CHAPTER 8

SUMMARY & CONCLUSIONS
I. Using semi-purified human peripheral nerve sonicate as antigen, leprosy sera were screened for presence of antineural antibodies. In LL, BL, BB, BT and TT categories of leprosy, measurable to high titres of antibodies were noted. The high frequency and high titres of the antibodies observed by us, suggest that there is a good humoral immune response directed to peripheral nerves in this disease. Both IgG and IgM classes of antibodies were detected; the frequency and titres of IgG being higher than that of IgM. These antibodies bound specifically to epitopes present in the antigens coated on the plate. The binding was evident with F (ab)\textsubscript{2} fractions also, showing lack of nonspecific reaction with Fc domain.

The presence of antineural antibodies was also observed in a primate model of leprosy, where animals developed signs and symptoms of nerve damage following infection with \textit{M. leprae}. Antibodies to nerve antigens appeared early and were detectable throughout the period tested.

The basis of generation of high levels of antineural antibodies in leprosy may be due to some cross-reactive antigen in the mycobacteria and the peripheral nerves. In ELISA and IIF, cross-reactivity was observed with two of the neural proteins, MBP and S100 screened against a panel of six different mycobacteria.
II. The antigenic determinants reactive with IgG and IgM type of antineural antibodies of patients were identified and characterised. The human nerve sonicate antigen was separated on the basis of the molecular weight using SDS-PAGE. On electroblotting, leprosy sera bound predominantly to 50-55 kDa, 85 kDa and 108 kDa molecular weight protein bands. The identity of these protein bands were checked with a panel of antibodies to known neural proteins such as myelin basic protein (MBP), S-100, glial fibrillary acidic protein (GFAP), fibronectin, neurofilament and vimentin. The 50-55 kDa band reacted with anti-S-100 and anti-GFAP antibodies, while 85 and 108 kDa could not be identified. Anti-MBP antibodies did not bind to any of the above three protein bands. However, in ELISA, the nerve sonicate antigen reacted with anti-MBP antibodies. Using purified MBP, we could detect anti-MBP antibodies throughout the leprosy spectrum and of interest was the presence of high levels of anti-MBP antibodies in some of the indeterminate forms.

The IgM type of antineural antibodies were predominantly directed against the lipid component of the peripheral nerves, specifically to two glycolipids (ceramide and galactocerebroside) and not to the neutral lipids or phospholipids.

III. The raised levels of antineural antibodies could have
functional significance. Indirect immunofluorescence (IIF) studies using in vitro cultured mouse Schwann cells and leprosy sera, showed binding of the antibodies to the node of Ranvier, inner lip of the myelin membrane and to the nerve fibres. To find out if these antibodies play a role in complement-mediated nerve cell lysis, cytotoxicity assays were carried out. Cytotoxicity was visualised on cultured Schwann cells or 33B Schwannoma cells on addition of leprosy sera followed by complement and staining with a vital dye such as eosin. Cells exhibited rounding off from bipolar form under the light microscope. Scanning electron microscopy showed a similar picture and in addition the cells appeared punched out. Cytotoxic effect was quantitated using neutral red colorimetric assay. Addition of immunoglobulins from both LL and TT leprosy sera with complement reduced the neutral red uptake of the cells significantly, indicating a reduction in the number of viable cells. No such effect was seen using a non-glial cell line such as Vero cells.

IV. Studies reported in Chapter 4 revealed wide prevalence of antineural antibodies in all categories of leprosy. Subsequent studies were therefore, directed towards use of this nerve antigen based ELISA for the serodiagnosis of leprosy.
Human nerve sonicate, purified nerve lipids and proteins were tested as antigens in ELISA. 309 sera of leprosy patients suffering from different forms of leprosy were examined, using whole nerve sonicate as antigen. The assay was positive for all the leprosy sera screened, while it was negative for 95 normal sera and 112 tuberculosis sera. Double-blind coded leprosy sera were obtained from different parts of the country, Varanasi (52); Chengalpattu (104); AIIMS-Delhi (25). The assay could detect all the leprosy cases, except for 2 obtained from Chengalpattu. The nerve antigen test was found to be sensitive and specific for leprosy and could be used as a diagnostic test for detection of all categories of leprosy patients. The above results were obtained from a large batch of antigen preparation. On subsequent preparations, however, we were not able to obtain the same degree of specificity and sensitivity, possibly due to instability or labile nature of the immunoreactive nerve proteins.

An ELISA using purified neural lipids such as ceramide and GalC and purified protein such as MBP was set up. Using ceramide, 80 to 82% of leprosy cases could be detected and with GalC, 70 to 75% of leprosy patients were positive. Using MBP as antigen, 80 to 90% of leprosy patients were positive, along with indeterminate and subclinical forms of leprosy. Using these antigens viz. ceramide, GalC and MBP
efforts are on towards making a kit for detection of leprosy.