ABSTRACT

Human lymphatic filariasis is caused mainly by infection with nematode parasites *Wuchereria bancrofti* and *Brugia malayi*, affecting about 130 million people worldwide. The current general method of diagnosis by microscopic examination of microfilariae in night blood samples is insensitive and tedious. Hence there is a need to develop sensitive and simple diagnostic tests that can detect earlier stages of active infection. The other main problem to be addressed is the treatment of lymphatic filariasis. The treatment for lymphatic filariasis relies mainly on the intake of Diethylcarbamazine and Ivermectin as chemotherapeutic agents. Diethylcarbamazine is primarily a microfilaricidal agent and its use is often accompanied by nausea and dizziness among the microfilaremics. Also there is emerging evidence for acquired resistance by parasites to Ivermectin. Hence there is a need to identify and develop better filaricidals that are safer and more effective.

Many attempts have been made to develop diagnostic kits using recombinant filarial antigens to detect filarial specific antibodies or to detect Circulating Filarial Antigen using monospecific or monoclonal antibodies. Use of polymerase chain reaction to detect specific parasite DNA sequences in the endemic population is also being investigated. Identification of parasite specific biochemical pathways that are vital to parasite growth and survival is essential. Inhibition of such pathways would lead to instantaneous growth arrest or death of the parasite, thus preventing the onset of filarial infection. Hence in the present study, an attempt has been made to identify,
characterize and develop a diagnostic antigen, and a putative chemotherapeutic target antigen.

The first part of the thesis discusses the identification and characterization of a promising diagnostic candidate gene, pRWbSXP-1 from *W. bancrofti* L3 cDNA library. pBSWbSXP-1 was identified by screening the cDNA library using pRBmSXP-1 as a DNA probe. This gene was cloned and expressed in T7 expression pRSET B vector. This has facilitated higher expression of this recombinant antigen as a histidine tagged protein for easy purification. The recombinant antigen was preferentially recognized by IgG4 isotype antibody of *W. bancrofti* infected MF patients. An antibody assay has been developed using this antigen. The sensitivity and specificity of antigen specific IgG4 antibody assay was comparable with commercially available Og4C3 Circulating Filarial Antigen assay (CFA). Antigen specific IgG4 antibodies could be detected from blood collected on filter strips, thus making it easier for field studies. Further, a sandwich antigen detection ELISA assay was developed for the identification of Circulating Filarial Antigen using monospecific antibody to purified recombinant pRWbSXP-1 antigen. This antigen detection assay was able to identify both Bancroftian and Brugian filarial infections and these results were comparable to Og4C3 antigen assay. The Og4C3 antigen assay could not be used for detection of CFA in Brugian filarial sera due to its narrow specificity to Bancroftian filariasis. pRWbSXP-1 assay could be used for the detection of active filarial infection in individuals harboring *W. bancrofti* or *B. malayi* infection.
The second part of the thesis deals with the isolation and characterization of a parasite specific Transglutaminase, which is vital for the growth and survival of the parasites. Transglutaminases are a family of enzymes that catalyze calcium-dependent covalent crosslinking of cellular proteins by establishing (γ-glutamyl) lysine isopeptide bonds. Transglutaminase is essential for the growth and development of the larval stages of parasites. In this context total RNA was isolated from *Brugia malayi* adult female parasite and the transglutaminase cDNA was isolated by 3' RACE PCR reaction and it was subcloned in pCR II TOPO TA cloning vector. Further the cDNA was recloned and expressed in pTrcHis B vector with N-terminal histidine fusion protein. Immunoreactivity was analyzed by western blotting using pre-immune and immune rabbit anti *Dirofilaria* recombinant transglutaminase sera. The native pTBmTGase was purified by Immobilised Metal Affinity Chromatography column and the enzyme activity was determined in a microtitre plate assay using S-(Biotinamido) pentylamine as substrate. Since this parasitic enzyme lacks homology to mammalian transglutaminase, designing a specific inhibitor may lead to development of potent therapeutic agents against the filarial parasites.

In conclusion *W. bancrofti* SXP-1 and *B. malayi* Transglutaminase cDNA have been identified and characterized. These genes have been cloned and expressed in *E. coli* as histidine fusion proteins. The pRWbSXP-1 protein was shown to be a valuable tool for the diagnosis of Bancroftian and Brugian filariasis, and *B. malayi* Transglutaminase can serve as a tool for further prophylactic studies and also as an excellent target for developing effective and safe chemotherapeutic or vaccine candidates.