OI 32

THE ROLE OF TH1 AND TH2 CYTOKINES IN ACUTE LEPROSY NEURITIS

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Study: To assess cytokine production in nerves from patients with acute neuritis (defined as tenderness and/or loss of function within the last six months). The clinical samples skin and nerve biopsies from 57 patients with acute neuritis (BT = 30, BL = 18 and LL = 9) were collected.

Immunohistochemistry was done on skin and nerve sections to detect the cytokine proteins IFN-γ, IL-6, IL-10, IL-12, IL-13, TNF-α, TGF-β and iNOS.

Results: Morphology: Granulomas were better defined and organised in nerve lesions. Cellular infiltration also more prominent in nerve. Th1 type cytokines (IFN-γ and IL-12) were present at high levels in skin and nerve. Nerves from LL patients had both low levels of IFN-γ and IL-12 and moderate levels of IL-6 and TGF-(3. Th2 type cytokines IL-6, IL-10 and IL-13 were present across the spectrum.

Comments: Nerve damage may occur through two mechanisms, a Th1 dependent mechanism in BT and BL patients and a Th2 dependent mechanism in LL patients.

OI 33

TNF PROMOTER GENOTYPE INFLUENCE TNF PRODUCTION IN LPS- BUT NOT *Mycobacterium leprae*- STIMULATED WHOLE BLOOD CELLS IN VITRO.

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Single nucleotide polymorphisms (SNP) on TNF promoter are associated with the risk and progression of infectious and inflammatory diseases. Mutations at position -308 in TNFα (TNF2) promoter gene might affect levels of the cytokine production that are central in the outcome and the natural course of leprosy. The study was set out to investigate the contribution of TNF2 SNP in the cytokine mRNA expression and protein secretion in vitro. Paucibacillary leprosy patients were genotyped by PCR-RFLP for the presence of TNF2 allele (carriers = 13, non-carriers = 21). Whole blood cells from these patients were stimulated with LPS (1ng/ml) and *M. leprae* (1μg/ml). To mRNA expression analysis, semi-quantitative RT-PCR was performed after 3h stimulation. TNFα mRNA did not show any differences among the patients analyzed regardless the stimulus or the genotype of the patients. Nevertheless, LPS induced an increased in TNFα secretion in TNF2 carriers as compared to non-TNF2 carriers at 6h only ($p<0,05$). In *M. leprae*-stimulated cultures no significant differences were achieved. TNF2 allele influence the increased production of LPS-stimulated TNFα production was time dependent and restricted at the protein levels suggesting a post transcriptional regulatory role associated to the promoter polymorphism.

MICROBIOLOGY & MOLECULAR BIOLOGY

OM&BM 1

ACCUMULATION OF NORFLOXACIN AND DAPSONE IN *M. smegmatis*

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Quinolones are being increasingly used as second-line agents in the treatment of tuberculosis caused by multidrug-resistant strains. Dapsone is the main component of the MDT regimen for leprosy. At this juncture adequate knowledge of the transport of these chemotherapeutic agents will be of help in the development of new agents. A preliminary study has been conducted at this Institute on the accumulation of norfloxacin and dapsone using modified fluorescence methods. By employing exogenous norfloxacin concentration of 10 ug/ml, a steady state concentration (SSC) of 100 ng of norfloxacin/mg cells, by dry weight was obtained for *M. smegmatis*. Adequate care was taken to nullify the errors due to drug adsorption to the cell surface and to maximise the desorption using several standardised washings in the buffer. The addition of either dinitrophenol (2.0 mM) or CCCP (150 uM) 10 minutes before or

after addition of norfloxacin did not affect drug accumulation suggesting an absence of energy involvement in its transport process. In a parallel study on dapsone accumulation, the drug accumulated to a level of 78-106 ng/mg cells of *M.smegmatis* (by dry weight) during 15-60 minutes of incubation at an exogenous concentration of 10 ug/ml. Further studies using other agents like ofloxacin, rifampicin and clofazimine and several other mycobacteria are being planned. The experimental conditions for each mycobacterial strain and each antimicrobial agent are to be suitably standardised in order to get useful information. The method, once standardised, will be applied to drug resistant strains so as to evaluate the role of efflux pump in the emergence of drug resistance.

OM&BM 2

AN *IN VITRO* MODEL FOR STUDYING THE EFFECTS OF *M. leprae* ON SCHWANN CELL/NEURON INTERACTIONS

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Globally, millions of leprosy patients suffer from irreversible nerve damage, resulting in disabilities or blindness as a consequence of infection with *Mycobacterium leprae*, an obligate intracellular pathogen. The mechanisms of nerve damage have not been fully elucidated due to a lack of a well-developed *in vitro* model which maintains the viability of *M. leprae* and closely mimics disease conditions. Therefore, an *in vitro* model was developed using freshly harvested nude mouse-derived *M. leprae*, rat Schwann cells and Schwann cell/neuron co-cultures incubated at 33^0 C, a conductive temperature for *M. leprae* viability. At 33^0 C, Schwann cells and mitogen-expanded Schwann cells appeared to be morphologically similar, express similar levels of Schwann cell markers and function in a comparable manner when seeded onto cultured neurons as those cells maintained at 37^0 C. *M. leprae* within Schwann cells retained 56 % of their original viability for at least 3 weeks post infection at 33^{0}C compared to only 3 % at 37^0 C. Infected cells exhibited morphological changes, gene expression alterations 33^0 C, but were capable of interacting with and myelinating neurons. Infected myelinated co-cultures maintained myelin sheath architecture and were morphological comparable to non-infected cultures at both temperatures. In conclusion, an improved model for studying the effects of *M. leprae* on Schwann cells has been described. Preliminary results using this model indicate that *M. leprae,* under the conditions specified, do not appear to have detrimental effects on Schwann cell functional capabilities in the peripheral nerve and suggest that the majority of the neuropathy observed in leprosy is most likely due to an aggressive immune response to infection within the nerve.

OM&BM 3

CARBOHYDRATE METABOLISM IN LEPROSY PATIENTS

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Leprosy infection is often accompanied by metabolic disturbances, and carbohydrate metabolism is no exception. To a large extent, parameters of carbohydrate metabolism depend on sex and age of the patients and reflect a metabolic state at the moment of taking samples. A state of carbohydrate metabolism was estimated in 150 leprosy cases, including 117 (78%) patients with multibacillary (MB) and 33 patients (22%) with paucibacillary (PB) forms of leprosy. Among the patients (of 45 to 93 years old) there were 75 males and 75 females. Carbohydrate metabolism was assessed by integral index of glycemia, i.e. glycolized hemoglobin (HbA1c) defined for the past 3 months by means of colorimeter method. Upper normal limit of HbA1c was 7,5%. In leprosy patients with normal carbohydrate metabolism level of HbA1c did not depend on either sex or age. Disturbances in carbohydrate metabolism were found out in 37 patients (25% of all studied), out of them there were 30 MB (81%) and 7 PB (19%) patients. Ratio of the patients with and without metabolic disturbances was approximately 1:3 in MB and 1:4 in PB leprosy. Diabetes II type was found out in 13 MB-patients (11%) and in 2 patients with PB leprosy (6%). Prevalence of diabetes in patients under observation was 10%. In one case diabetes preceded the development of PB-leprosy, and in other cases sugar disease occurs against the background of leprosy process. Latent disturbances of carbohydrate metabolism were detected in 15% of cases observed. Thus, the data obtained showed a higher prevalence of carbohydrate metabolic disturbances in leprosy patients (to 11%) as compared with that in general population, necessitating further investigations to elucidate their possible causes and mechanisms.

OM&BM 4

COMPARISON OF PCR MEDIATED AMPLIFICATION OF DNA AND THE CLASSICAL METHODS FOR THE DETECTION OF M*ycobacterium leprae* IN LEPROSY PATIENTS AND CONTACTS

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Traditional staining and microscopic examination techniques for the detection of *Mycobacterium leprae* and Polymerase chain reaction (PCR), DNA amplification of a 531-bp fragment of the *Mycobacterium leprae* specific pra gene were compared on different clinical specimens on 60 leprosy patients attending the Sanatorium of Fontilles and divided for the purpose of the study in. multibacillary patients (MB) with positive Bacteriological Index (BI), 30 MB patients with negative BI and, 10 paucibacillary (PB) together with 4 non-leprosy patients as controls.

The results in the multibacillary BI positive group show a good correlation between practically all methods and specimens, most techniques detecting 100% of the cases.

The results in the MB negative group reveal that a combination of test (humoral response to D-BSA, together with PCR biopsy and PCR post biopsy swab) are the most sensitive in some cases of this group for monitoring leprosy patients who have completed chemotherapy. In the paucibacillary group no level of positivity was detected by conventional or PCR methods.

The prevalence of antibodies to *Mycobacterium leprae* antigens in serum was measured together with the presence of *Mycobacterium leprae* DNA in the nose and lepromin status in a group of 43 contacts of leprosy patients. Two individuals were found to form a potential high risk group.

OM&BM 5

DETECTION OF ANTILEPROTIC DRUG (S) INDUCED DNA DAMAGE IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES BY THE ALKALINE SINGLE CELL GEL ELECTROPHORESIS

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Multidrug treatment (MDT) is the WHO recommended method of treatment for leprosy. In MDT, dapsone and rifampicine are effective chemotherapy followed by other frontline drugs like clofazamine, and ofloxacin. These drugs are reported to induce cytogenetic damage in different test systems. Our previous studies indicated higher incidence of DNA strand breaks, chromosomal aberration, and micronucleus frequency in the peripheral blood lymphocytes of leprosy patients treated with MDT. Therefore, to clarify the possible role of components of MDT in inducing DNA damage in leprosy patients, in this study, the induction of DNA damage by antileprotic drugs (dapsone, rifampicin, clofazamine, minocycline and ofloxacin) and subsequent repair was investigated by the alkaline comet assay in human blood lymphocytes. Lymphocytes isolated from leprosy patients and healthy individuals were treated with increasing concentrations of antileprotic drug(s) for varying duration of exposure and subjected for the comet assay. Metabolic activation/inactivation of the drugs was studied by incorporating rodent liver microsomal activation system (S9-mix). DNA damage data in lymphocytes of leprosy patients were compared with that of health individuals.

OM&BM 6

DETECTION OF *Mycobacterium leprae* NASAL CARRIERS BY POLYMERASE CHAIN REACTION IN SINGLE LESION LEPROSY PATIENTS

Silva, M.H.M., Castro, F., Visconde, A.M., Sousa, A.L.O.M., Rebello, P.F.B., Gomes, M.K., Nararashi, K., Sacchetim, S.C., Costa, M.B., Stefani, M.M.A., Martelli, C.M.T. and Gillis, T.P.

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Objective: Detect *M. leprae* DNA by PCR in nasal swabs among Brazilian single skin lesion paucibacillary leprosy patients (SSL-PB).

Methods: 259 newly detected SSL-PB leprosy patients, negative baciloscopy, were recruited in 3 endemic regions. 155 nasal swabs and 134 skin biopsies were collected before ROM therapy, snap-frozen and stored (liquid nitrogen) for *M. leprae* DNA detection by PCR. After DNA extraction each specimen was amplified, undiluted and at 1:5, using pairs of primers for a 360 bp *M. leprae* specific fragment and products detected by slot blot hybridization using digoxygenin-labeled 212bp DNA probe. Specimens were coded and tested blinded to patient's characteristics at IPTSP/Brazil in collaboration with National Hansen's Disease Programs.

Results: In nasal swabs, *M. leprae* DNA was detected in 9.7% (15/155) of SSL-PB. Higher positivity (14.1%) was found among specimens from patients living in the North region compared with samples from Southeast and Central Brazil, compatible with endemic levels. No association was found between patient's characteristics or presence of household leprosy contact with PCR positivity. There was no agreement between positivity of *M. leprae* DNA PCR in skin biopsies and nasal swabs (Kappa=0.07).

Conclusion: Detection of *M. leprae* DNA in nasal swabs from SSL-PB patients may reflect exposure in endemic areas without agreement with bacilli detection in skin biopsy by PCR. *TDR/WHO grant 981007*

OM&BM 7

DISTINGUISHING VARIANTS OF *M. leprae* LABORATORY STRAINS.

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Genotyping has practical application in outbreak investigations and variant classification of cultured strains. Though remarkably little variability has been noted among *M. leprae,* in recent times a few loci for allelic diversity have been identified. These include mainly small insertion sequences and tandem repeating elements. At least one of these, the TTC triplet occurring in the putative sugar transporter pseudogene, has been found to occur at variable copy numbers in different clinical isolates. To better understand the suitability of this and other VNTR markers in differentiating variant strains of *M. leprae,* we examined a battery of 12 *M. leprae* isolates derived from leprosy patients in different regions of the United States, Brazil, Mexico, and the Philippines, as well as from wild nine-banded armadillos and the Sooty Mangaby Monkey. The stability of the TTC VNTR was compared among the individual isolates as well as to those from bacilli obtained on subsequent passage in nude mice and armadillos. Copy numbers for the TTC repeat ranged from 10-15 among the isolates tested. No regional clustering was noted and all of the U.S. isolates showed a variable number of repeats. Strains derived from wild animals were not identical. Greatest variability in TTC was seen over long term passage with the Thai-53 strain, which has been maintained continuously in nude mice for many years. Thai-53 TTC copy number varied markedly over 8 passage intervals. However, the TTC VNTR genotype of most individual strains remained relatively constant for isolates passaged outside man for fewer than 12 generations. In addition, the TTC VNTR genotype of these strains tended to remain constant when passaged through an alternate animal host, the experimentally infected nine-banded armadillo. Even though the TTC VNTR occurs in a non-coding region of the *M. leprae* chromosome, its apparent stability among most short term passaged isolates suggests that it has utility for differentiating laboratory strains of *M. leprae,* and may be useful in assessing drift amongst isolates carried in long term culture.

OM&BM 8

EVALUATION OF TNF-α AND IL-10 SINGLE NUCLEOTIDE POLYMORPHISM (SNPS) AMONG HIV/*M. leprae* CO-INFECTED AND LEPROSY PATIENTS

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The effective immune response against pathogens depends on an interaction of different cells and molecules from which pro and anti-inflammatory cytokines like TNF-α and IL-10 have a fundamental role. Thus, up or down regulation of these genes can influence clinical manifestations and outcome of several diseases including aids and leprosy. Recently, several SNPs have been described in cytokine genes and associated with gene expression and a number of diseases. Although the mutant -308A TNF-a allele have been associated with protection in leprosy, no polimorphic TNF alleles was associated to outcome in HIV infection. The aim of this study was to evaluate the possible association of promoter SNPs on TNF-α (-238, -308) and IL-10 (-819, -1082) positions with the outcome in HIV/ *M. leprae* co-infected compared with leprosy patients. Twenty one co-infected patients classified as multi (10) and paucibacillary (12) MB/PB leprosy were evaluated besides a group of 300 leprosy patients (210 MB and 90 PB). The results indicated that for the TNF-α polymorphisms the frequency of -238A was higher in the co-infected group compared with leprosy patients (p=0,04) corroborating with previous studies in which this allele was associated with the more severe MB forms of leprosy. For the IL-10 polymorphisms only the -819T allele showed an increased frequency in co-infected patients ($p = 0,01$). Frequencies of -308 and -1082 did not show difference between groups. However, horizontal analysis of the co-infected group shows the higher frequency of -1082A (related to the down regulation of IL-10 gene) linked with a low frequency of -308A (related to the up regulation of TNF-α gene) suggesting a combination of genetic factors probably associated with susceptibility for the co-infection HIV/ *M. leprae*ng laboratory strains of *M. leprae,* and may be useful in assessing drift amongst isolates carried in long term culture.

OM&BM 9

FURTHER STUDIES ON *M. leprae* - PERIPHERAL NERVE PROTEIN INTERACTION AND THE ROLE OF THE 25 kDa GLYCOPROTEIN MYELIN P_0.

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The invasion of Schwann cells and axons by *M.leprae* results in demyelination and axonal degeneration leading to motor, sensory and autonomic nerve damage and disfigurement which is the hallmark of leprosy. Other workers shown that tissue proteins such as fibronectin, β integrin, laminin-2 and αdystroglycan are involved in *M. leprae* - target tissue binding. Our earlier biochemical studies have revealed that a 25 kDa glycoprotein of the peripheral nerve has an affinity for *M. leprae* and is involved in binding. This glycoprotein is a major phosphorylated protein of the human peripheral nerve. Its molecular weight, carbohydrate content and phosphorylatable nature are similar to myelin P_O.

The present study is an immunological confirmation that this protein is the myelin P_0. The 25 kDa phosphorylated protein was confirmed as myelin P_0 by the following experiments – dot blot assays, immunoprecipitation, western blot and by immuno-histochemistry using monoclonal antibodies to P_0 and the HNK-1 epitope.

Since myelin P_O is a peripheral nerve specific protein, it could be one of the key target molecule for *M. leprae* binding/internalisation and may also explain the neural prediliction of *M. leprae*

OM&BM 10

GENE EXPRESSION IN *Mycobacterium leprae*

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The genome of *M. leprae* has been completely sequenced and annotated. 1604 open reading frames and 1104 pseudogenes have been identified, however, the minimum gene set required for growth and survival (transcriptome) has not been defined. We have developed a protocol for *M. leprae* RNA purification and obtained RNA from two strains of *M. leprae* (T-53 and 4089). The expression of approximately 5% of the potential transcriptome was analyzed using RT-PCR. cDNA was produced using 1 ug of RNA from each strain, random hexamers and reverse-transcription (RT). Gene transcripts were amplified from cDNA using PCR with primer sets flanking several potentially functional families. The cDNA from both strains was amplified and results demonstrated that genes encoding several enzymes including those involved with, folic acid synthesis, iron utilization, cofactor biosynthesis, gluconeogenesis, degradation of phosphorous compounds, degradation of DNA, detoxification, synthesis of mycolic acids, modification and maturation of ribosomes, synthesis of RNA, glycolysis, glyoxylate bypass, and genes containing secretion motifs or encoding stress proteins, and several genes with unknown functions were transcribed in both strains. These data have provided the first insight into the transcriptome of *M. leprae*. However, not all genes were expressed in both strains. Comparative analysis of gene expression theses strains will be discussed in greater detail. It is anticipated that this analysis along with cDNA array analysis will help to identify a larger set of functional genes in *M. leprae* which will potentially help us to understand the minimal requirements for growth and replication of this pathogen. This information may lead to the identification of new drug targets, skin test antigens and to identify factors that allow this pathogen to evade the immune system and destroy peripheral nerves.

OM&BM 11

GENETIC SUSCEPTIBILITY TO ERYTHEMA NODOSUM LEPROSUM (ENL) IN LEPROSY

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Erythema nodosum leprosum (ENL) is a distressing complication, experienced by up to 40% of lepromatous leprosy patients, which is characterized by severe systemic symptoms, including fever, painful cutaneous lesions, and neuritis, which often result in permanent nerve damage. The determinants and mechanisms underlying the onset of reactional states, progressive nerve damage and the regulation of immunity in these patients are not well understood.

Aim: To investigate the role of genetic factors in leprosy patients in their propensity for developing ENL.

Methods: We have recruited over 950 Nepali individuals, including both leprosy patients and their first-degree relatives. DNA was obtained from blood samples taken from each of these participants, and the SSO technique used to estimate the prevalence of polymorphisms in a number of candidate genetic loci: specifically, HLA-DR, TNF-á and Vitamin D receptor genes.

Results: Our results indicate that while the genetic loci under investigation may play a role in a patients' susceptibility to ENL, other factors may also have an effect. We will present data with regard to our analyses of the incidences of polymorphisms at these loci in each of the groups studied.

Conclusions: We have applied a rapid technique to determine the prevalence of specific genetic polymorphisms among leprosy patients and their first-degree relatives. In addition, the establishment of a large databank of DNA from patients susceptible to ENL will be an important resource for future studies.

OM&BM 12

GENOMIC DIVERSITY IN *RPOT* GENE OF *Mycobacterium leprae* AND GEOGRAPHIC DISTRIBUTION IN LATIN AMERICA

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In the study to establish the genotyping of *Mycobacterium leprae*, two genotypes of the *rpoT* gene were detected among isolates. Some of them showed the *rpoT* gene with 4 copies of 6 base tandem repeats and other isolates harbored 3 copies of 6 base tandem repeats in the gene. Most striking finding was the apparent dominant distribution of the 6bp 4 tandem repeat genotype of *M. leprae* in the main island of Japan and Korea. In contrast, almost all isolates from other regions in the world revealed 3-copy type. It is clear 6bp 4 tandem repeat genotype spread in Japan in concordance with the migration of the people from Korea to Japan. Biased distribution of each genotype in the world led us to imagine the spread of the leprosy concordant with the migration of Mongoloids to Latin American countries as revealed for other microorganisms. Geographic distribution of different *rpoT* genotypes of *M. leprae* isolated in Paraguay, Peru and Mexico was investigated in connection with human prehistoric migration. All *M. leprae* genotype of *rpoT* gene isolated in Paraguay and Peru showed three tandem repeats of 6bp. On the contrary, isolates from Mexico showed the 6bp 4 tandem repeat genotype. It seems that the *M. leprae* distributed in Mexico was carried by the movement of Mongoloid but the bacilli in two South American countries is originated in another source.

OM&BM 13

Mycobacterium leprae DNA DETECTION BY POLYMERASE CHAIN REACTION FOR EARLY LEPROSY

Sousa, A.L.O.M., Castro, F, Silva, M.H., Rebello, P.F.B., Gomes, M.K., Nararashi, K., Sacchetim, S.C., Visconde, A.M., Costa, M.B., Martelli, C.M.T., Stefani, M.M.A. and Gillis, T.P.

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Objective: To detect *M. leprae* DNA by PCR in skin biopsies among Brazilian single skin lesion paucibacillary leprosy patients (SSL-PB) prior to one dose ROM therapy.

Methods: 259 newly detected SSL-PB leprosy patients, negative baciloscopy, were recruited in 3 endemic regions from 97/98 and followed-up for 3 years. Before drug intake, 4 mm punch skin biopsies were collected for conventional histopathology. In a subgroup of 134 patients, half of the skin biopsy was snap-frozen and stored (liquid nitrogen) for *M. leprae* DNA detection by PCR. After DNA extraction (phenol/chloroform/ isoamyl alcohol) each specimen was amplified, undiluted and at 1:5, using pairs of primers for a 360 bp *M. leprae* specific fragment. Products were detected by slot blot hybridization using digoxygenin-labeled 212bp DNA probe. Specimens were tested blinded to patient's characteristics at IPTSP/Brazil in partnership with National Hansen's Disease Programs.

Results: 43.3% (95%CI 34.8-52.1) of *M. leprae* DNA positivity was detected among SSL-PB, representing an increase of 37.3% (50/134) bacilli detection when compared to the rare bacilli found in histopathology readings (12/134). There was an increased positivity trend with age ($p<0.01$). Patients with skin lesion on the face, Mitsuda negative (<5mm), anti PGLI negative were independently associated with positivity.

Conclusion: *M. leprae* DNA by PCR was a valuable tool for diagnosis confirmation among early paucibacillary leprosy patients and to explore prediction factors of disease progression.

TDR/WHO grant 98100

OM&BM 14

NASAL PRESENCE OF *Mycobacterium leprae* AND MUCOSAL IMMUNITY IN HOUSEHOLD CONTACTS OF LEPROSY PATIENTS

Mrs. S. P. Madhale, Dr. R.S. Jadhav, Miss A. Fernando, Miss V.S. Shinde, Ravindra R Kamble, Dr. V. K. Edward, Dr. J.R. Rao and Prof. W.C.S. Smith on behalf of MILEP-2 Study Group*

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Transmission of leprosy in the household contacts (HC), as reflected in new case detection rate does not appear to be affected significantly in the post-MDT era. Incidence rates have been reported 8-10 times higher in the HC than the general population. Major route of transmission of *M. leprae* is thought to be mainly through the respiratory system with nose as the site of initial infection. The aim of the study was to see the mucosal immunity and exposure to *M. leprae* in HCs of patients and non-contacts (NC) to understand transmission. The principal methods employed for this were the polymerase chain reaction (PCR) to detect small quantities of *M. leprae* DNA and measurement of mucosal immunity by ELISA. 201 subjects out of 3035 were identified as HCs. Saliva samples and nasal swab were collected from subjects to carry out this study. Overall analysis of all the samples shows that the percentage of PCR positivity is almost same in HC (2.3%) and NC (2.5%). Similarly in both groups 68% of the subjects show mucosal immune response. Both the groups show similar pattern of exposure to *M. leprae* with PCR positivity peak seen in monsoon. Amongst the household contacts, females show higher PCR positivity (3%) than males (1.5%). The difference in the PCR positivity in noncontacts in males (2.2%) and females (2.8%) is relatively small. Exposure to *M. leprae* is likely to be followed by immunity in most individuals, which is consistent with wide spread transmission of *M. leprae* producing transient nasal carriage and the development of a mucosal immune response, which may be protective.

OM&BM 15

PATTERN & SIGNIFICANCE OF PARASITAZATION OF ENDOTHELIAL CELLS IN LEPROSY: MORPHOLOGICAL & INVITRO STUDIES.

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Previous studies have implicated the role of the endothelial cell in the dissemination of leprosy. In this paper we present a detailed morphological study of 17 skin biopsies (4 BT, 3 BL & 10 LL) in which acid fast bacilli were found in the endothelial cells and relate it to other morphological features in the biopsies.

Among the 17 biopsies in whom bacilli were present in the endothelial cells; bacilli were also prevent in the nerves in 13 biopsies, in the macrophage in 16; smooth muscle in 10 and in the sub epidermal zone in 2 biopsies. Bacilli were present also in the walls of the blood vessels in 5 biopsies and in the lumen in 1 biopsy. Interestingly there was 1 biopsy in a BT patient in which bacilli were present only in the endothelial cells and absent elsewhere in the section.

In vitro studies on *M. leprae*-endothelial cell interaction were carried out using immortalized endothelial cell lines. The short term cultured endothelial cells were isolated and phosphorylated with gamma P_{32} ATP. *M. leprae* binding studies were carried out on nitrocellulose blot. Preliminary experiments suggest that there is a phosphorylated glycoprotein receptor (55 kDa) on the endothelial cells that interacts and binds to *M. leprae*.

These morphological and in vitro studies suggest that *M. leprae* has an affinity for endothelial cells which it parasitizes. The organism is then probably released into the blood stream resulting in its dissemination to distant sites of prediliction in the body

OM&BM 16

PERSISTERS IN LEPROSY AFTER MULTIDRUG TREATMENT IN MB PATIENTS

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With the Multi-Drug Treatment (MDT) of leprosy, the results have been satisfactory all over the world. However, the presence of drug sensitive viable organisms is well recognized in MB leprosy. These persisting bacilli have special significance due to their relapse potential. This study has been initiated to gain an overview of this problem and follow the trends in multibacillary cases treated with MDT. In this study, biopsies for Mouse Foot Pad (MFP) have been obtained from MB patients treated with (i) standard MDT +Minocycline + Ofloxacin for 12 months, (ii). Standard MB MDT after 12,24 and 36 months Bacilli harvested from the biopsies were inoculated in to mouse foot pad and estimation of bacillary ATP levels by bioluminescence assay as per established methods. Available results indicate that despite reduction in viability after MDT, viable persisters are detected even beyond one and 2 years of treatment. There has not been much change in the trends over the last 5–10 years. It would be important to carry out such surveillance in larger number of MB cases to know the trends and the resultant relapses.

OM&BM 17

PROTECTION OF MICE AGAINST *Mycobacterium leprae* INFECTION BY A DNA VACCINE ENCODING *M. lepraE* ANTIGEN 85A AND MUTANT MURINE IL-12

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Female BALB/c were administered one of three DNA vaccines: *M. leprae* DNA: Ag85A; DNA: Ag85A + wild-type murine DNA: IL-12w; and DNA: Ag85A + mutant murine DNA: IL-12m. Expression of Ag85A by the preparation of DNA: Ag85A had been confirmed by specific stimulation of IFN by murine spleen cells before it was employed in this experiment. Control mice were administered saline or the empty vector; live BCG served as a positive control. The mice were injected into the posterior tibial muscles with 200 g/dose/mouse of one of the preparations on four occasions four weeks apart, except for BCG, only two doses of which were injected. Four weeks after the last dose, the mice were challenged with 5000 *M. leprae* into a hind foot pad, and the organisms were harvested approximately five months later. The results of the harvests are summarized in the table. As shown by the control group, the results of the harvests demonstrate that the inoculum employed included only a small proportion of viable organisms. BCG appears to have conferred modest protection. Only the mixture of the DNAs encoding Ag85A and IL-12m conferred protection, whereas the mixture of the DNAs encoding Ag85A and IL-12w appears to have enhanced the infection.

Material	Median no. AFB/foot pad (x 10^5)	P
Control	1.06	0.00001
BCG	0.488	0.038
Vector	1.69	0.075
DNA::Ag85A	3.90†	0.240
DNA::Ag85A +DNA::IL-12w	4.57	0.041
DNA::Ag85A +DNA::IL-12m	0.266	0.0197

OM&BM 18

SCHWANN CELL GENE EXPRESSION PROFILE IS MODULATED BY *Mycobacterium leprae*

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The primary effects of *Mycobacterium leprae* invasion on the physiology and metabolism of Schwann cells, and to whar extent these effects might be related to the progressive, irreversible degenerative nerve damage observed in leprosy, are poorly understood. In this study, we have applied differential display PCR and DNA microarray techniques to identify genes selectively expressed or repressed in Schwann cells in response to *M. leprae* infection. Schwann cell lineage ST-8814 was cultured and incubated with *M. leprae* isolated from armadillo and from human biopsies between 1 and 24 hours. Complementary DNA synthesized from RNA isolated from these cultures was used for differential display RT-PCR reactions and hybridizations against oligonucleotide chips. Currently bands identified in polyacrilamide gel electrophoresis as differentially expressed have been cloned and sequenced for subsequent northern blot and real time PCR confirmation. Images of microarray hybridizations have been acquired using the Gen Pix software. The cluster analysis has been performed using the Tree View software. Preliminary results indicate that *M. leprae* is able to alter the gene expression profile of *in vitro* cultured Schwann cell.

NIH, WHO/TDR, sponsored this work.

OM&BM 19

SIMPLIFIED REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION FOR DETECTION OF *Mycobacterium leprae* IN SKIN SPECIMENS

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Diagnosis of leprosy based on detection of *Mycobacterium leprae* RNA remains a complicated process. To simplify the detection procedure, a one-step RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) was established and evaluated for its potential in rapid detection of leprosy patients. The assay relies on the extraction of *M. leprae* RNA, and single-tube reactions of reverse transcription, followed by PCR amplification. Using *M. leprae*-specific primers targeting 171-bp fragment of the *M. leprae* 16s rRNA gene, the RT-PCR designed for convenience, and reproducibility resulted in detectable *M. leprae* in both slit skin smears and skin biopsies. The assay was specific for *M. leprae* in comparison with results obtained from *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. The use of digoxigenin-label DNA enhanced the pos-

itive signal of the amplified RT-PCR product. The method could detect less than 10 CFU of mycobacteria in analyzed samples indicating the sensitivity of the test. In the initial application, diagnostic results were obtained from 24 leprosy patients. Of these, 20 were multibacillary (MB) and 17/20 patients were positive for 16s rRNA of *M. leprae* in skin specimens. The assay particularly useful since slit skin smears negative in staining for acid fast bacilli were positive by RT-PCR. The method has also been evaluated for its potential to help monitor bacterial clearance in leprosy patients during chemotherapeutic treatment. We propose that this form of RT-PCR gives values in term of its simplicity and sensitivity to identify *M. leprae* in skin specimens especially when acid-fast bacilli are not discernable. The usefulness of RT-PCR in detection of viable leprosy bacilli needs to be extensively explored.

OM&BM 20

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) OF TNF-α AND IL-10 GENES AND SUSCEPTIBILITY TO LEPROSY AMONG HOUSEHOLD CONTACTS.

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The interindividual variations in the host response to a certain pathogen are one of the most important variables for the determination of susceptibility and severity of the disease, which is the result of environmental effects against the background of genetic factors. Thus, identification of such factors, which are somehow associated to a higher or lower susceptibility, is of fundamental importance for the prediction of development or establishment of the disease. The aim of this study was to evaluate the possible association of the SNPs at positions -238 and -308 of the TNF-α and -819, -1082 and -2849 of the IL-10 genes among household contacts of leprosy patients.

Two hundred and sixty seven household contacts were enrolled in this study from which 67 became patients and 200 remained as healthy contacts. The results showed no statistic difference on the distribution of carriers and non-carriers of the -238A allele among sick and healthy contacts. For the -308 position, the number of carriers was significantly higher among sick in comparison to healthy contacts ($p < 0,01$). Moreover, when analyzed through the clinical spectrum of leprosy, all the -238A carriers developed multibacillary (MB) forms of the disease whereas 73,3% of the -308A carriers developed the paucibacillary (PB) forms. Regarding the IL-10 SNPs, the allelic frequency of the -819T was significantly higher in the healthy and -1082A in sick contacts ($p < 0,01$ for both). Analysis according to the clinical forms revealed an increased frequency of the -819T carriers in the PB forms when compared to the MB ($p < 0,01$).

The present data suggest that SNPs of cytokine genes could be used to screen contacts of leprosy patients as a prognostic marker of diseases susceptibility and severity

OM&BM 21

SITE OF ENTRY: AN IMPORTANT FACTOR IN THE GROWTH AND DISSEMINATION OF *M. leprae* IN MICE.

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Sixty eight thymectimized and irradiated mice were randomized and 36 inoculated intra-dermally in the flank and 36 in the footpad. In each of these two groups four different concentrations of *M. leprae* inoculation were used namely 10^7 in 0.1 ml, 10^6 in 0.1 ml, 10^5 in 0.1 ml and 10^4 in 0.1 ml. Mice were sacrificed at the 6th, 8th, 12th and 15th month and growth of *M. leprae* at the site of inoculation was estimated. Internal organs were subjected to histo-pathological examination.

The 10^4 in 0.1 ml inoculum did not promote growth in mice injected in the flank but growth was seen in all mice that were inoculated in the foot-pad. In all other groups there was growth of *M. leprae* but it was quantitatively more in the foot-pad inoculated animals than in the flank inoculated ones. Further, growth in the foot-pad inoculated mice was associated with disseminated of *M. leprae* to the internal organs while such dissemination was not seen in flank injected mice.

We conclude that in mice, entry of *M. leprae* through a relatively cooler entry point (foot pad) allows better growth of *M. leprae* locally, needs smaller doses of inoculum to promote growth and allows dissemination of the organism to the internal organs. The site of entry of *M. leprae* and the dose may have a role in determining whether the person will be infected or not.

OM&BM 22

STUDIES ON NASAL TRANSMISSION BY *M. leprae* SPECIFIC GENE AMPLIFICATION

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Nose has been considered as an important portal of exit and entry in leprosy. Due to continued high incidence rates in leprosy, there is a great need to understand the sources and spread of *M. leprae*. This study has been carried out to study the nasal positivity on in leprosy cases by using *M. leprae* specific PCR. Nasal scrapings were collected from leprosy cases across the spectrum. These were from untreated as well patients treated with standard MDT for varying duration. These scrapings were suspended in TE buffer, decontaminated and DNA was extracted by a physiochemical procedure already established at the laboratory. Gene amplification was carried out by using a system targeting 36 kD gene (Hartskeerl et al 1989). Amplicons were analysed by gel electrophoresis and southern blot hybridization. PCR positivity was been analysed in relation to type of disease and duration of treatment. Positive results were observed in a section of PB cases (classified according to current WHO criteria) and most of MB cases. This positivity persisted for varying periods after treatment. The relevance of these findings will be discussed keeping in view the potential application of this approach in studying the transmission of leprosy.

OM&BM 23

STUDIES ON STRAIN VARIATION BY *M. leprae* USING TTC REPEATS

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There is a great need to develop molecular markers for eliciting the strain variation among *M. leprae* for understanding the dynamics of transmission of leprosy. This study has been carried out to study the strain variation in leprosy cases by using TTC repeats as markers. Biopsies were collected from leprosy cases across the spectrum. These biopsies were homogenized and DNA was extracted by a physiochemical procedure already established at the laboratory. TTC regions were amplified by using the primers and procedure described by Shin et. al, (2000). Amplicons were analysed by gel eletrophoresis. The polymorphism observed in the size of amplicons has been analysed in relation to geographical distribution, type of disease and possible sources. The relevance of these findings will be discussed in context of potential application in the molecular epidemiology of leprosy. Such techniques become very important due to persistent high incidence rates seen in our populations.

OM&BM 24

SUBCLINICAL TRANSMISSION OF *M. leprae*: OCCURENCE OF NASAL PCR POSITIVITY AND MUCOSAL IMMUNITY – ANALYSIS IN SCHOOL CHILDREN

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Background: The transmission of leprosy is less well understood. Infection from sub-clinical sources could play an important role than from active clinically apparent cases. Most of the individuals in high leprosy endemic areas have immunological evidence to *M. leprae*. Thus the first exposure, probably exposure in childhood is important and also, is the related mucosal immune response to characterize the immune status of the individual.

Aim: To define the means by which *M. leprae* is transmitted and the development of immunity in school children in a population in which multidrug therapy had been used for more than 10 years.

Materials and Methods: Three villages in South Maharashtra, where leprosy is endemic were selected. These villages were comparable in size, socio-economic status and prevalence of leprosy. The principal methods employed in this study were the PCR to detect small quantities of *M. leprae* DNA, and measurement of mucosal immunity by assay of salivary IgA.

Results: 633 school children (26% of the total population) were analysed for the presence of *M. leprae* DNA and mucosal immunity. Analysis of the data show that the incidence of nasal PCR positivity (PCR+) in school children and rest of the population (ROP) is same (2.7%) whereas IgA positivity is 61% and 70% respectively. PCR+ percentages in school children and ROP in monsoons is 3% and 4% respectively as compared to 1.6% (school children) and 0.7% (ROP) in the summer months. In the group (5-9 years) the PCR+ percentage in household contacts is higher (7.7%) than 10-14 years group (1.3%). A significant difference in PCR+ percentage is observed in males (1.9%) and females (3.7%) in 5-9 years group.

Conclusion: Results suggest though there is no obvious differences in between the groups, the exposure and mucosal immunity to *M. leprae* is affected by seasons and shows marked variation in males and females

OM&BM 25

THE *Mycobacterium leprae* HLP PROTEIN: A PUTATIVE ADHESIN THAT BINDS MULTIPLE EXTRACELLULAR MATRIX COMPONENTS.

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Recent reports have identified a 21 kDa histone-like protein (Hlp) as a laminin-binding protein of the *Mycobacterium leprae* cell wall (Shimoji et al, Proc. Natl. Acad. Sci 96: 9857-9862, 1999; Marques et al, Microbes & Infection 2: 1407-1417, 2000). The C-terminal domain of Hlp (also known as ML-LBP21) contains Ala/Lys-rich repeated motifs, which are also found in the heparin-binding hemaglutinin (HBHA), a major adhesin of *M. tuberculosis*. These repeated sequences constitute the heparin-binding site of HBHA, suggesting that *M. leprae* Hlp might also interact with glycosaminoglycans (GAG). In this study, we have further characterized the interaction of Hlp with laminin-2 and other extracellular matrix components. To map the functional binding sites of Hlp, truncated recombinant fragments corresponding to the N-terminal (rHlp-N) and the C-terminal (rHlp-C) domains of the protein were produced by a PCR cloning strategy. The capacity of recombinant Hlp and truncated proteins to interact with extracellular matrix components was investigated using a solid phase-based assay. In these assays, soluble laminin-1 and -2 were able to bind in a dose-dependent manner to rHlp and rHlp-C, but not to rHlp-N. rHlp and rHlp-C were also able to bind heparin and collagen I, III and IV, but not fibronectin. These observations suggest that the Ala/Lys-rich sequences present in the C-terminal half of *M. leprae* Hlp constitute the binding sites to extracellular matrix proteins. The capacity of Hlp to interact with other extracellular matrix components expands the potential role of Hlp as adhesin in mycobacterial pathogenesis. Currently, *in vitro* adherence assays are under way to evaluate the role of Hlp, collagen and GAG in the interaction of *M. leprae* with Schwann cells and epithelial cells.

This work was upported by FAPERJ, WHO/TDR and NIAID, NIH.

OM&BM 26

THE USE OF POLYMERASE CHAIN REACTION (PCR) IN LEPROSY RESEARCH AND CONTROL

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The PCR is a sensitive and specific technique, that allows the detection of minute amounts of DNA in a matter of hours. Since the development of the first PCR assay for the detection of *Mycobacterium leprae* more than 10 years ago, the technique has been used on a whole range of samples, varying from biopsy material and nose swabs from patients and contacts to dust samples from the environment.

The PCR has been used to investigate a variety of matters of clinical and epidemiological importance. We have now a better insight into the spread of leprosy in the society, because PCR made it possible to show that the presence of the bacterium on the nasal mucosa is widespread in the population. Also, PCR and another amplification technique, NASBA, have been used to monitor the presence of *M.leprae* DNA and RNA during and after treatment.

This presentation will give a critical overview of the possibilities, applications and achievements of molecular amplification techniques and the way in which they have influenced and will influence leprosy research and control.

OM&BM 27

THREE-COLOR IMMUNOFLUORESCENT STAINING TO IDENTIFY *M. leprae* WITHIN ENDOTHELIUM OF HUMAN PERIPHERAL NERVE.

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Studies in an animal model have suggested that *M. leprae* enter peripheral nerves by colonizing epineurial blood vessels and lymphatics, gaining access to the endoneurial compartment by passing through the vascular endothelium. To evaluate this possibility in human lesions, where excision and dissection of major nerve trunks is not possible, we have developed a method to assess endothelial involvement of cutaneous nerves in skin biopsies.

Archived, paraffin-embedded skin biopsies from HD patients were selected based on lepromatous classification (LL or BL) and presence of at least one large cutaneous nerve. Schwann cells were identified using rabbit anti-S-100, biotin-goat anti-rabbit, and streptavidin-Alexa-Flour-350; bacilli were identified using guinea pig anti-*M. leprae* and FITC-goat anti-guinea pig; endothelium was identified using rhodamine-*Ulex europaeus* -1 (UEA-1). Examined under appropriate filters, this allowed positive identification of nerve (blue), *M. leprae* (green), and endothelium (red). Images were captured by digital photography and superimposed using Adobe Photoshop software.

Preliminary results from 5 biopsies indicate that the endothelium is infected in 29% of blood vessels associated with nerves, and 32% of blood vessels not associated with nerves. At this time, the sample is too small for differential analysis of infection of vessels at different levels of the dermis. The method appears to offer a sensitive means of positive identification of these and other structures that may be involved in vascular endothelial infection of nerves in HD.

OM&BM 28

UNIQUE METABOLIC PROPERTIES OF *Mycobacterium leprae*

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The sequencing of the genomes of several microorganisms, including *Mycobacterium leprae* and *Mycobacterium tuberculosis* has been reported in recent years. *M. tuberculosis* contains a full complement of genes needed for survival and independent growth. On the other hand, *M. leprae* is deficient in genes coding for many biosynthetic enzymes, that makes the organism incapable of independent growth and survival, contradicting the claim that *M. leprae* is a competent bacterium. The finding explains the obligate intracellular parasitism of the organism and failure of attempts for over a century to culture the bacterium in chemically defined media. In addition, *M. leprae* was found to possess unique genes, not found in *M. tuberculosis.* These genes code for enzymes characteristic of the Hansen bacterium. We have discovered a unique enzyme activity, *o*-diphenoloxidase, in *M. leprae.* The enzyme is not present in *M. tuberculosis* or any other mycobacteria, including *M. lepraemurium* recovered from infected mouse tissues. It acts on phenolic substrates like 3,4-dihydroxyphenylalanine and related compounds, converting them to quinones. No rational explanation has been available for the unusual affinity of *M. leprae* for the Schwann cells of peripheral nerves, and for the hypopigmentation of skin lesions. Both Schwann cells, and melanocytes of the skin contain tyrosine hydroxylase that generates 3,4-dihydroxyphenylalanine (dopa), metabolized by the bacteria. Tyrosine hydroxylase occurs in the adrenal medulla that synthesizes dopa, epinephrine and norepinephrine from tyrosine. We found that adrenal medulla is a preferred site for early multiplication of *M. leprae.* In tuberculoid HD (Hansen's Disease) where the bacteria are restricted to specific areas of the skin, there is hypopigmentation of skin lesions. Melanocytes continually generate trace amounts of dopa, which is converted to melanin pigment. *M. leprae* diverts the substrate for its own metabolism, which prevents pigment formation. In melanocyte cultures, granules of melanin can be observed. When we added live *M. leprae* to such cultures, pigment production was suppressed. In lepromatous condition where the bacteria are distributed diffusely, only hypo-pigmented mottling results. The quinones generated by the bacteria can undergo reversible oxidation-reduction, helping in the utilization of other metabolites by the bacilli. Mycobacteria in general, can synthesize their own ATP. *M. leprae,* on the other hand, possesses a mechanism for the active transport of ATP from the surrounding milieu. ß-Lactamase is a constitutive enzyme in mycobacteria, including *M. tuberculosis.* But *M. leprae* unexposed to ß-lactam antibiotics showed no ß-lactamase; bacteria recovered from experimentally infected armadillos treated with Bicillin (penicillin G benzathine), to control secondary infections, contained active ß-lactamase. The enzyme activity persisted when these bacteria were used as inoula to infect other armadillos, which received no bicillin treatment subsequently. Once the enzyme is induced, it is not lost when the inducing agent is withdrawn; the phenomenon is referred to as de-repression. A potent ß-lactam-ß-lactamase inhibitor combination, UNASYN, was bactericidal to *M. leprae* and *M. tuberculosis,* even resistant to other drugs. The compound could serve as an effective alternative drug for treating HD patients.

OM&BM 29

USE OF PCR IN THE RAPID DIAGNOSIS OF RIFAMPICIN RESISTANCE IN LEPROSY

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As rifampicin is the major bactericidal drug used in MDT therapy of leprosy, it is essential that resistance trends be monitored. The established method of assessing drug resistance, using culture in the mouse footpad, has recently been augmented by the development of a rapid PCR detection method.

Aim: To test for defined mutations in the *M. leprae* RNA polymerase â chain gene (*rpoB*), and to correlate these with drug resistance in the mouse footpad system.

Methods: A novel PCR based technique was used to examine bacteria obtained from skin biopsies from MDT defaulters or non-responders, and from samples which had previously been passaged in the mouse footpad. *M. leprae* DNA was extracted from these and a set of oligonucleotide probes immobilized on a nylon membrane used to probe for mutations associated with rifampicin resistance. The test combined positive and negative controls and used chemiluminesence for detection.

Results: A number of samples were found to have the rifampicin resistant genotype in the PCR assay. We will present data on all of these *M. leprae* strains genotyped for rifampicin resistance and tested at full (10mg/kg) and half (5mg/kg) doses in mouse footpad cultures.

Conclusions: While the rapidity of PCR based methods is a major advantage over MFP, the validation of genotype methods of detecting drug resistance in leprosy is critical for their wider use in monitoring this important problem.

OM&BM 30

VIABILITY OF *M. leprae* IN LEPROMATOUS PATIENTS AFTER COMPLETION OF 12 MONTHS OF MULTI-DRUG THERAPY.

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The Seventh WHO expert committee had recommended shortening the duration of multi-drug therapy (MDT) to 12months from 24 months for multibacillary (MB) patients. We carried out a study to determine whether viable bacilli can persist in the body of treated MB patients after 12 months of MDT. 34 untreated lepromatous patients who had an initial average bacterial index (BI) of 3+ or more were enrolled in the study. At the end of 12 months of MDT, skin biopsies were obtained from a site, which displayed the maximum number of bacilli on skin smear examination. An *M.leprae* concentrate was prepared from each of the biopsies and inoculated into the footpads of five thymectomized and irradiated (T900r) mice. The preparation of innoculum, method of inoculation, harvesting and counting of M.leprae from the footpad tissue was done using the method described by Rees. Harvesting was done at 6th, 9th and 12th month. Skin histopathological examination was also done on 32 patients on completion of 12 doses of MDT. In nine (26%) out the 34 biopsies *M.leprae* continue to exist in the footpads of T900r mice. These nine patients had an initial average BI of 4+ or more at the time of starting MDT. Histopathologically, resolving granulomatous lesions were found only in eleven (34%) of the 32 skin biopsies at 12 months. Skin smears at the completion of 12 months of MDT showed a fall of one log BI or more in only 18 (56%) patients. This study demonstrates that at the completion of 12 doses of MDT, a considerable proportion of MB patients with initially high average BI, harbor bacilli. It is possible that these are dead bacilli, not yet absorbed by the tissue. Long-term follow up of these patients will reveal whether these bacilli are alive or not. It may be necessary to maintain these mice for longer periods to study the behavior of persisting bacilli.

OPERATIONAL ASPECTS OF ELIMINATION

OOA 1

ACTIVITIES OF THE TASK FORCE IN THE ACCELERATION OF THE ELIMINATION OF LEPROSY IN BRAZIL

Vera Andrade – WHO

Tadiana Maria Alves Moreira – Secretary of Health of Rio de Janeiro State

Gerson Fernando Mendes Pereira – Ministry of Health

Marcos Virmond - Institute Lauro de Souza Lima

Gil Soares - PAHO

Artur Custódio de Souza – Movement for the reintegration of leprosy affected persons (MORHAN)

The strategy to encourage municipal health secretaries to be committed to the elimination of leprosy, by increasing coverage of MDT services, is a conjoint initiative of the National Council of Municipal Health Secretaries (CONASEMS) and WHO with support from the Technical Area of Sanitary Dermatology of the Ministry of Health, MORAHN and PAHO. To establish such strategy CONASEMS has created in 1998 the Task Force for Accelerating the Elimination of Leprosy (GT/HANSEN/ CONASEMS), which aim is to identify practical solutions at the local level within the available structure and resources of the basic heath system. At the methodological level it is stressed the need to strengthen the participation of various social and institutional partners, involving mainly the municipal managers and the community. At the political level, after including the issue of elimination in the agenda of local managers, it was created adequate condition to increase the coverage for diagnosis and treatment of leprosy with the additional outcome of a political profit to the local manger due to the success of eliminating leprosy from his municipality. In august 2001 the project has covered 52% of the municipalities through the country (2898 municipalities in 14 states), out of them 38% are priority municipalities for the MoH, mainly in the north and northeast region. In Tocantins, Piaui and Rio de Janeiro the process of decentralization is in its stage of consolidation. To support the decon-