Filarial infections cause spectrum of clinical manifestations in infected individuals. Induction of allergic inflammatory responses is also a characteristic feature of parasitic infections. Several crude parasitic proteins have been shown to cause histamine release, a potent mediator in allergic reactions but their exact mode of action on the host cells has not been demonstrated. Molecular mechanisms in allergic inflammatory reactions in parasitic diseases are poorly understood due to lack of well-characterized allergen molecules. Recombinant DNA technology has provided avenues to identify antigens of interest and produce them in sufficient quantities. Several recombinant antigens identified have diagnostic (Dissanayake et al 1992; Theodore et al 1993; Chandrashekar et al 1994; Rao et al., 2000) and prophylactic potential in filariasis (Li et al 1993; Taylor et al 1995; Wang et al 1997; Gregory et al., 2000). A few recombinant allergens (Allen et al 1995; Lobos et al 1996) have also been identified to study the pathogenesis developed during filarial infections.

The first part of the thesis discusses about the characterization of TCTPs from the filarial parasites W. bancrofti and B. malayi. Immunoscreening of the cDNA libraries of filarial parasites with TPE sera resulted in the identification of γ-glutamyl transpeptidase from B. malayi (Lobos et al 1996). This antigen has been shown to induce IgE (Lobos et al 1992) suggesting a role
for such antigens in induction of filarial pathogenesis. Another study used recombinant antigens BpL4, glycoprotein (gp29), heat shock protein (hsp) 70 and filarial chitinase to examine their role in the induction of inflammatory responses in jirds (Rao et al 1999). Among these recombinant antigens, gp29 has been shown to mediate significant pulmonary granulomatous response compared to other antigens after 28 days post infection in jirds. Primary infections of *B. pahangi* have been shown to elicit granulomatous response within and around lymphatic vessels that is subsequently down regulated (Klei et al 1997).

Through the filarial genome project, various filarial specific recombinant antigens have been produced and their functional characteristics have been evaluated at our institute (Rao 1998; Rao et al 2000). In the present study, Translationally controlled tumor protein (TCTP) homologue was cloned from the filarial parasites *W. bancrofti* and *B. malayi*. Based on the various functions reported for TCTP proteins, parasite TCTP may play a vital role in the cellular process such as growth, calcium homeostasis, drug target and in host pathogenesis. The previously described human counterpart has been shown to have histamine releasing function on basophils from allergic individuals (Wantke et al 1999) and also activate eosinophils (Escura et al 2000). Teshima et al (1998) has shown that mouse TCTP mediates severe eosinophilia in peritoneum of antigen-sensitised mice. These proteins are suggested to play role in late phase reactions in allergy. Hence the filarial TCTPs were studied in detail to assess their role in allergic and inflammatory reactions in the filarial infections.

The second part of the thesis discusses about the characterization of *B. malayi* L3 phage displayed antigens. The effective control of filariasis lies in
the identification of a candidate vaccine. The widely used choice of drugs for this disease are DEC and Ivermectin. There is a possibility of parasites becoming resistant to these drugs in due course and a emerging drug resistant for Ivermectin has been reported (Clark et al 1995; Ming et al 1998). Hence there is a need to develop a suitable chemotherapeutic agent that is effective against these parasites. Immunoprophylaxis using antigens of the parasites is one of the methods to develop vaccine for the control of this disease. Several crude or fractionated antigens of the parasites have been used to study their prophylactic efficacy in animal models (Kazura et al 1986; Chenthamarakshan et al 1995; Frank et al 1996).

Immunoscreening the cDNA library of *B. malayi* using sera from infected individuals identified an antigen that provide partial protection in jirds (Wang et al 1997). Another study using immunoscreening the *O. volvulus* larval cDNA libraries (Lizotte-Waniewski et al 2000) led to the identification of potential vaccine and drug target candidates. However, the proteins expressed in the cDNA libraries do not assume native form and hence all the proteins may not be effectively identified by immunoscreening. Hence a novel T7 based phage display system in which proteins are effectively folded was used to clone the *B. malayi* L3 cDNA to identify immunodominant antigens by panning with EN sera.

4.1 CHARACTERIZATION OF TCTP FROM *B. MALAYI* AND *W. BANCROFTI*

4.1.1 Sequence analysis of filarial TCTP

The sequence analysis of Bm- and Wb-TCTP showed significant identity with mammalian HRFs/TCTP and TCTP from wide range of species
such as plants, yeast, parasites, earthworm etc., suggesting that TCTP is a ubiquitous gene with a possible house keeping function of the cell. Previously Bm-TCTP (alternately known as Bm-tph-1; Accession no. U80971) was reported from L3 stage of *B. malayi* (Gregory et al 1997). However, its characterization and functional activities were not reported in that study and subsequently till now no information is available on this. In the present study a similar gene was cloned from L4 stage of *B. malayi* and mf stage of *W. bancrofti* and was designated as Bm-TCTP and Wb-TCTP respectively.

Presence of TCTP is also reported from nematodes such as *Strongyloides ratti* (accession no. 14494508) and *C. elegans* (Bini et al 1997), protozoan parasites such as *P. falciparum* (Bhisutthibhan et al 1999), *Trypanosoma brucei* (Haghighat and Ruben 1992) and *Borrelia burgdorferi* (accession no. AAK69458). The only reported function of TCTP from the parasites is available from malarial parasite *P. falciparum* that it is a target for the antimalarial drug dihydroartemisinin (Bhisutthibhan et al 1998). The existence of TCTP like molecules in the parasites may have functions essential for its survival and initiating pathology in the host. Bm- and Wb-TCTP sharing a high homology with its human counterpart (Human TCTP/HrHRF) suggests filarial TCTPs may also have possible histamine inducing activity from basophils and mast cells. Analysis of nucleotide and amino acid sequences between Bm- and Wb-TCTPs showed that there is 95% homology at the gene level and 98% homology at the protein level. This may explain the high cross reactivity of anti Bm-TCTP with Wb-TCTP protein in immunoblots. However, the amino acid at two positions in Wb-TCTP predicted amino acid sequence differed significantly from Bm-TCTP. First, at 54th aa position, Tyrosine (Aromatic and hydrophilic amino acid) is substituted for alanine (hydrophobic amino acid) in Wb-TCTP. Second at 124th aa position in
Wb-TCTP, glutamine is substituted for proline and may have structural significance.

The absence of obvious hydrophobic region and signal peptidase cleavage sites at N-terminal regions of Bm- and Wb-TCTP suggest that it is a cytoplasmic protein. The presence of TCTP in cytoplasm has been reported in human and mouse cells (Bohm et al 1991; Sanchez et al 1997). *Plasmodium falciparum* TCTP has been localized to be present in cytoplasm, outer membranes and food vacuolar membranes surrounding the heme-rich food vacuole where they are likely to be bound to heme (Bhissutthibhan et al 1999). Other studies also showed that TCTP to be present in the culture supernatants of monocytes, macrophages, epidermal keratinocytes and basophil leucocytes (MacDonald et al 1995; Katz and Taichman 1999; Nielsen et al 1998; Teshima et al 1998). It is interesting to note that despite lack of signal sequence, these proteins are secreted with a possible novel secretory pathway suggested for IL-1β and thioredoxin (Rubartelli and Sitia 1991) or alternatively may be released during apoptosis of the cell. These studies showed that TCTP might be both a cytoplasmic and a secretory protein.

4.1.2 Expression of Bm- and Wb-TCTP in T7 expression system

The expressed Bm- and Wb-TCTP in BL21(DE3) pLysS host in the present study resolved at 28 kDa on SDS-PAGE, which was 3 kDa higher than the calculated molecular mass of the protein along with the 6X-Histidine tagged-fusion protein (Figures 3.9A and 3.9B). Similar anomalous migration was observed with human TCTP that resolved in SDS-PAGE corresponding to an apparent molecular mass of 24 kDa instead of calculated molecular mass of 19.5 kDa (Sanchez et al 1997). Further, addition of 1mM Ca$^{2+}$ to the gel during
1-D electrophoresis has been shown to induce a 10% faster migration rate for the human TCTP without affecting the migration behaviour of other proteins. Calcium binding protein, calreticulin from *D. immitis* has been shown to migrate with an apparent molecular mass of 56 kDa instead of 43 kDa protein (Tsuji et al 1998) due to the physico-chemical properties of the protein. This aberrant property of migration of proteins on SDS-PAGE is thought to be a common property for calcium binding proteins like TCTP.

4.1.3 Humoral immune response analysis to Bm- and Wb-TCTP

Antibody responses to various filarial antigens have been studied in humans and in animals. Individuals exposed to the filarial infections induce differential antibody response to the parasites leading either to protection or pathogenesis (Hussain et al 1987). In this present study, humoral immune response to recombinant Bm- and Wb-TCTP were assessed in clinical groups of bancroftian filariasis. The results show that TCTP specific IgG or IgE antibodies were not present in *W. bancrofti* infection. Some possible explanations can be offered for this. a) Filarial TCTP may not be an immunodominant antigen in its native form b) anti Bm- and Wb-TCTP antibodies may be in trace levels in patients and it could not be detectable by ELISA. However the positive control recombinant Wb-SXP-1 antigen reacted with the patients sera at IgG and IgE levels under the same conditions (Figures 3.10 and 3.11).

It has been implicated that major histocompatibility complex (MHC) limits responsiveness to nematode antigens in infection (Tomlinson et al 1989; Christie et al 1992). Some of the secreted and somatic antigens of *Ascaris suum* lack responsiveness in infection could elicit responses in Freund’s
adjuvant assisted immunization in mice and rats (Christie et al 1992). This is supported from our study that Bm-TCTP administered with Freund’s adjuvant induced a significant antibody response in mice (Figure 3.12).

4.1.4 Stage specific expression of TCTP in *B. malayi*

Bm-TCTP expression was differentially expressed in various stages of the parasite. The preferential expression of TCTP in the post infective stages of the parasite may have a possible role in the parasite development in the host. Presence of Bm-TCTP in the ES products of microfilariae suggests that filarial TCTPs are also secretory proteins like human and mouse TCTPs (MacDonald et al 1995; Katz and Taichman 1999; Nielsen et al 1998; Teshima et al 1998). The secreted Bm-TCTP may have extracellular function of inducing histamine release from basophils and mast cells thereby causing allergic and inflammatory reactions in filarial infections.

The absence of TCTP in the infective stages of the parasite may be probably because of translational suppression of TCTP mRNA. *P. falciparum* TCTP is also differentially expressed in various stages with highest expression in trophozoites followed by food vacuoles of mature trophozoites and in ring stages (Bhisutthibhan et al 1999) suggesting that parasite TCTPs are translationally regulated. Similar observations were made in human and rabbit with differential expression of TCTP in various organs but no expression in kidneys (Thiele et al 2000). Guillaume et al (2001) has shown that rat and human TCTP is differentially expressed in various cells of testis with the highest expression in spermatogonia, somewhat less in isolated leydig, resident macrophage, peritubular and sertoli cells, weakly in the primary spermatocytes but completely absent in spermatids.
Interestingly, dimeric and oligomeric forms of TCTP were also observed in adult stages of *B. malayi* (Figure 3.14A). The existence of Lupas coiled coil structure from amino acid residues 92-126 in both the filarial TCTPs may be involved in the formation of stable dimers and oligomers. These findings are supported by a recent publication showing that the malarial TCTP also forms dimers and oligomers (Bhisutthibhan and Meshnick 2001). Yoon et al (2000) has also shown that rat TCTPs self interact and form oligomers. Existence of dimeric and oligomeric forms of TCTP in *B. malayi* may have implications in the biological functions.

4.1.5 Tyrosine phosphorylation of *B. malayi* TCTP

It is shown that TCTP is a growth related protein (Bohm et al 1989), expressed highly in dividing cells (Woo and Hawes 1997) and in apoptosis (Baudet et al 1998). Actively dividing cells and cells undergoing apoptosis may be correlated with tyrosine phosphorylation of proteins. Monomeric form of TCTP was tyrosine phosphorylated in mf and adult female stages (Figure 3.14B). Interestingly the secreted TCTP from mf was also tyrosine phosphorylated. The presence of monomeric form of TCTP in adult females may be due to developing mf in the embryo. The phosphorylated TCTP might be associated with the growth of the initial stages of the parasite. Phosphorylation of monomer and dephosphorylation of dimer may have some implications in the regulation of cellular process of the parasites. For example, phorbol myristate acetate (PMA) stimulation is known to stimulate cells through protein kinase C (PKC) for increased protein phosphorylation. This is correlated with increased expression of TCTP in macrophages after treatment with PMA, Lipopolysaccharide (LPS) or interferon-gamma (IFN-γ) (Walsh et al 1995).
4.1.6 Calcium binding of Bm- and Wb-TCTP

The present study has shown that filarial TCTPs are calcium-binding proteins. Calcium signals are very essential to regulate a wide range of activities in eukaryotic cells. Calcium ions play important role in cell division, motility, metabolism and secretions. Calcium is also shown to maintain the conformation of several proteins necessary for the exposure of hydrophobic patches that can bind to physiological ligands. Specific calcium binding proteins (CaBPs) have evolved to sensitize cells to calcium signals for various cellular process. In filarial parasites little is known about the calcium regulatory pathways.

The calcium binding property of Bm- and Wb-TCTP (Figure 3.15) may have implications in calcium pathways in the parasites. A 22-kDa calcium binding protein from *T. brucei* highly identical to the mouse TCTP has been reported (Haghighat and Ruben 1992). Human TCTP is also shown to be calcium binding protein (Sanchez et al 1997; Xu et al 1999) and its apparent molecular weight is 20% higher than the protein deduced from the cDNA sequence. The presence of human-TCTP in cytosol as that of *T. brucei* CaBP suggest that these properties are consistent with the calcium binding properties. Calcium binding of *P. falciparum* TCTP (Bhisutthibhan et al 1999) has been correlated with its association in food vacuoles, since much of the calcium in malaria appears to be concentrated in food vacuoles. The secretion of calcium binding proteins, Bm- and Wb-TCTP by the parasites may have role in chelating the extracellular calcium and could modulate the cellular responses of the host. The intracellular calcium binding function of Bm- and Wb-TCTP may have role in maintaining the calcium homeostasis and cellular signalling pathways of the filarial parasites, which may be essential for its survival.
4.1.7 Histamine releasing activity of Bm- and Wb-TCTP

Histamine is one of the potent mediators in allergic inflammatory reactions attracting eosinophils and neutrophils at the site of inflammation. The source of histamine is mast cells and basophils in which it is stored as preformed granules. The activation of mast cells and basophils by various factors lead to the release of preformed mediators such as histamine and tryptase and newly formed mediators such as sulfidoleukotrienes (LTs) and prostaglandin D2 (PGD2) (Madeline 1998).

The ability of Bm- and Wb-TCTP to release histamine from mouse, rat mast cells and from basophils of normal and allergic human subjects suggest that they act across species barrier (Figures 3.16, 3.17 and 3.18). Similarly, these proteins induced histamine release from whole blood of patients with bancroftian filariasis (Figure 3.19). Bm-TCTP required 100 times higher dosage than Wb-TCTP for induction of histamine release from rat mast cells and 4 times higher dosage on human basophils from normal and allergic subjects and whole blood of patients with bancroftian filariasis. This variation in dosage requirement for filarial TCTPs may be due to structural differences between them. Proline, a helix breaking amino acid is present in Bm-TCTP instead of glutamine in Wb-TCTP at 124th aa position. This difference in amino acid substitution may contribute to the structural variation and its binding affinity on the target cells for its action.

A histamine releasing factor from human lymphocytes was also reported to release histamine from mast cells of various species such as mouse-peritoneal mast cells, hamster and rat-peritoneal and pleural mast cells, guinea pig-mesenteric and pulmonary mast cells (Blaszczyk et al 1987). However, it
has been reported that human TCTP causes histamine release only from basophils of allergic individuals and not from normal individuals or any other species (Wankte et al 1999).

4.1.8 Eosinophil infiltration of Bm-TCTP in mice

Murine models of ovalbumin-induced eosinophil infiltration are available to study the antigen induced late phase reactions (Russo et al 1998; Taylor et al 1998). These models can be used to study the antigen-induced pathogenesis in sensitised mice. Administration of Bm-TCTP in ovalbumin-sensitised mice induced marked infiltration of eosinophils and neutrophils in the peritoneal cavity (Figure 3.20) suggesting that filarial TCTPs have a role in development of eosinophilia and its associated pathogenesis. The role for TCTP in eosinophilia pathogenesis is strengthened from the studies with mouse p26 HRF (Mouse TCTP) (Teshima et al 1998). This mouse TCTP has been shown to induce significant eosinophilia in the peritoneal cavity of ovalbumin-sensitised mice. Further, Human TCTP has also been shown to activate eosinophils of the patients and caused chemotaxis (MacDonald 1993; Escura et al., 2000). These findings suggest that filarial TCTPs may be partly responsible for allergic manifestations observed in filarial patients.

4.2 IDENTIFICATION OF PHAGE DISPLAYED ANTIGENS FROM L3 STAGE OF B. MALAYI

Phage display systems offer an excellent screening system using a specific ligand of interest. The peptides or proteins that are displayed on the surface of the phage are properly folded and in native form can be efficiently recognized by the ligand. However, the proteins expressed in conventional
λ ZAP cDNA libraries are not properly folded and may not be in the native form. Hence identification of ligand specific clones becomes difficult. In phage display systems, the expressed proteins and its gene are linked together. Hence isolation of the desired gene becomes much easier. M13 phage display system has been extensively used for cloning and expression of foreign genes. The potential disadvantage with this system is that the protein has to be transported through the periplasmic space and limits with high molecular weight proteins. A novel T7 bacteriophage display system has been developed in which assembly of the phage proteins take place in the cytoplasm and lysis take place within few hours after infection of the host. This property makes T7 display system extremely robust for selection and screening of recombinant proteins.

Identification of novel and immunodominant antigens from infective stage of *B. malayi* and targeting them will lead to block in the further development of the parasite in the host. *B. malayi* genome is being well characterized than *W. bancrofti* and animal models are also available for this parasite to evaluate the protective efficacies of the identified antigens. Individuals who are exposed to the infection but not harbouring the infection are consider to be protective individuals or normal individuals (EN). These individuals have better immune response and mount antibodies to the infective stage of the parasite probably may provide protective immunity in them compared to other groups in the endemic area. Hence in the present study, EN sera was used as a ligand to identify putatively protective antigens of the infective stage of *B. malayi*.

Phage display system has been widely used in malarial disease for protective immune response in mice, epitope mapping and for enhancing the immune response of the cloned antigens (Willis et al 1993; Heal et al 1999;
However this system has not yet been used in filariasis. A novel T7 phage display system has been used to identify immunodominant antigens of the infective stage of *B. malayi* by panning with EN sera. This method was found to be equally efficient compared to differential immunoscreening employed to identify immunodominant antigens of the parasite (Dissanayake et al. 1992; Lizotte-Wanieński et al. 2000). Random DNA sequencing of three immunoreactive clones resulted in the identification of N-terminal partial clones of previously reported proteins such as *B. malayi* Abundant larval transcript-2 (BmALT-2; Accession no. U84723), *B. malayi* Venom vespid allergen homolog (BmVAH; Accession no. AF042088) and *Brugia malayi* Thioredoxin peroxidase-2 (BmTPX-2; Accession no. Q17172). The C-terminal cloning site in the T7 phage display vector (T7 select 1-1b) and the internal restriction site in those genes had *Hind* III restriction enzyme site and probably might be the reason for their partial lengths. However, since these clones were identified based on the immunoreactivity with EN sera, were subjected for further analysis.

### 4.2.1 Partial characterization of identified phage displayed antigens

T7BmALT-2 shared significant homology with ALT family of proteins from *B. malayi* (BmALT-1; Accession no. U57547), *W. bancrofti* (WbALT-1; Accession no. AF084553), *C. elegans* (Accession no. CO8A9), *D. immitis* (Accession no. U29459) and *O. volvulus* (Accession no. U29576). Interestingly ALT family of proteins are shown to be present in nematodes and no known homolog in the mammalian host has been reported. The presence of N-terminal hydrophobic regions and signal peptidase recognition site (SES) suggest that it is a secretory protein. BmALT-2 has been shown to be expressed only in infective stages of the parasite (Gregory et al. 2000). The stage specific
expression of the protein may have some role in the development of the parasite. The prophylactic potential of its closely related protein *B. malayi* ALT-1 (BmALT-1) from the same infective stage has been evaluated and shown to provide 70% protection in Jirds (Gregory et al 2000). Similar observations were obtained from our study using BmALT-2 in mice model (Sabarinathan 2000). Epitope mapping of this antigen using phage display system will aid in the enhancement of the protective efficacy in animal models.

T7BmVAH shared significant similarity with *W. bancrofti* VAH (WbVAH; Accession no. AF109794) and *O. volvulus* vespid venom antigen, designated as activation associated secreted protein -1 (Ov-asp-1) (Tawe et al 2000). T7BmVAH identified by panning with EN sera suggests that the EN individuals mount antibody response to this antigen in the initial stages of infection, which probably could provide protective immunity in them. Sequence and phylogenetic analysis of Ov-asp-1 by Tawe et al (2000) suggests that these proteins form a filarial specific protein family related to both the vespid venom antigen 5 and the vertebrate CRISP/TPX family of proteins. A role in generating angiogenic response in the corneas of naive mice by this protein has been shown (Tawe et al 2000). Thus these proteins may play a role contributing to the inflammatory responses in the filarial infections. Further work is in progress to evaluate the protective efficacy of T7BmVAH in animal models.

T7BmTPX-2 identified from the phage display library using EN sera suggests that apart from its pivotal antioxidant property (Klimowski et al 1997; Ghosh et al 1998; Lu et al 1998), it may also be an immunodominant antigen inducing antibodies in the putative immune individuals. Parasite TPX-2 is essential for detoxification of oxygen free radicals produced by it and the host. Hence targeting this enzyme will be lethal to the parasite. The possible role for
BmTPX-2 in inducing protective immune response is strengthened by the identification of TPX from cDNA library of *D. immitis* using serum from dogs vaccinated by chemotherapeutically abbreviated *D. immitis* larval infections (Klimowski et al 1997). *B. malayi* TPX-1 has been shown to be transcribed in all the stages of the parasite and to be localized in the cells of the hypodermis and lateral chord suggesting a role for TPX in counteracting oxygen free radicals derived from endogenous and exogenous sources (Ghosh et al 1998). TPX is also shown to be excretory-secretory products of *B. malayi* (Ghosh et al 1998) and eggs of schistosomes (Williams et al 2001). OvTPX has been shown to expressed in higher intensities during differentiation of infective L3 stage compared to the other stages of the parasite (Lu et al 1998). Another role for TPX in granuloma formation in schistosome infections has been shown by inducing significant production of IFN-gamma, IL-2 and IL-5 and essentially no IL-4 in CD4<sup>+</sup> cells from mice (Williams et al 2001). An immunodominant liver stage antigen-1 (LSA-1) of *P. falciparum* displayed on the surface of bacteriophage capsids has shown to induce immune response in mice with significant upregulation of IFN-gamma (Heal et al 1999). Hence the use of BmTPX-2 displayed on the surface of the phage in protective studies of animal models will be an attractive tool.

Phage displayed antigens T7BmALT-2, T7BmAHA and T7BmTPX-2 were used in ELISA to analyse the humoral immune response in different clinical groups from an area endemic for bancroftian filariasis. These antigens showed a similar pattern of reactivity to the clinical groups. The reactivity of these antigens was highest with EN individuals followed by CP and MF individuals (Figures 3.29A, 3.29B and 3.29C). These results are in accordance with the previously reported BmALT-1 in which antibodies to this protein were found in individuals of Endemic normals and microfilaremics.
(Gregory et al 2000). Similar observations were made by Sabarinathan (2000) using purified BmALT-2 in patients with bancroftian filariasis. The effective recognition of phage displayed antigens by the putative immune individuals suggests that antigens displayed on the surface of the phage retains its immunogenic properties and can be used for further characterization. Thus antigens with three different properties of abundantly expressed genes of the parasite displayed on the surface of the phage specifically recognized by EN sera may provide a tool in understanding the host immune responses generated by these antigens. Their possible prophylactic capability is being evaluated in animal models. Further work is in progress to identify novel antigens of interest from the phage display library useful for diagnostic, immunological and prophylactic studies.

4.3 CONCLUSION

- Filarial TCTPs were cloned in pRSET B as Histidine tagged fusion protein and sequence analysis of Bm-TCTP and Wb-TCTP showed significant similarity with mammalian TCTPs and other reported TCTPs. The recombinant proteins were expressed in BL21 (DE3) pLysS as 28-kDa proteins and purified on IMAC columns.

- Humoral immune response to Bm- and Wb-TCTP in patients sera at both IgG and IgE level did not show any significant reactivity. However, the Immunogenicity of Bm-TCTP was shown in mice. Anti-Bm-TCTP antibodies specifically recognized Wb-TCTP on immuno blots confirming the high similarity of filarial TCTP as evidenced from its high sequence similarity.
• Expression analysis of Bm-TCTP in various life stages of *B. malayi* showed that the protein was differentially expressed. Bm- and Wb-TCTP has calcium-binding activity and monomeric forms of *B. malayi* TCTP in mf and adult female stages were tyrosine phosphorylated. Bm- and Wb-TCTPs has been shown to have histamine releasing activity from mouse and rat peritoneal mast cells, from human basophils and whole blood of patients with bancroftian filariasis.

• In order to identify putatively protective antigens, BmL3 λ ZAPcDNA was cloned in T7 phage display vector and biopanning of the phage displayed library with EN sera resulted in several specific clones. Randomly three clones were sequenced and the clones showed significant similarity with *B. malayi* Abundant larval transcript-2 (BmALT-2), *B. malayi* Vespid venom allergen homolog (BmVAH) and *B. malayi* Thioredoxin peroxidase-2 (BmTPX-2). These clones were of N-terminal partial length clones due to the presence of internal *Hind* III site.

• Humoral immune response to the phage displayed antigens (T7BmALT-2, T7BmVAH and T7BmTPX-2) showed high degree of reactivity with EN individuals compared to CP or MF individuals.
4.4 FUTURE DIRECTION OF THE WORK

a) Identification and blocking the receptor for filarial TCTP on its target cells will be useful in reducing the pathogenesis caused during the filarial infections.

b) Further work is in progress to evaluate the immune response and protective efficacies of phage displayed antigens viz., T7BmALT-2, T7BmVAH and T7BmTPX-2 in jirds. Epitope mapping of these antigens by phage display system will aid in the enhancement of immune response and protective efficacy in animal models.