CHAPTER 5

CONCLUSION

It is evident that while developing immunodiagnostic tests for infectious diseases, the use of relatively purified antigens is bound to give much more specific results than a group of antigens. In the present study the initial screening of the λ *W. bancrofti* genomic library by the filarial specific antibodies (15 KDa specific) obtained from patients sera, resulted in the selection of specific recombinant epitopes which do not cross react with other parasitic sera. The expressed recombinant protein of the 2 Kb *W. bancrofti* DNA insert of the clone pGT7 was considerably more specific in the detection of microfilaremic status at the IgG4 subclass level.

The development of monoclonal and polyclonal antibody reagents to these recombinant antigens have paved way to detect and to quantify the circulating filarial antigens in patients sera. The presence of antigen in the patients' sera revealed the active filarial infection. The increase in antigenemia indicated the degree of infection.

Certain normal individuals from endemic region, showing the presence of filarial specific antibodies or antigen may indicate an occult infection hitherto undetected by parasitological night blood examination. Of these antibody/antigen "positive" normal individuals, 14 to 43% were confirmed parasitologically by repeated direct microscopic examination or by membrane concentration method. This interesting observation of detecting the occult infection in the normal population from the endemic regions needed further study in order to develop a diagnostic reagent suitable for detecting the early infection.
In summary, this thesis contains the following salient features, claims and observations.

- Affinity purification of the *S. digitata* cuticular surface antigens. 4 peptides immunoreactive with patients sera, of which a 15 KDa peptide was highly specific. It reacted only with filarial sera and was feebly reactive or non reactive with other non filarial or non helminthic parasite sera tested.

- Bancroftian filarial antibodies from patients sera captured by the above 15 KDa *S. digitata* peptide was eluted and successfully employed to immunoscreen the *W. bancrofti* genomic library.

- Out of the 28 λ positive clones isolated, a single clone (pGT7) specific for filariasis was recloned in a pMAL vector system to over express and to produce a fusion peptide of 105 KDa enabling an easy purification by a single step maltose affinity chromatography. It was possible to obtain highly specific recombinant fusion protein, free from host *E. coli* proteins.

- The purified recombinant filarial protein (pGT7) was used to capture specific filarial antibodies (especially IgG4) in patients sera for the diagnosis of filarial infection.

- Twenty eight out of 50 endemic normals showed filarial specific IgG4 antibodies of which 14% were having microfilaria in the peripheral blood, on repeated night blood sampling or by membrane concentration method indicating that the test can be used for the detection of occult filarial infection.
A murine monoclonal as well as polyclonal antibodies were developed against the purified recombinant filarial antigen to detect the circulating filarial antigen in patients sera.

Both polyclonal and monoclonal antibodies detected the antigen present in Mf carriers (symptomatic and asymptomatic). However while detecting the antigen in the Tropical Pulmonary Eosinophilia individuals, the monoclonal antibody detected only half (40%) of what the polyclonal could detect (80%).

Of the endemic normals 28% were positive for circulating antigen (7/25) by the polyclonal antibody detection assay. Of the 7 positive individuals 3 subjects (43%) showed Mf in peripheral blood after concentration of the night blood by membrane filtration.

A significant correlation was observed between the parasite antigen levels and the blood microfilaria counts among the Mf carriers. This information of the parasite antigen levels will be an ideal monitor to indicate the degree of active infection and in the follow up of chemotherapy.

Unlimited and continuous availability of specific antigens through recombinant technology and immortalizing specific antibody producing clones through hybridoma technology had provided the means of working with defined reagents useful for large scale seroepidemiological surveys.

This has also opened up the direction in which our search is presently underway in terms of locating other antigens that may elicit a differential but clear immune response at different subclass levels. The availability of such antigens will also enable us to look at the interleukin network and to understand the nature of anergy encountered in parasitic infections in general and filariasis in particular.