follow up activities, insufficient smearing and staining was seen in only 8% respectively 2% of the slides and correlation of BI values occured in 93%.

We conclude that the experience in the Western region of Nepal demonstrates the importance of tracing problems and providing practical training in the area of slit skin smears in order to improve the performance in the peripheral laboratories.

Green Pastures Hospital, P.O. Box 28, Pokhara - 33701, Nepal Phone : 00977-61-20342, 23099, 28162

Fax : 00977-61-20430

Email : gph@inf.org.np

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ENDOTHELIAL CELL INFECTION IN THE PATHOGENESIS OF LEPROMATOUS NEURITIS

David M.Scollard, *Gregory McCormick & Joe Allen* National Hansen's Disease Centre at LSU, USA

M. leprae is the only bacterial pathogen that infects peripheral nerves, often leading to defomity and thus to the stigma associated with leprosy. The mechanisms responsible for localization of M.leprae to peripheral nerves and therefore responsible for initiating specific nerve injury, have eluded detailed investigation due to the serious limitations in the biopsy of human peripheral nerves and the lack of good animal models.

Recent studies have revealed that experimentally infected armadillos develop a neuropathy which closely resembles human lapromatous neuritis. Evidence from this model suggests that an early event in the infection of peripheral nerves by M. leprae is the infection of endothelial cells of the epineurial blood vessels and lymphatics.

In order to study the mechanisms of infection of endothelial cells, we have examined the interaction between human umbilical vein endothelial calls (HUVEC) and M. leprae in vitro. HUVEC bound and ingested freshly obtained M. leprae and control BCG in a time and concentration-dependant manner. Uptake increased slowly, peaking at 18-24 hr. for M. leprae, and at 12-18 hr for BCG. Optimal uptake of M. leprae requires a ratio of bacilli: HUVEC of approximately 100:1. Assays using radiolabelled bacilli have revealed that uptake is slightly accelerated with heat killed or aldehyde fixed

M. leprae. Ultrastructural and confocal microscopic studies have indicated that some bacilli are internalized soon after binding to HUVEC. This suggests that the delay in uptake is probably due to low levels of binding initially by mechanisms which may be upregulated after prolonged exposure to mycobacteria.

We propose that M.leprae first colonize the surface of nerves, infecting lymphatic and vascular endothelium, then extend inward along blood vessels into the endoneurium. This is consistent with many previous reports of endothelial cell infection in leprosy, but contrasts sharply with the classical view that the bacilli initially bind and enter Schwann cells, ascend within the nerve, and explode outward . The possibility that nerve involvement by M.leprae begins with endothelial cells offers new approaches to understanding the pathogenesis of nerve injury in leprosy, viewing this as a dynamic sequence of adhesion, immunologic and inflammatory processes involving endothelium. The identification of specific mechanisms in this pathway of infection may offer new opportunities for intervention to treat or prevent nerve injury in this disease, and thus to prevent permanent nerve loss and subsequent deformity.

Department of Pathology, Laboratory Research Branch, National Hansen's Disease Centre at LSU, Baton Rouge, LA, 70894, USA

Phone : 001-225-3465769

Fax : 001-225-345786

Email : dscoll1@isu.edu

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CAN DNA HOMOLOGY BE THE ABSOLUTE YARDSTICK TO IDENTIFY M.LEPRAE?

Dr.A.N.Chakrabarty, *Dr.SujataG.Dastidar, Dr.A.Sen& Dr.M.Ganguli,* Calcutta University College of Medicine, Calcutta

Before the nucleic acid characteristics of the leprosy bacilli (LB) could be adequately understood, there was a general belief that all LB possibly belong to a single genetic type, and identification of isolates of LB would depend on their conforming to this type. Studies on DNA relatedness of LB strains, however, revealed a wide diversity among different isolates. These genetic diversities could be due to the leprosy bacillus being prevalent for thousands of years over widely separated geographical regions like India, China, Egypt, Africa, Europe, as well as, the New World with chances of segregation and multi-centric evolution. These genotype differences are similar to those existing among the salmonellae, the plague bacilli, cholera vibrios and the tubercle bacilli; these differences in leprosy bacilli correlate well with distinct clinical diseases these types or subtypes produce. These often have a geographical segregation. In recent times, application of numerous taxonomic parameters showed that the so called M.leprae forms a dense cluster of human pathogenic strains, yet are divisible into numerous genetic subtypes, as evident from the work of different workers. These had thrown new light on several distinctly different biological types, e.g. those with long/short generation time (slow/fast growers) those with low or high yields in vitro; slow or fast growers in the mouse footpads; multibacillary, clinical LL or TT types; lucio type, histoid type, alopecia type, hyperbacillary single nodule type, pure nuritic

type, ulcerating type and skin pustule type; xanthene/hypoxanthene utilising type. These distinct but stable biotypes confirm the wide genetic variability within the cluster called leprosy bacillus which actually comprises many heterogenous subtypes.

The clinical diversities observed seem very distinct, stable and well demarcated geographically. Such distinct clinical diversities should be determined by and reflected in genetic diversities of the leprosy bacilli. Predictably, when LB isolates from diverse sources were compared to the Reference strains, marked differences were found among different strains ; moreover, the different reference strains used by different workers, varied widely among themselves, disproving the earlier dogma that all LB basically belong to a single genetic type. The reference strain(s), thus lost their value and purpose as single yardstick(s) for identifying LB of different origin. Another complicating problem was that the DNA hybridisation intensities of LB strains varied according to the DNA region tested, and had therefore caused much confusion, which seemed

Continued on next page natural as thus far only 50% of the DNA domains of the LB had been explored, leaving remainder as blind areas.. Specific probes of adequate number are lacking, which prove their point of diversity further. Thus, the lack of sufficient homology among different LB is not surprising, as DNA-wise the LB isolated from different human/animal/or geographical sources may be greatly heterogenous, but sub-divisible into different types. Other basic tests will serve as gold standard to identify LB.

Calcutta University College of Medicine, P.O.Box No.16409, Calcutta - 700 020 Phone : 0091-33-2232041, 2232084, 378486

Fax : 0091-33-2486604

Email : madhabi@giasc101.vsnl.net.in

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USE OF REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR) FOR THE DETECTION OF MYCOBACTERIUM LEPRAE IN THE SLIT SKIN SMEARS OF LEPROSY PATIENTS

Jadhav R.S., *Shinde V.S., Kamble R.R., Edward S, Rao J.R. & Edward V.K.* Richardson Leprosy Hospital, Miraj, Maharashtra

Bacterial Index from the slit skin smears is a commonly used parameter for the clinical evaluation and management of leprosy. Its relevance for understanding prognosis of patients on treatment has been extensively debated, as it does not give a very clear idea of the viability of the bacteria in patients under treatment. As the treatment duration in multibacillary (MB) cases is reduced, it is important to know the bacteriological status of the patient at the end of the treatment.

Reverse transcription polymerase chain reaction (RT-PCR) was used to detect M.leprae in slit skin smear samples of leprosy patients. For this we extracted RNA using Trizol reagent (Life Technologies, UK) from the slit skin smear samples from 13 leprosy patients. The RNA preparation was used for the RT-PCR. Mycobacterium leprae specific primers for the fragment of 16S ribosomal RNA gene were used in the amplification (Ryon et al. J.Clin.Microbio. 1998,36(5), 1352-56). Samples from seven patients showed amplification by RT-PCR. Of these, two were new cases, four were suspected relapse cases and one was under treatment. Other six patients whose smear samples did not show any amplification by RT- PCR were on MB-multi drug therapy. The usefulness of the technique needs to be more extensively explored for studying viability of M.leprae, efficacy of treatment and presence of other mycobacteria in the slit skin smear samples.

Stanley Browne Laboratories, Richardson Leprosy Hospital, Miraj - 416 410, Sangli District, Maharashtra

Phone : 0091-233-211213

Fax : 0091-233-211708

Email : sblabtlm@vsnl.com

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SIMPLIFIED PCR DETECTION METHOD FOR NASAL MYCOBACTERIUM LEPRAE

Jadhav R.S. *Macdonald M on behalf of MILEP-2** Richardson Leprosy Hospital, Miraj, Maharashtra

Aim : The purpose of this study was to determine the accuracy and specificity of the simplifred PCR method for the detection of nasal Mycobacterium leprae and its suitability for application to a larger epidemiological study of M.leprae transmission in rural India. We also wished to confirm that the nasal M.leprae was not present in nasal swabs from a non-endemic population.

Methods : DNA was extracted from nasal swabs and analysed by PCR, and M.leprae specific amplicons detected by means of a novel peptide nucleic acid (PNA)-ELISA method. The supply of laboratory consumables and overall detection procedure were simplified and standardized by the use of PCR Ready to Go beads.

Results : Of the total 219 nasal swabs tested, 213 swabs were collected from different individuals and six swabs were spiked with M.leprae DNA. The swabs were coded and analysed blindly by PCR using two different sets of primers (for pra and rlep) separately and subsequent PNA ELISA and blot. After decoding it was found that all the positive swabs had been spiked with M.leprae DNA whereas all other swabs were negative. No false positive results were obtained. These results confirm the negativity of swabs from a non-endemic country.

Conclusion : The PNA ELISA and use of manufactured PCR beads simplifies the PCR detection of

M.leprae to a point suitable for application in large scale epidemiological studies.

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Stanley Browne Laboratories, Richardson Leprosy Hospital, Miraj - 416 410, Sangli District, Maharashtra

Phone : 0091-233-211213

Fax : 0091-233-211708

Email : sblabtlm@vsnl.com

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CONFIRMATION OF DIAGNOSIS IN DIFFERENT FORMS OF LEPROSY BY PCR AND GENE PROBES

V.M.Katoch, *H.B.Singh, K.Katoch, M.Natrajan, V.D. Sharma, D.Singh, R.Das, D.S. Chauhan & K.Srivastava* Central JALMA Institute For Leprosy, Agra

The development of PCR assays for detection of M.leprae directly from clinical specimens was expected to provide a major help to improve the diagnostic capabilities. Over the last 5-7 years our laboratory has used various DNA as well as in-house standardized rRNA targeting PCR methods as well as rRNA targeting probes for the detection of M.leprae in the clinical specimens (mainly biopsies). Various clinical groups included in these studies are well established clinical forms, indetenninate as well as suspicious cases. DNA as well as rRNA from the biopsy specimens were extracted by a physiochemical procedure adapted at the laboratory. Besides assessing the hybridization with rRNA targeting probes, PCR assays using different gene targets were performed by established systems. Analysis shows that RNA probes are mainly relevant in MB cases (including low bacillated specimens) as well as a section of PB specimens for monitoring of treatment. On the other hand, different PCR assays were constantly found to be useful in early atypical forms with positivity ranging from 50-70% in these specimens in different series of patients investigated in the laboratory. The need to have PCR laboratories (may be at least in referral centres/institutes) needs to be seriously considered for improving the diagnostic capabilities in the new millenium.

Central JALMA Institute For Leprosy, Tajganj, Agra - 282 001 Phone : 0091-562-331751 - Extension 206

Fax : 0091-562-331755

Email : useng@nde.vsnl.net.in

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APPLICATION FOR PCR ASSAYS TO RELAPSED LEPROSY CASES

H.B.Singh, *V.D.Sharma, K.Katoch, M.Natarajan, A.Shakya, Archna, D.Singh, R.Das, D.S.Chauhan, K.Srivastava & V.M.Katoch* Central JALMA Institute For Leprosy, Agra

PCR techniques have been earlier reported to be correlated with viability in leprosy and other diseases. We had observed similar trends earlier. From our earlier studies as well as some published reports it has been inferred that PCR assays can be used to monitor the progress of leprosy cases under treatment and also relapsed cases. However, as the disease profile is constantly changing, there is need to continue these studies using different PCR methods. In this extended study, the biopsies from clinically relapsed leprosy cases have been processed for extraction of nucleic acids. A DNA targeting PCR assays described by Hartskeerl et al (36 kDa) and Pattyn/ Cox et al (rDNA) have been used to investigate these specimens. PCR product was analysed on the agarose gels as well as by southern blot hybridization. Positive amplification was observed in majority of the specimens which belonged to paucibacillary spectrum. All the multibacillary specimens were PCR positive by both the methods. Better sensitivity with probe hybridization was observed. PCR method targeting 36 kDa was found to be more sensitive than the rDNA targeting method of Cox/ Pattyn et al. Overall, both the PCR techniques appear to be usefull for investigating the relapsed leprosy cases currently coming to clinics.

Central JALMA Institute For Leprosy, Tajganj, Agra - 282 001 Phone : 0091-562-331751 - Extension 206

Fax : 0091-562-331755

Email : useng@nde.vsnl.net.in

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DELAYS IN PRESENTATION FOR TREATMENT IN LEPROSY

Donald Benjamin, The Leprosy Mission Hospital, Allahabad

Objectives : To prevent disability amongst those affected by Leprosy and encouraging them for early presentation and treatment.

Design : Prospective Observational study. 30 new cases were interviewed, assessment done clinically and bacteriological. The data included demographics, first symptom and individual patients first action behavior, etc.

Setting : Out patients Department, The Leprosy Mission Hospital, Naini, Allahabad

Participants : Out patients visited to Hospital first time.