Streptomyces coelicolor* which are also found in *Mycobacterium tuberculosis* and *M. leprae*. Data show that inappropriate overexpression of these genes affects the growth rate and colony morphology. Moreover, the *M. tuberculosis sigF* gene is specifically induced upon entry into a stationary phase in BCG. Hence, the genes are likely to govern segments of the mycobacterial adaptation response to the stationary phase and, potentially, latency. The existence of sporulation regulator gene homologs in these pathogenic mycobacteria supports the hypothesis that latent tuberculosis and leprosy may involve spore-like bacillary states.—[Department of Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD, U.S.A.]

Goto, M., Matsuoka, M., Kitajima, S. and Matsushita, Y. A trial to establish animal models of leprous neuritis -transfer of spleen cells to *M. leprae*-inoculated nude mice.

During the treatment of leprosy, mononeuritis multiplex often occurs accompanied by reversal reaction, which may result in irreversible damage to the extremities and eyes. In order to establish a suitable model of leprous neuritis, spleen cell transfer to a previously *Mycobacterium leprae*-inoculated nude mouse was done.

Nine athymic BALB/c nu/nu mice (NM4-1 to 9) were inoculated with *M. leprae* (Thai53) 1×10^6 in the foot pads and kept for 13.5 months. Five-week-old BALB/c mice were immunized with *M. leprae* (Thai53) by foot pad injection, and their spleen cells (2×10^7) were transferred to each nude mouse interperitoneally (NM4-3,4,8,9). Nonimmunized BALB/c spleen cells were also transferred to nude mice (NM4-1,2,6,7). Two days (NM4-1 to 5) and 22 days (NM4-6 to 9) after the spleen cell transfer, the nude mice were sacrificed by paraformaldehyde perfusion for histological study.

Compared with the nontransferred nude mouse (NM4-5), all transferred mice showed decreases in foot pad size, especially when immunized spleen cells had been transferred. Histologically, transferred nude mouse foot pads showed short, spindle-shaped macrophages containing less acid-fast bacilli than in the macrophages of nontransferred mice. In some immunized, spleen cell transfer mouse (NM4-4,9) foot pads, perineural cuffing and intraneural growth of the spindle macrophages were noted. These histological findings closely resemble those of human leprous neuritis, especially in borderline lepromatous leprosy with reversal reaction. On the other hand, spleens of immunized spleen cell transferred nude mice (day 2: NM4-3,4) showed swelling and granuloma formation although acid-fast staining and anti-BCG immunohistochemistry were negative. In conclusion, this experimental system could become a useful animal model for leprous neuritis.—[National Leprosarium Hoshizuka-Keiaien, Kagoshima; National Institute for Leprosy Research, Tokyo; Department of Pathology, Kagoshima University Faculty of Medicine, Kagoshima, Japan]

Izumi, S., Hatta, M., Matsuoka, M., Budiawan, T. and Kawatsu, K. Epidemiological study on *M. leprae* infection and distribution of *M. leprae* in the environment of the highly endemic area of South Sulawesi, Indonesia.

Despite the rapid decline in the number of registered leprosy patients and in prevalence due to the increasing coverage with multidrug therapy, the number of new cases of leprosy in the endemic countries remains at the level of 530,000 per year in 1996. The large discrepancy in the declining rates between prevalence and incidence and the rapid decline of prevalence and slow decline in incidence raises questions about the dogma that the main source of infection of *Mycobacterium leprae* is the untreated multibacillary patient.

In 1995, we conducted an epidemiological survey in a leprosy-endemic village in South Sulawesi, Indonesia, using serological techniques and the nose-swab polymerase chain reaction (PCR) technique. The following results were obtained: 1) Significant numbers of general inhabitants in the endemic areas are infected with leprosy bacilli without direct contact with leprosy patients. 2) The rate of infection as well as antibody titers in household contacts are significantly higher than non-household contacts. 3) Seropositivity shows clustering in families. 4) The leprosy bacilli are distributed in the environment of endemic areas. The bacilli may infect humans through the nasal mucosa. 5) The number of leprosy bacilli in the environment varies by season, suggesting the possibility of natural reservoir(s) of *M. leprae*.—[National Institute for Leprosy Research, Tokyo, Japan; Hasanuddin University, Ujung Pandang, Indonesia]

Maeda, S. and Kashiwabara, Y. Purification, characterization of a phospholipase B of the membrane fraction of *M. lepraemurium* and cloning of the gene.

The phospholipid deacylating enzyme was solubilized from the particulate (mem-

brane) fraction of *Mycobacterium lepraemurium* with Triton X-100 and sodium cholate and purified 1100-fold to a homogeneous state by five-step chromatography. The purified enzyme was composed of a single polypeptide chain and the molecular mass, estimated by SDS-PAGE, was 37 kDa. From a comparison with the degradation patterns of phosphatidylcholine (PC) labeled at the *sn*-1 position or *sn*-2 position as the substrate, this enzyme appears to hydrolyze the *sn*-1 position of PC first and then the remaining fatty acid in the *sn*-2 position. This result also shows that the phospholipase has both activities of phospholipase A_1, and lysophospholipase in one protein, and the purified enzyme could be classified as a phospholipase B. Anti-phospholipase B antibody produced in rabbits reacted only with the protein of 37 kDa and inhibited the activity depending on the dose. By using this anti-phospholipase B antibody, we tried to isolate the gene encoding phospholipase B of *M. lepraemurium*. After a third screening, two kinds of positive phages which have 500 bp or 350 bp DNA inserts were obtained. The Southern blot analysis showed that the 4.5 kbp DNA digested by *Kpn*I and *Sma*I could be hybridized with the isolated 500 bp DNA fragment. From the nucleotide sequence of the 4.5 kbp DNA, one open reading frame was revealed with GTG as the start condon, which coded for 348 amino acids. The molecular weight of phospholipase B calculated from the DNA sequence was 37 kDa, coinciding with the molecular mass determined by SDS-PAGE of the purified phospholipase B.

Using the DNA fragment of the gene of phospholipase B from murine leprosy bacilli, the DNA fragment of *M. leprae*, which had very high homology to the gene of phospholipase B of *M. lepraemurium*, has been obtained. Sequencing of the DNA fragment thus obtained from the *M. leprae* genomic DNA is now in progress.—[National Institute for Leprosy Research, Tokyo, Japan]

Matsuo, E., Komatsu,A., Maekawa, S., Sidik, H., Sumiishi, A., Inoue, T. and Furuno, Y. On the molecular pathological significance of β-glucuronidase binding protein (BGBP) in leprosy and related diseases, and the lower anti-BGBP antibody titers in leprosy and tuberculosis patients compared to healthy individuals.

Beta-glucuronidase binding protein (BGBP) is a molecule synthesized by several parasitic microorganisms, such as *Mycobacterium leprae* as well as *M. avium-intracellulare, M. tuberculosis, Candidae* and the hepatitis B virus, which allows these microorganisms to utilize the host's enzyme to cleave appropriate substrates in host tissues. The inability of the host to neutralize the activity of BGBP, therefore, may facilitate parasitism. The present study was conducted to compare the actual differences in anti-BGBP IgG titers in leprosy and tuberculosis patients versus healthy individuals.

Sera were obtained from 69 healthy individuals; 25 indeterminate, 33 lepromatous, 14 borderline and 21 tuberculoid leprosy patients; and from 36 active tuberculosis patients. The BGBP, which is cross-immunoreactive with that of *M. leprae*, was purified from *Bisum sativum* and was bound to amino-type ELISA plates with the aid of aldehyde. The positive control was the serum of a healthy individual which showed the highest BGBP titer previously. The sera were diluted 50 times with PBS containing methylmannoside. The rest of the procedure was the standard indirect method. The immune reactions were developed with *o*-phenylenediamine and hydrogen peroxide solution. The data were calculated as the percentage of absorbance of each serum compared to that of the positive control. A summary of the data in terms of the means and standard deviations for the groups of patients described above are: 81.19 ± 22.67, 49.85 ± 15.69, 40.55 ± 17.44, 46.50 ± 15.66, 40.28 ± 15.42 and 49.06 ± 19.59, respectively. These data indicate lower anti-BGBP antibody titers in leprosy and tuberculosis patients compared to those of healthy individuals. Although the standard deviations of the titers were large for yet unknown reasons, the results clearly indicate a diminution in the ability of leprosy and tuberculosis patients to fully develop antibody against BGBP compared to healthy people.—[Department of Pathology, Kyorin University School of Medicine, Tokyo, Japan]

Nakanaga, K., Nomaguchi, H. and Matsuoka, M. Detection of 65-kDa *M. leprae* heat-shock protein messenger RNA (mRNA) by RNase protection assay.

Rapid identification and measurements of the viability of *Mycobacterium leprae* are important in the context of the diagnosis and chemotherapy of leprosy. However, currently no system is available for the cultivation of *M. leprae* in axenic media or in tissue culture systems. Because of the very short half-life of prokaryotic mRNA, the presence of mRNA may be an efficient indicator of the viability of *M. leprae*.

The RNase protection assay (RPA) is a valuable method for the detection and quantitation of specific mRNAs. In this experiment, RPA was applied in order to formulate a 65-kDa *M. leprae* heat-shock protein (hsp65) mRNA detection system.

RPA was performed with an antisense hsp65 RNA probe labeled with ^{32}P which was generated as a transcript RNA. Total RNA or polyA(+)mRNA extracted from hsp65-expressing mouse cell lines were hybridized with an antisense RNA probe. After digestion with RNase, protected RNA: RNA hybrids were analyzed by urea-PAGE. By RPA, *M. leprae* hsp65 mRNA was detected from these *M. leprae* hsp65-expressing mouse cell lines. To compare with northern hybridization analysis (NH), the sensitivity of RPA was 10 times higher than that of NH. Since there is approximately 50% homology between the *M. leprae* hsp65 gene and the gene of mitochondrial P1 or hsp60, NH required higher stringency washes. However, there was no need to consider wash conditions in the case of RPA.

Total RNA was successfully isolated from *M. chelonae* and *M. marinum* which had been grown on the surface of Sauton medium. The total RNA sample extracted from *M. leprae*-infected nude mouse foot pads showed, not 23s or 16s, but 28s and 18s mammalian ribosomal RNA bands in formaldehyde agarose gel electrophoresis.

Although there is more than 85% homology between *M. leprae* and other mycobacterial hsp65 genes, RPA was specific enough to demonstrate shorter RNase protected bands on the *M. marinum* and *M. chelonae* lanes after urea-PAGE analysis. Because of the unusual structure and the resultant low permeability of the mycobacterial cell wall, it is difficult to obtain intact mRNA from *M. leprae*. However, the specific detection system based on hsp65 mRNA may help our efforts toward *M. leprae* mRNA isolation and the development of *M. leprae* viability assays.—[National Institute for Leprosy Research, Tokyo, Japan]

Nomaguchi, H., Yogi, Y., Nakanaga, K., Kawatsu, K. and Okamura, H. Cytokine production with hsp65 in mice infected with *M. leprae*; diverse roles of hsp65 in the immune responses.

We have previously described that the mycobacterial heat-shock protein 65 (hsp65) was an inducer of the Th-1 type of cytokines in cultured splenocytes isolated from BALB/c mice pre-inoculated with *Mycobacterium leprae*. In this present study, we compared the ability of hsp65 to induce gamma interferon (IFN-γ) in BALB/c and NOD mice. The NOD mice spontaneously develop a diabetic syndrome which, in many respects, resembles human Type 1 insulin-dependent diabetes mellitus (IDDM).

Spleen cells of control and *M.leprae*-inoculated mice were stimulated with hsp65 (5 μg/ml), and the proliferative responses of the culture were scored in terms of thymidine incorporation. The lymphocyte proliferation (SI) was positive in the control and in the *M. leprae*-inoculated BALB/c and NOD mice, and the SI was especially high in the NOD mice in terms of the Tes-Tape test (+1).

The culture supernatants of splenocytes from *M. leprae*-inoculated BALB/c mice in the presence of hsp65 showed a high titer of IFN-γ, but no production from the NOD mice. However, the production of IFN-γ from NOD mice was recovered by the addition of anti-interleukin 10 (IL-10) in the culture. These results suggest that IL-10 may be produced by stimulation with hsp65 in NOD mice, and the production of IFN-γ may be suppressed by IL-10. IL-10 production was then studied in splenocyte cultures of NOD mice by stimulation with hsp65. A high titer of IL-10 production was detected

in the NOD mice by the stimulation with hsp65, but not in the BALB/c mice. Thus, the hsp65 shows a diverse role in different circumstances.—[National Institute for Leprosy Research, Tokyo; Hyogo College of Medicine, Nishinomiya, Japan]

Okamura, H., Nagata, K., Tamura, T., Tsutsui, H., Kawada, N., Yogi, Y., Nomaguchi, H. and Fujiwara, H. Effect of phenolic glycolipid-I (PGL-I) on cytokine expression inmacrophages.

Recent work has tried to understand the pathogenesis of various diseases in terms of abnormalities in Th1/Th2 balance in which various cytokines are involved. Recently, we cloned a new cytokine, interferon gamma (IFN-γ)-inducing factor (IGIF), that is produced by activated macrophages. IGIF, together with interleukin 12 (IL-12) was considered to be involved in the differentiation of Th1 cells and, hence, in the development of cellular immunity. IGIF also was shown to strongly augment the functions of the Fas ligand of T or natural killer (NK) cells, suggesting that it is involved in inflammatory tissue damage or in AICD of lymphocytes.

Phenolic glycolipid-I (PGL-I), a unique antigen of *Mycobacterium leprae*, has been used for the detection of antibody against *M. leprae*. However, its biological activities are not fully elucidated; it has been shown to suppress T-cell proliferation or macrophage functions. Here, the biological activities of IGIF were introduced and the effects of PGL-I on the expression of IGIF, IL-12 and IL-10 were compared. It was shown that PGL-I decreased the mRNA levels of IGIF and IL-12, but not of IL-10, in activated macrophages. PGL-I may suppress the cytokines involved in development to TH1 cells.

IGIF was discovered as a factor stimulating T cells to produce IFN-γ, but one of its notable actions is the marked synergism with IL-12 on IFN-γ induction. IGIF induces higher levels of IFN-γ than IL-12 alone, while the combined use of low concentrations of these cytokines induces much higher levels. The underlying mechanism for this synergism is not clarified. The promoter regions of the IFN-γ gene may have the binding sequences for transcription factors activated independently by these cytokines, or IL-12 may augment or activate the receptor for IGIF on T or NK cells.

Another important biological activity of IGIF is the ability to augment the functions of the Fas ligand on T or NK cells. IGIF augmented the expression of the Fas ligand on Th1 cells and their cytocidal activity against Fas-transfectant, A-1 cells, at the same time. IGIF exhibited the same action on established NK cells. However, IGIF had no such effect on TH2 cells. Since IGIF has been proved to be involved in the development of liver damage to the mouse model for fulminant hepatitis, it may exert its abilities in a Fas-mediated manner or through activation of the cytokine network, including IFN-γ or tumor necrosis factor.

Both IGIF and IL-12 were not expressed in resident peritoneal macrophages, but were strongly expressed in the liver Kupffer cells or peritoneal exudate cells of *P. acnes*-treated mice. It was of interest that the addition of a high dose of PGL-I (40 μg/ml) to the culture of these cells quickly diminished the expression of these cytokines; whereas PGL-I did not affect the expression of IL-10. In addition, IFN-γ production induced by mitogens in spleen cells was suppressed by the addition of PGL-I to the culture. The *M. bovis* PGL failed to suppress the expression of IGIF and IL-12 in macrophages, suggesting that the structure of the carbohydrate moiety of PGL-I has important roles in the biological activities of PGL-I.

Although these observations about the action of PGL-I need further analysis, it is probable that PGL-I affects the development of cellular immunity by inhibiting the production of cytokines involved in it.—[Department of Bacteriology, Hyogo College of Medicine, Nishinomiya; Faculty of Medicine, Osaka City University, Osaka; National Institute for Leprosy Research, Tokyo; Nara University, Nara, Japan]

Prigozy, T. I., Sieling, P. A., Clemens, D., Porcelli, S. A., Stewart, P., Kronenberg, M. and Modlin, R. L. CD1-Mediated presentation of *M. leprae* lipoarabinomannan to T cells requires uptake by the mannose receptor and transport to a late endosome.

Recent studies have begun to reveal new mechanisms by which T-cell recognition may occur, suggesting that other solutions to this problem have been incorporated into the immune systems of vertebrates during evolution. In particular, our studies have contributed to the finding that the CD1 antigen processing pathway is involved in the presentation of nonpeptide antigens to T cells.

Analysis of two *Mycobacterium leprae*-reactive, CD1b-restricted T-cell lines demonstrated that both recognized a major mycobacterial cell-wall constituent known as lipoarabinomannan (LAM). This molecule belongs to the family of lipoglycans, and is composed of a hydrophobic lipid-containing phosphatidyl inositol group attached to a large and complex hydrophilic heteropolysaccharide. For the two T-cell lines analyzed, studies using chemically modified LAM and related compounds showed that both carbohydrate and lipid components of the antigen were required for presentation and/or T-cell recognition. Furthermore, these two T-cell lines differed in their ability to recognize LAM purified from different species of mycobacteria. This presumably reflects a significant level of specificity in CD1-restricted T-cell responses, probably arising from subtle variations in the lipid and carbohydrate moieties. These CD1-restricted mycobacteria-specific T cells can release interferon gamma (IFN-γ) and show cytolytic activity upon contact with mycobacteria pulsed target cells. These activities suggest a proinflammatory role for these T cells in type 1 cellular immune responses which are required for immunity to intracellular pathogens. The CD1 system may significantly enhance the ability of the T-cell system to detect intracellular parasites like *M. leprae* and *M. tuberculosis*, leading to activation of intracellular killing mechanisms and lysis of infected cells serving as a reservoir of such pathogens. Although so far only mycobacteria have been demonstrated to harbor CD1-restricted antigens, this mechanism could potentially apply to many other microbial pathogens which express a range of unique lipid or glycolipid antigens.

In more recent work, we have established that the mannose receptor (MR), a pattern recognition molecule involved in the innate immune response, is an integral component of CD1b-mediated antigen presentation to effector T cells in the adaptive immune response. Mycobacteria-reactive and CD1b-restricted T cells secrete proinflammatory cytokines and are cytotoxic, indicating an effector role in cell-mediated immunity. The blockade of both antigen presentation and the transport of LAM to late endosomal compartments, by inhibition of the vacuolar- H+ ATPase, supports a model in which LAM first is routed via the MR into early endosomes and subsequently trafficks into late endosomal compartments containing CD1b. During intracellular infection, LAM is present in endosomal/lysosomal compartments, and it should be directly available to the CD1b antigen presentation pathway. However, as mycobacterial infection progresses, the maturation and acidification of the phagosome is inhibited, thereby preventing CD1b presentation of LAM. In this situation, endocytosis of extracellular LAM via the MR by uninfected, bystander macrophages could be critical in the generation of antibacterial cell-mediated immunity. MR-binding lipoglycans are present in a variety of bacterial and protozoan pathogens, and we therefore speculate that the pathway of MR-mediated uptake leading to antigen presentation by CD1 could be critical in the generation of T-cell responses against microbial pathogens.—[Division of Dermatology, UCLA School of Medicine, Los Angeles, CA, U.S.A.]

Silbaq, F. S., Cole, S. T. and Brennan, P. J. Progress toward sequencing the genome of *M. leprae.*

Conscious of the enormous potential benefits to be derived from knowledge of the *Mycobacterium leprae* genome, we have been working with Dr. S. T. Cole to complete the task. The recombinant DNA cosmid, L373, was the source of 400 different recombinant clones of M13. The sequence of 104 clones with base peaks well separated were assembled by the Sequencer Program (Gene Codes Corporation). Fifty-two clones are organized in 19 different contigs with a total size of 13,600 bp. The remainder of the clones (52 in number) did not overlap with any of the above different

contigs. Comparison of the DNA sequence against the DNA and protein data bases revealed 18 clones with significant homology to genes or proteins in the data bases and 86 clones which had no significant matches. The 18 clones that match in the data base are:

Four overlapping clones, *L55, L-59, L-121* and *L-151,* that had high homology (63% identity at the DNA level) with a 34-kDa protein of *M. paratuberculosis* (EMBL accession number X68102). Clone *L-96* DNA sequence from *M. leprae* coded for the complete sequence of tRNA-Ala. This sequence shows 80% and 70% identity with the corresponding gene of *Micrococcus luteus* (EMBL X55101) and *Escherichia coli* (EMBL X66515), respectively. This is the second tRNA-Ala gene of *M. leprae* and shows relatively low homology (66%) with the first tRNA-Ala, that located at cosmid b1770 (EMBL Z70722). Clones *L-53, L-102, L-102.1, L-92,* and *L-190* of *M. leprae* show significant similarity with the *purH* and *purN* gene product to *Bacillus subtilis* (EMBL J02732) and *Homo sapiens* (EMBL X54199). Clones *L-124.1, L-187,* and *L-201* show significant homology with the hypothetical 34-kDa protein of *M. paratuberculosis* (EMBL Z23092) and the heat-shock protein/serine protease (*htrA* gene) of *Campylobacter jejuni* (EMBL X82628). Clone *L-209* showed high homology with acetyl-coenzyme A carboxylase/carboxyl transferase of *M. tuberculosis* (EMBL Z19549) but not to the *M. leprae* isolog gene (EMBL X63470). Clones *L-107* and *L-249* showed high homology at the amino-acid level with succinyl-CoA synthetase alpha chain (sucD) of *Coxiella burnetii* (EMBL U07789). Clone *L-114* showed homology with the *Anabaena* sp. *moeA* gene (EMBL U34309) and with the gene product from *E. coli* (EMBL M21151) involved in the biosynthesis of demolybdo-cofactor (molybdoptrin) necessary for molybdo-enzyme synthesis. Clone *L-168* showed high homology with UDP-glucose pyrophosphorylase (*gtaB*) (GenBank L43967) of *Mycoplasma genitalium.* This gene involved in the glycosylation of teichoic acid is closely linked to *gtaA* in *B. subtilis.*—[Colorado State University, Fort Collins, CO, U.S.A.; Institut Pasteur, Paris, France]

Steele, W. S., Williams, D. L. and Gillis, T. P. Molecular characterization of dapsone resistance in *M. leprae.*

A *Mycobacterium leprae* gene encoding the putative dihydropteroate synthase (dhps) enzyme (*folP*) has been identified and sequenced from the *M. leprae* cosmid library. We have sequenced this gene from other strains of *M. leprae,* both dapsone (DDS) resistant and DDS susceptible, and have performed comparative sequence analysis of this gene with other known prokaryotic and eukaryotic *folP* genes. Amino-acid sequence analysis indicated that the putative *folP* gene of *M. leprae* is similar in size and domain composition, strongly suggesting that this is *M. leprae folP.* Good homology in the region thought to be involved in PABA binding was observed in the *M. leprae folP* as well. No apparent sequence differences were noted in the *folP* genes from DDS-resistant and -susceptible strains, suggesting a lack of correlation between specific mutations and DDS resistance in the strains tested. RFLP analysis of DDS-resistant and-susceptible strains showed a single predominant restriction fragment of equal size and intensity, suggesting that in the strains tested DDS resistance could not be attributed to the dhps gene amplification. Work is in progress on promoter analysis of this gene as well as biochemical characterization of *folP* from *M. leprae* and *M. smegmatis.*—[Department of Microbiology, Louisiana State University, Baton Rouge, LA; Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Suzuki, Y., Katsukawa, C., Tamaru, A. and Makino, M. Application of recombinant 85 complex of *M. leprae* for serodiagnosis of leprosy: overexpression of MPT51-like protein (MPL51) of *M. leprae* in *E. coli.*

In the course of our study, we have cloned and analyzed the genes coding for the antigen 85 (Ag85) complex. From the results, 85 complex genes of *Mycobacterium leprae* have turned out to form a gene family like that of *M. tuberculosis.* In

further study of the Ag85 complex, we have performed an analysis on the downstream region of the Ag85 gene, and found an open reading frame (ORF) coding for a peptide possessing homology with MPT51 of *M. tuberculosis*. MPT51 is an example of a secretion protein of *M. tuberculosis*. This protein possesses high identity at its N-terminus with the major secreted protein of the Ag85 complex. In addition, anti-MPT51 anti-serum generated by immunizing rabbits with purified MPT51 recognizes 85B of *M. tuberculosis*. From these facts, MPT51 was considered to be a member of the Ag85 complex gene family.

In our current study, we have constructed an overexpression system for MPL51 in *Escherichia coli* using pTrx-Fus as an expression vector. By using the recombinant MPL51 (rMPL51) overexpressed in *E. coli*, Western blot analysis was performed. Only 1 out of 20 sera exhibited reactivity against rMPL51; the remaining 19 had no reactivity. Similar observations were obtained even when we analyzed the antibody titer using an ELISA system.

The reason why almost all sera had no antibody response against rMPL51 is not clear. Two hypotheses are suggested by these results: 1) Post-translational modification may be necessary for MPL51 to achieve antigenicity. 2) There are no sequential epitopes on the MPL51 that are recognized by patient sera. The reason why almost all sera had no antibody against rMPL51 will be further investigated.—[Departments of Pathology and Microbiology, Osaka Prefectural Institute of Public Health, Osaka, Japan]

Tanaka, S., Matsui, S., Taguchi, H. and Miyoshi, I. Immunological study of infection against retrovirus (HTLV-1) in leprosy.

In Japan most leprosy patients are expatients; however, they have been forced to live in isolation for over 40 years in leprosaria until this year. It is possible that tumor-related viruses (HTLV-1, HBV, HCV) as environmental infectious agents could easily infect patients with leprosy. We studied leprosy patients at the National Leprosarium Kuryu Rakusenen in Japan to see whether there was an association between leprosy and HTLV-1 infection, and we compared leprosy with hepatitis B (HB) and hepatitis C (HC) virus infection. Samples from leprosy patients (395) were collected from 1993 to 1994 at Kuryu Rakusenen, Gunma Prefecture, in the middle of Japan. There were 216 male and 179 female cases. They were over 47 years old (mean age 71). Controls (9602) were blood donors over 50 years old from Gunma Prefecture (6554 males and 3048 females).

All sera (leprosy and control) were screened for HTLV-1 by enzyme immunoassay (HTLV-1 EIA; Eizai). All sera positive or doubtful by at least one of the two techniques were studied by immunofluorescence assay (IF) and by Western blot. Antigens and antibodies to HBs were screened with sandwich immunoassay (Daina Screen) and detected by IMX EIA. Antibodies to HCV were detected by passive hemagglutination assay (HCV PHA; Dainabot). Statistical analyses were conducted with the chi-squared test.

The prevalence rate of HTLV-1 antibodies in leprosy patients (8.4%) was significantly higher than in controls (0.62%) in Gunma Prefecture. The seroprevalence rate of lepromatous leprosy (L form) was double that of tuberculoid leprosy (T form) (9.3% and 4.1%, respectively). HBs antigen-positive patients were all L form (3 males, 2 females). The HB antibody prevalence rate in leprous patients (22.5%) was significantly higher than in controls (4.64%). However, the seroprevalence rate of the L form (20.8%) was significantly lower than the T form (31.8%). The HCV antibody prevalence rate with leprosy (32.0%) was significantly higher than the carrier rate of blood donors (1.97%). No significant differences were found in the prevalence rates between the L form (33.2%) and the T form (27.2%). The results suggest that easy infection with HTLV-1 in leprosy is similar to that with HBV and HCV. There was no significant difference in the prevalence rates of antibodies to HTLV-1 and HCV between the L form and the T form of leprosy.—[National Leprosarium Kuryu Rakusenen, Gunma; Kouchi Medical School, Kouchi, Japan]

Walker, L. L., King, C. H. and Shinnick, T. M. Cultivation of *M. leprae* in primary cell cultures.

No system is currently available for the cultivation of *Mycobacterium leprae* in axenic media or in tissue culture systems, although the bacilli can be maintained in a metabolically active state in axenic media for several weeks. Last year, we presented the results of preliminary studies to grow *M. leprae* in primary murine cell cultures.

In our present work, acid-fast bacilli (AFB)-positive cultures were established and maintained from foot pads of mice infected with 22 different isolates of *M. leprae*. In addition, 18 lines of spleen, inguinal lymph node, or tendon cells were successfully infected with bacilli from mice inoculated with 14 different strains of *M. leprae* (a subset of the 22 strains). Although many of the cell lines/organ cultures became unstable after several months of cultivation, most cultures could be maintained for 7 to 8 months; the longest a culture remained AFB-positive was 14 months. In these cultures, the bacilli appeared to be located within the eukaryotic cells, and the number of bacilli per culture increased with a doubling time of 30 to 40 days. After several months of culture, the bacilli were typically found in clusters of 2-5 bacilli within a eukaryotic cell, although clusters containing 8-20 bacilli were not rare. The cultivated AFB were shown to be *M. leprae* by staining characteristics, polymerase chain reaction amplification studies using two *M. leprae*-specific primer sets and a *Mycobacterium* genus-wide primer set, and high-performance liquid-chromatographic analysis of mycolic acids. The bacilli in these cultures appeared to be viable because the number of bacilli recovered increased with time and because of their staining characteristics, that is, the bacilli were evenly stained over the entire bacterial surface, which is indicative of a viable cell.

Bacilli from 6 of the 10 cultures displayed 100- to 1000-fold increases after inoculation into mice, and $>10^6$ bacilli were usually recovered from the infected mice. These 6 cultures included ones based on cells derived from lymph nodes and tendons. In general, the bacilli from the *in vitro* cultures multiplied in the foot pads at a rate and extent consistent with the same strains maintained in regular mouse passage, and the bacilli recovered from the foot pads were identified as *M. leprae* as described above. Of the 4 cultures that did not display growth in mice, 2 had no detectable bacilli in the inoculum; the other 2 had detectable AFB in the inoculum but no AFB were detected after 221 and 252 days in mice. Overall, the studies are encouraging for the development of a system for the cultivation of *M. leprae* and for studies of its intracellular replication.—[Tuberculosis/Mycobacteriology Branch, Division of AIDS, STD and TB Laboratory Research, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.]