Poster Presentations
Immunology and Molecular Biology

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**Evolutionary Analysis of Mycobacterium leprae Strains by Using Two Variable-Number Tandem Repeats from Uttar Pradesh, India**

Mallika Lavania1, Kiran Katoch1,2, Ram Das1, DS Chauhan1, HB Singh1, VD Sharma1, P Sachan2, S Sachan2 and VM Katoch1
Department of Microbiology and Molecular Biology, Medical Unit-I, National JALMA Institute for Leprosy & Other Mycobacterial Diseases (ICMR), Agra, 282001, Model Rural Health Research Unit, Ghatampur, Kanpur, India

E-mail: dscchauhan01@yahoo.co.in

**Introduction**: Leprosy is a chronic disease **caused by infection with Mycobacterium leprae**. One difficulty in studying the mode of transmission of leprosy is the small amount of variation initially reported in bacterial genomic DNA. PCR-based amplification methods have been developed to overcome the problem of limited sources of genomic DNA. In general, these methods are sufficient for end-point molecular evolutionary studies. **Methodology**: To investigate genetic diversity in a bacterial population, we measured the copy numbers of simple sequence repeats, or microsatellites, in Mycobacterium leprae from patients of Uttar Pradesh, where pockets of endemicity have been observed. One microsatellite loci containing trinucleotide (TTC) or a minisatellite with hexanucleotide repeats (rpoC) were amplified from biopsies from fifty patients, and the copy numbers were analyzed by sequence analysis. **Results**: Extensive diversity was observed in 50 patients, but closely related profiles were found for members of a multicase family and nearby areas likely to share a common transmission source. Different TTC genotypes were distributed among residents at the same dwelling in villages. A limited discriminative capacity of the TTC polymorphism in the epidemiological analysis implies the need of searching other useful polymorphic loci for detailed subdivision of clinical isolates. **Conclusion**: The inclusion of more loci in typing schemes is likely to improve the discrimination of this approach. In addition to further exploration of microsatellite diversity, it will be important to search for other forms of genetic variation suitable for strain typing. Important goals will be to identify typing systems capable of providing reliable information about the transmission dynamics of M. leprae and to use these to assist in the search for interventions that will reduce the number of new cases of leprosy. **Keywords**: M. leprae, Evolutionary analysis, VNTR

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**Assimilation of M. leprae Specific PCR Testing into Routine Clinical Work**

L Gilead1, S Ulj-Bat1, R Wexler1 and L Valinsky2
1Israel Hansen's Disease Center, Israel Ministry of Health and the Department of Dermatology, Hadassah University Medical Center, Jerusalem, Israel, 2The Molecular Biology Laboratory of the Israel Ministry of Health

E-mail: lonas@hadassah.org.in

**Introduction**: The lack of an objective diagnostic test for leprosy, independent of clinical manifestations, is responsible for late diagnosis in many cases and is considered a cause for further spread of the disease. We have applied PCR for identifying *M. leprae* from slit skin smears, nose swabs and skin biopsies of human leprosy patients, family members and other contacts. **Methods**: Samples taken from subjects suspected as having leprosy, leprosy patients and contacts were examined using two methods: Ziehl-Nielsen stained samples were examined under a light microscope. DNA was extracted from the same samples. The PCR target was the *M. leprae* specific repetitive elements (RELP). **Results**: Out of 52 subjects tested, 36 were found PCR positive. Of these 6 were patient contacts with no clinical evidence of leprosy. Lepromin testing was positive in 5 of them, and the one subject with negative lepromin was diagnosed 6 months later as suffering from sub-clinical LL. PCR was positive in all microscopy positive smears, and in more than 50% of microscopy negative smears. **Conclusions**: The detection of several new positive contacts carrying *M. leprae* DNA that couldn't be detected by clinical criteria, underlines the effectiveness of the PCR assay. Immunological correlation is required for the application of PCR results to the clinical diagnosis. The assimilation of PCR testing into our routine clinical work proved feasible and effective.
A Simple Method for Detecting Drug Resistant *Mycobacterium leprae* Based on DNA Microarray

Masanori Matsuoka1, Kin Saw Aye2, Kyaw Kyaw3, Esterina Virtudes Tan4, Ma Victoria Balagon4, Paul Saunderson4, Masanoo Makino5, Chie Nakajima6 and Yasushiko Suzuki6

1Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan; 2Department of Medical Research, Yangon, Myanmar; 3Central Special Skin Clinic, Yangon General Hospital, Yangon, Myanmar; 4Leonard Wood Memorial, Cebu, The Philippines; 5National Leprosy Hospital Okumyono, Okayama, Japan; 6Department of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan

E-mail: matsuoka@nih.go.jp

A simple method to detect mutations of the genome of *Mycobacterium leprae* which confer resistance to key drugs for leprosy, was exploited on the basis of a reverse hybridization system. A series of oligonucleotide probes corresponding to each mutation in *folP1*, *rpoB*, and *gyrA* for dapsone, rifampicin and ofloxacin resistance, respectively, were selected and fixed on a glass slide as capture probes, to develop a DNA microarray, termed the Leprosy Drug Susceptibility test DNA microarray (LDS-DA). Mutations in clinical isolates of *M. leprae* were successfully discriminated by the LDS-DA. Feasibility studies were conducted to evaluate the performance of the LDS-DA in two developing countries, Myanmar and the Philippines, where the leprosy burden is relatively high. The high concordance of results obtained by this method with the results of nucleotide sequencing, strongly support the applicability of the LDS-DA as a drug susceptibility test, in place of the conventional time-consuming procedure. This is the first report of the development and evaluation of a rapid and simple method for the simultaneous susceptibility testing of three front-line drugs for leprosy. **Keywords**: Drug resistance, Dapsone, Rifampicin, Ofloxacin, Mutation, Reverse hybridization.

Possible Changes on Leprosy Classification Using ML Flow Test in Minas Gerais - Brazil

MAF Grossi1, S Burher-Sekula2 and CMF Antunes3

1Secretaria da Saúde de Minas Gerais; 2Universidade Federal de Goiás; 3Universidade Federal de Minas Gerais - Brazil

E-mail: crda@grossi.com.br

This is a descriptive study of epidemiological, clinical and laboratorial data on Leprosy, correlating sex, age, number of skin lesions and nerves involved, WHO impairment grading, skin smears and type of health facilities with the ML Flow result and WHO classification. It was carried out from October 2002 to March 2004, involved 1,072 patients in 13 municipalities of Minas Gerais State. Patient with positive BI and/or positive serology was classified as MB. The association of seropositivity with the variables was analyzed through logistic regression. The seropositivity (50.7%) was correlated with patients over 14 (OR:2.6), >5 skin lesions (OR:7.5), >1 nerve involved (OR:2.4) and positive BI (OR:5.5 to BI<2; OR: 19.12 to BI≥2). From 2000 to 2004, the MB rate decreased from 78.1% to 65.8% in Minas Gerais. The difference between PB and MB rate in participating and non-participating health services was statistically significant, indicating a direct and beneficial impact on this endemic in Minas Gerais. **Keywords**: Leprosy, classification, serology, skin smear.

Uptake of Clofazimine by Mouse Peritoneal Macrophages and the Effect of the Drug on Lysosomal Enzyme Synthesis

K Venkatesan, Nirmala Deo and D Bisht

National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Taj Ganj, Agra 282001, INDIA

E-mail: venkatesan_s2@rediffmail.com

Objective: To study the uptake of clofazimine (3-[p-chloroanilino]-10-p-chloroophenyl2,10-dihydro-2-isopropylimino phenazine) (CAS 54-85-3), an anti-inflammatory as well as antmycobacterial drug, by mouse peritoneal macrophage cultures exposed to various concentrations of 272 to evaluate the effect of accumulated drug on the lysosomal enzyme levels in the macrophages. **Materials and Methods**: Peritoneal cells were harvested from normal unstimulated Balb/c mice (weighing 20-25 g) and cultured by standard method. The cultures were exposed to clofazimine at concentrations of 0.1, 0.3, 0.5, 1, 2.5, 5, 10 and 25 µl/ml for 72h. Cycloheximide at a concentration of 0.5 µg/ml was added in parallel cultures treated with 0.3 and 0.5 µg/ml of clofazimine respectively. Clofazimine concentrations in the cell lysates were determined by the HPTLC-densitometric method. The activities of two lysosomal enzymes - á-galactosidase and á-glucuronidase were assayed in the cell lysates. The protein concentrations of the cells were also determined. **Results**: The intracellular clofazimine content ranged from 0.06 to 7.80 µg/ml corresponding to exposure concentrations. Uptake occurred equally well with both dead cells. Exposure of macrophage to 0.1-1.0 µg/ml resulted in significant increase in the activity of both the enzymes although the increase was only moderate with 0.1 µg/ml concentration. Cycloheximide, at 0.5 µg/ml concentration, inhibited the clofazimine-induced increase in the level of both enzymes in the macrophage cultures exposed to 0.3 and 0.5 µg/ml of clofazimine. **Conclusion**: Clofazimine accumulates in significant amounts inside the macrophages and induces the de novo synthesis of lysosomal enzymes at concentrations close to therapeutic plasma levels of 0.3-1.0 µg/ml.
Genetic Analysis of Mexican Mycobacterium leprae Strains

Iris C Estrada, M Matsuoka1, Fafutis-Morris M2, S Estrada-Parra2 and L Estrada3
1Leprosy Research Center, National Institute of Infectious Diseases, 4-2-1, Aobacho, Higashimurayama-shi, 189-0002 Tokyo, Japan
2CIINDE, Department of Physiology, CUCS, University of Guadalajara, Federalismo, Norte, 3102 Guadalajara, Jalisco 44220, Mexico
3Dept. de Inmunología, Escuela Nacional de Ciencias Biológicas, IPN, Prol. Carpio y Plan de Ayala s/n. Miguel Hidalgo, Mexico DF, Mexico 11340
E-mail: lestrada5@hotmail.com

Introduction: We have shown that the genotype with four copies (4c) of the six-base tandem repeats in the rpoT gene was predominant in Mexico, and different to isolates from Peru and Paraguay, which presented the 3c genotype. This different distribution might reflect the effect of human race movements on the spread of leprosy. Here, we analyzed more strains from the Northwest of Mexico, for rpoT and SNPs genotypes. As well as mutations relevant to drug resistance (DR). Methodology: DNA from clinical samples was amplified by PCR with primers for the rpoT, SNPs and fopP, gprA and rpoB genes for DR. Products were analyzed by electrophoresis (rpoT) or direct sequencing (SNPs and DR). Results: of 10 samples analyzed, seven showed the 3c rpoT genotype, whereas only three the previous predominant 4c pattern. SNPs genotype from five samples was type 3 (CTC), showing correlation between the SNP and rpoT genotype. No mutations for DR were found in five samples analyzed. Conclusions: as expected, Mexico has several M. leprae genotypes. Further work to characterize strain from Eastern Mexico is under way. Although no DR strains were identified, sentinel surveillance should be impose guaranty good implementation of treatment.

Key words: M. leprae, genotypes, rpoT, SNP, drug resistance.

Gene Expression Profile and Evaluation of Unique Hypothetical Unknown Proteins of Mycobacterium leprae by Using Quantitative RT-PCR

Hee Jin Kim, Kalyani Prithiviraj, Nathan A Groathouse, Patrick J Brennan and John S Spencer
NIH, NIAID Contract Leprosy Research Support and Maintenance of an Armadillo Colony Post Genome Era*, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523, USA

Diagnosis of leprosy is a major obstacle to disease control, and has been compromised in the past by the lack of specific markers. Using bioinformatics to compare the genome of M. leprae with other mycobacterial genomes in databases, up to 142 putative open reading frames (ORFs) have been identified as potential unique M. leprae proteins called hypothetical unknowns. Because of massive gene decay and prevalence of pseudogenes, it is unclear if any of these proteins are expressed or are immunologically relevant. To evaluate whether these genes produce a functional protein, we have performed cDNA based real time quantitative PCR to investigate the expression status of these ORFs. Out of the 142 ORFs, 23 showed significant level of expression compared to ESAT-6 (ML0049). Of these, 16 were cloned and expressed in E. coli, and polyclonal antiserum raised to these were used to detect the native proteins in the subcellular fractions of M. leprae by inmunoblotting. Candidate proteins identified in this manner are being screened for reactivity by serology and cell mediated response assays for their potential use in diagnostic development.

Recognition of Purified Microbial Products and M. leprae in Human Schwann Cells is Mediated by Toll-like Receptors

Rosane Barbosa de Oliveira
E-mail: rosane.doliveira@umassmed.edu

We investigated the expression and function of Toll-like Receptors (TLRs) in human Schwann cells (SC), are an important cellular target in M. leprae infection. The human Schwann cell line (ST88-14) was found to constitutively express TLRs 4, 6, 5, 1, 2, 7, 8, 3 and 10 (in quantitative order) as assessed by real-time quantitative PCR; TLR9 mRNA was not assessed. The mRNA of MD2 and CD14 were well expressed. Surface expression of TLRs 1, 2, 4, 6 and CD14 was confirmed by FACS. We investigated the distribution of fluorescent chimeric of TLRs in transfected SC by confocal microscopy. TLR4 endosomal recruitment was observed within 4 hours of exposure. In the absence of LPS, TLR4 positive endosomal structures were not detected. TLR4 expressing endosomes were observed in Schwann cells transfected with TLR2 or TLR9 within 3 hours of exposure to live Mycobacterium leprae. Schwann cells also were activated by a variety of other TLR ligands, consistent with their known TLR expression. Furthermore, live M. leprae induced the expression of IL-6 and TNF from the SC line. Quantitative PCR revealed that TLR4 mRNA levels were upregulated after LPS or live M. leprae stimulation. The neural lesion in leprosy is specifically related to M. leprae infection. The activation of TLRs in the innate immune response to M. leprae recognition seems to be a likely cause of nerve damage in leprosy.
The Utility of Measuring Serum Markers in Leprosy Patients During Multi Drug Treatment (MDT)

EA Silva¹, A IyerR², S Ura³, JR Lauris³, B Naaś³,⁴, PK Das³ and F Vilani-Moreno¹

¹ Instituto Lauro de Souza Lima, Bauru, SP, Brazil; ² Department of Pathology, Academic Medical Centre, Amsterdam, The Netherlands; ³Faculdade de Odontologia da USP, Bauru, SP, Brazil; ⁴ Department of Dermatology Leiden University Medical Centre, Leiden, The Netherlands

Dra EA Silva, Dep of Immunology ILSL Rodovia Comandante João Ribeiro de Barros Km 225CEP: 17034971
E-mail : elianesi@yahoo.com.br

We measured anti-PGL-I antibody, neopterin, and C-reactive protein (CRP) levels in serial serum samples from leprosy patients during MDT. Untreated leprosy patients, 15 multibacillary (MB) and 10 paucibacillary (PB), participated. The bacterial index (BI) in slit-skin smears was determined at diagnosis and blood samples collected at diagnosis and after 2, 4, 6 and 12 months of MDT. PGL-I antibody and neopterin were measured by ELISA and CRP levels by the latex agglutination method. PGL-I antibody and neopterin levels were higher in MB than PB patients, which correlated with their BI. The levels of CRP did not differ significantly between MB and PB patients. Eight patients developed reversal reaction and five developed erythema nodosum leprosum (ENL) during follow-up. Anti-PGL-I and neopterin levels were no higher in reactional patients. ENL patients had higher CRP levels than non-reactional MB patients. The PGL-I antibody levels declined significantly during MDT, in contrast to neopterin and CRP levels. PGL-I antibody and neopterin levels appear useful in distinguishing MB from PB patients. PGL-I antibody levels are useful in MB patients on MDT. CRP levels have some value in the detection of ENL reactions. Keywords: PGL-I antibody, Neopterin, C-Reactive Protein, Multi Drug Treatment, Leprosy.

IL-10 and NOS2 Modulate Cellular Dynamics in Leprosy Granulomas

Linda Benton Adams
National Hansen's Disease Programme Laboratory Research Branch, School of Veterinary Medicine Louisiana State University, Baton Rouge LA USA
E-mail : ladams1@lsu.edu

Mycobacterium leprae (ML) induced granuloma formation and maintenance depends upon T cells and macrophages and their respective cytokines. IL-10, a key regulatory cytokine, is generated by T cells and macrophages and inhibits IFN-γ production by T cells. IFN-γ is crucial for macrophage activation and induces NOS2 generation of nitric oxide, an important macrophage effector molecule. We have previously shown that NOS2 knockout (NOS2-/-) mice generate an enhanced granulomatous response to ML and exhibit features of borderline tuberculoid leprosy. In this study, experimental leprosy was evaluated in C57BL/6 (B6), IL-10-/-, NOS2-/- and IL-10/NOS2 double knockout (10NOS2-/-) mice using both the Shepard model and the modified Lepromin Test model. Growth of ML was controlled in all of the strains. However, NOS2-/- and 10NOS2-/- mice exhibited increased FP induration and T cell infiltration into the FP granuloma compared to B6 and IL-10-/- mice. ML antigen-specific, IFN-γ generating CD4+CD44+ and CD8+CD44+ T cells were augmented in the NOS2-/- FP, and these cells were enhanced even further in the 10NOS2-/- FP. Thus, IL-10 and NOS2 can modulate the intensity of the cellular response in the ML granuloma and may play a role in regulating the pathologic granulomatous response at the tuberculoid end of the spectrum.

Pain Control of Leprosy Chronic Neuritis with Cyclosporine A is not Associated with Th1 or Th2 Cytokine Plasma Levels

Claudio Guedes Salgado¹,²,³, Pedro Augusto Fiel Cabral¹,², Terezinha de Jesus de Araújo Filha², Moisés Batista da Silva¹ and Carlos Alberto Vieira da Cruz²

¹Dermatomeumology Laboratory UEPF/UFPA/Comodrigo Candia, Marituba, Pará, Brazil; ²Dr Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará, Marituba, Pará, Brazil; ³Institute of Biological Sciences, Pará Federal University, Belém, Pará, Brazil
E-mail : csalgado@ufpa.br

Leprosy chronic neuritis (LCN) is difficult to control in patients who do not respond well to prednisone, and there are few alternative drugs. Cyclosporine A (CyA) can control pain in these patients inhibiting the production of anti-NGF, which blocks NGF, a nociceptive, anti-inflammatory protein produced after nerve injury. Following our previous studies (Lepr Rev (2006) 77, 121-129), we now evaluated the presence of two Th1 cytokines, TNF-α and IFN-γ, and two Th2 cytokines, IL-4 and IL-10, plasma levels in these patients before and after CyA therapy. Plasma levels of these four cytokines were within normal limits when compared to control subjects, while the patients were taking only prednisone or after three months of 5mg/Kg/day CyA, after the complete removal of prednisone from the drug regimen. The mean period of time of LCN patients taking prednisone was 18 months. All but 2 of 15 patients had no pain complaints after three months of CyA use. These results suggest Th1/Th2 cytokines have no involvement in controlling LCN. The production of antibodies against NGF or other neurotoxins may be responsible for these patients pain, which can be controlled by CyA. Acknowledgements: This work was supported by Secretaria Executiva de Saúde Pública do Estado do Pará (SESPA); by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); by Financiadora de Estudos e Projetos do Governo Federal, Ministério da Ciência e Tecnologia (FINEP 1460/03) and; by Secretaria de Ciência, Tecnologia e Insumos Estratégicos do Ministério da Saúde do Brasil (SCTIE, MS).
Isolation of *Mycobacterium leprae* from Human Skin: Ultrastructure and Immune Response

Denis Vieira Gomes Ferreira¹, Moisés Batista da Silva¹, Jorge Pereira da Silva², José Antônio Picanço Diniz³, Ubirajara Imbiriba Salgado⁴ and Claudio Guedes Salgado¹,³,⁶

¹DermatolImmunology Laboratory UEPF/UFPA/Paracari/Marajó Institute, Marituba, Pará, Brazil, ²Institute of Health Sciences, Pará Federal University, Belém, Pará, Brazil, ³Ultrastructure Laboratory, Evandro Chagas Institute, Belém, Pará, Brazil, ⁴Dermatology Service, Pará State University, Belém, Pará, Brazil, ⁵Dr Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará, Marituba, Pará, Brazil, ⁶Institute of Biological Sciences, Pará Federal University, Belém, Pará, Brazil

E-mail: csalgado@ufpa.br

The *in vitro* culture of *Mycobacterium leprae* is still a challenge, which imposes restrictions for research on leprosy. Using a new enzymatic technique we can isolate and purify *M. leprae* from skin biopsies of multibacillary (MB) patients, allowing us to evaluate the bacilli ultrastructure and their interaction with immune cells. After diagnosis, a punch biopsy is done and the skin is kept in a disperse-RPMI medium for 5 days. After that, the specimen is submitted to two cycles of centrifugation. The first, at low speed, to remove debris, and the second, at high speed, to recover *M. leprae*, which is used for Scanning Electron Microscopy (SEM) analysis or interaction with macrophages previously isolated from peritoneum of BALB/c mice. SEM demonstrated homogeneous, 1.5x0.8mm, rod-shaped bacilli, with a rough surface, very similar to *M. tuberculosis* in culture. After 6h interaction of *M. leprae* with macrophages, supernatant levels of TNF-α raised from 2.4pg/ml to 98.2pg/ml, while IFN-γ had no difference. The experiments were performed in triplicate, and always in comparison to culture of macrophages alone. These results suggest this new technique can isolate and purify *M. leprae* directly from human skin, and these bacilli can be used in different experiments. **Acknowledgements**: This work was supported by Secretaria Executiva de Saúde Pública do Estado do Pará (SESPA); by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); by Financiadora de Estudos e Projetos do Governo Federal, Ministério da Ciência e Tecnologia (FINEP 1460/03) and; by Secretaria de Ciência, Tecnologia e Insumos Estratégicos do Ministério da Saúde do Brasil (SCTIE, MS).

A Study on Various Approaches for Classification of Leprosy Patients into Multibacillary and Paucibacillary Groups

Om Parkash, Avnish Kumar and Richa Pandey

Immunology Laboratory, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj Ganj, Agra-1, India

E-mail: om1234@gmail.com

A strategy for simplified classification method (based on presence acid fast bacilli in skin smear) into multibacillary (MB) and paucibacillary (PB) was initiated in 1982, followed by other approaches like lesion counts and serology. In this investigation we have compared the performance of all the three foregoing methods employing data obtained from 81 leprosy patients. On classifying leprosy patients according to lesion count criteria, 55.8% (29/52) of MB patients and 89.7% (26/29) of PB patients were positive with the serological test. On the other hand, the sensitivity of the skin smear to classify lesion based MB patients was 17.3% (9/52) and for PB patients it was 96.6% (28/29). Regarding serological approach, the sensitivity of the serology to classify smear positive MB patients was 80.0% (8/10) and for smear negative PB group it was 66.2% (47/71). Thus, serological assay was able to detect highest proportion (24/71; 33.8%) of MB patients in the PB group classified by the other assays. However, there existed a poor agreement (kappa value varied from 0.105 to 0.394) among all the approaches (lesion count versus skin smear; lesion count versus serology; skin smear versus serology). Keeping the above information in view, we wish to suggest that follow-up studies need to be conducted to understand the efficiency of various classification approaches in preventing the occurrence of relapse after treatment. The method permitting the occurrence of lowest relapse would be worth adapting for its future use. **Keywords**: leprosy, serology, classification, treatment, management.
Lipopeptide (Lipok) of Mycobacterium leprae Activates Antigen Presenting Cells and Type-I T-Cells

Maeda Yumi, Toshiki Tamura, Yasuo Fukunomi, Masanori Kai and Masahiko Makino
4-2-1 Aobacho, Higashimurayama, Tokyo 189-0002, Japan
E-mail: yumi@nih.go.jp

Previously, we have identified one of the lipoproteins (LpK) of Mycobacterium leprae. Triacylated lipopeptide having the N-terminal 13 amino acids of LpK (lipoK) was synthesized and used to study the immunological relevance to leprosy. LipoK stimulated human monocyte derived dendritic cell to induce the production of Th1 cytokines IL-12 and TNF-α. Also, lipoK activated M. leprae infected dendritic cells and synergistically induced cytokine production. And this induction of cytokine was blocked by the pre-treatment of these dendritic cells with anti-TLR2 antibody. Significant up-regulation of HLA-ABC, HLA-DR, CD86 and the activation marker CD83 on these lipoK pulsed M. leprae infected dendritic cells was observed. Moreover when these activated dendritic cell were co-cultured with autologous CD4+ or CD8+ T cells, significantly high production of IFN-γ was seen. The effect of lipoK on monocyte derived macrophages was similar and the induction of IL-12 production was TLR2 mediated. LipoK activated M. leprae infected macrophages and induced the production of cytokines. The results indicate that lipoK could be an effective mediator of host defenses and a tool to find optimal immunotherapeutic strategies to fight against leprosy. Keyword: leprosy, lipopeptide, dendritic cells.

Adaptative Hormones in Pathogenesis of Leprosy Neuritis

ES Balybin
Leprosy Research Institute P0414057 N. Ostrovskogo p, 3, Astrakhan, Russia
E-mail: nil@astmail.astranet.ru

The aim of the investigation is to study the role of adaptative hormones in pathogenesis of leprosy neuritis. Methods: 95 LL patients were observed, 18 of them with acute leprosy neuritis, 77 had no such signs during long period of observation. Using radio-immunologic methods there were defined the levels of hypothysis hormones in blood plasma (adrenocorticotrophic—ACTH), tirotropic (TTH); main hormones of adrenal cortex (cortisol, aldosterone). Acuteness of leprosy neuritis in most patients was predicted by significant increase of level of ACTH, TTH in comparison with beginning data, exhaustion of reserve of corticosterone function of adrenal cortex. The probability of development of such complication increased in synchronic increase of aldosteron level. In this situation the main mechanism may be non-specific stressor; overcooking, physical overpressure or psychic trauma. In some patients after physiologic load there were found decrease of ACTH production instead its sharp increase. Such patients had severe course of leprosy neuritis. Received results witnessed the special role of some adaptative hormones, including neuropeptides, in pathogenesis of neuritis. Key words: leprosy, neuritis, adaptative hormones.

The Role of Tumor Necrosis Factor Production by Leukocytes in Formation of Visceral Complications in Leprosy Patients

AV Naumov, LV Saroyants and MN Dyachina
Leprosy Research Institute, 414057, N Ostrovskogo p, 3 Astrakhan, Russia
E-mail: nil@astmail.astranet.ru

The aim of investigation is to clear up the role of the tumor necrosis factor alfa (TNF-α) production by leukocytes stimulated by M.leprae antigens in formation of visceral complications of leprosy. Leukocytes of 40 patients with lepromatous leprosy were cultivated in presence of antigens from M. leprae represented as sonicated M. leprae purified from infected tissues by Draper method. In culture supernatants there were detected the concentration of TNF-α by ELISA method. The level of cellular production of TNF-α was higher in patients with clinical and laboratory features of liver dysfunction. Some of these patients had increase of M.leprae antibodies in blood. These results witnessed that some leprosy patients could demonstrate hyperreaction of leukocytes to M. leprae which leads to intensified production of TNF-α, inflammation and liver fibrosis. The possibility of such mechanism realization in leprosy is evident for taking into consideration the ability of M. leprae to persist in liver. The received results may be used in working out the ways of cytokine therapy in leprosy. Key words: leprosy, leukocytes, tumor necrosis factor.
The Effect of Conjugal Leprosy and Genetic Susceptibility on Vaccine Efficacy Estimates

M Patricia Joyce
Centers for Disease Control and Prevention, Atlanta, GA, USA
E-mail: ev44@cdc.gov, pjoyce@cdc.gov

Despite advances in treatment and disability prevention, new case rates of leprosy have been slow to decline. Numerous studies have shown potential candidate vaccines for primary immunophylaxis. Estimates of genetic susceptibility to leprosy have been made from observational reports in familial settings using descriptive data. Risk of secondary transmission is presumed higher in family and household contacts. Conjugal transmission risk has been estimated between 1-10%, and generally thought to occur in 3-5% of spouses exposed to untreated lepromatous disease. Higher rates on conjugal leprosy have been described in populations with traditional familial intermarriage, possibly increasing the likelihood of genetic susceptibility and increased risk of infection within the population. Vaccine efficacy (VE) is calculated by comparing attack rates for a condition in vaccinated and unvaccinated groups. Genetic susceptibility or resistance to leprosy may affect VE calculation similar to that seen with differential susceptibility based on prior immunization or immunity following previous infection. Vaccine trials for leprosy should consider underlying genetic resistance to infection in study subjects. Development of markers for genetic resistance to leprosy might exclude the human population unlikely to benefit from immunophylactic measures, and provide susceptible individuals for whom a vaccine could prevent primary acquisition of this infection.

Description of HLA Alleles and Cytokines SNPs in Leprosy/AIDS Co-infected Patients: Preliminary Results

PS Rosa, SMRS Usó, CPM Carvalho, EVC Marcos, FC Souza, S Ura and RAMB Almeida
Instituto Lauro de Souza Lima; Rod comite Joanio Ribeiro de Barros Km 225, Bauru, sp: 17034-971, Brazil
E-mail: prosa@islal.br

Literature shows the role of HLA and SNPs on development of complex diseases such as leprosy and AIDS; however, there isn’t data about these markers in leprosy/aids co-infected patients. Therefore, HLA alleles class I and II (-A,-B,-Cw, -DR e -DQ) and SNPs of cytokines (TNF-α, TGFβ1, IL-10, IL-6 e INFγ) were identified to check the status of these markers in co-infected patients. Methodology: PCR-SSP, One Lambda Inc. (USA) commercial kit, was utilized for typing of 09 leprosy/aids co-infected patients between 1981 and 2006. Results: HLA alleles associated with both aids and leprosy were identified, but there was predominant association with aids. In respect to SNPs for cytokines, we also found compatible associations with both diseases, however, associations with leprosy were more frequent. Conclusion: HLA alleles in co-infected patients seem not to influence the clinical spectrum of leprosy, while SNPs of the evaluated cytokines are compatible with the clinical forms of leprosy. The presence of these markers didn’t influence the clinical manifestation of any of the diseases in the studied co-infected patients, further studies are necessary to confirm this data. Key-words: leprosy, AIDS, HLA, SNPs, co-infection.

Serological Markers to Identify Nerve Damage in Multibacillary Leprosy Patients in the INFIR Cohort Study in India

Rupendra Jadhav1,2, Lavanya Suneetha1, Karuna Devi Sagili1, Meher Vani1, Renuka Raju1, Vidya Gauri Shinde1, Ravi Kamble1, Peter Nicholls2, Diana NJ Lockwood4 and Suji Suneetha4
1 London School of Hygiene & Tropical Medicine, London, UK, 2 Nireekshana –ACET, Hyderabad & Formerly, Blue Peter Research Centre, Hyderabad, India, 3 Blue Peter Research Centre, LEPRRA India, Hyderabad, India, 4 Stanley Browne Laboratories, Miraj, Maharashtra, India
4 Department of Public Health, University of Aberdeen, Scotland; UK, 2 Presenting Author: Dr Rupendra Jadhav, Stanley Browne Laboratories, Miraj, Maharashtra, India
E-mail: rupendra.jadhav@llm.india.org

Introduction: The INFIR Cohort Study and was designed as a comprehensive project to identify predictors of reactions & nerve function impairment (NFI) in leprosy. The aim of the study was to look at various serological markers in MB leprosy patients and to evaluate their role as potential markers to predict reactions & NFI. Methodology: 303 untreated multibacillary leprosy patients from two centers in North India were the study subjects. The serological parameters tested in the serum at the time of intake of patients (baseline) were- antibodies to the M. leprae antigens PGL & LAM and antibodies against nerve component S100. The methods for serological reactions were basically Indirect Enzyme Linked Immunosorbert Assay. Results: Results reveal that mycobacterial antibodies have a significantly different immunological response across the Ridley-Jopling (RJ) classification. Antineural antibodies to S100 showed a significant increase as compared to antileishmanoid antibody. Serological measurements in patients with and without old and new sensory and motor nerve function impairment showed that PGL- IgG, LAM-IgG1 and S100 antibody were significantly associated with old sensory NFI. Conclusion: These results reveal that the antibody response to mycobacterial and nerve antigens are in a dynamic flux and collectively contribute to nerve function impairment in leprosy. Keywords: PGL, LAM, S100, Leprosy, Nerve damage.

[203]
Effect of Methyl Prednisolone on TNF-α Release in Cultured PBMC's of Treated Leprosy Patients

Karuna Devi S,1 Renuka Raju,1 Anandaraj MPJS,1 Sujai Suneetha2 and Lavanya M Suneetha2
1 Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, A.P, India, 2 Nireekshana ACET, Hyderabad, A.P, India
E-mail: drkarunas@gmail.com

Introduction: Glucocorticosteroids exert anti-inflammatory and immune suppressive effect by inhibiting the expression of cytokines and adhesion molecules. Steroids are widely used to treat leprosy reactions and prevent nerve damage. The present study was carried out to evaluate the in-vitro effect of steroid on TNF-α production in cultured PBMC's of treated leprosy patients. Methodology: PBMC's were isolated from 16 leprosy patients and 7 controls and were cultured and stimulated with Methyl Prednisolone (MP), Vitamin D3 & EB 1089 at optimum concentrations. TNF-alpha was estimated by Sandwich ELISA. Results & Discussion: 89% of patients showed >50% inhibition & 11% showed <50% inhibition with methyl prednisolone. Inhibition patterns of Vit D3 and its analogue EB 1089 were similar in patients; majority of them showing >50% inhibition. Vit D3 showed a significant difference in response. The level of inhibition of TNF-α using methyl prednisolone is high and shows no difference between patients and controls. 11% of patients showed <50% inhibition- which may be related to steroid non-responders. Though Vit D3 shows less inhibition, it shows significant difference between leprosy patients and normal controls which could be due to variation in Vit D3 metabolic pathway. Conclusion: Methyl Prednisolone shows good inhibition and is the first choice in immunosuppressive therapy. Vit D3 could be an additional anti-inflammatory therapeutic agent in leprosy. Keywords: Methyl Prednisolone, TNF-α, PBMC, Leprosy.

Auto Antibodies to Myelin Protein Zero (MPZ) in Leprosy Affected Individuals

Renuka Raju1, Karuna Devi S1, PP Reddy1, Sujai Suneetha2 and Lavanya M Suneetha2
1 Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, A.P, India, 2 Nireekshana-ACET, Hyderabad, A.P, India
E-mail: drrenukar@gmail.com

Introduction: Autoimmunity to nerve components has been implicated in various peripheral neuropathies and leprosy (Vardhani etal, 2003). Myelin Protein Zero (MPZ) is an intrinsic, structural membrane glycoprotein that comprises 40%-80% of peripheral nerve myelin. The present study was undertaken to determine whether leprosy nerve damage is associated with loss of myelin and production of antibodies to its components. Methodology: MPZ was isolated from peripheral nerve by Con A column chromatography and the protein was coated onto the ELISA plates. Antibody to MPZ was quantitated in serum by Indirect ELISA in 88 long standing treated leprosy affected individuals. Demyelination in leprosy nerve was evaluated by Immunohistochemistry using monoclonal antibodies to the extracellular domain of MPZ. Results & Discussion: The results showed that treated leprosy patients have significantly higher antibody levels (1.45 ± 0.57) as compared to normal subjects (0.48 ± 0.35). Immunohistochemical staining for Myelin P0 carried out on leprosy nerves, showed various stages of myelin degeneration in leprosy nerves, correlating with nerve histopathological observations. Immunohistochemical studies of Myelin P0 suggest that it is a good marker for demyelination and can be used for leprosy and other peripheral nerve degenerative diseases. Conclusion: The finding of this study suggests that MPZ could be an important molecule in the pathogenesis of neuropathy. Keywords: Autoimmunity, Myelin P zero, Leprosy, Neuropathy.

Intracellular Killing of M. leprae by Biological Chimeric Nanoparticles – A Novel Hypothesis

Pooria Gill1 and Amir Ghaemi2
1 Tarbiat Modares University, Tehran, I.R. Iran and 2 Golestan University of Medical Sciences and Health Care, Gorgan, I.R. Iran
E-mail: pooriagill@yahoo.com, ghaemiamodares.ac.ir

Introduction: Leprosy is a chronic infectious disease caused by Mycobacterium leprae and is still a major health problem in several countries of Asia, Latin America, and Africa. Global efforts to control leprosy by intensive chemotherapy have led to a significant decrease in the number of registered patients. However, drug resistance has been reported since 1964 for dapson, 1976 for rifampin, and 1996 for ofloxacin. To prevent the emergence and transmission of multidrug-resistant (MDR) leprosy and treat existing cases of MDR leprosy, it is necessary to establish more efficiently drugs using novel technologies. Methodology: Construction of biological chimeric nanoparticles for killing of intracellular M. leprae can be used as a novel approach in Nanobiotechnological researches for drug design. These nanoparticles are constructed using the killer mycobacteriophages targeted for specific receptors on the cells containing mycobacteria. Consequently, the nanoparticles can arrive to intracellular microorganisms and kill them in their positions, efficiently. Conclusion: This methodology can be considered not only for treatment of leprosy, but also for construction of novel drug delivery systems for other infectious diseases. The chimeric particles will also improve our knowledge about the production of novel antibacterial agents against MDR strains of other fastidious bacteria. Key words: Chimeric Nanoparticle, Mycobacteriophage, Nanobiotechnology, M. leprae.
**P-89**

The Level of Immunoglobulins (IgA, IgM, IgG), Lysozyme and pH in Saliva in Patients with Leprosy, Compared with a Group of Matched Control Subjects

U Demirel, S Badur, R Vigit and G Can

Association to Fight Against Leprosy, Istanbul, Turkey

There are currently 2599 registered leprosy patients in Turkey. Our surveys indicate that the leprosy patients who live in Turkey have the worst oral health condition. Also whole saliva is a very important factor in the homeostasis of the mouth. Antimicrobial agents (antibody and non-antibody) present in human saliva protect oral tissues by a variety of mechanisms. **Materials and Methods**: This study was conducted in the dental clinic at Istanbul Leprosy Hospital. The control group was selected from healthy people who is non-leprosy employee in Istanbul Leprosy Hospital. The aim of the present study was to determine the level of lysozyme, IgA, IgM, IgG and pH in the saliva collected from a total of 30 patients with leprosy. The patients were categorized according to type of leprosy (25 LL, 5 BL). 10 patients with LL leprosy and two patients with BL leprosy had severe type II (ENL) reaction. **Results**: There was no difference in IgG, IgA and Lysozyme levels in the saliva between the leprosy and healthy groups. However, the mean values of IgM was significantly higher in saliva from patients with leprosy diseases compared with healthy controls. Also salivary pH level with healthy group was significantly higher than leprosy group. Salivary immunoglobulins (IgA, IgM, IgG), Lysozyme and pH levels in the leprosy patients were not significantly among the LL and BL groups. **Conclusions**: Most of the leprosy patients in Turkey live in the rural areas. Nearly all patients are very poor; The incidence of deformities in our patients is high, excluding them from regular employment and a source of income. It is important that we consider all possibilities to develop, implement and manage oral health programs for patients with leprosy. Also oral health status of economically and socially disadvantaged leprosy patients must be improved more importance in Turkey in the 21st century. **Key Words**: leprosy, IgA, IgM, IgG, Lysozyme, pH.

**P-90**

Thalidomide Does Not Interfere with the Activation of Complement

E Shannon and F Sandoval

National Hansen's Disease Programs, Laboratory Research Branch, Baton Rouge, LA, USA

Thalidomide is effective treatment of erythema nodosum leprosum (ENL). Its mechanism is not known. Many of the histological features of ENL, especially the detection of bacterial antigen, antibodies, complement, and neutrophils are suggestive of an immune complex hypersensitivity reaction. If ENL is initiated by immune complexes, activation of complement will facilitate inflammation. We investigated thalidomide's effect on complement. Serum from six individuals was incubated with zymosan or with a highly-purified-viable preparation of *M. leprae*. The *M. leprae* were harvested from nu/nu mice, treated with alkali, washed, and sonication. The serum-zymosan or serum-*M. leprae* preparations were incubated with 2 to 4 µg/ml thalidomide. [These concentrations are within the concentrations achieved in vivo for the treatment of ENL]. The CH50 titer of the serum was determined. The control preparations of zymosan and *M. leprae* activated complement. There was no significant difference between the CH50 Units in the controls and the CH50 Units in any of the concentrations of thalidomide assayed. Thalidomide does not appear to disrupt the pathophysiological contribution that may be made by complement in the ENL reaction. **Key words**: ENL, Thalidomide, Complement.

**P-91**

Loop-Mediated Isothermal Amplification (LAMP) for the Detection of *Mycobacterium leprae* from Slit-Skin Fluid

S Bishwa Raj

Anandaban Leprosy Hospital, P.O. Box 151, Kathmandu, Nepal

E-mail: anandaban@fmmnepal.org

For the establishment of a genetic diagnostic tool for *Mycobacterium leprae*, the LAMP assay was performed with simple freeze and boil DNA extraction from the slit-skin fluid. A total of 39 clinical specimens of *Mycobacterium leprae* from slit skin fluid of newly diagnosed patients were used in the LAMP assay. The sensitivity and specificity was compared with slit-skin smear results. A set of four primers comprising two inner primers and two outer primers, that recognized 6 distinct regions on the targeted RLEP gene sequences (GenBank accession number X17151 (RLEP1), X17152 (RLEP2), X17153 (RLEP3)) of *Mycobacterium leprae* (Mukai et al. 2006, US-Japan Cooperative Medical Science Program) were used for LAMP assay (Notomi et al., 2000, Nucleic Acids Res). **Key words**: LAMP, isothermal amplification of mycobacteria.
Localization of FOX P3 (Forkhead Box Protein P3) in Human Leprosy

Mehervani Chaduvula, V Vijayalakshmi and Indira Nath
Blue Peter Research Center, LEPRO Society, Hyderabad, India
E-mail: mehervani@bprleprasociety.org

Introduction: T cells exerting negative modulation of immune responses are currently designated as T regulatory cells (Treg) and have been characterised to be CD4+ CD25+ cells with a specific transcriptional factor FOXP3 (Forkhead box protein P3). The newly defined T regulatory (Treg) cells would have immediate relevance for understanding the leprosy spectrum and T cell unresponsiveness in leprosy. This study aimed to localize the expression of FOXP3 in paraffin embedded skin lesions of leprosy.

Methodology: Serial sections from formalin fixed paraffin skin tissue of leprosy cases were studied for the localization of T cells and natural T reg cells using CD3 and FOXP3 markers by immunohistochemistry. Super sensitive - Horseradish peroxidase (HRP) method was used.

Results and Conclusion: Intense nuclear stain of FOXP3 indicative of T reg cells was observed mainly in the borderline tuberculoid as compared to lepromatous leprosy skin lesions. Key words: T regulatory cells, FOXP3, CD3, human leprosy.

Known Leprosy Contact Among HIV Infected Patients

Patricia Duarte Deps, CG Gripp, RL Aragão, MP Andrade, DG Gripp, RM Loureiro and BL Alves
Federal University of Espirito Santo, Vitória-ES, Brazil
E-mail: pddeps@uol.com.br

Introduction: Leprosy in HIV+ patients is not so frequent even in Brazil, where both diseases are endemic. However, after HAART introduction for HIV+, their immune condition has changed and those patients should be observed carefully looking for leprosy mainly when they report leprosy known contact (KLC). The objective is to evaluate the KLC and its importance among HIV+ patients.

Methodology: A cohort study was carried through in Vitória, Brazil, with 153 HIV+ patients. They answered a questionnaire and blood was collected from those that reported KLC. Anti-PGL-1 was evaluated among them by ML Flow.

Results: Of the 153 studied patients, 48% were women and 52% were men. The mean of age was 42 and the mean of CD4 account was 418. HAART was being used by 116 (76%) and 37 (24%) not. KLC was reported by 31 (20%) and 122 (80%) not. Three patients (13%) from 23 tested showed ML Flow (+), however they had no clinical signs for leprosy. They are using HAART. Conclusions: HIV+ patients with KLC and ML Flow (+) should be monitored for leprosy onset, once they have two conditions that can be considered as risk factors for leprosy: KLC and antibodies anti-PGL-1 production. Key words: HIV, ML flow, known leprosy contact.

Comparison Between Two Rapid Tests for Anti PGL-1 Serology

Grassi AB1, Sampaio LH1, Martelli CMT1, Cho R2, Osakam L1, Buhrer S1 and Stefani MMA1
1 Tropical Pathology and Public Health Institute, Federal University of Goias, Goiânia, GO, Brazil; 2 Yonsei University, Seoul, South Korea; 3 KIT Biomedical Research, Amsterdam, The Netherlands
E-mail: mstefani@iptsp.ufg.br

This cross-sectional study compared leprosy rapid tests detecting (1) IgM against PGL-1 (ML-Flow test, KIT, Amsterdam, Netherlands) or (2) IgM, IgG and IgA against PGL-1 (ML-ICA, Yonsei University, South Korea). Whole blood and serum from untreated multicellular (MB; n=50) and paucibacillary (PB; n=48) leprosy patients (Reference Center for Diagnosis and Treatment, Goiânia, central Brazil), MB household contacts (HHC; n=35) and healthy endemic controls (EC; n=50) were tested. Leprosy patients were classified by dermato-neurological evaluation, bacillary index (BI) and histopathology. Median age was 29 years, 39.9% males. MB patients had BB-BL-LL leprosy (median BI=2.0), PB patients had TT-BT leprosy.

### Anti PGL-1 Positivity (%)

<table>
<thead>
<tr>
<th>Category</th>
<th>Whole Blood</th>
<th>Serum</th>
<th>Whole Blood</th>
<th>Serum</th>
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<tr>
<td>MB</td>
<td>84%</td>
<td>94%</td>
<td>48%</td>
<td>90%</td>
</tr>
<tr>
<td>PB</td>
<td>31%</td>
<td>44%</td>
<td>0</td>
<td>19%</td>
</tr>
<tr>
<td>HHC</td>
<td>0</td>
<td>31%</td>
<td>3%</td>
<td>20%</td>
</tr>
<tr>
<td>EC</td>
<td>0</td>
<td>2%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Strong seropositivity (≥ 2) in ML-Flow and ML-ICAS in MB patients was 22% and 26% and in PB leprosy 8% and 15%. ML Flow positivity in whole blood and serum of MB and PB patients was higher than ML-ICA positivity. Funding: The Heiser Program for Research in Leprosy and Tuberculosis for IDEAL Consortium. Key Words: anti-PGL-1 rapid tests.
A New Mycobacterium Species Causing Diffuse Lepromatous Leprosy

John S Spencer¹, Hee Jin Kim¹, Richard A Slayden¹, Mary Fafutis Morris², Iris Estrada Garcia¹, Varalakshmi D Vissa¹, Patrick J Brennan¹, R Geetha Nair³ and Xiang Y Han²

¹Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, ²Universidad de Guadalajara, Centro Universitario de Ciencias de Salud, Guadalajara, Jalisco, Mexico, ³Department of Laboratory Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, TX NIH, NIAID Contract N01 AI-25469
E-mail: John.Spencer@colostate.edu

Diffuse lepromatous leprosy (DLL) with Lucio’s phenomenon was originally described over 150 years ago in Mexico by Lucio and Alvarez. It is characterized by a distinctive necrotizing skin reaction in non-nodular leprosy, and affects individuals primarily in the Sinaloa and Jalisco provinces of Mexico, Costa Rica, and countries in the Caribbean. From an individual who was diagnosed with DLL at a clinic in Phoenix, AZ, mycobacteria were purified from skin lesions, lung, and lymph nodes, and DNA was isolated for genetic typing. The antibody titer to the M. leprae-specific PGL-I was very high (50% endpoint, 1:1,500), although reactivity to 6 out of 10 M. leprae recombinant proteins indicated an unusual pattern for a lepromatous patient. Sequence analysis of genes from two individuals with DLL revealed that the 16S rRNA gene diverged by 2.1% and contained a unique sequence, while five other genes mismatched by 6–14% when compared to that of the TN strain of M. leprae. Phylogenetically, using evolutionary trees for a number of sequences, it appears that this mycobacterium evolved from a common ancestor with M. leprae that had branched off from other mycobacteria. These findings may have implications for the research, diagnosis and treatment of DLL. Key words: diffuse lepromatous leprosy, Lucio’s phenomenon, 16S rRNA.

Application of New Serological Test for Leprosy in Vietnam

Kai Masanori, Nguyen Phuc Nhu Ha, Yasuo Fukutomi, Yuji Miyamoto, Yumi Maeda, Tetsu Mukai, Nguyen Thanh Than and Masahiko Makino

Department Microbiology, Leprosy Research Center, National Institute of Infectious Diseases, 4-2-1 Aobacho, Higashimurayama, Tokyo 189-0002, Japan
E-mail: mki@nih.go.jp

Since early detection and rapid medication is important for leprosy control, serodiagnosis is very useful to detect Mycobacterium leprae infection. We have established a new serodiagnostic test using major membrane protein-II (MMP-II) as antigen for detecting leprosy infection. So, in this study, we conducted serological survey of leprosy patients, their contacts, healthy individuals living in the central part of Vietnam. Although Vietnam attained the WHO’s leprosy elimination target of less than 1 per 10,000 population in 1995, the number of newly detected cases in the year 2006 was still 666. We measured anti-MMP-II IgG antibody level and anti-PGL-I IgM antibody level in sera from 224 leprosy patients, their contacts and 211 healthy controls by enzyme linked immuno-sorbent assay (ELISA). The percent positivity by MMP-II ELISA was 83% for multi-bacillary leprosy (MB). For pauci-bacillary leprosy (PB) where cell-mediated immunity predominates, 4.7% showed positive results. Although, the positive rate of PGL-I ELISA were almost similar in both MB and PB to those of MMP-II ELISA. These results suggest that MMP-II antibody detection would facilitate diagnosis of leprosy.

Type 2 Reaction of Leprosy Hospitalized Patients at a University Hospital: Study of Immunological Mechanisms and their Clinical Expressions

Sueli Carneiro, Fernanda Torres, Bruna Martins, Vivian Balassiano, Maria Katia Gomes and Marcia Ramos-e-Silva

Sector of Dermatology HUCFF/UFRJ and Post Graduation Program, School of Medicine, Universidade Federal do Rio de Janeiro, Brazil

Introduction: Review of 133 files of Leprosy patients presenting type 2 reaction, admitted to our University Hospital, from 1979 to 1990, to assess clinical, epidemiological, immunological and laboratorial data. Results: Fifty percent of the patients were between 21 and 40 year-old, and the majority was female (57.9%). Lepromatous form was seen in 72.9% and borderline in 25.5%. Erythema nodosum late reactions were more frequently seen in the lepromatous form (71%) than in borderline form (44%). There was lower incidence of polymorphic erythema (13% and 32%, respectively). Lucio’s phenomena were observed in 15.4% of lepromatous patients and in 23.6% of borderline patients. Immunoglobulin profile was similar in both groups. Articular commitment was seen in 55.6% of the cases. Conclusion: The combination of exams and effective clinical and immunological evaluation can help to monitor and to prevent the disabilities and neural injury. Key words: Type 2 Reaction: Leprosy; Immunology.
Expression of Coronin-1 in Mouse Schwann Cell Line (SW 10 Cell)

Chung Eun Yeum, Jae Hyung Kim and Gue-Tae Chae
Institute of Hansen's Disease, Department of Pathology, College of Medicine, The Catholic University of Korea, 505, Bampo-Dong, Seol, Korea
E-mail: guetae@catholic.ac.kr

It is already known that M. tuberculosiis and M. bovis BCG induces expression of Coronin (TACO, tryptophan aspartate-containing coat protein), which is recruited into phagosome membrane depends on lipid raft of cholesterol during phagosome formation process. Thus, TACO suppresses phagosome-lysosome fusion when the mycobacterium invade into host. We tried to find out whether Coronin-1 protein will be influenced with survival of Mycobacterium leprae(M. leprae) in phagosome. The expression of Coronin-1 protein was observed at both tissue section of leprosy patients and nu/nu mice foot pad injected with wild M. leprae by immunohistochemistry using home-made rabbit originated polyclonal Coronin-1 antibody. The expression and accumulation of Coronin-1 protein was observed on phagosomal membrane infected with live M. leprae, markedly different from dead M. leprae that showed scant or few Coronin-1 protein with immunofluorescence stain and immunogold labeling, in vitro assay with murine macrophage Raw 264.7 cell line. We also applied the Coronin-1 expression in murine Schwann cell line (SW10) infected with M. leprae, but the Schwann cell could not expressed Coronin-1 protein in spite of phagocytosis of M. leprae. It suggests that Coronin-1 expression appears unique function of macrophages. Keywords: Leprosy, Mycobacterium leprae, Coronin-1, Schwann cell

Vasomotor Reflex Alteration and PGL-I Antibodies Aid the Early Diagnosis of Leprosy

Ximena Ilarramendi, AM Sales, R Vital, N Duppre, RB Teles, N Piccolo, JAC Nery and EN Sarno
Oswaldo Cruz Institute, FIOCRUZ, Laboratório de Hanseníase, Av Brasil 4365, Manguinhos Rio de Janeiro,RJ, 21040-360
E-mail: ximena@ioc.fiocruz.br

Early detection of leprosy remains one of the major challenges in the control of the disease. Dysautonomia occurs in leprosy neuropathy and indicates early nerve lesion. On the other hand, the presence of anti-PGL-1 antibodies has been considered as a measurement of disease in contacts. At the Leprosy Clinic, FIOCRUZ, Rio de Janeiro, active leprosy detection is performed by contact vigilance. A group of 25 co-prevalent cases (ie. contacts with leprosy on their first evaluation following their index case diagnosis) was evaluated. A high prevalence (20%) of both altered skin vasomotor reflex (44%) and presence of anti-PGL-1 antibodies (46%) was observed on diagnosis. All of the patients were PB (diagnosed as indeterminate or borderline-tuberculoid), and such a pattern is not expected in this group of patients. Studying autonomic alterations in leprosy patients and their contacts has provided new information to explain the evolution of leprosy neuropathy. Contact examination is fundamental for early detection of leprosy. Keywords: contacts, co-prevalent case, skin vasomotor reflex, PGL-I antibodies, autonomic neuropathy

Detection of Mycobacterium leprae Short Tandem Repeats (STR) Strain Loci Distribution From Nasal Secretions of Untreated MB Cases

Hema Newton 1, Gift Norman 1, Joyce Ponnaaya 1, Tom P. Gillis 2, Abraham Joseph 1 and Job CK 1
1 Schieffelin Institute of Health Research and Leprosy Center, Karigiri, Tamilnadu 632 106, India 2 Laboratory Research Branch, National Hansen's Disease Program, Baton Rouge, LA, USA
E-mail: norman@htf.yahoo.co.in

This pilot study was undertaken to study the distribution of Short Tandem Repeats (STR) loci of M. leprae from nasal secretions of untreated smear positive MB cases. The study was carried out on untreated MB patients registered SIHR&LC, Karigiri from 2005-2007. Nasal swabs from untreated smear positive MB cases were collected in Specolysin Reagent (a concentrate of Dithiodibiotol) and Mycobacterium leprae DNA was extracted using DNasey blood and tissue kit method (Qiagen). Distributions of Multiple sets of Mycobacterium leprae STR loci were studied by PCR-Direct Sequencing to identify different strain types of M. leprae. 13 sets of Short Tandem Repeats (STR) loci of M. leprae had been selected which provide partial typing capable of differentiating M. leprae isolates among 50 potential polymorphic STR identified in recent publications worldwide. The characterisation will define the transmission pattern among population from an endemic origin. The results will be reported.
M. leprae Inhibits Apoptosis in THP-1 Cells by Downregulation of bad and bak and Upregulation of mcl-1 Gene Expression

Zahra Hasan, Mussarat Ashraf, Ali Tayyebi and Rabia Hussain
Department of Pathology, Microbiology, The Aga Khan University, Karachi, Pakistan
E-mail: zahra.hasan@aku.edu

Virulent Mycobacterium leprae interfere with host apoptotic mechanisms. We investigated M. leprae and non-pathogenic M. bovis BCG induced expression of apoptotic genes in THP-1 monocytes. M. leprae did not induce apoptosis in THP-1 cells in contrast with BCG. BCG-induced cell death was accompanied by characteristic apoptotic DNA laddering in cells. Non-viable BCG had a limited effect on host cell death suggesting that BCG-induced apoptosis was a function of mycobacterial viability. M. leprae also activated lower levels of TNF-α secretion and mRNA expression than BCG. A time course of infection illustrated that M. leprae reduced Bad and Bak mRNA expression by 18 h post-stimulation, with a further decrease at 48 h. M. leprae infection resulted in downregulation of gene expression ratios, Bad/Bcl-2 mRNA by 39% and Bak/Bcl-2 mRNA by 23%. In contrast, live BCG increased Bad/Bcl-2 mRNA (29%) but had a negligible effect on Bak/Bcl-2 mRNA. Heat killed BCG induced only a negligible (1-4%) change in mRNA expression of either Bak/Bcl-2 or Bad/Bcl-2. Additionally, M. leprae upregulated the expression of anti-apoptotic gene Mcl-1 while, BCG downregulated Mcl-1 mRNA. We propose that M. leprae restricts apoptosis by downregulation of Bad and Bak, and upregulation of Mcl-1 mRNA expression.

Laboratory Estimation of Intoxication in Leprosy

AK Ayupova, NG Urylapova and AA Juschenko
Leprosy Research Institute, PO 414057, N Ostrovskogo p, 3, Astrakhan, Russia
E-mail: nil@astmail.astranet.ru

The degree of intoxication in leprosy was studied with the help of clinical degidration of blood serum (BS) effective in estimation of character and severity of pathologic changes in patient’s organism. Additionally it was determined the content of BS molecules of middle mass, universal indicator of endogene intoxication using the method of UV-spectrophotometry in diapason of waves length from 290 to 320 nm. There were observed 87 patients with leprosy (12 in active stage, 75 — in regress) at the age of 40 and 87. Control was BS of donors. The morphologic picture of BS witnessed moderate level of intoxication. Structural markers of intoxication toxic platelets were observed in 82 patients. Maximal number of toxic platelets was observed in BS of leprosy patients with neurotropic ulcers. Results of morphologic analysis were confirmed by data of biochemical observation of deproteinized supernatant of BS characterized by increased level of middle mass molecules with length of wave from 248 to 280 nm. It is known that the length of waves from 240 to 248 nm characterized the presence of substance of catabolic origin, 278-286 nm — parts of proteins containing aromatic aminoacids. So, the results demonstrate moderate degree of intoxication in leprosy and underline the fact that mostly it depends on complications of leprosy. Key words: leprosy, intoxication, laboratory diagnostic.

ML Flow Serologic Test Versus Bacilloscopy Results in Newly Detected Leprosy Patients

Sandra Lyon1, Ana Claudia Lyom2, Rozana Castorina da Silv3, Silvia Helena Lyon de Moura1; Maria Aparecida de Faria Grossi3, Samira Bührer-Skula4 and Manoel Otávio da Costa Rocha5
1Sanitary Dermatology Service, Hospital Eduardo de Menezes – Fundação Hospitalar do Estado de Minas Gerais – Belo Horizonte, Brazil;
2Post-Graduate Program in Health Sciences: Infectology and Tropical Medicine – Universidade Federal de Minas Gerais – Belo Horizonte, Brazil;
3State Coordinator of Leprosy Control in Minas Gerais, Brazil; 4Universidade Federal de Goiás, Brazil.
E-mail: dclp@vsnl.com

Introduction: The aim of this work was to compare ML Flow serologic test and bacilloscopy results in leprosy patients. Methodology: A group of 135 patients with newly detected leprosy at a reference service in Sanitary Dermatology in Brazil was submitted to bacilloscopy (registered as bacillary index – BI) and ML Flow test (registered qualitatively and semi-quantitatively) at admission. Results: ML Flow test was positive in 57% of patients; bacilloscopy was positive in 35.9% of cases. Patients with more than five skin lesions had five times the chance of having positive ML Flow test result when compared to other patients. ML Flow result was positive in 100% of patients with BI > 2+ and in 93.5% of patients with positive bacilloscopy. Patients with BI > 2+ had more than three times the chance of having a positive serologic test in high category (4+) in comparison to those who had low BI. Conclusions: There is a strong correlation between the results of serology and bacilloscopy results, which suggests that the ML Flow test may become a useful tool in clinical classification of leprosy. Key-words: Leprosy, ML Flow serologic test, bacilloscopy, diagnosis.
Oxidative Stress- Trend in Lepra Reaction

N Chhabra, SN Bhattacharya, A Singal and R Ahmed
Department of Dermatology, University College of Medical Sciences, Guru Teg Bahadur Hospital, Delhi-110095
E-mail: chhabra.narmata@gmail.com

Background: Lepra reactions develop through abrupt changes in host parasite immunologic balance and resultant acute clinical exacerbations. Reactive oxygen species play a significant role in pathogenesis of leprosy and Multi-drug therapy demonstrably tends to normalize the resultant oxidative stress. Rifampicin and Dapsone have antioxidant action while clofazimine has both pro-oxidant and anti-inflammatory action. However, data regarding oxidative stress in lepra reactions and effect of drugs used in its treatment is lacking. Objectives: Aim of study was to assess oxidative stress in lepra reactions and determine its change with treatment of the reaction. Methods: From Dec 2006 to Mar 2008, a prospective study is being conducted in 40 patients with lepra reactions. MDA, FRAP and Reduced Glutathione were measured and comparisons of oxidant status and anti-oxidant capacity by assay of these parameters at inclusion, after 4 weeks of initial therapy (following standard guidelines including MDT, NSAIDS and cortico-steroids), and yet again 4 weeks after clinical remission were performed. Statistical analysis using repeated measure ANOVA and with Tukey's test was performed. Results: Upcoming trends in this study under process show no significant difference in oxidative stress parameters in tuberculoid and lepromatous pole with reaction. Oxidative stress is towards higher side in patients with active nerve damage. After 4 weeks of follow up, oxidative stress parameter (MDA) continues to increase and anti-oxidant level (FRAP, Glutathione) continues to decrease despite treatment. With the clinical remission of reaction, all these parameters show a significant improvement. Conclusions: Oxidative stress correlate well with nerve damage and improves after successful completion of treatment of reaction. Key words: oxidative stress in lepra reaction, MDA, FRAP, Glutathione, NSAIDS, Corticosteroids.

Mycobacterium leprae as a Unique Leprosy Research Reagent

James L Krahenbuhl and Ramanuj Lahiri
Laboratory Research Branch (LRB) of the National Hansen's Disease Programs (NHDP), Baton Rouge, LA, U.S.A
E-mail: jkrahenbuhl@hrsa.gov

The absence of a reliable source of M. leprae suitable for leprosy research has been a major obstacle in the study of this disease for >130 years. Highly viable (and irradiated) purified M. leprae are needed to address the complex immunological and cell biology questions that define the fascinating relationship between M. leprae and its host cells and shed light on the unique pathogenicity of leprosy. The NHDP-LRB has devoted considerable effort to routinely providing M. leprae as a reagent and characterizing the concept of “viability” in M. leprae harvested and purified from the nu/nu mouse foot pad. We have developed a “programmed passage” schedule permitting the harvest of billions of bacilli weekly that are 80-90% viable, a level unprecedented in the history of leprosy research. We have made >500 express shipments worldwide to over a dozen researchers. Qualified researchers are invited to request this valuable research reagent from the NHDP. Shipments of 1 – 2 billion can be handled routinely. Key Words: M. leprae, Mouse foot pad, Purified, Viable, Provision.

Potentialities of Combined Usage of Peroxidase – Containing Horseradish Root and Lodide for Therapy of Experimental Leprosy

AK Maslov and SA Luchnova
Leprosy Research Institute, 414057, N Ostrovskogo p, 3 Astrakhan, Russia
E-mail: nil@astrmail.astranet.ru

Aim: A study therapeutic effect of horseradish root combined with potassium iodine in experimental leprosy. Material and methods: CBA mice, inoculated with M. leprae isolated from leprosy patients. Determination of neutrophil myeloperoxidase activity, blood count and assessment of hepatic function by AST and ALT – activity, M. leprae count in mice foot-pads. Results: combined therapy per os had a higher antimicrobial effect as compared to monotherapy with peroxidase – containing horseradish root. Combined administration of horseradish root and iodine activated myeloperoxidase in blood neutrophils, had antiinflammatory action, stimulated cell-mediated immunity, showed no signs of anemia and toxic effects of mice liver. Conclusion: proposed approach to therapy of experimental leprosy increases activity of myeloperoxidase system, which is one of the main bactericidal systems of phagocytes and might be used in complex therapy of leprosy. Key words: experimental leprosy, horseradish root, potassium iodine, phagocytic activity, haemogram.
Viable Mycobacterium leprae Does not Induce Apoptosis in Infected Murine Macrophages

Ramanuj Lahiri, Baljit Randhawa and James L. Krahenbuhl
Laboratory Research Branch, National Hansen's Disease Programs, Baton Rouge, LA, U.S.A
E-mail: krah@nhsa.gov

The principal host cells of M. leprae are macrophages and Schwann cells. A number of studies have reported that M. leprae induces apoptosis in their host cells. This is not consistent with our studies with murine macrophages where even a heavy intracellular burden of M. leprae had essentially no adverse effect. We suspect that the purity and viability of M. leprae used might be the cause of these divergent findings. In this study we explored the effect of freshly harvested nude mouse foot pad derived live and irradiated M. leprae in inducing apoptosis in infected macrophages. Apoptotic cells were determined by annexin V and TUNEL assays and also by staining for cells with activated Caspases. We also studied heavily infected macrophages from infected nude mouse foot pads. To further assess the role of M. leprae viability in induction of apoptosis, we incubated cells infected with viable bacilli at 37°C, as M. leprae rapidly loses viability at 37°C. Our results indicate that viable M. leprae does not induce apoptosis. However, loss of M. leprae viability induces apoptosis in infected macrophages. Key Words: M. leprae, Apoptosis, Macrophage, Viability.

Analysis of Plasma Proteome of Mycobacterium leprae Infected Mice

Bini Ramachandran1, CS Suribabu2 and K Dharmalingam1
1Department of Genetic engineering, School of Biotechnology, Madurai Kamaraj University, Madurai, India; 2Central Leprosy Teaching and Research Institute, Chengalpet, India
E-mail: kdharmanlgam@iim.com

Introduction: Plasma proteome profiling allows better understanding of the physiological state of the organism. Such profiles also provide the foundation for identification of candidate protein markers for the differential stages of the system. Since the onset of M. leprae infection in humans cannot be determined with confidence, studying the early responses to infection in human system is restricted. Footpad inoculated mice could be a good model system to study the early host responses to M. leprae infection. Methodology: Biopsy derived M. leprae was inoculated to the footpads and blood was collected at intervals from sixth month after infection. The plasma proteome was analyzed using two-dimensional electrophoresis and compared with appropriate controls. Differentially expressed proteins were identified by MALDI-TOF mass spectrometry. Results: Significant change in the expression levels of acute phase proteins like apolipoprotein A1 and haptoglobin was found in infected mice. The expression of the isoforms of apolipoprotein A1 was also differentially regulated. One of the isoforms was absent in plasma collected after 6-7 months of M. leprae infection; however this isoform was restored in the later stages of infection. Conclusions: A generalized inflammatory response to multiplication of M. leprae could be demonstrated by examining the plasma proteome. The ongoing study may help to provide new insights into the host-pathogen interactions in leprosy and in the identification of early responses to infection. Key words: Plasma proteome, mice footpad inoculation, acute phase proteins.

Functional Analysis of sHSP 18 Antigen of Mycobacterium leprae

S. Shibajrak, N. Lin, EA Rehna and K Dharmalingam
Department of Genetic Engineering, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamilnadu- 625021, India
E-mail: kdharmanlgam@iim.com

Introduction: HSP18 is one of the major T-cell antigens of M. leprae, presumably a Heat shock protein. We have cloned and expressed the gene of HSP18 in E. coli and examined its functions in vitro and in vivo. Materials and Methods: sHSP18 gene of M. leprae was amplified from biopsy-derived cDNA of leprosy patients, cloned and expressed in pQE31 vector. Histidine tagged recombinant protein was purified with Ni-affinity chromatography. The purified proteins were run on a non-denaturing polyacrylamide gel (7.5%) to check self aggregation. In vitro cross-linking of the sHSP18 protein was also done using Gluteraldehyde (1%). For analyzing the effect of sHSP18 on thermal aggregation of E. coli soluble proteins, the cell extracts with or without HSP18 were heated at 100°C for 0, 20, 30, 40 and 60 min and centrifuged at 10,000 x g for 5 min at 25°C. The residual proteins were visualized by SDS-PAGE. The preheated HSP18 (100°C) was analysed for in vitro chaperon assay using Smal restriction Enzyme. Results: In the native gel analysis sHSP18 showed self aggregation and appears as a monomeric structure of 100-200 kDa size. The time course of cross-linking with 1% gluteraldehyde shows that the 18HSP proteins are forming oligomeric structures of dimeric, trimeric, hexameric and nonameric forms. The M15/pQE3 1/18HSP cells demonstrated thermotolerance at 47.5°C. It also prevents thermal aggregation of E. coli soluble proteins. Conclusions: sHSP18 antigen of M. leprae, a small heat shock protein with conserved α-crystallin domains, forms multimers and we also could demonstrate for the first time, the in vitro chaperon activity of this protein. Key words: M. leprae, Heat shock Protein, Chaperons.
Study of Cross Reactivity of M. leprae Reactive Salivary IgA with Other Environmental Mycobacteria

RR Kamble, VS Shinde, SP Madhale and RS Jadhav
Stanley Browne Labs, Richardson Leprosy Hospital, Sangli-Miraj Road, Miraj 416410, Maharashtra, India
E-mail id: ttimirmiraj@ttimirindia.org

Multidrug therapy has significantly reduced leprosy prevalence in most of the endemic countries. But the incidence is decreasing very slowly. Majority of the endemic population in exposed to Mycobacterium leprae but very few develop disease. This could be because of the development of protective immunity against the pathogen. Humoral mucosal immune response has been suggested to be quite important in the protective immunity. Presence of M. leprae reactive antibodies in the saliva has been reported earlier. As the endemic population is also exposed many environmental bacteria, we tested saliva from 121 subjects for the presence of antibodies against other mycobacteria like M. smegmatis and M. phlei. We also checked for the cross reactivity of the antibodies. Saliva samples were cross-reacted with these two mycobacteria prior to testing M. leprae reactive antibodies by ELISA. In 59 subjects (48.76%) original saliva and cross reacted saliva showed same absorbance values (antibody levels) suggesting no cross reactivity. 26 subjects (21.49%) showed less than 25% drop in the O.D. values whereas 21 subjects (17.4%) showed 25 to 50% drop after reacting saliva with the mycobacteria. 15 subjects (12.4%) showed more that 50% drop in O.D. The data suggest that though large proportion of subjects had M. leprae reactive antibodies in their saliva that did not cross react with other mycobacteria tested, there were some subjects where salivary antibodies showed cross reactivity to different mycobacteria. Key words: cross reactivity of M. leprae, mycobacteria.

Study of Cytokine Response Against Panel of Purified M. leprae Antigens by Whole Blood Assay in Subjects Residing in a Resettlement Village of Cured Leprosy Patients

RR Kamble, VS Shinde, SP Madhale and RS Jadhav
Stanley Browne Labs, Richardson Leprosy Hospital, Sangli-Miraj Road, Miraj 416410, Maharashtra, India
E-mail id: ttimirmiraj@ttimirindia.org

Mycobacterium leprae being an intra cellular pathogen, cell mediated immunity is very important in the clinical outcome of leprosy. Manifestation of the disease is correlated with the level and type of cell mediated immune response. The main objective of this study was to analyse TNF-α and IFN-γ production by T-cells when challenged with different M. leprae purified antigens in subjects with known exposure. 52 subjects residing in resettlement village of cured leprosy patients were included in the study. Whole Blood assay studies were undertaken in which the blood was placed in culture and was challenged with 35kDa antigen, whole M. leprae cells, M. leprae cell wall antigen, M. leprae cell wall antigen inus LAM and cytosolic fraction. T-cell derived cytokines TNF-α and IFN-γ were measured by ELISA. It was observed that challenging the lymphocytes with 35 kDa antigen, M. leprae cell wall antigen minus LAM and the cytosolic fraction resulted in increased levels of IFN-γ whereas challenge with 35kDa antigen and M. leprae cell wall antigen resulted in increased levels of TNF-α. Key words: cytokine responses, cured leprosy patients.

Genotyping of M. leprae and Study on Families with Multi-Cases

Liu Jian, Wang Zheng, Wen Yan, Tian Xiuju, Li Huanying and Weng Xiaoman
Capital University of Medicine affiliated Beijing Friendship Hospital, Beijing Tropical Medicine Research, Institute, Beijing, PR China 100050
E-mail: wengxiaoman@sina.com

Objective: Strain typing with MLVA on 7 VNTR loci (1) was applied to 84 M. leprae isolates from Qiubei County, Yunnan Province. The genotype of the isolates were traced to their village of origin and relationship to the index case, in multi-case families. Methods: All members in the family with a confirmed case were screened annually for leprosy and also the immediate neighbors of the index case (map). Phenogenetic analysis through application of PAUP 4.0, the isolates were grouped into A, B, C, D, E strains according to the allelic range of 9, 11-13, 15-26 and >26 on the (GTA)9 locus. Results: The isolates with nine copies on (GTA)9 are in Group A. The isolates from five multi-case families were from two northern townships GZ & GH of Qiubei. They belong to Groups A and B. One-fourth of the isolates (21/84) belong to cluster A and 8.3% (7/84) to cluster B. Conclusion: Genotype of isolates within a family are usually identical or nearly identical, but not between families. These results indicate that M. leprae belonging to Group A is the prevalent strain in Qiubei during this period.
Cloning of Fragment 16S rRNA of Mycobacterium leprae in E. coli

Jadhav RS, Kamble RR, Shinde VS and Katooch VM
Stanley Browne Labs, Richardson Leprosy Hospital, Sangli-Miraj Road, Miraj 416410, Maharashtra, India
E-mail: ttmiraj@ttm.com

Decline in prevalence of leprosy in last few years has raised hopes of elimination and eventually its eradication. But the problems like early detection, determining efficacy of the treatment and differentiating relapses from the reactions can linger on and will also have a serious impact on the actual state of the disease in the community. Development of a tool, which could help answer questions related to M. leprae viability will aid to some extent effective elimination and also subsequent eradication of the disease. The present work aims at developing quantitative PCR technology using ribosomal RNA genes as a target. As a part of the strategy we used directional cloning method to clone a DNA fragment (171bp) coding 16S rRNA in E.coli SURE strain using pGEM 3Z plasmid vector. This fragment can be amplified using M.leprae specific primers. We also have cloned part of same fragment with internal deletion of 60 bp. The clones were screened by blue/white colony screening method using X-gal and IPTG and by colony PCR. Presence of fragment in the plasmid was confirmed by restriction digestion of the plasmid. The recombinant plasmids can be used to generate RNA in vitro which subsequently can be used as internal controls as well as standards in RT-PCR reaction for the quantization and quality control purpose.

Serum Proteome Analysis of Leprosy Patients: Search for Disease Biomarkers

Nishma Gupta, NP Shankernarayan and K Dharmalingam
Department of Genetic Engineering, School of Biotechnology, Madurai Kamaraj University, Madurai-625021, India. Voluntary Health services, Sakthi Nagar, Erode, India

Introduction: Validated disease specific biomarkers could help in early diagnosis, monitoring disease state, sub typing of disease and treatment determination efficacy. Discovery of biomarkers in leprosy would provide a unique approach to understand the disease and also provide better insights about disease predisposition in leprosy. The proteome of M.leprae has recently been examined, but studying the effect of M.leprae infection on host proteome has not yet been attempted. Methods: Serum proteome of leprosy patients across the spectrum and also reactional cases were examined and compared with that of healthy controls. After depleting the high abundant proteins, serum proteins were separated using high resolution 2D gels. Differentially expressed proteins were quantified using ImageMaster 2D Platinum software and identified by MALDI-TOF mass spectrometry. Results: Significant increase in one of the isoforms of a2 chain of haptoglobin, an acute phase protein, was observed in ENL condition. In addition, Hp 0-0 phenotype (representing absence of haptoglobin) was detected in 21.4% of the ENL patients undergoing treatment, which on follow up examination showed typeable phenotype, thus showing a condition of acquired anhaptoglobinemia (Gupta, N., Shankernarayan, N. P. and Dharmalingam K. (2007) Serum proteome of leprosy patients undergoing Erythema nodosum leprosum reaction: Regulation of expression of the isoforms of haptoglobin. Journal of Proteome Research 6:3669-3679). We also observed increase in another acute phase protein Orosomucoid (also known as alpha-1-acid glycoprotein) in the serum of ENL patients. The study was extended to follow up examination of the same patients to check for response to the treatment. Changes in these two markers were shown to be correlated to the disease stage and they also respond to the treatment given. Conclusion: Haptoglobin negative condition reverted back to a typeable haptoglobin phenotype after the patients were released from treatment. Interestingly the precursor protein of haptoglobin was also absent from the serum of all these patients. Validation of these observations will help in developing clinical assays for monitoring the disease course and treatment efficacy. Keywords: ENL, Haptoglobin, Leprosy, Orosomucoid.

Nerve Conduction Velocity in Normal and M. leprae - Infected Armadillos

Richard Truman
National Hansen Diseases Programma, Louisana State University, Baton Rouge, LA, USA
E-mail: rtruman@lsu.edu

The nine-banded armadillo (Dasypus novemcinctus) is the only other animal, besides humans, that are naturally infected with Mycobacterium leprae. Armadillos, like humans, exhibit the full clinical spectrum, making it an excellent model for the study of disease progression in leprosy. Infection and subsequent loss of function of peripheral nerves is a hallmark of leprosy. Previous studies have shown that the armadillo is a good morphological model of leprosy neuritis, the present study aimed to correlate loss of nerve function to the degree of infection. Change in the rate of impulse conduction velocity is the most important manifestation of abnormal nerve function. Hence, we determined nerve conduction velocity (NCV) in normal and infected armadillos, by surface stimulation of the posterior tibial nerve, distally at the ankle and proximally at the knee, and subsequent recording of compound muscle action potential. The mean distance between the two stimulation points were 4.47 ± 0.32 cm. Our results show that uninfected armadillos have a mean NCV of 87.21 ± 14.41 m/sec, with a mean distal latency of 2.26 ± 0.23 msec and a mean proximal latency of 2.79 ± 0.26 msec. Late stage infected animals had a longer latency and hence slower NCV.