CHAPTER 1

INTRODUCTION

Human lymphatic filariasis is a debilitating tropical parasitic disease caused by the parasites *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. The existence of this disease has been reported as early as 70 AD by the Indian physician Sushruta and at various periods by several physicians of different parts of the world (Bancroft 1877; Manson 1899). Bancroftian filariasis is prevalent throughout the tropics whereas the brugian infection is confined to certain parts of South East Asia (Figure 1.1). Co-existence of both brugian and bancroftian infections are reported from certain parts of the world like Kerala, South India. 90% of the individuals infected with *W. bancrofti* infection and the remaining 10% suffer from brugian filariasis. At present 120 million people are infected by this disease (Michael et al 1996) and several millions of people are under the risk of infection (WHO 2000). In India alone more than 45 million people are infected with *W. bancrofti* and *B. malayi* infections respectively.

The main factor for this global increase in number of infected individuals is because of the poor sanitation in the urban endemic areas. The vectors that transmit this disease are mosquitoes of the species Anopheles, Aedes, Culex and Mansonia. The infected individuals show clinical spectrum such as elephantiasis of the extremities, lymphoedema, lymphangitis, hydrocoele, chyluria and occult filarial tropical pulmonary eosinophilia. The disease causes morbidity leading to socio-economic burden on the individuals.
Figure 1.1 Areas endemic for lymphatic filariasis
The effective control of filariasis lies in the early diagnosis, treatment of the infected individuals particularly, the microfilaremics (MF) and follow up of infections using suitable diagnostic tools. At present there are two commercially available diagnostic kits (Og4C3 and AD12 monoclonal antibody based) to detect circulating *W. bancrofti* antigen in the infected individuals. The widely used drugs of choice for the treatment of filariasis are Diethyl carbamazine (DEC), Ivermectin and albendazole. DEC is used for the treatment of microfilaremics, Tropical pulmonary eosinophilia, acute filarial adenolymphangitis and hydrocoele. There is no permanent cure reported for the clinical exhibits apart from the surgery. Ivermectin is particularly useful in the treatment of loiasis or onchocerciasis whereas albendazole is shown to be microfilaricidal against *W. bancrofti* (Jayakody et al 1993). The use of these drugs are cost effective and is suitable for mass treatment in the endemic areas. Per-person cost per year in India ranges between US $0.05 and 0.08.

The successful elimination of filariasis has been implemented in China, Malaysia, Korea and in certain islands of the Pacific (Ottesen et al 1997). DEC is widely used in India for the control of lymphatic filariasis while in the African countries several parasitic diseases such as lymphatic filariasis, onchocerciasis and loiasis co-exists and these drugs are used either separately or in combination for the effective treatment. These drugs are filaricidal to only certain stages of the parasite and hence suitable chemotherapeutic agents should be developed for this complex life cycle stages of these parasites. The control of the disease also lies in the control of the mosquito, as this is a primitive host for these parasites. Development of chemotherapeutic agents that target all the stages of the parasite in the humans will be an attractive and effective choice of drug for the treatment of filariasis.
1.1 OVERVIEW OF THE THESIS

Parasitic infections potentiate non-specific IgE and causes allergic inflammatory reactions leading either to pathogenesis such as Tropical pulmonary eosinophilia (Ottesen and Nutman 1992), Mazotti reaction in onchocerciasis (Bird et al 1980) or protection as reported in Schistosomiasis (Hagan et al 1991). The treatment of this disease with DEC often results in allergic inflammatory reactions due to the death of the microfilariae (Haarbrink et al 1999). These allergic reactions can be treated with administration of antihistaminics in addition to the intake of DEC (Titanji et al 1983). Histamine is one of the potent mediators of allergic inflammatory reactions released from mast cells and basophils. Several allergens, which induce histamine release, have been reported from the filarial parasites (Genta et al 1983; Nielsen et al 1991; Muralidhara et al 1992; Munoz et al 1999) and a few recombinant allergens have been characterized (Allen et al 1995; Lobos et al 1996; Santiago et al 1998) but the direct demonstration of histamine release has not been reported. The molecular mechanisms of the allergic inflammatory reactions in filarial infections are poorly understood because of the lack of well-characterized allergen molecules.

The major impediment in advancing the filarial research is the non-availability of the parasitic material and lack of well-characterized animal models. The Gerbils are the well-established animal model for \textit{B. malayi} in which the parasite passes from L3 to adult and used for studies in lymphatic pathology (Klei et al 1987). Murine models allow limited development of the parasite and are being used to study acquired resistance to L3 (Bancroft et al 1993). The only reported model for \textit{W. bancrofti} is the infection in silver leaf monkeys (Palmieri et al 1984) and serves as a tool for immunological
studies. The development of eosinophilia in mice (Egwang and Kazura 1990) can give some insights into understanding of the mechanisms of allergic inflammatory reactions caused by filarial infections.

Histamine releasing factors (HRF)/p23/Translationally Controlled Tumor Protein (TCTP) has been cloned from human and mouse and shown to release histamine from basophils (MacDonald et al 1995). Identification of a homologue of this protein from the filarial parasites and development of allergen immunotherapy can reduce the pathogenesis caused by these parasites. TCTP is reported from parasites such as *B. malayi* (Gregory et al 1997), *Plasmodium falciparum* (Bhisutthibhan et al 1998), *Plasmodium yoelii* (Walker et al 2000) and *Trypanosoma brucei* (Haghighat and Ruben 1992) but the histamine release has not been studied.

The treatment of human lymphatic filariasis lies in the administration of the drugs DEC, Ivermectin and albendazole. These drugs are largely macrofilaricidal and side effects of these drugs are reported. Parasites may also become resistance 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 and safe against these parasites. Identification of protective antigens, enzymes and hormones essential for parasite survival and targeting them will lead to develop novel targets for the control of the parasite. Immunoprophylaxis using antigens of the parasite is one of the methods to control this disease. Most of the immunoprophylactic studies of filariasis has been evaluated in animal models. Several crude and purified protective antigens from various stages of the parasite have been identified and shown to provide protection to different extent (Kazura et al 1986; Chenthamarakshan et al 1995). BmALT-1 cloned from
L3 stage of the parasite affords significant protection (Gregory et al 2000) than any of the other filarial recombinant antigens in Gerbils (Li et al 1993; Taylor et al 1995; Wang et al 1997). Targeting the antigens of infective stage (L3) of the parasite will lead to block in the further development of the parasites. Several genes have been shown to be upregulated in this stage of the parasite, which are essential for its survival and establishment in the host (Martin et al 1996; Gregory et al 1997). The antibodies to these upregulated gene products may confer protection in putative immune individuals (EN) in the endemic area. Immunoscreening the cDNA library of the infective stage of the parasite with EN sera resulted in identification of several putative protective antigens (Lizotte-Waniewski et al 2000).

Phage display system is a novel technology to express the foreign DNA in its native form (Smith 1985; Crameri and Suter 1993). The peptides or proteins are expressed as part of the viral coat protein and hence are believed to assume the native structure and allows enrichment using antibodies or other ligands (Parmley and Smith 1988). Genes expressed in conventional λ ZAP cDNA libraries do not assume native conformation and hence identification of desired products becomes difficult (Thorpe et al 1984). Phage display techniques have been shown to be useful for epitope mapping of immunodominant antigens, immunization studies, prophylactic studies, isolating ligands for drug discovery (Christensen et al 2001). Cloning of parasite genes in phage display system and panning with suitable ligand will lead to identification of novel antigens that may play a role in disease development. In the present study, a novel T7 bacteriophage display system has been used to clone the cDNA of infective stage (L3) of *B. malayi*. The peptides displayed on the surface of the phage will be useful to identify the peptides that bind to keratinocytes of the skin, endothelial cells and the antigens that mount immune response in putative immune individuals.
The specific objectives of the present study are:

a) Identification and characterization of Translationally Controlled Tumor Protein (TCTP) from the filarial parasites *W. bancrofti* and *B. malayi*.

b) Demonstration of biological function of TCTP- Histamine release assay using mouse and rat peritoneal cells, whole blood and human basophils.

c) Identification and characterization of putative protective antigens from *B. malayi* L3 phage display library.

As part of the ongoing Filarial Genome project at our centre, the ESTs of L4 cDNA library of the *B. malayi* was randomly sequenced and several clones were deposited in the GenBank (Rao 1998). One of the clones shared significant homology with mammalian TCTPs (Human, mouse and rabbit) and was named as Bm-TCTP. The first described TCTP was observed in Ehrlich ascite tumor cells and was reported to be growth related protein and its expression was shown to be translationally regulated (Bohm et al 1989). Subsequently TCTP was reported from various organisms with several properties such as calcium binding, heat stability (Walsh et al 1995; Sanchez et al 1997), having both intracellular (Gachet et al 1997; Bhishutthibhan et al 1999) and extracellular functions (MacDonald et al 1995). Human and mouse TCTP (HRFs/p23) have been cloned and they release histamine from basophils (Wantke et al 1999). Other functions such as target for artemisinin, an endoperoxide antimalarial drug for plasmodium (Bhishutthibhan et al 1998) and its association with development of chemoresistance in melano carcinoma.
(Sinha et al 2000) have also been reported. Taken together, suggest that TCTP is a multifunctional protein.

The thesis work is divided into two parts. The first part of the thesis describes the cloning and characterization of Bm-TCTP and Wb-TCTP from the filarial parasites. The identified Bm-TCTP gene in pBluescript was not in correct reading frame and was cloned in pRSET B at BamHI and EcoRI sites for high expression. A homologue of TCTP from W. bancroftii was also identified from mf cDNA library using Bm-TCTP forward primer and T: promoter as reverse primer. The gene was cloned in pRSET B in frame at BamHI and HindIII sites. The authenticity of the gene was confirmed by sequencing on both the strands with plasmid DNA templates using cycle sequencing kit from New England Biolabs, MA, USA or on ABI 377 automated sequencer using Dye-terminator sequencing kit of Perkin-Elmer.

Further, Bm- and Wb-TCTP genes were expressed in BL21 (DE3) pLys S as Histidine tagged fusion protein of approximately 28 kDa and was purified with 8M urea by Immobilized Metal Affinity Chromatography (IMAC). Humoral immune response to Bm- and Wb-TCTP with clinical sera by ELISA and Western blot showed that these proteins do not contain significant antibodies in the infected individuals. However, the immunogenicity of these proteins was demonstrated in mice. Immuno blot analysis of Bm-TCTP and Wb-TCTP with mouse anti-Bm-TCTP specifically recognized these proteins. Expression of TCTP in various life stages of the B. malayi was studied by western blot with anti-Bm-TCTP sera. TCTP expression was observed in the post infective stages of the parasite such as adult male, female, microfilariae (mf) and its ES product. Radioactive calcium overlay assay showed that Bm-TCTP and Wb-TCTP are calcium-binding proteins. Bm-and
Wb-TCTP induced histamine release from mouse and rat peritoneal mast cells, human basophils and whole blood of individuals obtained from filarial endemic area. Further, Bm-TCTP induced significant eosinophilia in the peritoneum of mice suggesting a possible role of filarial TCTPs in allergic inflammatory reactions.

The second part of the thesis describes the construction and characterization of phage displayed antigens from L3 stage of B. malayi using putative immune individuals/Endemic normals sera (EN). The effective control of the disease and its associated pathogenesis lies in the identification of a candidate vaccine. A novel system, T7 bacteriophage display system was used to identify putative protective antigens of the B. malayi L3 by biopanning technique using EN sera as a ligand. In this system, the proteins were expressed as a fusion to the capsid protein and displayed on the surface of the bacteriophages so that the screening of the antigens becomes much easier than that of the conventional λ ZAP libraries (Yamamoto et al 1999).

The λ ZAP cDNA library of BmL3 was cloned in T7 bacteriophage display system (T7 select 1-1b) at Eco RI and Hind III sites. The non-specific phages of the phage library was removed by absorption with Non endemic normal sera (NEN), microfilaremic (MF) and Chronic pathology patients (CP) sera. The absorbed library was biopanned with EN sera to identify specific antigens. The DNA of the EN specific phage antigens were cloned in either pPCR-script Amp SK (+) / pSTBlue-1 vectors for sequence analysis. The clones were sequenced on both the strands to obtain the authenticity of the gene. Biopanning of the library with EN sera resulted in the identification of BmALT-2 (B. malayi Abundant Larval Transcript-2), BmVAH (B. malayi Venom vespid allergen homologue) and BmTPX-2
These sequences have an internal \textit{Hind} III site and hence the clones were of partial length from this phage display library. Humoral immune response analysis of phage displayed antigens by ELISA with various groups of clinical sera showed predominant reactivity with EN individuals followed by CP and MF.

1.2 REVIEW OF LITERATURE

1.2.1 Filarial parasites

The filarial parasites \textit{W. bancrofti} and \textit{B. malayi} are classified in the family Onchoceridea, super family Filarioidea. The filarial nematodes are closely related to the Ascaridida such as Toxocara and Ascaris and Oxyurida such as Enterobius. The filarial parasites have mosquito as intermediate host and human as definitive host to complete its life cycle. The adult parasites dwelling in the lymphatic vessels undergo oviviparous reproduction and release millions of microfilariae from the female. The microfilariae follows a nocturnal periodicity in peripheral blood (Gupta et al 1990).

The other parasites that infect human of importance are \textit{Onchocerca volvulus}, \textit{Loa loa}, \textit{Mansonella perstans} and \textit{Mansonella ozzardi}. The adult worms of \textit{O. volvulus} and \textit{L. loa} reside in the subcutaneous tissues and the microfilariae released from adult parasites results in severe visual impairment, calabar swellings, hypersesoinophilia and allergic manifestations (WHO 1992).

1.2.2 Life cycle of the parasite

The life cycle of the filarial parasites are complex involving two hosts for its life cycle completion (Figure 1.2). The larval developments take place in
Female worms release microfilariae (mf) at If. Develops into L4 and adult worms in the lymphatics. 

Infective stage L3 enters the skin through mosquito bite when mf are ingested by mosquito during blood meal. Molts to L3 in mosquito. 

Figure 1.2 Life cycle of human lymphatic filarial parasites 
*Wuchereria bancrofti* and *Brugia malayi*
the mosquito host; larval and adult developments take place in the human host. The prime mosquito host for the transmission of the filarial parasites are Anopheles, Culex, Aedes and Mansonia sp. When infective mosquito bites during the blood meal, L3 stage of the parasite enter the body through the skin and subsequently molt to the L4 stage within 9-14 days post infection and become adult worms in a period of 6-12 months in the lymphatic vessels. The adult worms live for 10 years, reproduce and female liberates microfilariae (mf), the first stage of the parasite in the blood stream. The mf is enclosed within a sheath, which is composed of complex of proteins, sulphated phospholipids, acid mucopolysaccharides and chitin. The mf is nocturnally periodic and during daytime it predominantly resides in the lungs and deep tissues (Spencer 1973). A sub periodic strain of Wuchereria is reported from Pacific region. The periodicity of mf can be altered by reversing the sleeping and working habits of the host (Mark 1987). During subsequent blood meal, mosquito ingests microfilariae from the blood. The microfilariae undergo differentiation to form oesophagus, intestine and rectum in the thoracic flight muscle of the mosquito. Further it undergoes molt to form L1 and L2 stage between 6-10 days. The infective form (L3) of the parasite is developed from L2 between 11 and 13 days. The L3 larvae migrate from flight muscles to the proboscis of the mosquito that will aid in the transmission of the L3 to the human host during feeding of the mosquito.

1.2.3 Morphology of the filarial parasites

The adult parasites have minute thread like structures with smooth cuticle. Female worms in general are larger (80-100 mm in length and 0.24-0.30 mm in diameter) than the male worms (40 mm in length and 0.1 mm in diameter) (Nanduri and Kazura 1989). Both adult male and female worms
have periodic annulations and numerous small spherical protuberances on its surface (Araujo et al 1995). The mf of the filarial parasites is sheathed and the length ranges from 244-296 μm for *W. bancrofti* and 177-230 μm for *B. malayi*. The mf of *B. malayi* has two terminal nuclei. The last terminal nucleus of *B. malayi* mf is small and is seen at the tip of the tail whereas *W. bancrofti* mf has body nuclei. The cuticle forms the exoskeleton of the parasites playing major role in the maintenance of morphology, motility, nutrition and protection from environmental factors. The thickness of the cuticle ranges from 0.2-0.5 mm in larvae and from 2-4 mm in adults. The infective L3 larvae measure 1.2 to 1.6 mm in length; the post-infective L4 larvae measure between 3 and 6 mm in length.

1.2.4 Clinical manifestations

The individuals residing in an area endemic to filariasis exhibit various clinical and parasitological manifestations. The clinical manifestations seen in lymphatic filariasis vary from one region to another and depend on the species of the parasite involved. The most frequently observed clinical disease in Tanzania of Africa is hydorcoele than that of lymphoedema and elephantiasis (Partono 1987).

The filarial Tropical Pulmonary eosinophilia is especially seen in India, Brazil and Malaysia. Hydrocoele and filarial scrotum is specifically caused by *W. bancrofti* and not by *B. malayi*. The classifications of clinical manifestations are mainly divided into asymptomatic, acute and chronic stages based on the immunological criteria (Ottesen 1984). The details of the stages are given below.
1.2.4.1 Asymptomatic microfilaremics (Endemic Normals, EN)

Healthy individuals residing in an area endemic for filariasis also termed as putatively immune individuals who do not harbour microfilariae in their blood, with no clinical symptoms of the disease. These individuals are negative for mf and circulating parasite antigens (Ramzy et al 1991). Immune responses to filarial antigens are higher in these individuals compared to other clinical groups (Ottesen et al 1977). A portion of normal individuals in endemic area are susceptible for filarial infection and become microfilaremics.

1.2.4.2 Asymptomatic microfilaremics (MF)

The majority of this infected individuals have large number of circulating microfilariae in the peripheral blood and have few overt clinical manifestations. The microfilaremia increases with age from childhood to adult (Brabin 1990). The microfilaremics show some degree of subclinical disease such as haematuria and/or proteinuria with low-grade renal damage (WHO 1994), gross damage of their lymphatics of the limbs (Freedman et al 1994) and occasional filarial fever. Filarial antigen specific hyporesponsiveness is the characteristic of these MF individuals (Mahanty et al 1997; Ravichandran et al 1997). Most of the microfilaremic individuals will become normal in due course. However very few of them develop clinical symptoms and develop filarial manifestations such as hydrocoele, chyluria, lymphoedema and elephantiasis.

1.2.4.3 Acute manifestations

Adenolymphangitis (ADL) and Acute Filarial Lymphangitis (AFL) are the acute manifestations in lymphatic filariasis. ADL is characterized by
recurrent attacks of fever associated with inflammation of the lymph node or lymph vessels, which is frequently seen in brugian filariasis than in bancroftian filariasis (Partono 1987). The lymphatic system of the male genitalia is frequently affected leading to funiculitis, epididymitis or orchitis, or to a combination of these (Pani et al 1995). The limb, breast or male genitalia are involved in acute attacks of ADL. A cord-like structure is associated with retrograde lymphangitis in the lower or upper limbs in AFL. The usual presenting feature is Funiculo-epididymoorchitis of acute attacks involving the male genitalia.

1.2.4.4 Chronic manifestations

The acquisition of infection generally occurs as early 2-3 years of age and the prevalence of microfilaremia increases with age during childhood. Though clinical manifestations of disease often remain hidden for years, internal pathology can develop in young children. Acquired lymphoedema of the genitalia and a case of lymphangiosarcoma of a lower extremity has been reported from a filarial infection in childhood (Murphy et al 2001). A low proportion of the infected individuals develop chronic signs of filariasis after the age of 15 years and majority of them do not have microfilariae and circulating antigen in the peripheral blood. These individuals show hypereponsiveness to filarial specific antigens than MF (Nutman et al 1987. Ravichandran et al 1997). In bancroftian infection, the major clinical manifestations include hydrocoele, chyluria, lymphoedema and elephantiasis. The secondary clinical signs of the chronic infection are skin fold thickening, hyperkeratosis, hypo- or hypertrichosis, pachydermia, pigmentary changes, chronic ulceration, epidermal and sub-epidermal nodules.
1.2.4.5 Tropical Pulmonary Eosinophilia (TPE)

TPE is caused by the filarial parasites *W. bancrofti* and *B. malavi*. This lung interstitial disease is characterized by cough, dyspnoea, wheezing symptoms similar to bronchial asthma, elevated levels of peripheral blood eosinophil count (>3000/µl) and high levels of filaria-specific antibodies (Nutman et al 1989). The other forms of this occult filariasis are associated with lymphadenopathy and hepatosplenomegaly (Beaver 1990). Other parasites which cause eosinophilic lung disease similar to TPE are Ancylostoma sp, Ascaris sp, *Strongyloides stercoralis, D. immitis* and *Trichenella spiralis*. In addition to parasites, other infections such as tuberculosis, coccidiomycosis and *Pneumocystis carinii* in AIDS are also found to be associated with pulmonary eosinophilia syndrome. Microfilariae are often absent in the blood and the dying microfilariae are surrounded by eosinophils at the sites of spleen, liver, lymph nodes or lungs (Spry and Kumaraswami 1982). TPE show immunological hyperresponsiveness especially to microfilariae or mf antigens (Ottesen et al 1979). It was also shown that activated eosinophils found in bronchoalveolar lavage fluid release oxygen free radicals and proinflammatory molecules (Rom et al 1990) leading to lung impairment. Treatment with diethylcarbamazine (DEC) has a rapid clinical response and decrease in the number of eosinophils. Intestinal worm infections also result in similar symptoms like TPE and can be treated with antihelmenthics such as albendazole and Ivermectin (Rocha et al 1995).

1.2.5 Diagnosis of lymphatic filariasis

The efficient treatment and control of filariasis lies in the development of easy diagnostic methods. Several diagnostic procedures are
available for the diagnosis of filarial infection. These include conventional parasitological diagnosis, lymphatic imaging techniques, immunological methods, recombinant antigen and DNA based diagnosis. Due to broad spectrum of filarial disease no single test meet all the requirements.

1.2.5.1 Parasitological diagnosis

The presence of parasite in the blood is identified by conventional night blood smear stained with Giemsa for microfilariae positivity is the most commonly used method for diagnosis of filarial infections. Other commonly used methods include counting chamber, membrane filtration, Knott's concentration techniques and DEC provocative day test (Denham 1995). Most of these tests need to collect blood in the night time due to the nocturnal periodicity of the mf, which is a non-compliance at the community level. These methods of diagnosis are insensitive if the mf count is low and when the mf sequestered at inaccessible sites (Wamae 1994).

1.2.5.2 Lymphatic Imaging Techniques

Imaging techniques such as lymphoscintigraphy, lymphangiography and ultrasonography involve use of modern instruments to detect parasite in the lymphatic system and trained personnel's are required to perform this. Lymphoscintigraphy has been used to assess lymphatic function of the filarial patients (Freedman et al 1994). The patients injected with radiolabelled colloid, albumin or dextran intradermally or subcutaneously collect in the lymphatics. The asymptomatic individuals are diagnosed with markedly dilated lymphatics and rapid lymph flow than normal lymphatics. The lymphatics of chronic patients with elephantiasis show lymph stasis with dilated lymphatic vessels.
dermal back flow and obstruction. Lymphangiography is used to study alterations in lymphatic function of filarial patients (Sen and Ellappan 1968). This technique is time consuming, invasive and can induce local morbidity aggravating the pathology.

Ultrasonography has been used to locate the adult *W. bancrofti* worm in the scrotal lymphatics of the infected patients using a 50 MHz sectorial transducer (Amaral et al 1994). This technique reveals lymphatic dilation with peculiar aleatory movements known as ‘filarial dance’ where the parasite is seemingly attached to the lymphatic endothelium (Suresh et al 1997). The demerits of this technique are its cost and requirement of sophisticated instrumentation.

1.2.5.3 DNA based diagnosis

Detection of DNA material of the parasite is a novel approach to diagnose a parasite infection. Polymerase Chain Reaction (PCR) and DNA probes have been used for the detection of DNA of *W. bancrofti* and *B. malayi*. Blood, plasma and paraffin-embedded tissue samples of the *W. bancrofti* infected individuals can be used for DNA analysis by PCR with high sensitivity (McCarthy et al 1996; Zhong et al 1996). These methods detect a DNA repeat (SspI) sequences of the parasite DNA that is highly specific for *W. bancrofti*. Another method which detects DNA sequence (pWb12) of a single *W. bancrofti* infective larvae or microfilariae from as little as 300 pg DNA material is also reported (Siridewa et al 1994). Detection of *W. bancrofti* in the mosquitoes to monitor infection rates in mosquito vectors has been developed (Dissanayake et al 1991). This method is based on nonradioactive probe that uses
chemiluminescent substrate. Identification of parasites in the mosquitoes will be useful in the control programs of the vectors in the endemic areas.

A number of methods have been reported for the detection of *B. malayi* parasite material. These methods include use of either DNA probe or PCR of the *B. malayi* DNA to identify the infection (Williams et al 1988; Lizzote et al 1994). Although these methods are highly sensitive, the costs of the reagents, requirement of sophisticated instruments make it unsuitable for large-scale field evaluation.

1.2.5.4 Immunodiagnosis

Immunodiagnostic methods are currently under evaluation for the diagnosis of filarial infections in addition to the conventional night blood smear. Immunodiagnostic methods can be classified as antibody and antigen based detection assays.

1.2.5.5 Antibody detection assays

Detection of antibodies to filarial antigens using heterologous and homologous antigens have been evaluated. Earlier studies were based on detection of filarial specific total Immunoglobulins by ELISA (Kaliraj et al 1981b); IFAT, IHAT and ELISA using *W. bancrofti* mf antigens (Dasgupta et al 1984). Assay for total antibodies gives information about the infection of the parasites but does not infer about the current or past infection status of the individual. IgG4 antibody assay has been shown to correlate with infection (Kwan-Lim et al 1990) and duration of infection (Mahanty et al 1994). This assay also increases the identification of cryptic infections.
Antifilarial IgG4 antibodies are developed against antigens other than phosphorylcholine (PC) (Scott et al 1987), which is responsible for much of the cross reactivity observed in the diagnosis of parasitic infections (Lal and Ottesen 1988). The levels of IgG4 are elevated significantly in MF individuals when compared to other groups (Hussain et al 1987).

The non-availability of the homologous antigens in filariasis has led to use of closely related helminth heterologous antigens in the application of diagnosis (Maizels et al 1985). The surface antigens of bovine filarial parasite *Setaria digitata* have been employed for the immunodiagnosis of bancroftian filariasis (Theodore and Kaliraj 1990). Secretory acetylcholinesterase (75 and 45 kDa) isolated from *Setaria cervi* microfilariae has been useful to diagnose *Setaria* filariasis (Sharma and Rathur 1999).

The use of homologous antigens against heterologous antigens for diagnosis of filariasis will enhance the sensitivity and specificity of the assay. The utility of *W. bancrofti* mf fractionated soluble antigens in the diagnosis has been evaluated by indirect haemagglutination test (IHAT) and enzyme linked immunosorbent assay (ELISA) (Kaliraj and Harinath 1982). Fractionated and albumin adsorbed antigen of *W. bancrofti* from urine of filarial patients have also been evaluated for its use in diagnosis (Ramprasad and Harinath 1995). The major disadvantage of the antibody detection assay is that it is not possible to distinguish between the past and current infection status.

Antigens produced through recombinant DNA technology can be efficiently used for the serodiagnosis of filarial infections. These proteins are devoid of PC antigens and can minimize the sera cross reactivity. The cloned
parasite antigens have been shown to detect anti-IgG4 antibodies in MF individuals (Dissanayake et al 1992; Theodore et al 1993; Chandrashekar et al 1994; Ramzy et al 1995; Rao et al 2000). Further, quantitation of IgG4 against recombinant antigens will be useful for chemotherapeutic follow up programmes in the endemic areas of filariasis.

1.2.5.6 Antigen detection assays

The conventional night blood smear involves cumbersome process of blood collection whereas the circulating antigen detection can be done with daytime blood samples. Further, the antigen detection assays infer the current status of infection. These advantages make antigen detection assays of diagnostic importance.

Polyclonal antibodies developed to *B. malayi* mf ES antigens (SEVA-FILACHEK) have been employed to identify occult filarial infections (Harinath et al 1996). A filarial antigen dipstick ELISA developed using antibodies produced to *W. bancrofti* microfilariae SDS soluble antigens was used to identify MF patients (Cheirmaraj et al 1992). A number of monoclonal antibodies have been developed against parasite antigens and were successfully used to identify circulating parasite antigens in the patients. Mab E34 raised against *W. bancrofti* mf ES antigen was able to detect filarial antigen associated with active infection (Reddy et al 1986). Monoclonal antibodies developed against phosphocholine, antigens from other related parasites and a recombinant antigen has been shown to detect circulating parasite antigens in bancroftian infections (Dissanayake et al 1984; Forsyth et al 1985; Lal et al 1987; Theodore and Kaliraj 1996). Although these tests are useful to diagnose filarial infections.
due to lack of high specificity for microfilaremic individuals, a suitable antigen detection system has to be developed.

At present there are two tests commercially available (Og4C3 and ICT card test) to detect bancroftian filarial infections with high sensitivity and specificity. Both these tests are based on monoclonal antibodies developed against heterologous antigens. The Og4C3 assay uses monoclonal antibodies directed against O. gibsoni adult antigen (More and Copeman 1990) whereas the ICT card test uses monoclonal antibody to AD12 of Dirofilaria immitis (Weil and Liftis 1987a). Both these tests were shown to be positive in daytime blood samples from infected individuals (Faris et al 1993). The circulating antigen detection assays are particularly useful in the assessment of treatment with DEC or Ivermectin in the infected patients (Eberhand et al 1997; Weil et al 1998).

1.2.6 Treatment of filariasis

1.2.6.1 Diethylcarbamazine treatment

The treatment and control of lymphatic filariasis relies on the administration of the drugs Diethylcarbamazine (DEC) and Ivermectin. The chemical name of DEC is 1-diethylcarbamyl-4-methyl piperazine, formulated and commercially sold under the trade names Hetrazan, Bonacide and Notezme. The salient features of this drug are its stability and less side effects. Treatment of microfilarems with DEC dramatically reduces microfilariae in the blood and reduces the opportunity for mosquito-borne transmission of the parasite.

The dosage treatment recommended by World Health Organization (WHO) to administer 6 mg per Kg of body weight in 3 equal doses for a period
of 3 days. An annual single dose of 6 mg per Kg of body weight has shown to be effective in reducing mf prevalence (Kimura and Mataika 1996). The target of DEC has been shown to be on the cholinergic receptors in the muscles of the parasite causing paralysis and dislocation of the parasite in the host (Langhman and Kramer 1980). It has also been shown that DEC causes alterations in the microfilarial surface membranes there by make the parasite susceptible for destruction by host defence mechanisms (Hawking 1979; Mackanzie and Kron 1985). The efficacy of the treatment in combination with other drugs is monitored by studying the level of circulating filarial antigen as an index (McCarthy et al 1995). DEC is not recommended for treatment in areas with coexistence of onchocerciasis or loiasis because of its adverse reactions.

1.2.6.2 Ivermectin treatment

Ivermectin, a macrolide antibiotic is obtained from a class of naturally occurring compounds having broad spectrum of antiparasitic activity. This drug is commercially available under the trade name Stromectol by Merck and Co., Inc. It disrupts the neuromuscular activity of the parasites by binding to glutamate gated chloride channels. This drug has been extensively used in the clinical trials of Onchocerciasis (Chodakewitz 1995). Ivermectin is particularly useful in areas of coexistence of loiasis and onchocerciasis. A single dose administration of 400 µg per Kg of body weight has been shown to be effective (Moulaia-Pelat et al 1994). The combined usage of Ivermectin and DEC simultaneously in a single dose in *W. bancrofti* infection has been shown to be effective in suppression of microfilaremia than either drug given alone (Ismail et al 2001).
1.2.6.3 Albendazole treatment

Albendazole remains the drug of choice for the treatment of most enteric nematodes such as enterbius, trichuris, ascaris and hookworm. The target of this drug is that it disrupts the microtubule formation by affecting the normal function of helminth’s gut cells and its ability to obtain nutrients. The repeated high dose of albendazole has been shown to have macrofilaricidal effect against *W. bancrofti* (Jayakody et al 1993). The hallmark of this drug is no clinical adverse reactions in the course of treatment was observed suggesting that it has a primary effect on the adult parasite, resulting in slow decrease in microfilaremia. The simultaneous administration of albendazole and Ivermectin has been shown to be effective in the reduction of microfilariae in bancroftian filariasis than does Ivermectin alone (Ismail et al 2001).

1.2.6.4 Other antifilarial treatments

Coumarin is another drug of choice that is being used to reduce lymphoedema as marketed by PHARM products pvt. Limited, Thanjavur, Tamil Nadu, India. An analogue of ubiquinone (2, 3-Dimethoxy-5-methyl1-1.4-benzoquinone) has been shown to produce irreversible paralysis in the adult parasites, in microfilariae of the bovine filarial parasite *Setaria digitata* and mf of *W. bancrofti* (Sivan and Kaleysa Raj 1999). Benzimidazole compounds have also been used in the treatment of cattle infected with *O. gibsoni*. These compounds bind to tubulin of the parasite and prevents the polymerisation into microtubules (Lacey 1990).

DEC is the widely used drug of choice for the treatment of *W. bancrofti* and *B. malayi* infections. However it has a limitation in its use in
areas with coexistence of onchocerciasis and loiasis. Ivermectin is particularly useful in such areas. Ivermectin in combination with DEC is shown to be effective in treatment of *W. bancrofti* infections. Albendazole is used for the treatment of most enteric nematodes such as Enterbius, Trichuris, Ascaris and hookworm. This drug has no side effects in the course of treatment. The repeated high dose of this drug has shown to be effective against *W. bancrofti* infections. The above drugs have its own merits and demerits for the treatment.

1.2.6.5 Disease management

The subclinical lymphatic damage of the microfilaremics can be prevented from the risk of developing clinically apparent lymphatic disease by avoiding lymph stasis through regular exercise, movement and elevation of the limbs. The external genitalia of elephantiasis have been shown to be in control by surgical excision of affected tissue and split skin graft (Ollapallil and Walters 1995). Hygiene and skin care needs to be given to prevent secondary bacterial infections in lymphoedema and clinical elephantiasis patients. Periodical cleaning of the affected limb and topical application of antibiotics results in suppression of adenolyphangitis (Shenoy et al 1995). The disease management of elephantiasis of the limbs can be done by the use of pneumatic pumps that are available from AMLA Mediequip, New Delhi, India. Complex physical therapy, heat treatment and nodo-venous anasthemosis are the other method of management of lymphoedema (Manokaran and Jamal 1996).

1.2.6.6 Vector control strategies

Apart from treatment of infected individuals the other important factor to be controlled is mosquito vectors. Insecticide impregnated bed nets can be
used to limit the vector-host interaction and spray of long-lasting pyrethroids can be especially toxic for the adult-stage of Culex and Mansonia mosquitoes (Vasuki and Rajavel 1992). The mosquitoes have developed resistance to the chemical DDT and newer methods have to be developed for the reduction in vector density. Use of biocides is a novel approach to control the spread of mosquitoes. Toxins isolated from *Bacillus thuringiensis* and *Bacillus sphaericus* have been used to control the mosquito vector (Porter et al 1993).

Centre for Biotechnology, Anna University is actively involved in the development of newer biocides isolated from *B. thuringiensis* and *B. sphaericus* for use in vector control strategies.

### 1.2.7 Immune response in filarial infections

The prime candidates for effective immune mechanisms against helminths are humoral and cellular components of the host immune system. The humoral arm of the immune system involves interaction of B cells with antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination. Effector T cells generated in response to antigen are responsible for cell-mediated immunity. Both activated Th cells and Cytotoxic T-lymphocytes (CTLs) serve as effector cells in cell-mediated immune reactions. Cytokines secreted by Th cells can activate various phagocytic cells, enabling them to phagocytose and kill microorganisms more effectively. This type of cell-mediated immune response is especially important in host defence against intracellular bacteria and protozoa. CTLs participate in cell-mediated immune reactions by killing altered self-cells and also play an important role in the killing of virus-infected cells and tumor cells. Lymphatic filariasis is a complex disease having three
broad areas such as tolerance, pathology and concomitant immunity. Different immune response pattern to the filarial antigens in infected individuals lead either to pathogenesis or protection.

1.2.7.1 Humoral immune response in filariasis

IgG is the predominant immunoglobulin, present in serum. IgG subclass differ in the number and location of disulphide bonds and the size of the hinge regions. In general, the amino acid sequences of the four IgG subclasses are over 95% identical. IgG1, IgG2 and IgG4 subclasses bind to staphylococcal protein A, but IgG3 does not bind. Except IgG4, all the other subclasses can fix complement. IgG1 is the major isotype antibody among the IgG subclasses. Elevated levels of serum IgG1 antibodies have been shown in chronic patients (Hussain et al 1992). The blocking activity of IgG1 in certain allergic reactions (Djurup and Osterballe 1984) suggest that increased levels of serum IgG1 level in chronic patients may contribute some control in the allergic reactions.

IgG2 antibody levels have been shown to be elevated in chronic pathology individuals and TPE when compared to microfilaremics and EN (Kumiawan et al 1993) could be important in the development of pathogenesis. IgG3 antibody responses to filarial antigens predominate in patients who develop lymphatic pathology (Hussain et al 1987). The implication of IgG3 as a factor in tissue damage in Onchocerciasis has been recorded (Cabera et al 1988). Filarial specific IgG4 is shown to be significantly elevated in the MF individuals compared to CP (Hussain et al 1987; Kumiawan et al 1993). IgG4 antibody is a monovalent antibody, which is unable to fix complement reaction by the classical pathway and hence it may lower complement mediated
parasite clearance in MF (Ottesen et al 1985). These antibodies are shown to be blocking agent for IgE recognizing the same antigens of the parasite (Hussain and Ottesen 1986) thereby preventing the allergic reactivity. The hyporesponsive state was also observed to transfer from microfilaraemic mothers to their children (Steel et al 1994) suggesting that hyporesponsiveness may also result from in-utero acquisition of tolerance to microfilariae antigens in chronically infected mothers. Elevated levels of IgE in the parasitic infections have been implicated in the allergic reactions. TPE individuals have elevated levels of filaria specific antibodies- IgG, IgM, IgA, IgG4 and IgE (Hussain et al 1992). The blocking function of IgG4 in the inhibition of allergic reactivity to parasite antigen in the sera of parasitized patients has been demonstrated (Ottesen et al 1981; Hofstetter et al 1982).

1.2.7.2 Cellular immune responses in filariasis

Upon antigenic stimulation, CD4$^+$ T helper (Th) cells differentiate into two distinct sub populations, the T-helper 1 (Th1) or T-helper 2 (Th2) cells, each producing unique cytokines and mediating separate effector functions (Mossmann and Coffman 1989). Th1 cells produce interleukin-2 (IL-2), tumor necrosis factor (TNF-α) and interferon-gamma (IFN-γ) thereby activating macrophages and inducing delayed type hypersensitivity responses and cell mediated immunity. Th2 cells produce IL-4, IL-5, IL-10 and IL-13 stimulating production of mast cells and eosinophils.

IL-2 and IFN-γ expression has been elevated in the PBMC of endemic normals significantly when treated with *B. malayi* adult antigens with the absence of IL-4 and IL-5 (Ravichandran et al 1997) showing that they have Th1
type of response. Studies in other parasitic infections indicate the development of Th1 response and production of IFN-γ was necessary for the control of murine infection with *Leishmania major* (Sadick et al 1990).

Th-2 type of immune response has been implicated for the hyporesponsive state of the MFs (Mahanty et al 1993). Generation of human Th2-type T-cell clones by excretory/secretory products of the parasites has been shown (Del Prete et al 1993). Elevated level of IL-10 is associated with antigen specific hyporesponsiveness in filariasis patients down regulating the Th1 response (Mahanty et al 1997; Ravichandran et al 1997). The mechanism underlying IL-10 mediated suppression of lymphocyte proliferation may occur through the ability of IL-10 to inhibit expression of MHC class II molecules or certain co stimulatory molecules on antigen presenting cells (de Waal Malefyt et al 1991). Th2 type of response was reversed in lymphocyte cultures when treated with anti-IL-10 antibodies to enhanced T cell proliferation and subsequent Th1 cytokine production (Pearlman et al 1993).

Several concepts have been suggested for the pathogenesis of lymphatic lesions in the filarial patients. Animal models have provided clear evidence that much of the pathology results from host’s immune response to the filarial parasites on the lymphatic tissue of the parasites themselves or the molecules they release. Immunodeficient mice infected with brugian parasites have shown to develop marked endothelial cell proliferation and lymphatic dilatation resulting in lymphoedema and elephantiasis in the absence of immune response to the parasite (Vincent et al 1984). Immunodeficient mice when reconstituted with immunocompetent cells from filaria-sensitized mice resulted in inflammatory reactions around the parasites leading to lymphatic obstruction, lymphoedema and elephantiasis (Vickery et al 1991). This suggests that two
distinct mechanism act to damage the lymphatic function of such infected animals. A mixed response of Th1 and Th2 has been implicated in CP patients (Ravichandran et al 1997). Class I MHC expression is shown to be upregulated by parasite driven lymphokines in endothelial cells of filarial patients leading to inflammatory events (Freedman et al 1989). The inflammatory cytokine TNFα has been shown to be produced by monocytes of chronic patients to filarial antigens (Raman et al 1999).

Cytokines such as IL-3, Granulocyte macrophage colony stimulating factor (GM-CSF) and IL-5 i.e. Th2 response has been implicated in the development of eosinophilia in humans (Coffman et al 1989; Mahanty et al 1993). Mitogen stimulation of PBMCs of eosinophilic patients induces high level of IL-5 expression at both the mRNA and protein level (Limaye et al 1990) suggesting that IL-5 for this effect. DEC treatment of eosinophilic patients showed decreased expression of IL-5 from PBMCs after 3 days of treatment (Limaye et al 1993) indicating that DEC treatment may have a role in regression of this disease. Administration of monoclonal antibody to IL-5 in parasitized mice has been shown to completely suppress blood eosinophilia and infiltration of eosinophils in the lungs (Coffman et al 1989). Thus the development of agents that inhibit IL-5 will be specifically useful in diseases such as tropical pulmonary eosinophilia, bronchial asthma or hypereosinophilic syndrome. The ability of rIL-12 in suppressing filaria-induced pulmonary eosinophilia in mice (Mehlotra et al 1998) gives some insights into the therapy in eosinophilia.

1.2.7.3 Parasite immune modulation

Lymphatic parasites survive for years in a complex immune environment by adopting various strategies of immune modulation.
A phosphorylcholine (PC) containing glycoprotein (ES-62), which is secreted by *Acanthocheilonema vitea* has been shown to possess immunomodulatory properties (Harnett et al 1999). The exposure of murine resting B cells to ES-62 affected number of signalling pathways associated with cellular activation and proliferation. The modulation of ES-62 requires PC to desensitize almost completely the cells to subsequent activation of the phosphoinositide 3-kinase and Ras-MAPK pathways via the BCR (Deehan et al 1998). The immunosuppressive effects of BmA antigen on Phytohemagglutinin (PHA) mediated T cell proliferation were shown to be phosphorylcholine containing antigens that were present in abundance in the filarial parasites and in the circulating filarial antigens (King et al 1992). The depressed mitogenic reactivity of the splenic lymphocytes from filaria-infected animals could be restored to normal by removing adherent cells (Portaro et al 1976; Lammie and Katz 1983).

1.2.7.4 **Parasite immune evasion**

Filarial parasites have evolved several strategies to evade the immune system of the host (Maizels et al 1993). Molecular mimicry and uptake of host antigens by the helminths are the widely used strategies for evasion. Most parasites secrete contrapsin, an antithrombotic serum serine protease inhibitors (Modha et al 1988) and low-density-lipoprotein, possibly causing the progressive loss of antibody binding site for immune evasion (Chiang and Caulfield 1989).

Antigenic variation as an immune evasion is observed in trypanosomes mainly through transcriptional regulation of surface glycoproteins (Maizels et al 1993). Many nematodes have a loose surface coat, which can be
readily prevented from immune attack (Blaxter et al 1992). Filarial parasites express or secrete antioxidant enzymes such as superoxide dismutase (Simurda et al 1988), Glutathione peroxidase (GPX) (Cookson et al 1992), Glutathione-S-transferase (GST) (Smith et al 1986) and Thioredoxin peroxidase (TPX) (Ghosh et al 1998; Lu et al 1998) to counteract the oxidative free radical damage produced by the host. Parasites block the effects of antibody directly or indirectly with surface or released proteases capable of degrading host immunoglobulin molecules (Auriault et al 1981). A several protease inhibitors secreted by filarial parasites have potential role in immune evasion (Tang et al 1994; Lu et al 1998). One of the strategies adopted by the parasites is to secrete homologues of host molecules to divert immune system. For example B. malayi secretes macrophage migration inhibitory factor (MIF) like protein with the potential to alter host immune response for its survival (Pastrana et al 1998). It is also shown that B. malayi secrete cystatin and serpin molecules (Maizels et al 2001). Cystatin (Bm-CPI-2) blocks papain and aspariginyl endopeptidase involved in the class II antigen processing pathways in human B cells whereas serpin (Bm-SPN-2) blocks two key proteases of the neutrophil essential for mediation of inflammation and innate immunity.

1.2.8 Allergic inflammatory and adverse reactions in filariasis

Allergic inflammatory reactions are characterized by elevated levels of IgE in the serum and expression of IgE receptors FceR I present on the basophils and mast cells. Cross-linking of antigens with IgE activates the basophils and mast cells and release of various vasoactive mediators such as histamine, prostaglandins and sulfoleukotrienes. Histamine plays a major role in inflammatory reactions by attracting the inflammatory cells such as eosinophils and neutrophils to the site of inflammation. The activated
eosinophils produce oxygen free radicals and release various factors that damage the surrounding tissues and cells. Tropical pulmonary eosinophilia (TPE) is an acute inflammatory interstitial lung disorder caused by dying microfilariae or mf antigens of *W. bancrofti* or *B. malayi* with markedly elevated number of eosinophils in the lower respiratory tract (Pinkston et al 1987). DEC treatment in *B. malayi* infected MF individuals has shown to cause systemic reactions with high fever, headache, pain in joints and muscles, dizziness and nausea (Haarbrink et al 1999). These adverse reactions are associated with elevated levels of inflammatory mediators particularly IL-6, LPS-binding protein and soluble TNF-R75. The severity of adverse reactions to DEC treatment in host is directly proportional to the intensity of the microfilariae and the release of biogenic amines such as histamine and serotonin from them (Saxena et al 1977). In onchocerciasis, treatment with DEC and Ivermectin results in Mazzotti reaction, an inflammatory response with an intense pruritis where mf is more in the skin (Bird et al 1980; Ottesen 1985; Zea-Flores et al 1992).

1.2.9 Histamine releasing factors (HRF)

HRF are defined as products of cells that are capable of interacting with basophils and mast cells to cause release of histamine (Theuson et al 1979; Kaplan et al 1985; MacDonald 1996). HRFs are produced by number of cells *in vitro* such as human mononuclear cells, alveolar macrophages and monocytes (Kaplan et al 1985; Haak-Fredscho et al 1988; Alam et al 1989). HRFs are known to activate basophils and mast cells to release leukotrienes. In addition cytokines such as IL-3, granulocyte macrophage colony stimulating factor (GM-CSF) and IL-1 have also induce HRF activity from basophils (Haak-Fredscho et al 1988).
Majority of the HRFs characterized so far are very heterogeneous and have molecular weight species ranging from 15 kDa to 50 kDa. HRFs mediate late phase reaction (LPR) (Cruikshank et al 1995), which is characterized by an initial event of mast cell degranulation and release of mediators that, in turn, attract and up regulate adhesion molecules. HRF- induced histamine release is classified in to IgE independent and IgE dependent histamine release. Cytokines of two large groups such as chemokines and interleukins fall under IgE independent histamine release.

1.2.9.1 Chemokines

Chemokines are a superfamily of small cytokine-like molecules ranging 6 kDa to 14 kDa that are classified according to the position of cysteine residues (Hedrick and Zlotnik 1996), which have the ability to mediate migration of cells of lymphoid origin. They are classified into two major groups of C-X-C family (two cysteines with an intervening amino acid) and the beta or C-C family (two adjacent cysteines).

C-X-C chemokines are further divided into subclasses based on the presence of an ELR amino acid motif immediately preceding cys-1 (ELR'). These chemokines are largely neutrophil chemoattractants. IL-8 is a prototype CXC chemokine molecule that attracts neutrophils but not monocytes in Boyden chamber assays (Yoshimura et al 1987). The other molecules of CXC family include Connective tissue activating peptide III (CTAP-III) and Neutrophil activating peptide-2 (NAP-2) (MacDonald 1993). The list of chemokines of C-C and CXC family and their histamine release are given in Table 1.1.
Table 1.1 Chemokines that cause basophil histamine release

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Histamine release</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C</td>
<td></td>
</tr>
<tr>
<td>MCP-4</td>
<td>+</td>
</tr>
<tr>
<td>MCP-3</td>
<td>+++</td>
</tr>
<tr>
<td>MCP-2</td>
<td>++</td>
</tr>
<tr>
<td>MCP-1</td>
<td>+/-</td>
</tr>
<tr>
<td>RANTES</td>
<td>+/-</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>+/-</td>
</tr>
<tr>
<td>C-X-C</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>+/-</td>
</tr>
<tr>
<td>CTAP-III</td>
<td>+/-</td>
</tr>
<tr>
<td>NAP-2</td>
<td>+/-</td>
</tr>
</tbody>
</table>

C-C chemokines are more active than CXC chemokines in stimuli for histamine release from basophils. Monocyte chemotactic protein (MCP)-3, which shares a high degree of homology with MCP-1, is both a chemotactic agent and a histamine-releasing agent (MacDonlad 1993). The gene for recently described C-C chemokine family, MCP-4 was cloned and expressed and its biological activities were determined (Uguccioni et al 1996). MCP-4 shares 56-61% sequence identity with MCP-1 and -3 and 60% identity with eotaxin, which causes chemotaxis of eosinophils (Jose et al 1994). Histamine release by MCP-2 from IL-3 primed basophils and calcium signalling in eosinophils and basophils were less potent than for MCP-1 (Weber et al 1995). A number of C-C chemokine receptors are being identified and a gene coding for a novel C-C receptor was cloned from the human immature basophilic cell line, KU812.
The chemokines that bind to this receptor are macrophage inflammatory protein (MIP)-1, RANTES and MCP-1 but not MCP-2. Chemokines have multiple receptors with varying specificity and complexity of biological functions (Kuna et al 1995). Thus chemokines act independent of IgE and exert their action solely on human basophils.

1.2.9.2 Interleukins

Interleukins are another group of IgE independent HRFs. IL-3 at higher concentrations causes direct histamine release from basophils in a subpopulation of allergic donors and at lower concentration it primes or augments the histamine release when stimulated with other agonists. Interleukins such as IL-1 (Massey et al 1989), tumor necrosis factor-α (TNF-α) and IL-6 (Kaplan et al 1991) have been shown to downregulate histamine release from basophils. A variety of other interleukins that do not posses the histamine release activity are IL-2, 4, 5 and 6 (Kaplan et al 1991). The histamine releasing activity of various cytokines are given in Table 1.2.

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
<th>Not studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 3</td>
<td>Interleukin 1</td>
<td>Interleukin 7</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>TNF-α</td>
<td>Various growth factors</td>
</tr>
<tr>
<td>Interleukin 8</td>
<td>Interleukin 2, 4, 5 and 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G-CSF, M-CSF, IFN-γ, TGF-β</td>
<td></td>
</tr>
</tbody>
</table>

GM-CSF resembles several physiochemical properties of IL-3 including the possibility of shared receptors. It enhances dose dependent
basophil histamine release and leukotriene release induced by the eosinophil product major basic protein (Sarmiento et al 1995). The factors that act on more differentiated cells such as granulocyte-colony stimulating factor (G-CSF) and macrophage-colony stimulating factor (M-CSF) do not have histamine-releasing function (Haak-Frendscho et al 1988). IL-8 is identical to monocyte-derived neutrophil activating peptide-1 (NAP-1) and has chemotactic functions for neutrophils and causes neutrophil activation and secretion (Yoshimura et al 1987). Platelet activating factor (PAF) requires extracellular calcium for the basophil histamine release and the augmentation of histamine release of PAF is enhanced by IL-3 (Columbo et al 1995). IL-3 regulates the ability of basophils to release histamine by altering the signal effectors such as the tyrosine kinases Lyn and Syk (Yamaguchi et al 1996). Thus IL-3 converted the non-histamine releasing basophils to histamine releasing basophils and the mechanism of this conversion is not known. The importance of IL-3 in basophil function is further supported by in vivo studies in rhesus monkeys undergoing IL-3 treatment in which it upregulated the number of high and low affinity receptors for IL-3 on basophils but not on mast cells or monocytes.

1.2.9.3 The IgE-dependent HRF

A number of HRFs act on basophils via surface-bound IgE and are reported from alveolar macrophages (Liu et al 1986), platelets (Orchard et al 1986) and from nasal washings (MacDonald et al 1987). Nasal lavage fluids contain histamine-releasing factor similar to previously described from macrophages, platelets, and from blister fluids obtained during the late cutaneous reaction. The nasal HRF is heterogeneous at an apparent molecular weight range of 15 kDa to 30 kDa (MacDonald et al 1987). The histamine release by nasal HRF is by interaction with basophil cell surface IgE. HRF
obtained from peripheral blood B cells (Alam et al 1989) release histamine from basophils of both allergic and normal subjects and appears to consist of both IgE and non-binding species. Macrophages are known to release several factors that modulate the humoral and cellular components of inflammatory reactions. A factor from the secretions of human lung macrophages release histamine from basophils and mast cells by binding to IgE (Schulman et al 1985). The macrophage supernatants appear to be a poor inducer of histamine release of mast cells than that of basophils. A unique HRF of molecular weight heterogeneity of two major forms of 41 kDa and 17 kDa has been purified and characterized from human mononuclear cells (Baeza et al 1988). An histamine releasing factor identified from human lymphocytes (Blaszczyk et al 1987) release histamine from mast cells of various species such as atopic and non-atopic asthmatics and from mouse, hamster, rat and guinea pig mast cells suggesting that some HRF exhibit cross species activity (Alam and Wyczolkowska 1985).

1.2.9.4 Histamine releasing factors of the parasites

High levels of IgE are induced in the helminth infections (Hussain et al 1981). Such infections results not only in the production of parasite-specific IgE but also parasite non-specific IgE (Turner et al 1979). IgE antibodies are involved in the mediation of allergic reactions by binding to receptors on the plasma membrane of mast cells and basophils. The attachment of allergen antigen to the membrane bound antibody or receptors leading to the release of histamine and other active substances. Metabolic antigens and somatic extract antigens of microfilariae and adult parasites of *W. bancrofti*, *B. malayi* and *D. immitis* induced histamine release from basophils of patients with Tropical eosinophilia than non eosinophilic individuals.
(Ottesen et al 1979). The metabolic antigens of microfilariae in particular are potent inducer of histamine release than other antigens suggesting that these antigens play a key role in allergic inflammatory reactions. Similarly in *Schistosoma mansoni* infections, itching or prickling sensation develops 4-10 minutes after exposure to the cercaria and subsequently erythematous macules appears.

The larval secreted antigens of *Toxocara canis* and *Ascaris suum* induces histamine release from human blood basophils (Nielsen et al 1991). Mazzoti and chronic inflammatory reactions are frequently observed in Onchocerciasis involving specific IgE and reactivity of mast cells and basophils to the parasite antigens. The soluble parasite extracts and cuticular collagen of *O. volvulus* induce more histamine from basophils in patients with Onchocerciasis than mansonellosis (Munoz et al 1999). The presence of reaginic antibodies to the human parasite *Strongyloides stercoralis* has been studied by histamine release from human basophils to the somatic larval antigens (Genta et al 1983). In human lymphatic filariasis the blocking antibodies IgG4 modulate immediate hypersensitivity responses (Hussain *et al* 1988). This is evident from the inhibition of histamine release from basophils by sera of patients with filariasis to parasite antigen (Ottesen et al 1981).

### 1.2.9.5 IgE inducing recombinant parasite antigens

Bm2325, a major IgE-inducing antigen of the filarial parasite *B. malayi* has been implicated in the pathology of TPE (Lobos *et al* 1992). IgE antibodies to Bm2325 from patients with TPE were used to identify a homolog of this antigen from *B. malayi* cDNA library and the cloned antigen was a
homolog of γ-glutamyl transpeptidase (Lobos et al 1996), a key enzyme essential in the synthesis and degradation of glutathione. Polyprotein allergens are a family of structurally homologous molecules from parasitic nematodes, which induce specific IgE in infected individuals. Recombinant gp15/400, a polyprotein allergen cloned from *B. malayi* has been implicated in pathogenesis of filarial infections (Allen et al 1995). In schistosomal infections, IgE antibodies have role in protection against the parasites (Dunne et al 1992). A recombinant 22kDa antigen of *S. japonicum*, a major target of the human IgE response has been cloned and explored as a candidate vaccine (Santiago et al 1998).

1.2.10 Translationally Controlled Tumor Protein (TCTP)

TCTP also designated as p21/p23/HRF was first identified as a growth associated protein of the Ehrlich ascite tumor (EAT) and mouse erythroleukemia cells (Chitpatima et al 1988; Bohm et al 1989). The synthesis of growth related proteins appears to be controlled at the post-transcriptional level when 3T3 cells stimulated with mitogens (Thomas and Thomas 1986) may be important for cell proliferation. TCTP expression is reported from several nontumoral cells such as erythrocytes, hepatocytes, macrophages, platelets, keratinocytes, erythroleukemia cells, gliomas, melanomas, hepatoblastomas and lymphomas (Sanchez et al 1997) suggest that TCTP may have a primary function essential for sustenance of most cell types. TCTP expression in various cells and organisms is modulated by several factors such as calcium (Xu et al 1999), Phorbol myristate acetate (PMA) (Walsh et al 1995), growth factors (Bommer et al 1994; Teshima et al 1998; Vercoutter-Edouart et al., 2001), heavy metals (Sturzenbaum et al 1998), interleukins (Nielsen et al 1998), environmental factors such as darkness (Sage-Ono et al 1998) and ammonium requirement (Bonnet et al 2000).
1.2.10.1 Translational regulation of TCTP expression

The growth associated protein TCTP of the EAT cells is preferentially expressed in the exponential phase of tumor. Expression of TCTP is under translational regulation controlled by its 5' untranslated region (UTR) (Bohm et al 1991). The identification of 5' UTR as a regulatory unit was studied by using expression of TCTP mRNA from EAT in cell-free systems of EAT and reticulocytes and wheat germ cell-free lysate. TCTP was efficiently translated in wheat germ cell lysates not in EAT cell free system and reticulocytes. This inhibition was abolished by the removal of 5'UTR region of the transcripts. The shifts in configuration of the 5'UTR region also determine the translation efficiency of TCTP (Chitpatima and Brawerman 1988). Two forms of TCTP mRNA was observed in sarcoma-180 ascites cells, one that is associated with ribosomes engaged in translation and the other in small particles unable to interact with ribosomes. It is postulated that interaction of mRNA with cytoplasmic factors strongly affects the conformation of 5' UTR region and that a particular conformation may be important for effective interaction with ribosomal particles during protein synthesis. Another translational regulation of TCTP in Ehrlich ascites tumor cells is mediated by cap-binding initiation factor complex, eIF-4E (Bommer et al 1994).

1.2.10.2 Intracellular functions of TCTP

Several important cellular functions of TCTP have been described. TCTP has been characterized from wide variety of species such as mouse (Chitpatima et al 1988), *Trypanosoma brucei* (Haghighat and Ruben 1992), alfalfa (Pay et al 1992), human (MacDonald et al 1995), *B. malayi* (Gregory et al 1997), *C. elegans* (Bini et al 1997), *Pisum sativum*
(Woo and Hawes 1997), rabbit (Thiele et al 1998), *P. falciparum* (Bhisutthibhan et al 1998), *Lumbricus rubellus* (Sturzenbaum et al 1998), rat (Kim et al 2000), *Schizosaccharomyces pombe* (Baxter et al 2000), *P. yoelii* (Walker et al 2000) and *Hydra vulgaris* (Yan et al 2000). TCTP is also shown to be present in cancer cells (Sinha et al 2000; Chung et al 2000) and in normal cells (Sanchez et al 1997) suggesting that it may have an important housekeeping function. Calcium binding property of TCTP reported from *Trypanosoma brucei* (Haghighat and Ruben 1992), human (Sanchez et al 1997), *Plasmodium falciparum* (Bhisutthibhan et al 1999) and rat (Kim et al 2000) implies that TCTP may play a vital role in signal transduction pathways and maintain a calcium homeostasis of the cell. TCTPs do not share properties with known families of calcium binding protein such as annexins, Ca$^{2+}$-ATPases, PKC enzymes and EF hand structures. The calcium-binding site of rat TCTP has been mapped to be between 81-112 amino acid residues of 172 amino acid protein (Kim et al 2000). This binding domain do not have any peculiar loop of calcium-binding motif such as CaLB domain and EF hand motif and it seems to be constituted of random coil regions neighbouring the helix suggesting that TCTP is a novel family of Ca$^{2+}$-binding protein. A role for TCTP/p23 in regulation of cell division is inferred from its tubulin and microtubule binding properties (Gachet et al 1997; Gachet et al 1999). Overexpression of TCTP in cells results in cell growth retardation and alterations of cell morphology by microtubule rearrangements and to an increase in microtubule mass and stability. Association of TCTP with mitosis in root caps of *Pisum sativum* is also reported (Woo and Hawes 1997). Several set of genes upregulate in cells undergoing apoptosis and TCTP is shown to be one of the genes that upregulate in C6.9 glioma cells during vitamin D-induced cell death program (Baudet et al 1998). Some of the upregulated genes encode ribosomal proteins
suggesting a possible involvement of the translational apparatus in this cell program. The exact role of TCTP in vitamin-D induced apoptosis has not been elucidated.

Drug resistance in parasitic diseases is a major public health problem encountered worldwide. Artemisinin are important new antimalarial endoperoxide drugs used for the treatment of malaria as they have developed resistance to the classical quinoline compounds. Identification of agents that mediate drug resistance will be of great importance to prevent the parasite to develop drug resistance. Treatment of *Plasmodium falciparum* infected erythrocytes with dihydro artemisinin labeled several malarial proteins, TCTP being the one (Bhisutthibhan et al 1998). TCTP reacts with artemisinin *in situ* and *in vitro* in the presence of hemin suggesting that TCTP may be an important drug target. TCTP from *Plasmodium yoelii* artemisinin resistant strain expressed 2.5 fold than the sensitive strain predicts that TCTP may also be a drug target of this parasite (Walker et al 2000). The artemisinin drug resistance in *P. yoelii* is multifactorial that the resistant parasites accumulate significantly less drug than the sensitive parasites and an altered expression of TCTP in the parasites. Development of frequent chemoresistance in melanoma carcinoma is a major problem in the treatment of skin cancer. Human melanoma cells (MeWo) treated with cytotoxic drugs vindesine, cisplatin, fotemustine and etoposide yields stable drug-resistant sublines with overexpression of several proteins (Sinha et al 2000). TCTP is one of the proteins associated with development of chemoresistance in MeWo cells. The over expression of this protein in the development of chemoresistance could lead to disturbances of signal transduction and interference with the chaperone system. High-level expression of TCTP in the earthworm exposed to the heavy metals in the soil is essential for the detoxification of the pollutants present in
the soil (Sturzenbaum et al. 1998) and serves as a biomarker for assessing the impact of such pollution on individual organisms.

1.2.10.3 Extracellular functions of TCTP

Presence of TCTP in secretions of several cell lines implies that it may have important extracellular functions. The reported extracellular functions of this protein are the histamine releasing activity on basophils (MacDonald et al. 1995; MacDonald 1997; Wantke et al. 1999) and activation of eosinophils (Escura et al., 2000). Human HRF (Human TCTP) was identified from the culture supernatants of U937 macrophage cell line and the protein sequencing showed 94% homology to mouse p21 as well as to human p23. Human TCTP do not have hydrophobic residues at the amino terminus of the protein, which suggests that they are not secretory protein. However, the presence of TCTP in the secretion of U937 cells may adopt a novel secretory pathway similar to IL-1 and thioredoxin (Rubartelli and Sitia 1991) or like IL-1 released during apoptosis (Hogquist et al. 1991). The secretions of human epidermal keratinocytes contain TCTP (Katz and Taichman 1999) may have role in allergic reactions of the skin. Keratinocytes also release a variety of cytokines, which have a localised as well as systemic inflammatory and immunological reactions (Schroder 1992). The presence of TCTP identical to the histamine releasing factor in the basophil leukocytes and its secretions after stimulation with IL-3 (Nielsen et al. 1998) indicates that TCTP is involved in the activation of cells. This is further strengthened by the synthesis of TCTP in human macrophage cell lines U937 after stimulation with LPS and PMA (Walsh et al. 1995). Stimulation of murine peritoneal macrophages with macrophage colony stimulating factor (MCSF) induced the secretion of TCTP (Teshima et al. 1998) identical to human histamine releasing factor.
(human TCTP). This mouse peritoneal macrophage TCTP has the capability to mediate eosinophilia and late phase allergic reaction in the mice.

1.2.11 Protective immunity in filariasis

The protection conferred in endemic normals has been shown to be from antibodies produced against surface of the third-stage larvae (L3). These individuals have been shown to recognize a distinct pattern of larval stage antigens when compared with MF individuals by immunoblot analysis (Freedman et al 1989). The stage specific antigens of the infective larval antigens may act as effective targets for a protective immunity in infected and uninfected individuals. Elevated levels of IgM and IgG have been shown in endemic normals of *W. bancrofti* infection (Bal and Das 1999).

The predominance of IgG3 subclass in the endemic normals is of interest considering the role of this subclass in various parasitic diseases. The elevated levels of IgG3 has been shown in the immune individuals of onchocerciasis (Stewart et al 1995), malaria (Bouharoun-Tayoun and Druilhe 1992) and in *Trypanosoma gambiense* (Takayangi et al 1991). The involvement of antibodies in the clearance of L3 by promoting adherence and killing of L3 by human leucocytes *in vitro* has been demonstrated (Higashi and Chowdhury 1970; Sim et al 1983a). IgG4 antibodies act as a blocking antibody to shield parasites from protective immune response (Hussain and Ottesen 1988). The endemic normals predominantly recognize 97, 55, 50 and 6 kDa antigens of *W. bancrofti* (Helmy et al 2000) and the use of these antigens in protective immune response studies will have implications in the development of vaccine.
1.2.11.1 Immunoprophylaxis in filariasis

The development of vaccine for filariasis is at present in the animal model stages due to difficulties with reproducibility, complex life cycle of the parasite, non availability of the *W. bancrofti* and *O. volvulus* parasite antigens. Most immunization studies are based on crude parasite antigens and attenuated larvae. Vaccination with radiation attenuated or chemically modified larvae have been found successful against experimental infections with *B. malayi* (Yates and Higashi 1985), *D. immitis* (Abraham et al 1988) and *O. volvulus* (Prince et al 1992). Immunisation with crude antigens of microfilariae (Kazura et al 1986) and infective L3 larvae (Lange et al 1993) or purified antigens from various stages of the parasite (Chenthamarakshan et al 1995; Frank et al 1996) have been found to provide varying levels of protection.

1.2.11.2 Importance of Recombinant DNA technology in Protection studies

The major impediment in the study of protective role of various antigens from the infective larvae is due to the lack of availability of the parasite material and well-characterized animal model system. With the advent of Recombinant DNA technology, the parasite genes can be cloned and sufficient amounts of the antigen can be produced. Several parasite genes have been cloned and their protective efficacy in animal models have been successfully demonstrated.

Chitinase cloned from microfilariae of *B. malayi* has been shown to be a promising candidate as transmission blocking vaccine (Fuhrman et al 1992). Paramyosin, (an immunodominant antigen derived from L3 stage of the parasite) immunized in jirds reduced the adult worm burden
and blood microfilaremia (Li et al. 1993). A multistage antigen SXP-1 cloned from *B. malayi* provides partial protection in jirds (Wang et al. 1997). An L3 stage specific antigen, Abundant larval transcript-1 (ALT-1) cloned from *B. malayi* has been shown to be a promising candidate vaccine for filariasis in jirds (Gregory et al. 2000).

Another novel method in immunoprophylaxis is cloning of parasite genes into DNA vaccine vectors and its direct administration in animals. Immunization of DNA vaccines has been shown to induce both humoral and cellular immune responses (Donnelly et al. 1997). DNA vaccines are simple, stable and can be produced in pure form in large scales. Parasite genes cloned in DNA vaccine vector has been shown to induce protective immunity in Schistosomiasis (Durpe et al. 1999), Leishmaniasis (Gurunathan et al. 1997) and Trypanosomiasis (Costa et al. 1998). These antigens were identified by immunoscreening the λ ZAP cDNA libraries using either putative immune individuals sera or vaccinated animal sera.

The potential disadvantage of λ ZAP cDNA libraries is that the cloned antigens expressed do not assume the native conformation and thus there is a possibility of missing several antigens during the immunoscreening of these libraries. Cloning of parasite cDNA in phage display systems aid in the easier expression, displayed on the surface of the phage in native form. The displayed antigens can be efficiently identified by panning with suitable ligand and can be characterized without any further modifications. An attempt has been made to clone the cDNA of infective stage (L3) of *B. malayi* in T7 phage display system and to identify putatively protective antigens from the library using EN sera as a ligand.
1.2.12 Phage display systems

1.2.12.1 M13 filamentous phage display system

Expression of foreign proteins on the surface of the phage makes an attractive tool for cloning and selection of proteins using a suitable ligand. Filamentous phage display systems have been developed and used mainly for the expression of peptides (Smith 1985; Parmley and Smith 1988; Scott and Smith 1990) and human growth hormone (Bass et al 1990). The peptides or proteins are expressed as part of the viral coat protein and hence are believed to assume the native structure and allows enrichment using antibodies or other ligands (Parmley and Smith 1988). The use of specific prokaryotic peptide leader/signal sequences such as the $omp$ A (Henning et al 1979) or the $pel$ B leader/signal sequence (Diolez et al 1986) allows the translated gene products into the periplasmic space of *E. coli*. The oxidizing milieu of the periplasmic microenvironment of the *E. coli* aid in disulfide bond formation and folding of gene products (Skerra and Pluckthun 1988). This secretory pathway and folding system is also employed by the filamentous phage for the production of its capsid protein as well as the cloned genes (Kang et al 1991).

The inherent difficulties of screening the conventional cDNA libraries are use of appropriate ligands such as antibodies (Mierendorf et al 1987; Snyder et al 1987) for the selection of desired gene products. The translated products from the cDNA libraries in prokaryotic host do not assume the native three-dimensional structure or correct disulfide bond formation or may be denatured when transferred to a solid support such as nitrocellulose. Hence the recognition of the ligand for the desired product becomes difficult (Butler 1992). The other disadvantage with the lambda ($\lambda$) phage based cDNA expression systems (Short et al 1988) and plasmid based systems (Sambrook et al 1989) is that they do not
allow for enrichment by ligands and hence a large number of clones has to be screened on solid-phase.

The most attractive feature of the filamentous phage display system (pJuFo) is that the displayed protein and the corresponding cDNA are physically linked and packed as one entity into a filamentous phage particle (Crameri and Suter 1993), which allows a direct selection and enrichment of the desired gene products. The pJuFo filamentous phage display system is a sensitive cloning system in which as few as $10^3$ to $10^4$ copies which translates into $10^{-19}$ to $10^{-20}$ mol of a product of a given recombinant phage is sufficient for cloning (Crameri et al 1994) whereas the other cDNA libraries require $10^{-10}$ mol of a specific product to be present for a successful detection with a ligand (Sambrook et al 1989). The biological limitation is that *E. coli* host for the propagation of filamentous phage lack post-translational modifications that often occur in eukaryotic systems.

Filamentous phage display systems are now widely used for mapping the immunodominant epitopes of the antigen to enhance better immune response (Parmley and Smith 1989). A putative protective epitope, T1 of immunodominant liver stage antigen-1 (LSA-1) of *P. falciparum* has been expressed with MS2 bacteriophage capsids and shown to induce immune response in mice (Heal et al 1999). This chimeric bacteriophage capsid stimulated a type 1-polarised response with significant upregulation of IFN-γ, a finding which corroborates naturally acquired resistance to liver stage malaria. This validates the use of phage capsid display of immunogenic determinants as a novel method of vaccination. Antibodies raised against a synthetic peptide corresponding to the amino-terminal 20 aminoacids sequence of *Taenia solium* paramyosin has been used for epitope mapping a 12-mer random peptide library.
(Gazarian et al 2000). Epitopes identified by this method will be useful in studying the complex host-parasite relationships in human and porcine cysticercosis.

The major difficulties of protective antigens cloned in prokaryotic or eukaryotic expression vectors is the purification and its stability. Further these antigens require adjuvants for mounting immune response to provide protection. Antigens displayed on the surface of the phages are easy to prepare and the coat proteins of the phage serve as adjuvants and hence can be efficiently used for prophylactic studies. Two major antigenic determinants of circumsporozoite protein of malaria parasite, *P. falciparum* has been cloned as N-terminal fusions to gpVIII of bacteriophage fd and shown to induce strong immunogenic response in mice (Greenwood et al 1991; Willis et al 1993). T-cell proliferation of BALB/c mice injected with recombinant phage particles in the presence or absence of Freund’s adjuvant showed no difference in the immune response. The ability of phage displayed epitopes to mount specific immune response, recruit helper T-cells and the lack of need for external adjuvants suggest that it will also be an inexpensive and simple route to the production of effective vaccines. Several epitopes of the viral pathogens such as Hepatitis B (Folgori et al 1994), herpes simplex virus-2 (Grabowska et al 2000) and human respiratory syncytial virus (RSV) (Bastein et al 1997) have been displayed on the surface of the phage and shown to induce protective immunity in animal models.

1.2.12.2 T7 bacteriophage display system

The properties of T7 bacteriophage have been used in the development of a novel phage display system. This system has the capacity to
display peptides up to about 50 amino acids in size in high copy number (415 per phage) and peptides or proteins up to 1200 amino acids in low copy number (0.1-1 per phage). The assembly of the phage takes place in the cytoplasm and mature phages are released by lysis of the cell. T7 bacteriophage has additional properties that it replicates more rapidly (2-3 hours) than either bacteriophage lambda (λ) or filamentous phage. This feature makes this system extremely robust for selection and screening of the desired gene products. Further, T7 phage withstands harsh conditions that inactivate other phages and remains stable.

T7 phage display system has been increasingly used for several studies. The icosahedral T7 phage displaying random peptides (Novagen, Madison, USA) was used as a model for drug and gene delivery vehicles in animals (Sokoloff et al 2000). Protein of carbohydrate binding property has been identified using T7 phage display cloning system (Yamamoto et al 1999). The cDNA of the HeLa cell transformant that stably expressed large amounts of blood group A antigens were cloned in this phage display system and biopanned using blood group H-specific glycoproteins led to the identification of galectin-3, a galactose/lactose-specific animal lectin of the galectin family. A receptor that is specific for phosphatidylserine on apoptotic cells has been cloned using this novel system (Fadok et al 2000). This gene transferred in B and T lymphocytes recognized and engulfed apoptotic cells in a phosphatidyl specific manner. Identification of natural product’s receptor provides an important link between its phenotype and cellular components. Screening of the T7 cDNA human brain library with biotinylated FK506 as a natural product affinity probe identified FKBP12 gene product of immunophilins (Sche et al 1999). T7 display system is also efficiently used for epitope mapping of the antigens. Monoclonal antibodies produced against polyomavirus large
T-antigens was used to characterize the epitopes of a heptapeptide library displayed by bacteriophage T7 (Houshmand et al 1999). Based on these various merits, this system was used to clone the cDNA of the infective stage of *B. malayi* to identify putative immunodominant antigens, which induce antibody response in endemic normals.