Biopsied peripheral nerves from ten leprosy patients (6 tuberculoid patients and 4 lepromatous patients) were examined from morphological aspect.

Light microscopial examination showed that the perineurium was markedly thickened by infiltrated cells in tuberculoid type and mycobacterium leprae in lepromatous type. Schwann cells markedly decreased in number and nerve fibre disappeared without regeneration in severe cases. In mild cases, subperineurial edema was present. The nerve fibre density was normal or mildly decreased. Ultrastructural examination showed the abnormalities of basal lamina on perineurial cells. The basal lamina of the perineurium completely disappeared in several cases. In mild cases, subperineurial edema was present. The nerve fibre density was normal or mild decreased. Ultrastructural examination showed the abnormalities of basal lamina on perineurial cells. The basal lamina of the perineurial disappeared in several cases and showed splitting even if the perineurial looked like the normal complete structure in light microscopy. Both types of leprous neuropathy had same changes with regard to abnormality of the basal lamina. There are many

M.leprae Schwann cells in fibroblasts and perineurial cells on the nerve of lepromatous patients, although few M.leprae in the nerve of tuberculoid patients. Previous studies indicated that the pathogeneris of leprous neuropathy was due to destruction of axon. This study provides that these abnormalities of perineurium are characteristic in both types of leprous neuropathy. The perineurium acts as a barrier between the interior of the nerve and extraneural fluid environment. The damaged perineurium light lose the normal function and allow tissue-damaging factors to enter the nerve resulting in degenerating nerve fibres.

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A STUDY ON THE REPRODUCTIBILITY OF TWO SPECIFIC SEROLOGICAL ASSAYS FOR DIAGNOSIS OF LEPROSY

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Diagnosis is the first step in the treatment and control of any disease, both in the individual as well as in the community. Over the years a variety of serological assays have been described for diagnosis of leprosy. Serum antibody competition test based enzyme linked immunosorbent assay (SACT-ELISA) and phenolic glycolipid based enzyme linked immunosorbent assay (PGLELISA) have been reported to be useful and have been studied widely. One of the important characteristics needed for an immunodiagnostic test, is reproducibility of the results. Regarding these two assays there is no such information available in the literature. Therefore, an attempt was made to find out variations (with-in and between the assays) in the results of these two tests. In the present report, the findings, in brief, for same have been described.

The reproducibilities of these two assays were estimated using sera with different levels of anti-Mycobacterium leprae antibodies. From the findings it appears that with-in assay reproducibility of SACT-ELISA is better for sera having low and middle levels of antibodies whereas with PGLELISA it was better with sera having high and low levels of antibodies. Between assay variations were not promising for both the assays. Regarding the percent positivities of both the assays, the PGL ELISA showed better reproducibility than SACT-ELISA. The results would be presented and discussed in details.

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SCREENING NEW LEPROSY ANTIGENS FOR POTENTIAL AS LEPROSY SKIN TESTS

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Elimination of leprosy will require new tools to identify trends in leprosy infection in the community. A leprosy-specific skin test could answer the critical question of how MDT programmes impact the transmission of leprosy. Present leprosy skin tests composed of fractions of the leprosy bacillus do not have the requisite specificity to detect leprosy exposure in communities with high levels of tuberculosis. We have demonstrated that levels of the cytokine interferon-gamma (IFN-) produced in a simple overnight whole blood culture with leprosy antigens are increased in healthy contacts of leprosy patients. The 35kD antigen (Triccas, 1996), the 45kD antigen (Vega-Lopez, 1998) and a newly expressed M. leprae homologue of the early secreted antigen of TB of 6kD (ESAT-6 ML) were employed in overnight whole blood assays and interferon-gamma was measured in supernatants. Short-term cultures were compared with longer (5-day) culture and with T-cell proliferation in Nepali leprosy patients, leprosy contacts and unexposed subjects. These data indicate the potential of these three relatively leprosy-specific antigens for leprosy skin tests.

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IMMUNOLOGICAL CHARACTERIZATION OF VACCINE

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We have developed the method to apply vaccine topically into mice. The immune response induced by vaccine showed differences among various routes of immunization, which were intramuscular injection, intranasal application, or topical application. Any immunization routes could induce strong specific immune response. Vaccine induced high levels of both humoral and cell-mediated immune activity against antigen in any immunization route. A high level of specific CTL response was also observed, and a high level of IFN- and IL-4 production was induced by even skin painting of vaccine. Adjuvant is one of the most important elements in developing an effective vaccine. The use of cationic liposomes may be helpful in this regard, because they are reportedly effective for enhancing immunization. High levels of both specific CTL and DTH by topical application were induced by coadministration of the vaccine with IL-12 expression plasmids and GM-CSF exression plasmids. The topical application of vaccine seems to induce both Th2 and Th1, but predominantly Th2 rather than Th1 cytokine response. These immune responses were well inhibited by intradermal injection of anti-I-A/I-E antibody. Therefore, topical administration of vaccine is one of effective routes because of a less expensive and less cumbersome method, and may be very useful for the prevention of infectious diseases.

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PATTERN OF CELLULAR PHENOTYPE IN LEPROSY

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Considering that leprosy may be associated with distinct patterns of immunological response, 51 individuals were investigated: 36 patients subdivided into groups with the lepromatous (N=12), tuberculoid (N=12) and reactional (N=12) forms and 15 normal controls.

Peripheral blood mononuclear cells from the individuals in the different study groups were separated and submitted to the in vitro lymphoproliferation test for 72 hours using the polyclonal mitogens, phytohemagglutinin (PHA) and concanavalin-A (ConA), and C-reactive protein (CRP), in parallel to the study of the phenotype of the cells in culture. The lymphoproliferative response was measured by thymidine incorporation and the percentages of CD4 and CD8 cells were obtained by flow cytometry.

Results : The various groups showed a different lymphoproliferative response to the mitogens. Lymphoproliferation was significantly reduced in the reactional group in the presence of Con-A and in the presence of CRP in combination with PHA or ConA, compared to control. The lepromatous group showed a reduction in the lymphoproliferative response in the presence of ConA alone or combined with CRP, but responded to PHA in a manner similar to that observed in the control and tuberculoid groups. In parallel to these results, there was a reduction in percent CD4 cells in all groups stimulated with PHA. CRP did not change the percentage of CD4 cells in culture in any group, but a tendency to an.increase in CD8 cells was observed in the lepromatous patients in the presence of this protein.

Conclusions : These results indicate differences in the patterns of lymphaproliferative response between the groups studied, depending on the different stimuli used and possibly resulting from the specificity of the mitogens for determined lymphocyte subpopulations that are functionally predominant. On this basis, we may attribute to the ConA stimulus the induction of proliferation of suppressor lymphocyte subpopulations in peripheral blood from lepromatous patients, which acted by limiting the lymphoproliferative response.

Continued on next page C-reactive protein alone did not induce lymphoproliferation in the groups under study, but reduced the lymphoproliferative response of tuberculoid patients when combined with PHA or ConA, and the response of reactional patients only when combined with PHA. These data suggest the possibility of CRP binding to lymphocytes since the presence of this protein modified the lymphoproliferative response of these groups. If we admit the existence of CRP receptors on the surface of lymphocytes, this condition seems to be different in the polar and reactional forms of leprosy. Together with other factors, CRP may locally or systemically participate in the acute inflammatory response of leprosy reactions, particularly in erythema nodosum of leprosy, and may also be involved in the mechanisms of cytotoxicity occuring in the cell immune response against the bacillus and possibly in the reverse reaction.

In parallel to literature reports, we suggest the possibility of an expressive involvement of CRP in an inter-

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.

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Mi 65

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

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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.

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

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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.

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