INTERNATIONAL JOURNAL OF LEPROSY

Volume 69, Number 4
Printed in the U.S.A.
(ISSN 0148-916X)

THIRTY-SIXTH U.S.–JAPAN TUBERCULOSIS-LEPROSY RESEARCH CONFERENCE

Omni Royal Orleans Hotel
New Orleans, Louisiana, U.S.A.
15–17 July 2001
sponsored by the
U.S.-Japan Cooperative Medical Science Program
National Institute of Allergy and Infectious Diseases
National Institutes of Health

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THIRTY-SIXTH U.S.-JAPAN TUBERCULOSIS-LEPROSY RESEARCH CONFERENCE

The 36th Research Conference on Tuberculosis and Leprosy of the U.S.-Japan Cooperative Medical Sciences Program was held at the Omni Royal Orleans Hotel in New Orleans, Louisiana, from 15–17 July 2001. Dr. James L. Krahenbuhl and his staff from the National Hansen's Disease Laboratory Research Branch organized the meeting. The U.S. Tuberculosis-Leprosy Panel was comprised of Drs. Clifton E. Barry, III, Thomas P. Gillis, Gilla Kaplan and David N. McMurray, with Dr. Philip C. Hopewell serving as the U.S. Panel Chairman. Drs. Kasuo Kobayashi, Masamichi Goto, Kiyoshi Takatsu and Hatsumi Taniguchi comprised the Japanese Panel with Dr. Masao Mitsuyama serving as the Japanese Panel Chairman.

The abstracts of oral presentations in the area of leprosy research are presented alphabetically by first author below, followed by poster presentations. Abstracts of presentations on tuberculosis research will appear in *Tubercle and Lung Disease.*

ABSTRACTS

Adams, L. B., Ray, N. A. and Krahenbuhl, J. L. Lack of superoxide production does not impair on effective host response to experimental *Mycobacterium leprae* infection.

Activated macrophages produce reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI), which are important in host resistance to pathogens. X-CGD mice, a model for X-linked chronic granulomatous disease, have a disruption in the $gp91^{phox}$ subunit of NADPH oxidase and cannot produce superoxide. When infected with *Mycobacterium leprae,* normal macrophages from X-CGD mice supported bacterial viability, whereas activated macrophages inhibited *M. leprae* metabolism and produced elevated levels of nitrite. Macrophage-mediated killing of *M. leprae* was abrogated, however, by incubation with inhibitors of RNI production. To determine if X-CGD mice would show an impaired immune response to *M. leprae in vivo,* we examined growth of the bacilli in mouse foot pads. Multiplication of *M. leprae* in X-CGD mice showed a similar pattern of growth throughout the infection as that observed in B6 mice. Thus, a deficiency in the production of powerful ROI by phagocytic leukocytes in X-CGD mice did not render them more susceptible to experimental leprosy.

Fukutomi, Y., Kimura, H., Toratini, S. and Matsuoka, M. Involvement of cAMP in IL-10 production by macrophages stimulated with *M. leprae.*

In response to *M. leprae,* mouse macrophages released cytokines, such as TNF-α, IL-10 and IL-1β, and Prostaglandin E2 (PGE2), a cyclooxygenase product. PGE2 was required for the induction of IL-10. Forskolin, which activated adenylate cyclase, enhanced IL-10 production and concurrent suppression of TNF production. Rolipram, which inhibits the activity of phosphodiesterase, similarly enhanced IL-10 and suppressed TNF. Neither forskolin nor rolipram enhanced the PGE2 production by *M. leprae*-stimulated macrophages. It is well known that PGE2 elevates cAMP in cells by stimulating adenylate cyclase. Phosphodiesterase represents the cAMP-hydrolysing activity. Therefore, it is likely that the elevation of cAMP triggers *M. leprae*-induced IL-10 production and the elevated production of cAMP, either directly or indirectly through the enhancement of IL-10 production, results in the suppression of TNF production.

Gillis, T. P., Elzer, P. H., Shurig, G. G., Rambukkana, A. and Krahenbuhl, J. L. Development of new vaccines for leprosy and tuberculosis.

We report here the results of two vaccine trials for leprosy and one for tuberculosis in BALB/c mice. The first vaccine was based on the *M. leprae* laminin binding protein-21 (ML-LBP 21) thought to be involved in *M. leprae*–Schwann cell interactions. The vaccine was given as a purified recombinant protein made in *E. coli* mixed with Freund's incomplete adjuvant. Lymphocyte transformation tests from immunized mice indicated sensitization to ML-LBP 21, however, immunized mice were unable to control the infection as judged by growth of *M. leprae* in the foot pad. The second vaccine trial compared protection afforded by *Brucella abortus* vaccine strain RB51 engineered to express either antigen 85A (Ag85A) or ESAT-6 cloned originally from *M. bovis* BCG and *M. bovis,* respectively. While each vaccine construct demonstrated protection over nonvaccinated controls challenged by the aerosol route with *M. tuberculosis,* the best protection was afforded through the co-administration of the recombinant vaccines yielding approximately 1 $\log_{10}$ protection. A separate group of mice vaccinated with the recombinant RB51 construct expressing Ag85A showed no protection when challenged with *M. leprae.*

Goto, M., Nomoto, M., Kitajima, S. and Yonezawa, S. Pathogenesis of silent neuropathy of leprosy—a morphometric

analysis of Pgp9.5 positive dermal nerves in autopsy.

Nerve damage should increase after effective leprosy chemotherapy. In a considerable number of cured cases, however, neuropathy continues to advance (silent neuropathy). To better understand its pathogenesis, a basic study of dermal nerves was performed. Paraffin sections of lower abdominal skin taken at autopsy from 9 control cases, 8 cured tuberculoid leprosy cases and 12 lepromatous leprosy cases were stained by Fite's acid-fast staining, anti-BCG and nerve specific anti-PGP9.5 immunohistochemistry. One of the lepromatous leprosy cases showed leprous neuritis. The remaining 19 cases showed no neuritis and all cases were negative for Fite and anti-BCG. Correlation of age and PGP9.5 positive area was studied. All the tuberculoid cases and 7/12 of lepromatous cases were within the normal range, however, 5/12 of lepromatous leprosy revealed a remarkable decrease in PGP9.5 positive area beginning at approximately age 60. Usually, the lower abdomen is spared from leprous neuropathy, but this study disclosed loss of abdominal dermal nerve in almost half of clinically cured lepromatous leprosy cases.

Hagge, D. A., McCormick, G., Scollard, D. and Williams, D. L. An improved model for studying *M. leprae*/Schwann cell interactions.

The viability of *Mycobacterium leprae* (*ML*) may have a major effect on the outcome of infection, therefore, an improved model for studying the effects of *ML* on Schwann cells (SC), its neural target, should include infection at 33°C. This temperature is a permissive temperature for short-term maintenance of *ML* viability, however, the effects of 33°C on *ML* viability within SC or on SC activity are unknown. The purpose of this study was to determine the effects of 33°C on neonatal rat SC and embryonic neurons and on *ML* viability within SC. Results showed that SC maintained at 33°C appeared to survive, expressed specific gene transcripts, and interacted with axons of embryonic neurons comparable to those maintained at 37°C. *ML* retained 58% of their original viability up to 21 days post infection within SC at 33°C versus 3.6% at 37°C. Preliminary experiments suggested that infection at 33°C leads to disruption of SC monolayers, but does not appear to grossly affect SC/axon interactions when infected cells were seeded onto axons of embryonic neurons. Therefore, we present an improved model for the study of *ML*/*SC* interactions.

Hagge, D. A., McCormick, G., Scollard, D. and Williams, D. L. The effect of *Mycobacterium leprae* infection on cell surface adhesion molecules of cultured rat Schwann cells.

Previously we have shown that *Mycobacterium leprae* (*ML*) infection of Schwann cells (SC) at an MOI = 100:1 at 33°C resulted in the loss of the typical SC 'swirled' monolayer morphology. SC appeared to retract from the monolayer into large cell aggregates and the expression of genes encoding the neural cell adhesion molecule (NCAM) and glial fibrillary acidic protein (GFAP) was altered in these cells. Since these molecules are important mediators of SC/SC and SC/axon interactions, these data indicated that adhesion molecules were affected by infection. However, when infected SC were seeded onto embryonic neurons, they were able to adhere, align, proliferate and myelinate axons. This indicated that infected SC could participate in nerve regeneration processes. To further investigate the effects of *ML* infection on SC adhesion molecules and SC/axon interactions, the expression of several other SC genes encoding surface adherence molecules was studied in SC cultures using RT-PCR and the effect of infection on intact, myelinated SC/axon units was studied using electron microscopy. Results showed that in addition to the alteration of NCAM and GFAP, the expression of N-cadherin gene was down-regulated and ICAM was up-regulated in cultured SC while expression of neural adhesion molecule LI was not altered. *ML* were found in the SC of these cultures but were not found in axons

or in neuronal cell bodies. In addition, infection did not appear to affect the ability of SC to maintain the architecture of myelin sheaths. These cells were able to maintain axonal contact in a comparable manner to that of uninfected cells. Therefore, *ML* infection did not appear to impair the functional capability of mature, myelinating SC, suggesting that other factors, such as an aggressive immune response, are responsible for nerve fiber destruction.

Haslett, A. J., Manandhar, R., Shrestha, N., Roche, P., Albert, M., Macdonald, M., Butlin, C. R. and Kaplan, G. Complex *Mycobacterium leprae* antigens stimulate interferon-gamma production by T cells bearing both gamma-delta and alpha-beta T cell receptors.

Erythema nodosum leprosum (ENL), a debilitating inflammatory complication of multibacillary leprosy, is associated with transient increases in *Mycobacterium leprae*-specific T cell immunity in patients otherwise anergic to *M. leprae* antigens. We quantified the *M. leprae*-specific T cell response in ENL patients utilizing the enzyme-linked immunospot (ELISPOT) technique. Following stimulation with MLSA-LAM, a delipidated cytosolic antigen obtained from *M. leprae,* a surprisingly high frequency of interferon-γ (IFN-γ)-producing peripheral blood mononuclear cells was observed. This response seemed disproportional to the modest lymphoproliferative responses that have been reported previously in ENL. Further flow-cytometry-based analysis of intracellular cytokine production showed that MLSA-LAM-stimulated IFN-γ production was predominantly by T cells expressing the γδ receptor (TCR-γδ), with a minimal contribution by TCR-αβ T cells. Similar patterns of IFN-γ production were noted in normal controls from leprosy endemic areas. Our findings suggest a possible role for TCR-γδ T cells in the immunopathogenesis of ENL.

Matsuoka, M., Kashiwabara, Y., Nakata, N., Bormate, A. B., Wiens, C. and Legua, P. Genomic diversity of *Mycobacterium leprae* and geographic distributions.

Geographic distribution of different *rpoT* genotypes of *Mycobacterium leprae* isolated in Paraguay and Peru was investigated in connection with human prehistoric migration. All *M. leprae* genotypes of the *rpoT* gene isolated in the two countries showed three tandem repeats of 6bp. No isolates were detected of the *rpoT* gene genotype with four tandem repeats of 6bp, which is dominant in Japanese and Korean isolates. Although it was assumed that leprosy was carried with ancient Mongoloids to Latin America, the history of the expansion of leprosy in this continent was obscure. Polymorphism caused by the 78 base insertion sequence was detected in one isolate based on the results of an arbitrarily primed polymerase chain reaction. The difference in nucleotide sequence at this position seemed not to be applicable for the genotyping since this unique insertion sequence was not generally found among strains of *M. leprae* except for one isolate. *M. leprae* isolates showed various genotypes of TTC repeat but the strain serially passed 7 or 11 generations in nude mice footpads showed the same genotype with bacterial material obtained from third generation strain. Genomic diversity of this region seemed to be useful for typing *M. leprae* isolates.

Ohyama, H., Ohira, T., Takeuchi, K., Nishimura, F., Hirosue, M., Kitanaka, M., Takashiba, S., Murayama, Y. and Matsushita, S. IL-12-induced IFN-γ productivity in T lymphocytes from humans with leprosy.

The objective of this study is to compare T cell responses to interleukin-12 (IL-12) among LL, TT leprosy patients and healthy subjects. The amounts of IFN-γ produced from T cells stimulated with PHA in the presence of IL-12 were measured. The polymorphism of the *IL-12RB1* gene was analyzed by using direct sequencing technique to determine the effect of the SNPs on IFN-γ production by T cells. The results of the study are as follows. I) All T cells

isolated from LL and TT patients produced small amounts of IFN-γ even ha the presence of IL-12, whereas IFN-γ production varied among T cells isolated from healthy donors. 2) Most subjects with leprosy carried the coding SNPs on the *IL-12RB1* gene. 3) Most activated T cells isolated from donors with the coding SNPs on the *IL-12RB1* gene produced small amounts of IFN-γ in the presence of IL-12. These results suggest that the difference of susceptibility to leprosy may be determined by the IFN-γ production from activated T cells in the presence of IL-12, and that the difference of IFN-γ productivity might be partially explained by the coding SNPs on the *IL-12RB1* gene.

Spencer, J. S., Marques, A. M., Lima, M. C. B. S., Junqueira-Kipnis, A. P., Truman, R. W. and Brennan, P. J. Characterization of B and T cell epitopes of the *M. leprae* homologue to ESAT-6.

ESAT-6 from *M. tuberculosis* is a well characterized immunodominant 6 kDa protein antigen found in the culture filtrate which was shown to elicit a very potent early IFN-γ response in T cells from *M. tuberculosis*-infected mice and humans. It has shown promise as a replacement for the PPD skin test since the genetic region that codes for this gene is deleted in all strains of BCG, and thus is not recognized by individuals who have been BCG vaccinated. The sequence of the *M. leprae* homologue to ESAT-6 shows only 36% homology at the protein level compared with that of *M. tuberculosis*. We decided to examine the level of immunologic cross-reactivity of the *M. leprae* ESAT-6 with its *M. tuberculosis* counterpart. Polyclonal antisera were obtained from mice immunized with the recombinant forms of both ESAT-6 proteins. The antisera raised against each ESAT-6 reacted only with the homologous protein. Synthesis of overlapping peptides for the *M. leprae* ESAT-6 allowed the identification of both B and T cell epitopes recognized by mice. In addition, using polyclonal and monoclonal antibodies specific for *M. leprae* ESAT-6, we were able to positively identify reactivity by Western blot in subcellular preparations of *M. leprae*.

POSTER PRESENTATIONS

Costa, M. B., Martelli, C. M. T., Stefani, M. M. A., Neto, F. F. C., Maceira, J. P., Schettini, A. P. M., Nery, J. A. C. and Scollard, D. M. Distinct histopathological patterns in single lesion leprosy: the Brazilian Multicenter Study.

Single lesion leprosy has been identified as a clinical entity by the WHO, proposing that this represents very early, paucibacillary (SSL-PB) disease, for which a single treatment with rifampin, ofloxacin, and minocycline (ROM) has been recommended. To determine whether clinical SSL-PB lesions are histopathologically homogeneous, biopsies of lesions from a multi-center ROM trial in Brazil were evaluated and correlations with Mitsuda skin tests and with circulating antibody to *M. leprae* PGL-1 were examined. Participants were recruited from clinics in 4 Brazilian States—Amazonas, Rondonia, Rio de Janeiro, and Goiás—from October 1997–December 1998. Punch biopsies were obtained from SSL-PB lesions, and H/E and Fite-Faraco-stained sections were classified by one pathologist (MBC), without any clinical information. A second pathologist (DMS) reviewed H/E sections of 15% of these, without knowledge of any prior findings. Biopsies were obtained from 278/299 clinically SSL-PB patients; 259 were consistent with leprosy, 7 of multibacillary disease; 12 had other diseases, and 6 were unsuitable for assessment. Lesions were classified into 5 groups: (1) well circumscribed epithelioid cell granuloma; (2) less circumscribed epithelioid cell granuloma; (3) mononuclear inflammatory infiltrate permeated with epithelioid cells; (4) perivascular/periadnexal mononuclear-inflammatory infiltrate; and (5) minimal or

no morphological alteration detected. The distribution of biopsies within these groups was as follows:

Group	1	2	3	4	5
Number	87	56	31	77	8
(%)	(33.6)	(21.6)	(12)	(29.7)	(3.1)

Nerve involvement was observed in 127 biopsies (49.8%), and rare acid-fast organisms were found in 17 (6.7%). Close agreement (81.6%; IC95% = 65.7–92.3) was observed between pathologists regarding SSL-PB leprosy. An association was observed between a positive Mitsuda skin test and patients in Group I ($p < 0.05$), but no correlation with anti-PGL1 IgM antibody were observed. Single lesion leprosy does not appear to be a homogeneous entity. It is not possible to determine that all such cases are actually early disease. Mis-diagnosis and mis-classification of some multibacillary cases may affect assessment of outcomes of ROM. Correlation of only Group 1 with Mitsuda skin tests, and lack of any correlation with serologic assays, may be important considerations in efforts to develop tests for the early diagnosis of leprosy.

Job, C. K., Jayakumar, J. and Gillis, T. P. Transmission of leprosy—a study of skin and nasal secretions of multibacillary leprosy patients and their contacts using PCR.

It has been asserted and generally held that dissemination of *M. leprae* is from nasal mucosa and not through the skin of infected patients. This widely held notion is based on studies by Pedley (Lep. Rev. 41:31, 1970, *Ibid* 44:33, 1973) in which the method used for collecting *M. leprae* from skin specimens may have been inadequate. In these studies *M. leprae* was collected from the skin by pressing a glass slide on the skin of lepromatous patients followed by staining and microscopic evaluation of the slide. In the present study we evaluated *M. leprae* in the unbroken skin of lepromatous patients using a different method for collecting skin specimens. A defined area (312 cm^2) of the skin from the posterior surface of both upper arms ($3 \times 12 \times 2 = 72$ cm^2) and the back of both sides of the chest ($12 \times 10 \times 2 = 240$ cm^2) was washed with 50 ml of sterile saline and the sediment obtained from the washings was examined by direct microscopy and PCR for *M. leprae* DNA. Biopsies of the skin were also obtained to further evaluate the location of bacilli in the skin. Results showed that 6 out of 10 untreated lepromatous leprosy patients examined histologically had acid fast bacilli in the keratin layer. By PCR studies it was found that 8 of 10 patients had *M. leprae* DNA in skin washings and 7 of 10 had *M. leprae* DNA on swabs obtained from the nasal mucosa indicating that both anatomical sites may contribute significantly to transmitting leprosy. Contacts of the untreated lepromatous cases were also tested for exposure by analyzing skin washing and nasal secretions by PCR. PCR analysis showed significant skin exposure (53% positive) and no nasal mucosal exposure in contacts prior to instituting treatment of the index cases. After one month of MDT 1 of 30 (3.3%) contacts tested positive for *M. leprae* by PCR from nasal secretions and a similar number of contacts were positive for *M. leprae* in skin washings. These data suggested that 1) nasal carriage of *M. leprae* was minimal and skin exposure significant in the contacts of lepromatous cases, and 2) following one month of treatment with MDT the lepromatous index cases did not continue to shed bacilli at rates equal to that observed prior to treatment based on finding *M. leprae.*

Krahenbuhl, J. L. and Randhawa, B. Vital staining of *Mycobacterium leprae.*

A major tool became available to cell biologists a dozen or so years ago with the development of fluorescent tracker dyes, which allowed the stable labeling of mammalian cells. The technology was based on the incorporation of highly aliphatic reporter molecules containing fluorochrome head groups into the lipid bilayers of cytoplasmic membranes. A key feature of these dyes is their retention. Once incorporated, they are trapped in the membrane by virtue of their inherent insolubility in aqueous solutions. In a variety of mammalian cells these dyes have been shown to be stable and non-toxic, permitting tracking of adop-

tively transferred cells *in vivo* without interfering with their function, for example cytotoxicity. The tracking dyes do not interfere with doubling times of labeled cells and the dye appears to be equally partitioned between daughter cells when a labeled cell divides. More recently, a number of reports have employed tracker dyes to label prokaryote cells such as yeasts and bacteria. Studies were carried out to determine if fresh, viable nu/nu mouse derived *M. leprae* could be labeled with fluorescent tracker dyes and employed *in vitro* and *in vivo*. Two tracker dyes, PHK26 (red) and PKH65 (green) were employed to label *M. leprae* at concentrations ranging from 1:250 to 1:1000 and yielded bacilli clearly fluorescent extracellularly and intracellularly in cultured mouse macrophages. The viability of labeled *M. leprae* appeared to be completely retained as determined by radiorespirometry, a quantitative measure of metabolism that we have shown to be directly correlated with viability. The viability of labeled leprosy bacilli was subsequently confirmed by titration of *M. leprae* in the mouse foot pad model of infection. Growth of *M. leprae* labeled at various concentrations of PKH26 was indistinguishable from the growth of control unlabeled bacilli. These findings complement our laboratory's goal of characterizing fresh, abundant nu/nu mouse derived *M. leprae* as a research resource and will offer an important tool to investigators interested in the intracellular interaction of the live (or dead) leprosy bacillus with subcellular components and organelles of its various host cells.

Stefani, M. M. A., Robbins, N., Martelli, C. M. T., Pereira, G. A. S., Gillis, T. P., Krahenbul J. L. and the Brazilian Leprosy Study Group. Cytokine profile and *Mycobacterium leprae* DNA detection in biopsies of single skin lesion paucibacillary (SSL-PB) patients.

Measurements of local cell-mediated immunity and the presence of *M. leprae* in single skill lesion paucibacillary (SSL-PB) leprosy patients were studied to establish the immunological and bacteriological characteristics of patients designated to receive single dose ROM therapy. mRNA for cytokines (IFN-gamma, IL12, IL10, IL4 and TNF-alpha) was quantitated from skin biopsies (n = 39) by real time PCR and the presence of *M. leprae* DNA was assessed using an *M. leprae*-specific PCR (ML-PCR) test. Cytokine results were expressed as a raw data ratio relative to 18S rRNA quantities found in each biopsy and values were $\log_{10}$ transformed to correct for skewed distributions. High levels of TNF-alpha, low levels of IL12 and an absence of IL4 were found in the majority of biopsies. Stratification of the SSL-PB patients into tuberculoid (TT), borderline tuberculoid (BT) and Indeterminate (I) showed that the highest values for IFN-gamma were among the TT group (median = 1.77) followed by BT (median = 1.08) and the I group (median = 0.02). ML-PCR results stratified by the same criteria showed TT lesions yielded a lower percentage of positivity (38.5%) for *M. leprae* as compared to BT lesions (83.3%). Taken together these results are in concordance with the highly organized nature of granuloma seen in TT and strong CMI in these patients. The lowest values for all cytokines was in the indeterminate group of patients correlating with lower cellularity and more undifferentiated infiltrate characteristic of indeterminate leprosy. A weak but significant correlation was observed ($r = 0.4$, $p = 0.01$) between IFN-gamma and IL12 in the 39 patients suggesting that IL12 may drive IFN-gamma production in these early, single lesions. A strong correlation was observed ($r = 0.7$, $p < 0.01$) between IFN-gamma and IL10 suggesting a possible regulatory mechanism for IFN-gamma via IL10 to avoid excessive macrophage activation and subsequent pathology. Finally, cytokine profiles indicated that SSL-PB leprosy patients are tuberculoid-like with strong CMI suggesting good prognosis after ROM treatment.

Scollard, D. M. Endothelial cell uptake of *M. leprae* is not altered by cell activation.

Infection of endothelial cells (EC) by *M. leprae* is well documented in diagnostic skin biopsies. Ultrastructural studies of pe-

ripheral nerves in experimentally infected armadillos have revealed conspicuous infection of epineurial and endoneurial EC, suggesting that *M. leprae* enter the endoneurial compartment through its vasculature. Binding and ingestion of *M. leprae* by human umbilical vein endothelial cells (HUVEC) has also been demonstrated. Uptake was slower than reported by EC for other organisms but was saturable in both time and dose-response studies, suggesting that binding is specific. We therefore sought to determine whether or not activation of EC would affect the binding and uptake of *M. leprae in vitro.* Primary HUVEC were isolated from umbilical cords and cultured at 37°C in gelatin-coated 96 well plates, or 13 mm cover slips, and used after they had become confluent (4–7 days). HUVEC were activated by addition of recombinant human TNFa, 50 ng/ml, or endotoxin (LPS), 1000 ng/ml. Activation was confirmed by a cellular ELISA assay for expression of ICAM-1 (CD54), VCAM-1, and E-selectin (CD62E). Fresh *M. leprae* were obtained from nude mouse footpads, ^{14}C-labeled by incubation in commercial medium (BACTEC) for 10–12 days, washed × 4 by centrifugation, and manually counted. ^{14}C-*M. leprae* were added to HUVEC at a ratio of 100:1. Unbound bacilli were collected on a filter-pad after vigorous washing using an automated cell harvester; bound and ingested bacilli were similarly collected after lysing the cells with 0.01% SDS. The total radioactivity of each fraction was determined by liquid scintillation counting. Uptake of ^{14}C-*M. leprae* was low at 3–6 hr, and became maximal at 18–24 hr. No difference was observed in cultures performed at 33°C vs 37°C, and no increase in uptake over baseline was observed with cells cultured at 4°C. Activation of cells by TNFa or LPS produced maximal increases in expression of adhesion molecules at different times, as previously described. Neither agent, however, produced any increase in uptake of ^{14}C-*M. leprae* in any combination of pre- and post-incubation regimens, from 3–18 hr. Similarly, no increase in uptake of labeled organisms could be demonstrated after pre-incubation with unlabeled *M. leprae* for 1–18 hr.

Shannon, E. J. and Sandoval, F. Thalidomide's ability to enhance correlates of T-cell activation *in vitro* is dependent on the stimulant.

Thalidomide's mechanism of action in arresting ENL is unknown. Reports on thalidomide's effect on lymphocytes are contradictory. The purpose of this study was to determine if thalidomide influenced activation of lymphocytes and to partially characterize the phenotype of the responding cell. Peripheral blood mononuclear cells (PBMC) were incubated in the presence or absence of thalidomide and then stimulated with SEA, anti-CD3, Con-A, or PHA. The culture supernatant was assayed for IL-2 and IFN-γ. The cells were harvested to assess proliferation. The thalidomide-treated-mitogen-stimulated group produced significantly more IL-2 than the control group. The PBMC treated with thalidomide and stimulated with anti-CD3 or Con-A produced more IFN-γ than the control group. The PBMC treated with thalidomide and stimulated with SEA or PHA were suppressed in their ability to incorporate [H^3]-thymidine; whereas, thalidomide enhanced the incorporation of [H^3]-thymidine when the PBMC were stimulated with anti-CD3. The PBMC were sorted by negative selection using micro beads conjugated with anti-human CD8 or anti-human CD4. When SEA or anti-CD3 were used to stimulate the thalidomide-treated PBMC or CD4+ or CD8+ cells, the PBMC responded best in the synthesis of IL-2 and incorporated more [H^3]-thymidine than the CD4+ which responded far better than the CD8+ cells. Among mitogen stimulated PBMC, thalidomide acts synergistically with the mitogen to stimulate the production of IL-2. The particular lymphocyte targeted by thalidomide is CD4+. The type of response depended on the nature of the stimulating agent.

Truman, R. Trends in viability and the histopathological response to live *M. leprae* after intradermal inoculation in leprosy susceptible and resistant armadillos.

We examined the granulomas formed in the skin of armadillos in response to intradermal inoculation of highly viable *M. leprae* and to killed leprosy bacilli. We found that the granulomas formed against live *M. leprae* were significantly larger than those produced to *M. leprae* killed by heat, gamma irradiation or by freeze/thaw. Among Mitsuda(–) animals (n = 20) granulomas involving viable bacilli ranged 2–12 times larger in size those made to killed *M. leprae,* but their cellular composition was little changed. Mitsuda(+) animals showed similar enhancement with little qualitative difference in cellular composition. We used Radiorespirometry (RR) and conventional mouse foot pad technique (MFP) to examine the viability of *M. leprae* recovered from these intradermal inoculations. *M. leprae* viabilities fell markedly after initial inoculation and then stabilized. Bacilli recovered from living-Mitsuda reactions showed a broad range of viabilities with variation according to the Ridley-Jopling classification. Highest viabilities were found among bacilli from multibacillary hosts and the lowest from among BT's. Over a six week period, the viability of intradermal *M. leprae* among most multibacillary animals tended to increase. They tended to decrease or remain very low among BT and other paucibacillary hosts. The pattern for intradermal *M. leprae* viability among leprosy resistant Mitsuda(–) animals (N = 4) resembled that seen for BT hosts, with initial viabilities waning over time. The trends for intradermal viability also correlated with the outcome of systemic infection. After intravenous challenge with 10×10^9 *M. leprae,* the LL-spectrum animals that had showed high intradermal viabilities developed signs of fully disseminated disease, while the Mitsuda(–) resistant and paucibacillary animals remained free of leprosy. Actively metabolizing bacilli may produce antigens that are not present among killed bacillary preparations, and better understanding of the *M. leprae* antigens involved in resistance to leprosy by armadillos could significantly benefit our ability to identify and treat potentially susceptible human contacts.