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THIRTY-SECOND U.S.-JAPAN TUBERCULOSIS AND LEPROSY RESEARCH CONFERENCE

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

The 32nd meeting of the U.S.-Japan Cooperative Medical Sciences Program (Leprosy Panel) was held in Cleveland, Ohio, U.S.A., under the auspices of the National Institute of Allergy and Infectious Diseases, the National Institutes of Health, and Case Western Reserve University, on 21–23 July 1997. Since the Leprosy and Tuberculosis Panels of the U.S.-Japan Cooperative Medical Sciences Program amalgamated last year, the format has changed. The Panels themselves now have Co-Chairs and two other members, equally representing leprosy and tuberculosis research. The Panel members are: Dr. J. J. Ellner (Co-Chair), Dr. D. N. McMurray and Dr. P. C. Hopewell, representing tuberculosis research on the U.S. side; Dr. P. J. Brennan (Co-Chair), Dr. T. P. Gillis and Dr. G. Kaplan representing leprosy research on the U.S. side; Dr. I. Tsuyuguchi (Co-Chair), Dr. C. Abe and Dr. M. Mitsuyama, representing tuberculosis research on the Japanese side; and Dr. T. Yamada (Co-Chair), Dr. H. Saito and Dr. Y. Fukotomi, representing leprosy research on the Japanese side.

Abstracts of presentations on leprosy are presented below. The abstracts of presentations on tuberculosis research will be published in *Tubercle and Lung Disease.*

Dr. Brennan, in the course of his scientific presentation, said that, in his experience, there was no apparent role for basic biological research in the context of the immediate goal of leprosy elimination. He said that he was speaking in the context of his active participation in the recent meeting of the WHO Leprosy Elimination Advisory Group and the Joint WHO-ILEP Workshop on Reaching Undetected Patients in Endemic Countries. However, he saw an important role in the post-elimination era,

when the goal of statistical elimination is achieved and as we strive for disease eradication or deal with the consequences of leprosy again becoming a neglected disease. He exhorted basic researchers to be fully and enthusiastically supportive of the elimination goal but to be visionary and to be prepared for subsequent events and to avail themselves of all forums to try to define a global research strategy.

ABSTRACTS

Steel, W. F., Williams, D. L., Dallas, W. S. and Gillis, T. P. The *folP* of *M. leprae* and dapsone resistance.

Biomedical evidence suggests that dapsone (DDS) resistance in *Mycobacterium leprae* is related to the enzyme dihydropteroate synthase (DHPS). We have investigated the relationship between DHPS and DDS resistance by characterizing the *folP* homolog from resistant and susceptible *M. leprae*. The *folP* from a DDS-susceptible strain *of M. leprae* was amplified by polymerase chain reaction (PCR), cloned into the TA cloning vector pCR-2.1 and the DNA sequence of the *folP* was confirmed by direct DNA cycle sequencing. The resultant recombinant plasmid was transformed into a DHPS temperature-sensitive mutant of *Escherichia coli* (MC4100ts3). Clones were obtained which complemented the folate pathway at 42°C, providing evidence that the *M. leprae folP* encodes the DHPS. Two possible mechanisms of DDS resistance in *M. leprae* were then investigated: 1) mutations in the *folP* homolog, and 2) mutations in the promoter region or open reading frame (ORF1) immediately upstream of the *folP* homolog. The *folP* of three DDS-resistant and two DDS-susceptible *M. leprae* strains were amplified by PCR, and the DNA sequence determined. The *folP* sequences from all strains were identical. To determine if mutations within promoter-like regions upstream of the ORF1 contribute to DDS resistance, a putative promoter region was identified based on sequence comparison with known mycobacterial promoters. This region was amplified by PCR, and the DNA sequence was obtained from a DDS-susceptible strain and compared to a known DDS-resistant strain. No sequence differences in this region were observed. In summary, these studies indicate that resistance to DDS does not appear to be associated with mutations in either the structural gene or the promoter regions of the gene encoding DHPS in *M. leprae*.—[Department of Microbiology, Louisiana State University, Baton Rouge; Molecular Biology Research Department, Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA; Molecular Science, Glaxo Wellcome Inc., Research Triangle, NC, U.S.A.]

Silbaq, F. S., TerLouw, S. M., Cho, S.-N. and Brennan, P. J. *M. leprae rpsL* and *rplR* genes.

We describe the sequences of two *Mycobacterium leprae* ribosomal genes: *rpsL* that encodes the S12 ribosomal protein (*str* operon) which binds to the 16S RNA and *rplR* that encodes the L18 ribosomal proteins (*spc* operon) which is one of three proteins that mediate the attachment of the 5S RNA into the large ribosomal subunit. The results show that the S12 and L18 ribosomal proteins are conserved between mycobacterial species and between mycobacteria and other prokaryotes. However, the *M. leprae rpsL* sequence reveals two ATG codons separated by an 87-bp fragment at the 5′ end. This fragment was absent from the corresponding genes of *M. bovis, M. intracellulare, Micrococcus luteus, Escherichia coli* and other prokaryotes. In spite of this 87-bp difference, the sequence of the *M. leprae rpsL* gene had a high homology to the *M. bovis rpsL* gene (87%). A similar homology was also found between *M. leprae* and *M. tuberculosis rplR* (83%). Expression of the *M. leprae rpsL* gene from

the two start codons produces two different proteins, 17 kDa (153 aa) and 14 kDa (124 aa), sizes that correlate with the deduced amino acid sequences from the two start codons. An oligopeptide was synthesized from the first 29 aa of the S12 protein, and serology tests with leprosy patient antibodies showed positive signals to the oligopeptide, i.e., the first 29 aa of the S12 ribosomal protein. The major implication of this work is that the *M. leprae* S12 ribosomal protein is bigger than that expected from the deduced amino acids of the *M. leprae rpsL* gene, published previously, and the corresponding genes from other prokaryotes. This difference may have a major effect on the slow growth rate and the low optimum temperature of *M. leprae.*—[Department of Microbiology, Colorado State University, Fort Collins, CO, U.S.A.]

Marques, M. A. M., Pessolani, M. C. V., Cho, S.-N. and Brennan, P. J. The mapping and identification of the expressed proteins of *M. leprae.*

Mycobacterium leprae purified from infected armadillos was disrupted by sonication and the subcellular fractions—membrane, cell wall and cytosol—were obtained by repeated differential centrifugation and sucrose gradient centrifugation. The subfractions were then submitted to the following: a) protein profiling by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE), and by bidimensional electrophoresis; b) glycolipid mapping by a modified silver staining procedure; c) monosaccharide profiling by gas-liquid chromatography; d) quantification of diaminopimelic acid (DAP) as a marker of cell wall by gas-liquid chromatography; e) reactivity to a set of monoclonal antibodies by immunoblotting; and f) determination of phenolic glycolipid-I (PGL-I) content as a capsule/"integument" marker by an ELISA. A total of 374 different major proteins were identified in the three fractions, with each fraction showing a unique and consistent protein profile. The previously described GroES (10 kDa), MMPI (35 kDa) and GroEL (65 kDa) proteins were detected preferentially in the cytosol, membrane and cell wall, respectively, and proved to be reliable protein markers of these compartments. The glycolipids, lipoarabinomannan, lipomannan and phosphatidylinositol mannosides were observed in all fractions. Analysis for monosaccharides and DAP provided excellent quality control in that there was no galactose (from the cell wall) in the cytosol fraction and little mannose in the cell wall (hence, LAM/LM is mostly membrane/cytosolic). DAP and PGL-I predominated in the cell wall fractions. Several of the new proteins from the cell wall were identified by N-terminal sequence analysis. This exercise provides us with added insight into the physiological functions of sub-cellular proteins.—[Colorado State University, Fort Collins, CO, U.S.A.; Oswaldo Cruz Foundation, Rio de Janeiro, Brazil]

Adams, L. B., Scollard, D. M. and Krahenbuhl, J. L. Reactive nitrogen intermediates regulate granuloma formation in *M. leprae*-infected mice.

Production of reactive nitrogen intermediates (RNI) by inducible nitric oxide synthase (iNOS) is a major antimicrobial mechanism of activated macrophages. C57BL/6 mice (B6), B6 mice given aminoguanidine (AG), an inhibitor of iNOS, and iNOS gene knockout mice (iNOS KO) were infected i.v. with *Mycobacterium leprae.* Liver tissue from B6 showed slow development of granulomas, with moderate-size, nonorganized, inflammatory infiltrates surrounding the bacilli. AG mice and iNOS KO mice exhibited large, focally organized granulomas containing multinucleated giant cells. CD4 and CD8 immunostainings were similar to human tuberculoid granulomas, with CD4+ cells dispersed throughout the granulomas and CD8+ cells located peripherally. iNOS mRNA was detected in granulomatous liver tissue from B6, but none was found in the iNOS KO mice. Interestingly, high levels of iNOS mRNA were detected in AG mice. *In vitro,* iNOS KO activated macrophages and B6 activated macrophages treated with AG produced negligible nitrite and could not kill *M. leprae.* Like activated B6 macrophages, though, activated iNOS KO macrophages generated tumor necrosis factor-

alpha (TNF-α). Intracellular mycobactericidal events may be suppressed *in vivo* in the absence of RNI and, in compensation, granuloma formation may be greatly enhanced. This suggests that production of RNI is an important regulator of cell-mediated immunity to *M. leprae*.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Ohara, N., Matsuoka, M., Nomaguchi, H., Yukitake, H., Matsumoto, S., Naito, M., Mise, K. and Yamada, T. Protection of the infection of *M. leprae* by ribosomal particle, culture filtrate, and α antigen from BCG.

Immunization of mice with the ribosomal fraction from ruptured *Mycobacterium bovis* BCG (BCG), the culture filtrate, and components of the antigen 85 complex (85A, 85B = α antigen, and 85C) remarkably reduced the multiplication of *M. leprae* in the foot pads of mice. This is the first reported case of the protective activity against *M. leprae* multiplication in mice of BCG ribosomal fraction and the culture filtrate. The inhibition was most evident with the 85A components of the antigen 85 complex. When the ribosomal proteins separated from ribosomal RNA were injected into mice, only a slight inhibition was observed. Ribosomal RNA alone did not inhibit at all, in contrast to the conclusion reported by Youmans and Youmans.—[Nagasaki University School of Dentistry, Nagasaki; Leprosy Research Center, National Institute of Infectious Diseases, Tokyo; National Institute of Health Sciences, Tokyo, Japan]

Ohyama, H., Matsushita, S., Hatano, K., Makino, M., Kato, N., Oyaizu, K., Takashiba, S. and Murayama, Y. Human T-cell responses to major membrane protein II (MMP II) derived from *M. leprae*.

M. leprae is reported to have two immunodominant outer membrane proteins, MMP I and MMP II, which were reported to be highly antigenic in leprosy patients. MMP II, a 22-kDa protein, was identified initially as a bacterioferritin of *M. leprae* by Pessolani, *et al.* Recently, it was reported that most leprosy patients showed a higher IgG titer in their sera than did healthy controls, and it was hypothesized that the immune responses to MMP II might reflect the immune system responses to *M. leprae* in leprosy patients.

In this study, we have evaluated T-cell responses to MMP II in nine subjects, including leprosy patients. Among them, peripheral blood mononuclear cells (PBMC) derived from one tuberculoid-type patient (DRB1*1501-DQA1*0102-DQB1*0602/DRB1*1101-DQA1*0501-DQB1*0302) showed remarkable proliferation when challenged with a mixture of each overlapping peptide covering the entire MMP II amino acid sequence. We established a MMP II-specific T-cell line (KT line) from PBMC in this tuberculoid patient and established the major T-cell epitopes of MMP II. This KT line reacted to two peptides, MMP II p67–87, including the DRB1*1501 binding motif, and MMP II p89–109, including the DRB1*1101 binding motif. MMP II p89–109 induced a much higher proliferative response in the KT line than did MMP II p67–87. Moreover, anti-DRB1 monoclonal antibody markedly inhibited MMP II-induced proliferation of the KT line. It is concluded that HLA-DRB1*1101 is one of the immune response (Ir) genes which induces a high T-cell response to MMP II.—[National Leprosarium Oku Komyo-En, Okayama; Department of Periodontology and Endodontology, Okayama University Dental School, Okayama; Division of Immunogenetics, Department of Neuroscience and Immunology, Kumamoto University Graduate School of Medical Sciences, Kumamoto-shi, Japan]

Goto, M., Matsuoka, M. and Kitajima, S.-I. Animal model of leprous neuritis by the transfer of CD4+ lymphocytes into *M. leprae*-inoculated nude mice.

In order to establish animal models of leprous neuritis and to analyze the pathogenesis, *Mycobacterium leprae*-inoculated BALB/C-nu/nu mice were challenged by *M. leprae*-immunized BALB/C mouse spleen-derived CD4+ cells, and the results

were compared with a naive BALB/C mouse spleen-derived CD4+ cell change model and a *M. leprae*-immunized whole spleen cell challenge model. In the cell transfer experiments, nude mouse food pads showed increased swelling. Histological examination of foot pad nerve fascicles showed infiltration of inflammatory cells and a decrease of the myelinated axons in the immunized CD4+ cell transfer mice and in the immunized whole spleen cell transfer mice. In the nerves, acid-fast bacilli showed fragmentation. In the naive CD4+ cell transfer mice, the nerve lesion was similar but mild. These results indicate that CD4+ lymphocytes play an important role in experimental leprous neuritis formation.—[National Leprosarium Hoshizuka-Keiai-En, Kaya-shi; Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Fukutomi, Y., Toratani, S., Matsuki, G. and Matsuoka, M. Involvement of nitric oxide in induction of anti-*M. leprae* activity of mouse macrophages and cytokines that induce or suppress the activity.

Mycobacterium leprae, the causative agent of leprosy, is an obligate intracellular pathogen that prefers the mononuclear phagocyte as its host cell. A major topic is the study of the mechanisms of macrophages to cope with *M. leprae* in order to understand host resistance to leprosy. In the present study, *M. leprae*-infected mouse macrophages were incubated *in vitro* in the presence of various cytokines followed by measurement of metabolic activity of the bacilli for evaluation of viability. Gamma-interferon (IFN-γ) addition to the culture induced activation of the macrophages accompanied by enhanced anti-*M. leprae* activity and accumulation in the culture medium of nitrite/nitrate which were stable products of nitric oxide generated by the activated macrophages. Released tumor necrosis factor (TNF) by macrophages in response to *M. leprae* was also indispensable in the induction of anti-*M. leprae* activity as a second signal with IFN-γ priming. T-cell growth factor-beta (TGF-β) inhibited both anti-*M. leprae* activity and nitric oxide production. Interleukin-10 (IL-10), on the other hand, could not suppress the activity. Rather, it enhanced the activity with the enhanced nitric oxide production. Concerning TNF production induced in macrophages by *M. leprae* phagocytosis, IL-10 suppressed both TNF protein production and TNF mRNA expression in contrast to the enhanced TNF production by TGF-β. From these results, we conclude that suppressed nitric oxide production induced by TGF-β could result in suppression of the anti-*M. leprae* response of macrophages. The amount of TNF-β released in the culture supernatant did not directly affect the extent of the activation.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Matsuoka, M., Gidoh, M., Kashiwabara, Y., Nagao, E. and Kinjoh, K. Rifampin-resistant isolates from Japanese leprosy patients and mutation in the *rpoB* gene.

Rifampin-resistant *Mycobacterium leprae* from Japanese patients suffering from a relapse were isolated in nude mouse foot pads. Susceptibility against antileprosy drugs was examined either by the mouse foot pad method or the Buddemeyer system. An isolate exhibited no difference in bacillary growth in the foot pad of the mice between nontreated mice, log 8.71 ± 0.197, and mice fed diets mixed with rifampin in a concentration of 0.01% at 50 weeks of infection. The isolate was regarded as rifampin resistant. The isolate was also resistant against KRM 1648, since no reduction of radioactivity was shown at any concentration examined in the Buddemeyer system. A susceptibility test for dapsone (DDS) analyzed by the foot pad method also indicated that the isolate was DDS resistant at a low level. Direct sequencing of the variant revealed a single base change from C to T in codon 531 of the *rpoB* gene, as shown in many other rifampin-resistant isolates. Another biopsy specimen was obtained from a patient suspected to be rifampin resistant and inoculated, 1.0×10^4/0.05 ml, into the nude mouse foot pads. Bacillary numbers in the foot pads of the control mice were log 8.74 ± 0.26 and log 5.78 ± 0.10 in the foot pads of the mice treated with rifampin. This isolate is re-

garded as rifampin resistant at a low level. Silent mutation, a single base change from C to T, was observed in codon 523 of the *rpoB* gene. No mutation was revealed at any rifampin-binding sites of the *rpoB* gene of this isolate. The clinical history suggested that this isolate is multidrug resistant, and another reason that confers multidrug resistance must be considered, such as discharge of the drugs by the efflux pump.

The banding pattern of PCR-SSCP was different between the variant and the wild type. A simple mutation detection method directed to the previously revealed hot spot in the *rpoB* gene is anticipated to be applicable for the detection of most rifampin-resistant cases in Japan. The result of the silent mutation indicated that the samples which harbor the mutation in screening tests, such as PCR-SSCP, must be determined by sequencing for rifampin resistance.—[Leprosy Research Center, National Institute of Infectious Diseases, Tokyo; National Leprosarium Okinawa Airaku-En, Nago-shi, Japan]

Okamura, H., Nomaguchi, H., Kawatsu, K. and Izumi, S. Relationship of leprosy types and the serum levels of IL-18.

Leprosy is classified into tuberculoid (T), borderline (B), and lepromatous (L) types according to pathological features. Recent investigations have clarified that there are biases in the cytokine types appearing in the lesions of the patients. Gamma-interferon (IFN-γ) appears dominantly in the lesions of T patients, and interleukin-4 (IL-4), -5 and -10 in L patients, although these cytokines are usually not detectable in the circulation. IL-18 (IFN-γ-inducing factor), a recently cloned cytokine, is produced by activated macrophages and induces IFN-γ, augments natural killer (NK) activity of splenocytes, and induces Fas ligand in T or NK cells. A marked synergism between IL-18 and IL-12 is observable for these actions. It has also been clarified that the IL-18 precursor has an unusual leader sequence, like IL-1β, and is processed by an IL-1β-converting enzyme (caspase 1) to the active form. Moreover, IL-18 has been proved to be produced by various types of cells other than macrophages, such as keratinocytes, osteoblasts, intestinal epithelial cells, and pituitary or adrenal cortex gland cells. Since IL-18 strongly induces the Thl cytokine IFN-γ, it was expected that IL-18 is expressed dominantly in the T type of leprosy. Thus, in the present study, the levels of IL-18 in the circulation of various types of patients were examined.

Human IL-18 was assayed using an ELISA kit (Hayashibara, Okayama, Japan). IL-10 and IL-12 were measured by the commercial ELISA kit, and the titration of anti-phenolic glycolipid-I (PGL-I) IgM antibody was carried out by an ELISA or an agglutination method. Unexpectedly, the serum levels of IL-18 were much higher in the blood of L patients than in that of T and B patients, which had the same levels as the healthy controls. Moreover, IL-18 levels in the circulation of L patients who were cured by successful treatment were significantly lower than those patients with active lepromatous leprosy. It was of interest that serum IL-18 levels were apparently in parallel with the levels of anti-PGL-I IgM levels. IL-10 and IL-12 in the blood were almost undetectable for any patient, and failed to be elucidated for their relationship to the leprosy types. It could be considered from these results that IL-18 is produced irrespectively of the dominancy of the Th subtypes. The patho-physiological roles of IL-18 have to be clarified. However, the long-existing IL-18 in the circulation of L patients may be related to the pathological aspects of leprosy. IL-18 has strong activity to induce the molecules concerned with tissue injury in T or NK cells. It also affects the secretion of some hormones. Thus, it is still probable that IL-18 is involved in the pathogenesis of leprosy. This cytokine may become one of the tools for the elucidation of the pathogenic mechanism of this difficult disease.—[Laboratory of Host Defenses, Hyogo College of Medicine, Nishinomiya; Institute for Hansen Disease, Tokyo, Japan]

Kobayashi, K., Gidoh, M., Kai, M. and Saito, J. Role of type 1 helper T-cell gamma-interferon-(IFN-γ)-inducing cytokines in experimental *M. leprae* infection in mice.

Cell-mediated immunity (CMI) participates in the host defense against mycobac-

terial infection. Both interleukin-12 (IL-12) and interferon-gamma-inducing factor (IGIF/IL-18), produced mainly by macrophages, play critical roles in the expression of CMI. To investigate the role of IL-12 and IGIF/IL-18 *in vivo,* we have analyzed the cytokine profile and bacterial growth in genetically susceptible and resistant mice infected with *Mycobacterium leprae.* The early expression of IL-12 p4O and IGIF/IL-18 at the site of inoculation was found in resistant mice 3–72 hr after infection, but not in susceptible mice. Both strains of mice did not show expression of IL-4. Replacement IL-12 therapy resulted in the reduction of bacterial counts in mice with established *M. leprae* infection. The results imply that susceptible mice exhibit a decreased type 1 helper T (Th1) lymphocyte response without a reciprocal increased Th2 response, and that the intrinsic defect of macrophages is responsible for the susceptibility. In addition, IL-12 therapy may be a possible rationale for treatment of *M. leprae* infection.—[Departments of Bioregulation and Microbiology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan]

Scollard, D. M., Gillis, T. P. and Williams, D. L. PCR assay for the detection and identification of *M. leprae* in the U.S.

The differentiation of leprosy from other cutaneous granulomatous diseases is routinely based on characteristic histopathologic features and the demonstration of *Mycobacterium leprae* by acid-fast staining. Increased ascertainment of other mycobacterial infections in the skin has made this task more difficult, but the distinction remains fundamental in selecting appropriate treatment. Experience with formalin-fixed and paraffin-embedded tissues, frozen tissues, and tissue lysates referred for *M. leprae*-polymerase chain reaction (PCR) during the past 4 years has been reviewed. PCR was performed using primers and probes previously developed to amplify a 360-bp fragment of the gene and for an 18-kDa protein of *M. leprae.* Among 32 biopsies, PCR was positive in 8/16 diagnosed as leprosy, and in 0/13 diagnosed as nonleprosy by histopathologic criteria. PCR also identified *M. leprae* in 1/3 biopsies in which acid-fast organisms were seen but leprosy was not diagnosed histologically. In a nonendemic population, the sensitivity and specificity of this technique recommend its use primarily to identify *M. leprae* when acid-fast organisms are discernible but atypical clinical or histopathologic features obscure the diagnosis.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Scollard, D. M., McCormick, G. T. and Allen, J. L. Early localization of *M. leprae* to epineural blood vessels.

Infection of the peripheral nerve by *Mycobacterium leprae* is the histopathologic hallmark of leprosy, and is a major factor in the deformity and social opprobrium of this disease, but the mechanisms by which the bacillus localizes to the peripheral nerve are not known. Nerve involvement in experimentally infected armadillos is very similar to that in man; notably, the early accumulation of bacilli in this model is to the epineurium. To identify the cellular location of epineural *M. leprae,* 1-cm segments of proximal portions of seven peripheral nerves from five infected armadillos were processed in Spurr resin for both high resolution light microscopy and ultrastructural examination. *M. leprae* were found in 18/47 specimens (38%), of which 16 (89%) were in the epineurium. The bacilli were usually located in the endothelium or in the adventitia of small blood vessels. *M. leprae* were not found in the epineural lymphatics. These results confirm earlier observations that *M. leprae* localize to the epineurium early in the pathogenesis of nerve involvement in leprosy, and support the hypothesis that this is due to interaction with vascular endothelium.—[Department of Research Pathology, Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA, U.S.A.]

Scollard, D. M., Gillis, T. P., Gormus, B. J., Lathrop, G. and Murphey-Corb, M. Simian immunodeficiency virus (SIV)-associated delay of CD4 cell accumulation at *M. leprae* sites *in vivo* is accompanied by a delay in lymphocyte responses *in vitro.*

The mechanism by which immunity to mycobacteria is deranged in HIV-infected individuals (and SIV-infected rhesus monkeys) is not known. Biopsies were taken at cutaneous *Mycobacterium leprae* inoculation sites in four SIV-negative control rhesus monkeys and eight SIV-positive monkeys with advanced SAIDS. The percentages of CD4 and CD8 cells were determined by immunostaining of frozen sections, and in blood by FACS analysis. Specific lymphocyte responsiveness to *M. leprae* by ^{3}H-thymidine after 6 days of culture with *M. leprae* sonicate antigen (MLS) was also measured. The percentage of CD4 lymphocytes peaked at 5 days in healthy, SIV-negative rhesus monkeys, but was delayed until 27 days in the eight SIV-positive animals with advanced SAIDS.

In four SIV-negative animals, lymphocyte responsiveness increased steadily from 3 weeks onward; all four were responsive at 15 weeks. In eight SIV-positive animals, responsiveness was not observed until 5 weeks (1/6 animals), and only 4/5 were responsive at 15 weeks. A delay has been observed in the early CD4 participation *in situ* in SIV-positive animals. Robust mechanisms of recruitment appear to remain in SIV-positive animals since the percentage of CD4 cells at inoculation sites ultimately reaches levels similar to the maximum in SIV-negative animals. The delay in CD4 recruitment appears to be associated with a delay in the initiation of specific, systemic immunity to *M. leprae* in SIV-positive animals. This suggests that early events in the response to mycobacteria, which may be critical in developing effective immunity, are disrupted by prior infection with SIV. *M. leprae* infection in rhesus monkeys may be a useful model with which to study the immunologic aberrations induced by retroviruses.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA; Tulane Regional Primate Center, Covington, LA, U.S.A.]

Shannon, E. J., Aseffa, A., Sandoval, F., Shitaye, A. and Webster, K. Thalidomide enhances the synthesis of IL-2 in ENL patients.

Background: There is a renewed interest in thalidomide due to its successful use in the treatment of dermatological conditions other than erythema nodosum leprosum (ENL). Although the exact mechanism(s) by which thalidomide attenuates inflammation is unknown, modulation of the immune response plays a role.

Design: Peripheral blood mononuclear cells (PBMC) obtained from patients during ENL (prior to treatment with thalidomide) were exposed for 2 hr to 4.0 μg/ml of thalidomide and stimulated with 50 ng/ml of staphylococcal enterotoxin A (SEA). After 16 hr the culture supernatant was assessed for interleukin-12 (IL-2) by an ELISA.

Results: Compared to SEA-stimulated cells there was an increase of 127 ± 17 pg/ml in IL-2 secreted by the thalidomide-SEA-treated cells. When compared to plasma from untreated patients, and after ingesting 300 mg of thalidomide, there was a mean increase of 5.4 pg/ml of IL-2.

Conclusion: Besides enhancement of IL-2 by thalidomide in ENL patients, we have observed thalidomide to enhance IL-2 in SEA-stimulated PBMC from HIV-seropositive patients and PBMC from healthy individuals. Ostraat, *et al.* (Transplant Immunol. 4:117, 1996) report similar IL-2 enhancement by thalidomide.—[Laboratory Research Branch, GWL Hansen's Disease Center at Louisiana State University, Baton Rouge, LA; Gondar College of Medical Sciences, Gondar, Ethiopia; Clinical Branch, GWL Hansen's Disease Center, Carville, LA, U.S.A.]