action with lymphocytes and bacillary antigens in the pathogenesis of leprosy reactions and in mechanisms of defense against the bacillus in the various clinical forms of the disease. Studies permitting the evaluation of CRP bound to the surface of lymphocytes from leprosy patients and to bacillary antigens in circulating blood, tissues and cells, and of the possible reactions associated with these mechanisms would contribute to the elucidation of these question.

Division of Dermatology, Faculty of Medicine of Ribeirao Preto, Sao Paulo University, Ribeirao Preto, Sao Paulo, Brazil

Phone : 0055-16-6330236

Fax : 0055-16-6330236

Email : ntfoss@fmrp.usp.br

Mi 65

THE USE OF MYELIN AND AXONAL STAINS TO ASSESS THE EXTENT OF NERVE DAMAGE IN NEURITIS OF LEPROSY

Mohd. Ismail *& Sujai Suneetha,* Lepra India, Hyderabad

Histopathologicai diagnosis of leprosy in skin & nerve biopsies is usually based on H&E stain to study the general morphology and modified Fite Faraco stain to identify Mycobacterium leprae. Since neuritis in leprosy precedes nerve damage, it is important also to evaluate the structural integrity of the nerve by using special stains for myelin and axon.

Solochrome cyanine is a myelin stain where intact myelin is stained brilliant blue and Glees Marshland, stains integral axons black. In this study we carried out regular H&E, Fite Faraco and these special stains - Solochrome and Glees on 10 nerves of leprosy patients in reaction (7BT, 3 BL) and were compared to normal nerve.

The results showed extensive demyelination of axons of BT leprosy nerves and relatively less damage in BL leprosy. Normal nerves showed structural integrity of myelin and axons. When the special stains were compared to H&E, the degree of damage of the nerve corresponded to the extent of endoneural inflammation.

In conclusion Glees & Solochrome stains give a direct & quantifiable evidence of the extent of nerve damage, which is difficult to assess in the regular H&E stain. The use of these special stains in conjunction with regular H&E and Fite stains can help in the individual neuritis patient management, the details of which will be discussed.

Lepra India, Blue Peter Research Centre, Cherlapally, Hyderabad - 500 301 Phone : 0091-40-7264547

Fax : 0091-40-7262571

Email : lepind@hd1.vsnl.net.in

Mi 125

EFFECT OF POLY UNSATURATED FATTY ACIDS IN EDIBLE OILS ON THE GROWTH OF MYCOBACTERIUM LEPRAE IN CBA MICE

Muruganand D, *Gigi Ebenezer, Shantha A & Job C.K,* Schieffelin Leprosy Research And Training Institute, Karigiri, Vellore District

Poly Unsaturated Fatty Acids (PUFA) / Essential Fatty Acids (EFA), supplemented in diet, are reported to mediate immune-suppression. Prostaglandin E2 a metabolite of PUFA also has a suppressive effect on the immune system. Mycobacterium leprae is known to scavenge fatty acids and other lipids for cell wall synthesis from the host cell and also to multiply more abundantly in immunologically suppressed hosts. Therefore it was planned to study the effect of PUFA on the multiplication of M.leprae within the footpads of normal mice. Coconut oil having a lower PUFA content and groundnut oil with higher PUFA were supplemented in the mice diet. Auto-oxidation of PUFA in vivo was inhibited by the addition of antioxidants.

Six groups of 5 CBA mice were used for the study. Group 1 animals were fed with 20% w/w of coconut oil, Group 2 with 20% groundnut oil, Group 3 and Group 4 in addition to coconut oil and groundnut oil were fed with the antioxidants, a-tocopherol acetate (500 ppm) and selenium (1 ppm) respectively. Group 5 was the control group fed with normal diet and Group 6 the controls for antioxidants. The footpads of the animals were inoculated with 1×10^{4} bacilli each and one animal in each group was harvested at 6th, 9th, 12th and 15th months.

A significant difference in the peaks of growth phase was observed. Mice fed with high PUFA content and antioxidants peaked by 6th month counting a significant high of 1.09×10^{6} bacilli/ml whereas low PUFA diet with antioxidant showed a peak at 12 th month (5.45×10^{5} bacilli/ml) indicating a slower growth rate. The observation confirms that PUFA may be utilized for the cell wall synthesis and at the same time its immuno suppressive activity favors the growth of the bacilli. Though it is a significant finding, it needs corroboration with a larger study.

Schieffelin Leprosy Research And Training Institute, Karigiri - 632 106, Vellore District, Tamil Nadu

Phone : 0091-416-274229

Fax : 0091-416-274274

Mo 337

DETECTION OF DAPSONE RESISTANCE MUTATION OF MYCOBACTERIUM LEPRAE FROM KOREAN LEPROSY PATIENTS

Seong-Beom Lee, Tae-Jin Kang, Se-Kon Kim & Gue-Tae Chae, Seoul

Though resistance to Dapsone (DDS) was confirmed by foot pad study of mice in 1964, more convenient in vitro method which circumvent the tedious and expensive in vivo test was not available. Recently Kai et al and Williams et al have reported independently that DDS resistant strains of M.laprae reveal missense mutations at highly conserved amino acid residues 53 or 55 in the folP1 gene. The missence mutations T53I, P55R, P55L suggests that this sulfone resistance-determining region (SRDR) of folP1 are responsible for the majority of dapsone resistance.

With use of primers which amplify the SRDR, we isolated two variant strains of M.leprae from Korean leprosy patients who are suspicious of resistance to dapsone by PCR-SSCP of the folP1 gene. Direct sequencing of the folP1 region of M. leprae variants revealed two missense mutations were identified. Two variants strains showed A to G and C to G substitutions at nucleotides 157 and 164, respectively. We screened the sulfone resistance-determining region of DHPS in 50 patients. The frequency of 157 and 164 guanine substitution was 11 (22%) patients and 6 (12%) patients, respectively, in our study population. The mutations at nucleotides 157 and 164 would substitute Thr to Ala at amino acid residue 53 and Pro to Arg at residue 55 of DHPS, respectively.

505 Banpo-Dong, Secho-Gu, Seoul - 137 701 Phone : 0082-2-5901320

Fax : 0082-2-5952241

Mo 354

DETECTION OF MYCOBACTERIUM LEPRAE BY PCR IN NASAL AND BUCCAL MUCOSAE IN LEPROSY PATIENTS AND HOUSEHOLD CONTACTS

Dr.I.M.B.Goulart, F.R.Ferreira, C.A.Pinheiro, D.S.Borges, G.Cunha, Dr.L.R.Goulart & ***Dr.N.T.Foss***

Sao Paulo University, Sao Paulo, Brazil

Leprosy is a disease of wide clinical and immunopathological spectrum, which causative organism, Mycobacterium leprae may occur in large amounts in host tissues without causing clinical signs and/or symptoms. The clinical manifestations correlate with distinct immunologic patterns, varying from a strong cell-mediated immunity to M.leprae with a predominantly Th 1-type pattern of cytokine production in tuberculoid leprosy, to an absence of specific cellular immune response to M.leprae antigens in lepromatous leprosy related to predominance of Th 2-type response and exacerbation of humoral immune response. Currently it is assumed that transmission occurs by the contact of susceptible individuals with untreated multibacillary patients, however it has been discussed the possibility that not only leprosy patients discharge bacillus, since leprosy bacillus were found in the nasal mucosa from household contacts of multibacillary patients. Because the M.leprae cannot be cultured in vitro and it is virtually impossible to assess exposure and the onset of the infection, the PCR holds promise as tool to detect subclinical infection with enough sensitivity and specificity for the use in epidemiological studies. In the present report the PCR was applied with a pair of primers described by Yonn et al., (1992) for detection of M.leprae in nasal and buccal mucosae of patients and its household contacts. The DNA of the specimens of nasal and buccal swabs was extracted using lysis buffer (Nacl 400mM; EDTA pH 8.0, 50mM; Tris-HCl, 25mM) and proteinase K (100mg/ml). The PCR was standardized according methodology proposed by Innis et al., (1990) to amplify a 372bp specific fragment from

M.leprae genome. The reaction results were visualized in 1.5% agarose gels stained with ethidium bromide. A family consisting of a 51 year old borderline tuberculoid patient and 5 household contacts was analyzed. Nasal swab specimens of patient and 4 (80%) of his household contacts was PCR positive, while buccal swab specimens was PCR positive on the patient and 1 (20%) of his household contacts. The difference of PCR positivity between nasal and buccal specimens reinforces the idea that the nasal mucosa is the main way of M.leprae transmission. The use of molecular biology in the detection of M.leprae, as in the genetic characterization of susceptibility will bring new insights for epidemiological research of the disease allowing to discuss the role of the healthy carrier in transmission of M.leprae and the early elimination of the infection source.

Division of Dermatology, Clinical Hospital - School of Medicine of Ribeirao Preto, Sao Paulo University, Ribeirao Preto, Sao Paulo, Brazil

Phone : 0055-16-6022446

Fax : 0055-16-6330236

Email : ntfoss@fmrp.usp.br

Di 04

IS DELAY IN TREATMENT AFTER DIAGNOSIS RELATED TO AN INCREASE IN DEFORMITY?

Y.Alban *& T.Amal Raj* The Leprosy Mission, Dayapuram, Sivagangai

OBJECTIVE : Over a period of 2 years from 1997 to 1999, many leprosy patients were reported, with impairments, but not at all treated with any anti leprosy drugs, in the district of Sahib Ganj - Bihar. This initiated us to find out the occurrence of impairment for untreated delayed leprosy patients.

DESIGN : This is a retrospective cross sectional study, in which 237 patients with impairments were interviewed and assessed for disability with W.H.O. grade