CHAPTER 1

INTRODUCTION

Human lymphatic filariasis is a mosquito-borne disease of the tropics caused by infection with the nematode parasite *Wuchereria bancrofti* and *Brugia malayi*. These lymph-dwelling nematodes of humans infect 128 million people globally: with *W. bancrofti* afflicting a total of 115 million and *B. malayi* infects 13 million individuals in 73 endemic countries (Ottesen and Ramachandran 1995). In 1995, the WHO identified filariasis as the world’s second leading cause of permanent and long-term disability among other infectious diseases. The problem is greatest in India and Africa affecting 45 and 40 million people respectively (Michael et al., 1996). It is estimated that at least one billion people are exposed to the risk of infection and the disease has already disabled 44 million. Like other parasites, the lymphatic filarial nematodes produce chronic infections inducing a broad spectrum of clinical manifestation. The infected individuals can be classified as (i) asymptomatic amicrofilaraemics (Endemic normals, EN) immunologically responsive individuals with no evidence for parasite, (ii) asymptomatic microfilaraemics (MF), who harbor circulating microfilariae (mf) but without any clinical signs of infection and (iii) symptomatic amicrofilaraemics individuals with chronic pathology (CP) who develop hydrocoele and chyluria, with chronic lymphatic obstruction and damage that eventually leads to an irreversible state of elephantiasis. A rare group of individuals exhibit acute hypersensitivity reactions following infection, produce elevated levels of both total and parasite specific IgE with clinical manifestations of Tropical Pulmonary Eosinophilia (TPE).

The other filariae that infect humans include *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans*, *M. ozzardi* and *M. streptocerca*. Adult worms of *O. volvulus* reside in the subcutaneous nodules and the presence of microfilariae (mf) in the skin and eye leads to severe lesions and visual impairment. *L. loa* provokes
temporary inflammatory swellings (calabar swellings), hypereosinophilia and allergic manifestations (WHO 1992).

In India bancroftian filariasis is prevalent in Andhra pradesh, Tamil Nadu, Pondicherry, Goa, Karnataka, Orissa, Madhya pradesh, Gujarat, Uttar pradesh, Bihar, Assam and West Bengal. Brugian filariasis in India is mostly found in Kerala, but scattered foci of low prevalence are reported in Orissa, Assam, Madhya pradesh, Andhra pradesh and Tamil Nadu. Diurnal subperiodic form of W. bancrofti is prevalent in Andaman and Nicobar Islands. Culex quinquefasciatus and Mansonia sp. mosquitoes are the vectors for W. bancrofti and B. malayi respectively the major causative agent. The overlap of W. bancrofti and B. malayi infection in certain areas of India like Kerala necessitated us to investigate and develop a diagnostic method for filariasis.

The morbidity, clinical manifestation and the treatment of lymphatic filariasis are given below.

1.1 MORBIDITY

Morbidity ranges from the acute inflammatory reactions, which may include adenolymphangitis (ADL) associated with fever and malaise, to chronic lymphatic disease, which includes hydrocoele in males and lymphoedema and elephantiasis in both sexes. Genital manifestations are common features in bancroftian filariasis but only rarely observed in brugian infections. Chyluria, renal disease and tropical pulmonary eosinophilia (TPE) are the other notable disease conditions caused by filariasis, particularly by bancroftian filariasis. Recent clinical studies suggest that asymptomatic but infected individuals (MF) may also suffer from profound subclinical lymphatic and renal pathology (Dreyer et al., 1992).
1.2 CLINICAL MANIFESTATIONS

1.2.1 Asymptomatic amicrofilaraemia: (Endemic ‘Normals’ EN)

In most endemic areas, a proportion of the population is mf negative and is devoid of symptoms of the disease despite life long exposure to infection. These individuals were negative for microfilaria and circulating antigens (Ramzy et al., 1991). More importantly the immune responsiveness to parasite antigens is significantly greater to that seen in individuals with microfilariae or lymphatic disease (Ottesen et al., 1977; Ottesen et al., 1984).

1.2.2 Asymptomatic microfilaraemia (MF)

Individuals with this form of filariasis are clinically asymptomatic and are unaware of the fact that they may have very large number of circulating microfilariae (mf). The presence of circulating antigens, in vitro filarial antigen specific lymphocyte hyporesponsiveness and decreased production of IL-2 and IFN-γ in response to parasitic antigen are the hallmarks of MF individuals. But the in vitro lymphocyte response of these individuals to non-parasitic antigen and mitogen are normal as with the other clinical groups (Nutman et al., 1987a). Recently it has been shown that such patients commonly have renal abnormalities, (haematuria and or proteinuria) which are clinically silent as is the microfilaraemia (Dreyer et al., 1992). Though these individuals develop occasional filarial fever, they ignore them assuming that it might be due to other routine bacterial or viral infection.

1.2.3 Symptomatic amicrofilaraemia (Chronic pathology CP)

In endemic areas, a smaller number of people develop chronic lymphatic disease, with damaged lymphatics and its dysfunction leading to lymphoedema. This state is initially transient and reversible but later becomes permanent resulting in irreversible state of elephantiasis. Other clinical manifestations of chronic filariasis include hydrocele and chyluria. Microfilaraemia is often absent in most
of these patients and are mostly negative for circulating filarial antigens. However in India, mf is observed in some individuals probably due to reinfection when living in an endemic area. Further these individuals have considerably more vigorous antigen specific T cell responses than do the asymptomatic carriers (MF) (Ottesen et al., 1977).

1.2.4 Tropical pulmonary eosinophilia (TPE)

Acute tropical pulmonary eosinophilia (TPE) is characterized by wheezing, pulmonary infiltrates, marked peripheral blood eosinophilia, and very high serum levels of filaria-specific antibodies (Nutman et al., 1989). It is an interstitial lung disease that results from hyper immune response to filarial antigen, one of the rare clinical manifestations of filariasis. These individuals have elevated levels of eosinophils that are highly activated. They demonstrate high level of total serum IgE and filarial antigen specific IgG and IgE antibodies. Although most individuals with acute TPE have a rapid clinical response to the standard course of diethylcarbamazine (DEC) with reduced cough and dyspnea, in some individuals the pulmonary disease progress to a chronic form that results in interstitial fibrosis and permanent loss of lung function (Ottesen and Nutman 1992). Recently a major IgE-inducing antigen Bm2325, of the filarial parasite B. malayi has been implicated in the pathology of TPE. This antigen is homologous to the entire precursor of the gamma-glutamyl transpeptidase (gamma-GT), a key enzyme in the synthesis and degradation of glutathione (Lobas et al., 1996).

1.3 OVERVIEW OF THE THESIS

An unequivocal diagnosis and monitoring efforts relied on microscopic detection of microfilariae in blood specimens where sampling had to be done at night time (because of the nocturnal periodicity of the parasite in the blood). Tests such as skin tests, complement fixation, haemagglutination inhibition and various other immunoassay have been employed to try to demonstrate the presence of anti-
filarial antibodies as an indicator of the filarial infection (Ambroise-Thomas 1974). Antibody-based assays using crude antigens or other antigens from animal filarial parasites were developed. But most of the assays share the major drawback of not being able to discriminate between past exposure and current infection and the magnitude of the response bears no relation to the parasite burden. Furthermore the usefulness of these antibody assays are limited by the problem of extensive cross-reactivity among the antigens of other helminthic parasites. Hence assays developed using homologous (\textit{W. bancrofti} and \textit{B. malayi}) antigens were preferred to heterologous antigens for developing specific immunodiagnostic techniques. The recent development in recombinant DNA technology provides hope for developing filarial specific protein for diagnosis.

By employing recombinant DNA technology gene libraries of parasite DNA have been constructed. The libraries were screened either with \textit{B. malayi} or \textit{W. bancrofti} infected patient’s sera or specific DNA probes to pick up antigen which are of diagnostic importance or which can be used for immunoprophylactic studies. Dissanayake et al., (1992) have used MF sera and screened the cDNA library of \textit{B. malayi} adult worms and has picked up an antigen (SXP-1) which is used in diagnosing individuals with patent infection. Ramzy et al., (1995) have used a recombinant clone BmM14 fused to glutathione-S-transferase (GST) to measure the IgG4 levels in the different clinical groups and have shown that the recombinant IgG4 levels do not correlate with the mf counts or antigen levels measured in bancroftian filariasis. Many attempts have been made to establish diagnostic kits using recombinant filarial antigens to detect specific antibodies (Dissanayake et al., 1992, Theodore et al., 1993, Chandrasekhar et al., 1994).

The better alternative to antibody detection is the antigen detection assay, which unequivocally demonstrate the presence of parasites in the host. Dissanayake et al., (1984) have exploited the antigenic cross reactivity of a monoclonal antibody directed against the eggs of a cattle filariad \textit{Onchocerca gibsoni} to detect \textit{W. bancrofti} antigen in sera from infected humans. An antigen
assay for early detection of filariasis has been developed by More and Copeman (1990) that is sensitive and simpler to perform than the previous cumbersome microscopic techniques. Recently using a monoclonal antibody (mAb) AD12.1 raised against a 200kDa protein, an antigen assay has been developed where they have shown that antigen positive EN had the same parasite antigens as the MF (Weil et al., 1996). Thus filarial antigenemia in endemic normals is a marker of prepatent infection and hence can be used to detect individuals in the early stage of infection.

These development in the field of diagnosis has helped us to use mAb-based antigen ELISA as a tool for early identification of the disease and further in studying the efficacy of control programs. McCarthy et al., (1995) have used a mAb based assay (Og4C3) to study the changes that take place following chemotherapy which measures the adult worm burden in the individuals.

Currently the mAb based Og4C3 assay and the ICT card test have been used widely for the early diagnosis of bancroftian filariasis. Though Og4C3 assay is most sensitive in detecting CFA levels from bancroftian filariasis, but it cannot be used for detection of active filarial infection from brugian filariasis. On the other hand the ICT card test is only a qualitative test and is found to be specific for bancroftian filariasis (Weil et al., 1997). Using these kits it is difficult to identify individuals who are having brugian filariasis especially in countries where dual infection is prevalent.

To overcome this problem, the present study was carried out where a recombinant antigen pRBSXP has been purified. An antigen /antibody based ELISA was developed for the detection of active filarial infection caused either by B. malayi or W. bancrofti. Further its utility to study the change in the IgG4 levels following DEC treatment has been analyzed.
1.3.1. Objectives of the study

a) To overexpress the recombinant filarial protein from pRBSXP clone, identified from the adult *Brugia malayi* cDNA library. The protein was purified using Immobilized metal affinity chromatography (IMAC).

b) To evaluate and compare the specificity of the commercially available monoclonal antibody based sandwich ELISA (Og4C3) with the IgG4 antibody based ELISA using recombinant filarial antigen (pRBSXP) for the diagnosis of active filarial infection and their use in chemotherapeutic follow-up studies using either sera or whole blood.

c) To develop an antigen based ELISA using monospecific antibodies raised against purified recombinant filarial antigen (pRBSXP) for diagnosis of lymphatic filariasis caused by *W. bancrofti* and *B. malayi*.

In the first part of the study the circulating filarial antigen (CFA) in the sera of different clinical groups of bancroftian filariasis was quantitated using a mAb based Og4C3 sandwich ELISA. All the MF individuals were antigen positive by ELISA whereas the CP and NEN individuals were negative. However two of the EN individuals showed the presence of CFA. These results indicate that this assay mainly used for active filarial infection from MF and also to identify the individual having prepatent or harboring juvenile filarial parasite in lymph node. Further a weak correlation was observed between the mf count and the antigen levels in the MF patients ($r = 0.835$). This indicates that mAb recognizes antigens released by the adult worm. This results reaffirms the earlier finding that neither the mf nor their products released by them following treatment have an affect on the CFA levels detected by Og4C3 mAb assay. Similar result has been observed by (Chanteau et al., 1994) who have shown that Og4C3 antigen level indirectly reflects the adult worm burden.
There was almost no change in the antigen levels in the day and night blood samples and hence this test could be performed using day blood samples \( (r = 0.732, p<0.05) \). To make this assay more applicable for field trials and participation of large numbers of individuals in the community based studies, blood samples were collected by venipuncture and on filter strips by finger prick from the same individuals and the CFA level was quantitated. There was a positive correlation between the CFA levels in the serum and the filter strip eluate of the same patient \( (r = 0.835, p<0.05) \) suggesting that the test can be carried out using either of them.

Development of a recombinant protein based IgG4 ELISA in the diagnosis of Bancroftian and Brugian filariasis. The filarial-specific recombinant clone (pRBSXP) was characterized in our laboratory (Rao 1998) and the cDNA of the clone was 489bp in length and coded for a protein that is about 23kDa in size. The protein was purified by IMAC and used for development of an antigen / antibody ELISA. The recombinant protein based IgG4 ELISA has been standardized to suit field survey conditions. Blood samples were collected onto filter paper by finger prick during daytime and the strips were stored at room temperature. It was then used to perform pRBSXP specific IgG4 assays and Og4C3 antigen assays. Recombinant pRBSXP specific IgG4 antibodies were detected in the filter paper elutes and the results were comparable to that of the Og4C3 ELISA. Hence these assays can be used for screening mass population with daytime samples and can replace the existing night blood survey.

Application of immunoassays for chemotherapeutic follow up studies in bancroftian MF individuals treated with DEC. Immunomonitoring of MF patients after DEC therapy is essential in an endemic area for arresting the transmission and prevention of pathology associated with clinical manifestations. In recent times ultrasonography has been used directly to assess the in vivo effect of antifilarial drugs on the adult worms (Dreyer et al., 1995). However this method is expensive and thus as an alternative to this technique we have used a monoclonal antibody based ELISA (Og4C3) and the recombinant antigen
(pRBSXP) based antibody ELISA in studying the changes that take place in MF following chemotherapy. Two groups of MF individuals living in different endemic areas in Chennai, India were studied. The first group was given the standard dose for 12 days (6mg/kg-body wt) and the second was given a single annual dose of DEC (6mg/kg-body wt). The results showed that there is a drop in the CFA levels on the 14th day and it was statistically significant, which is followed by a significant increase at one month and the levels subsequently remained high (p<0.05) when compared with the pretreatment levels in both the groups. The change in the antigen levels in short duration maybe because the DEC has some affect on the adult worm like immobilization and thus prevents the release of either secretory or excretory antigen that is recognized by the mAb of the assay. At the same time the pRBSXP specific IgG4 levels were measured in both the groups. A remarkable increase in IgG4 antibody levels after treatment with DEC was observed. The significant increase was noticed from the 14th day onwards and it remained high up to six months (p<0.05). Hence the recombinant antigen specific IgG4 antibody response might indirectly reflects the state of active ongoing infection in the MF individuals.

Thus Og4C3 antigen or recombinant antigen (pRBSXP) based antibody assay is more advantageous over the conventional methods, like routine night blood smear or membrane filtration in studying the efficacy of different dosage of DEC. Further this study has proved that the single annual dose is as effective as the standard dose of 12 days treatment and may be a suitable regimen for mass chemotherapy programs for the control of filariasis.

Development of sandwich ELISA (antigen detection) using antibodies raised to recombinant filarial antigen (pRBSXP). The Og4C3 antigen assay is able to detect only individuals with *W. bancrofti* thus, an attempt is made to develop an antigen detection system using antibodies raised to recombinant antigen to detect individuals with active infection either due to brugian or bancroftian filariasis.
The recombinant protein (pRBSXP) was overexpressed and purified using Immobilized metal affinity column (IMAC). Antibodies were raised against the purified recombinant protein in mice and rabbits. Using the monospecific antibodies, an pRBSXP antigen capture ELISA was developed. The pRBSXP antigen levels were measured in the sera of different clinical groups of bancroftian filariasis. Asymptomatic microfilaraemic group (MF) from bancroftian and brugian filariasis individuals exhibited the highest percentage of positive reactivity of 30/34 (88.23%) and 25/30 (83.33%) respectively.

In conclusion it was possible to use Og4C3 ELISA in field conditions using daytime blood samples collected filter strips and thus the survey can be carried out even during daytime. In addition antibody/antigen ELISA have been developed using recombinant antigen pRBSXP to diagnose individuals with active infection caused by bancroftian or brugian filariasis. Moreover the production of the recombinant protein is cost effective and can be obtained in large amount in the laboratory. Further the assay developed in this study will be of immense help in diagnosing filarial infection in endemic areas, monitoring filariasis control programs and to study the immunological changes that take place in response to different drugs or in combination following treatment.

1.4 REVIEW OF LITERATURE

1.4.1 Life Cycle of Filarial Parasite

The life cycle of filarial parasite involves two hosts namely the human, which is definite or the permanent host while the mosquito is an intermediate host. In humans the adult worms live in the lymphatic system for up to 17 years, where they viviparously produce blood-dwelling microfilariae (mf) (Leeuwin et al., 1962). It is long and slender with blunt ends. Male worms vary from 20 to 40mm in length in *W. bancrofti* and *B. malayi* while the female worms are 60 and 100mm in length respectively. The microfilariae are ingested by mosquitoes, penetrate the stomach wall, grow and metamorphose through stages L2 and L3 within the
thoracic flight muscles and get transformed into sausage-shaped bodies of 240 to 250micron in length. After approximately 10 days, the L3 migrate to the salivary glands, proboscis and associated structures from which they are injected into the next host. In humans, the L3 migrates through the deep tissues, molting to the L4 and then an adult. The adult then penetrates the lymphatics (Fig 1.1).

1.4.2 Vectors

There are many mosquito species, which act as vectors for the three different types of filarial nematodes. Specifically, the most important vectors of *W. bancrofti* are *Culex quinquefasciatus*, *Anopheles gambiae*, *An. funestus*, *Aedes polynesiensis*, *Ae. Scapularis* and *Ae. pseudoscutellaris*. *B. malayi* is vectored by *An. barbirostris*, *An. sinensis*, *An. donaldi* and several species of *Aedes* and *Mansonina*. *B. timori* however is transmitted by *An. barbirostris*.

1.4.3 Pathogenesis and clinical disease

The asymptomatic form (MF) is most often characterized by the presence of thousands or millions of larval parasites (mf) in the blood and the adult worms are located in the lymphatic nodules. The presence of adult worms in the lymphatics causes lymph node and duct dilation leading to lymph retention in the area drained by the affected nodes and vessels. Studies using lymphoscintigraphy have shown the diffuse structural lymphatic with lymph vessel dilation in MF individuals (Freedman et al., 1994). This structural damage to the lymph vessel walls maybe caused by the whiplike action of the motile adult worm (Case et al., 1992) or due to the effect of parasite secretory product on lymphatic endothelial cell integrity (Kaiser et al., 1990). Moreover the studies done by Dreyer et al (1992) have demonstrated that the asymptomatic microfilaraemic individuals had haematuria and/or proteinuria even before treatment with DEC. The specific mechanisms underlying these renal abnormalities maybe either mechanical damage to the glomeruli by microfilariae themselves or immunological damage by immune complexes, which have been deposited in renal glomeruli. The immune response is
Infected person

Microfilariae develop into infective larvae in 8-12 days

Microfilariae are released into lymphatic system and enter blood stream

Infective larvae enter human through bite wound

Mosquito ingests microfilariae during blood-meal

Adult worms mature in about 9 months and mate in lymph vessels and nodes

Adult worms cause obstruction of lymphatic system, leading to swelling and sometimes elephantiasis

Fig 1.1 Life cycle of filarial parasite
specifically downregulated in individuals with active infection (Maizels et al., 1991). The end of immunological anergy coincides with the disappearance of microfilariae and a rapid increase in the lymph duct hyperplasia, edema and fibrosis.

Adenolymphangitis (ADL) is one of the important clinical manifestations of lymphatic filariasis. The cause of ADL reaction has been attributed to the results of a reaction to parasite products released either by the adult worm or the mf (Ottesen 1984) or it may due to the secondary infections, which continuously invade the damaged lymphatic system (Jamal and Pani 1990). This generates repeated trauma resulting in acute episodes of local inflammation involving skin, lymph nodes and lymphatic vessels and subsequent lymphatic damage. Lymphatic damage which includes lymphadenitis, lymphedema, chyluria hydrocele in progressive and is linked to the host immune response. This leads to elephantiasis, i.e., solid non-pitting edema (permanent swelling) fibrosis and hyperplasia (excess growth) of the affected organs: like foot, leg, vulva, male genital and rarely the female breast. Certain superficial bacterial infections and mycotic skin infections especially of the foot giving fissuring, lichenification nodular, papillomatous and verrucous (wart-like) skin changes leading to gross disfigurement (Shenoy et al., 1995).

1.4.4 Clinical Management of Elephantiasis

The gross morbidity in elephantiasis can be prevented especially during the early stage of the disease by proper foot care and hygiene. Careful cleansing with antibiotic soaps and topical creams can be extremely helpful in healing the infected surface areas and in both slowing and reversing much of the overt damage that has occurred already.

1.5 Diagnosis

Wamae (1994) has reviewed the various methods of diagnosis. Based on this the various types of diagnosis has been discussed below.
15.1. Parasitological Diagnosis

Microscopy remains as a simple method for diagnosis of parasites and can be rapid. The most widely used method for the diagnosis of filarial infections is the examination of blood for microfilariae by the conventional night blood smear. The microfilariae typically exhibit nocturnal periodicity (i.e., they remain sequestered in the capillary beds of the deep organs during the day and appear in the peripheral circulation at night, coinciding with the biting habits of the vector mosquitoes). The other methods of parasitological diagnosis are Giemsa staining of night blood smears, membrane filtration, Knotts concentration and DEC provocative day test techniques have been reviewed by Denham et al., (1995). All these methods have certain practical problems. The membrane filtration is sensitive technique but requires large volume of blood to be examined. However community opposition to venipuncture make the technique impractical for large-scale field use in many parts of the world. The knott's concentration test requires the concentration of 1mL of blood by centrifugation, which can be used, as an alternative where membrane filters is not available (Knott 1939). But this method is also time consuming and requires the use of a centrifuge. However microfilarial tests are inconvenient for the survey teams because the nocturnal periodicity of microfilaria necessitates the night blood collection.

1.5.2. DNA based Diagnosis

Polymerase chain reaction (PCR) represents a powerful tool in studying parasitic infections since it is an efficient way of amplifying the target DNA by several thousand folds. Moreover they yield information on the current infection status and are independent of the host's immune competence. The DNA sequences are present in all developmental stages of the parasite and many allow the identification of a single filarial worm, either adult or microfilaria in the definitive host or a developing larva in the intermediate host. Moreover the nucleic acid probe based assays is always specific for the corresponding parasite DNA and hence species specific and helps to differentiate morphologically similar species. Species-specific DNA probes have been developed for B. malayi, W. bancrofti,
Onchocerca volvulus, and Loa loa (Nutman et al., 1994). Highly repeated sequence is generally used to make the most sensitive DNA probes in Onchocerca volvulus (Zimmerman et al., 1994). This is because the highly repeated DNA sequences are generally non-coding and evolve more rapidly than rest of the genome thus making them as potential targets for genus and species specific identification by DNA probes and PCR. Such DNA based diagnostic methods have been recently developed for detecting parasitic DNA in blood samples and also in vector population.

A 320 bp Hha I repeat sequence was identified from B. malayi genome (McReyonolds et al., 1986). Oligonucleotide probe derived from this repeat element was capable of specifically detecting 200 pg of B. malayi DNA (Williams et al., 1988). Another repeat element pBm15 was cloned from Sau3A digest of B. malayi (Sim et al., 1986a). When labeled with $^{32}\text{P}$, the probe pBm15 was able to detect single infective larva of B. malayi (Sim et al., 1986b). Alternate methods of detection based on chemiluminescence combined with specific amplification of the target molecule by PCR are the focus of study in the recent years (Nutman et al., 1994). A PCR assay has been developed using the Hha I repeat DNA sequence of B. malayi for detection of parasite in blood samples (Lizotte et al., 1994). Another PCR assay based on the 195 bp genus specific repeat sequence from W. bancrofti was shown to detect one L3 in pools of mosquitoes (Nicholas et al., 1996). It has been reported that this assay could detect as few as one mf per ml of human blood samples (Zhong et al., 1996). This assay was further modified into a microtitre plate based method for rapid evaluation of field samples (McCarthy et al., 1996). Recently PCR assay has been developed from a 969 bp repeat sequence pWb12 that detect W. bancrofti DNA in human blood samples, hydrocele fluid, and in mosquito vector (Siridewa et al., 1996). PCR amplification of myosin-like myofibrillar proteins of W bancrofti using specific primers detected DNA in a species-specific manner from as little as 16 pg of isolated DNA or from one microfilaria. Thus several of these DNA based assays are promising for the diagnosis of filarial infection and are currently in the research phase and its field
applicability in the endemic areas will require further validation. The DNA based techniques have their own disadvantage like requirement of personnel with technical capabilities and good laboratory infrastructure to carry out the assay. This is of more importance to prevent contamination of reagents with target DNA and hence avoid false positive cases.

1.5.3. Immunological Methods

The low sensitivity and the difficulty in performing the parasitological diagnosis procedure have called for alternative diagnostic methods for this disease. The use of sensitive membrane filtration technique (Nuclepore Pleasanton, CA) requires venipuncture, which decrease the patient’s acceptability. In addition individuals within a population may be infected with male or female adult worms that produce no microfilariae. Thus an ideal diagnostic method for human filariasis is needed to facilitate surveillance activities, to monitor control efforts and to evaluate new drugs and vaccines. Basically the diagnostic method should fulfill the below mentioned criterion

1. Identify individuals who are infected or have been exposed to infection
2. It should exhibit no diurnal fluctuation in sensitivity.

1.5.3.1 Antibody based assays

The classical methods were based on skin test or serological determination using complement fixation (CFT), diffusion in gel (GD), latex agglutination and indirect haemagglutination (IHAT) to assess antibodies generated by the host. Using *W. bancrofti* mf antigens Kaliraj *et al.* (1981a) have developed an IFAT, IHAT and ELISA for the diagnosis of filariasis and the efficiency of the tests were compared. They have found that ELISA technique was simple and sensitive technique over the other methods of detection. Thereafter significant progress was made in the measurement of antibodies in filariasis using filarial antigens that were obtained by rearing *B. malayi* and *B. timori* worms intraperitonially in jirds. Using these antigens the classical antibody assay was replaced with labelled reagent assays such as immunofluorescent antibody test (IFAT), enzyme linked
immunosorbent assay (ELISA) Kaliraj et al. (1981b) and immunoradiometric assays (IRMA) which have been developed and were highly sensitive in detecting very low levels of antibodies.

Studies done by Ottesen et al., (1985a) have shown that in filariasis the presence of isotype and subclass dominance is a prominent feature of the immune response. Thus the examination of subclass antibody levels by Hussian et al., (1987) have shown that the most significant differences were noticed in the levels of IgG4 in MF patients which was 17 times higher than those observed in CP. Lal and Ottesen (1988) developed an ELISA where IgG4 levels to the soluble B. malayi antigens were measured to enhance the specificity of the assay for serodiagnostic studies.

1.5.3.2 IgG4 antibody based assays

The major hallmark with filarial nematode parasites is the unusually high levels of filarial specific IgG4 (Ottesen et al., 1985a). Recent studies have shown in MF that high levels of IgG4 are associated with circulating filarial antigen (Dimock et al., 1996). Hence active infection appears to promote IgG4, limits the levels of IgE and avoids stimulation of IgG2 and IgG3. IgG4 based assays have been developed for the diagnosis of individual with active infection either using the crude antigens or recombinant antigens. Lal and Ottesen (1989) have shown that IgG4 antibody assay using phosphorylcholine (PC) epitopes are useful since, humans appear to be generally incapable of mounting IgG4 response to phosphorylcholine (PC) epitopes. Moreover amicrofilaraemic patients with clinical filariasis who go undetectable by routine parasitological methods are detected by the assay. Recently it has been shown that using soluble B. malayi antigen antifilarial IgG4-ELISA could detect 4.6 times more positive cases (microfilaremic) than the microfilaria detection done by night blood smear (Rahmah et al., 1994)
1.5.4. Heterologous or homologous antigens based antibody assays

Heterologous antigens: These broadly include soluble whole worm antigens or microfilarial (mf) antigen extracts, excretory secretory (ES) antigens and circulating immune complex antigens (CIC).

1.5.4.1 Excretory Secretory antigens (ES)

Nematode ES products have been advocated as potentially useful for the detection of antibody because they appear to be more species specific than crude somatic extracts (Kaushal et al., 1984). The release of macromolecules by parasites into their environment both in vitro and in vivo has been reported in human lymphatic filariasis (Kaushal et al., 1982). These excretory-secretory products (ES) have been studied with respect to function, vaccination potential, pathogenicity, and ability to serve as antigen targets for diagnostic tests. ES antigens are released by the living adult worm and thus may induce higher antibody titres than the somatic extracts of the worms. Due to the non-availability of sufficient parasite material from W. bancrofti, a heterologous ES antigens from B. malayi has been used in the diagnosis of bancroftian filariasis. Malhotra and Harinath (1984) have shown that the antibodies to L3ES antigen was more pronounced in TPE individuals possibly because these are the first antigens that the immune system is exposed. In contrast the MF individuals had high levels of antibodies to the mf ES antigen suggesting that once the microfilaremia state is established the immune response to mf ES becomes stronger.

Recent studies have been done where the cDNA library of B. malayi was screened with the antibodies raised to ES antigen and two important diagnostic recombinant protein (Bm12 and Bm14) have been identified (Kumari et al., 1994). These antigens were highly species specific and that it is helpful to detect individuals before the onset of patent infection. Monoclonal antibodies K3AE7 and K3BDS raised against excretory-secretory (ES) antigens of S. digitata were shown to be promising in the diagnosis of W. bancrofti infection (Dhas and Raj 1995). The Wb E34 monoclonal antibody raised against W. bancrofti microfilarial
excretory secretory (mf ES) antigen was reported to be useful in detecting the filarial antigen in *W. bancrofti* and *B. malayi* infected sera (Reddy et al., 1989). Further, filarial antigen detection system 'SEVA-FILACHEK' developed based on *B. malayi* mf ES antigen can detect occult filarial infections (Harinath et al., 1996)

1.5.4.2 Circulating Immune complex

The study of circulating immune complex (CIC) becomes important since they are capable of immunomodulating the immune responses (Barnett 1986). Circulating immune complex (CIC) have been found to occur in most of the parasitic diseases but their role in *in vivo* is not well understood. CIC are formed in circulation or tissues as a result of interaction between the exogenous or endogenous antigens and their corresponding antibodies. Such complexes are common in filariasis patient's sera although the antigens they contain are largely undefined (Au et al., 1981). Earlier studies have demonstrated the presence of immune complex antigens in filariasis patients using polyclonal antibodies raised against adult *Setaria digitata* worm (Dissanayake et al., 1982). They have shown that the polyclonal antibodies were reactive with the antigens derived from the CIC and were able to bind to the adult worms but not to the microfilaria. It was further demonstrated that, specific immune complexes (IC) were found to occur in 30-40% of the clinical filarial patients and only in 3-7% of the microfilaremic carriers. Studies done by Prasad and Harinath (1988) have shown certain fraction of the antigens from IC namely (IC-9) which was similar to the mf ES antigen in its antigenic determinant showed elevated IgG response in patients with chronic pathology (CP) with respect to MF. It can be therefore used to follow the MF carriers with elevated IgG response to IC-9 fraction to determine whether or not they will develop clinical manifestations. Lunde *et al.*, (1988) have shown the presence of IC in filariasis patients. It was shown that the IC from all clinical group of filariasis contained a 200kDa glycoprotein when probed with rabbit anti *BmA* antibody. Currently monoclonal antibodies (mAb 2G01) ELISA has been developed to detect the CIC in filariasis patients. It has been found that this mAb reacts with three antigens of *W. bancrofti* mf sheath and it is stage specific
(Kobayashi et al. 1997) and suggesting that the mf play an important role in CIC formation in filariasis.

1.5.5. Homologous Antigens

These antigens can be obtained either by recombinant DNA technology or by fractionation of the crude worm extracts. These antigens are preferred over the heterogeneous antigens since they exhibit diminish cross reactivity among the different nematode species. Kaliraj et al. (1981c) had fractionated human filarial serum (FSI) by DEAE- Sephadex A-50 column chromatography and the antibody was used in detecting circulating antigen in filarial sera by counter immunoelectrophoresis (CIEP) and the indirect haemagglutination test (IHAT). They have shown that all the MF patients had circulating filarial antigen by CIEP but none of the other sera from those with helminths, showed the presence of CFA. Further the surface antigens of the bovine filarial parasite Setaria digitata were extracted by using EDTA and were purified by affinity chromatography using antibodies obtained from chronic human filarial sera. It was observed that, the purified antigen showed sensitive and specific reactions in ELISA for the detection of antibodies in filarial sera and showed least cross reactivity with other parasitic infections compared with the crude antigens (Theodore and Kaliraj 1990).

1.5.5.1 Recombinant antigens based assays

The new generation of immunodiagnostic tests will permit individual epitopes to be used as targets, although indications are that a ‘cocktail’ of such epitopes will be necessary to cover wide individual variations in antibody response among human populations. DNA technology has provided an avenue for purifying substantial quantities of specific parasite antigens, which is a prerequisite for any strategy aimed in identifying diagnostic antigens.

A Brugia malayi genomic DNA library was constructed by Arasu et al. (1987) in bacteriophage vector λgt11 and was screened for species-specific clones. Two recombinant clones derived from genomic expression library of B. malayi
were recognized by two distinct immunoglobulin classes. One clone containing part of the myosin tail region was recognized at IgG level whereas another collagen like clone was recognized at IgE levels (Werner et al., 1989). Differential immunoscreening of *B. malayi* cDNA library, (Dissanayake et al., 1992) a novel parasite antigen SXP was identified. It was observed that about 80% of the MF and 33% of CP contained IgG antibodies to recombinant SXP (Dissanayake et al., 1994). Chandrasekhar et al., (1994) identified and cloned an antigen BmM14 by screening a *B. malayi* cDNA library. The recombinant BmM14 specifically reacted with sera from filarial patients and encoded a 130 amino acid protein, which had an endoplasmic reticulum targeting sequence. The BmM14 antibody test was positive for >90% of sera from MF and 60% for CP (Ramzy et al., 1995). A recombinant clone pGT7 selected from genomic expression library of *W. bancrofti* mf in λgt11 (Raghavan et al., 1991) was shown to specifically recognize IgG4 isotype antibodies by MF (Theodore et al., 1993). Raghavan et al., (1992) have isolated a recombinant antigen, WbN1 from the genomic expression library of *W. bancrofti* and found the sequence similarities with myosin. Further they have demonstrated the presence of this antigen in the muscle of the adult filarial parasite and microfilariae.

Thus several recombinant antigen based assays have been developed and in view of the complexity of the disease, the more recombinant antigens developed, the greater are the chances, to arrive at an efficient, early, and stage specific diagnosis of this debilitating disease.

1.5.6. Antigen Assays

Since it is not possible to differentiate between current and past infection based on antibody assays, parasite antigens detection has been focussed on the patient’s blood and other body fluids (Dissanayake et al., 1982; Reddy et al., 1984; Hamilton et al., 1984). Several homologous and heterologous antigen preparations have been used and monoclonal antibodies have been produced which appears potentially useful for filarial antigen detection.
Several investigators demonstrated circulating antigens in filariasis using monoclonal antibodies. Earlier workers like Au et al., (1981) have demonstrated the present of circulating worm antigens in 93% of sera from humans with malayan or bancroftian filariasis by a double antibody sandwich enzyme-linked immunosorbent assay, using rabbit antisera to B. pahangi adult worms. Paranjape et al., (1986) have demonstrated the presence of circulating antigen in patients with bancroftian filariasis by immunoradiometric assay (IRMA) using rabbit polyclonal antisera labelled with 1-125. Forsyth et al., (1985) have used mAb Gib13 raised against Onchocerca gibsoni egg and mf antigens in an IRMA assay to detect circulating antigen in the sera of bancroftian filariasis. Another mAb, E34 raised against W. bancrofti mf ES antigens was able to detect filarial antigen associated with active infection (Reddy et al., 1986). A mAb raised against a major 200 kDa circulating antigen was directed against phosphocholine (PC) epitopes of W. bancrofti. Though this PC determinant itself is not "filarial specific" its abundance in PC bearing filarial antigen in circulation makes it a potentially useful target for immuno-diagnosis (Lal et al., 1987). Zheng et al., (1987) had utilized a polyclonal rabbit antifilarial antiserum as capture antibody, and a monoclonal antibody to identify, circulating parasite antigen in bancroftian and brugian filariasis patients. It was observed that 95% of the MF sera were having circulating antigens whereas only 60% of the MF with hydrocele or elephantiasis and 15% of the EN had circulating antigens. Using antibodies raised against W. bancrofti microfilarial SDS soluble antigen filarial antigen dipstick ELISA was developed and was positive in MF patients (Cheirmaraj et al., 1992). Recently circulating filarial antigen (CFA) has been detected by using a mAb Og4C3 directed against antigen of O. gibsoni in a sandwich ELISA. This antigen was detected only in those patients infected with bancroftian filariasis but not B. malayi, B. timori, O. volvulus or Loa loa and is a marker of Wuchereria bancrofti adult worm infection (More and Copeman, 1990). Poly and monoclonal antibodies derived from pGT7 were used in a sandwich ELISA for the detection of circulating parasite antigens (Theodore et al., 1996). Ramzy et al., 1991, have evaluated the
performance of antigen detection in the sera from an endemic area of *W. bancrofti* using mAb raised to *Dirofilaria immitis*. Yet another mAb raised against *W. bancrofti* L3 larvae recognized 93kDa antigen (Burkot *et al.*, 1996). This mAb did not react with nine other nematode species or two vector species and thus appears to be a promising immunodiagnostic agent. Recently a rapid form of filarial antigen card test was developed by ICT diagnostics, Balgowlah, New south Wales, Australia based on AD12.1 mAb (Weil *et al.*, 1987a; Weil *et al.*, 1997) and was found to be specific for bancroftian filariasis.

Several groups have used the levels of circulating filarial antigen levels (CFA) as an index to study the efficacy of the drug or its combination with other drugs (Day *et al.*, 1991; McCarthy *et al.*, 1995) in various control programs. Studies have reported that reduction of CFA level after repeated treatments showed the elimination of *W. bancrofti* infection, as monitored by CFA clearance, with annual treatments of DEC combined with ivermectin (Nicolas *et al.*, 1997).

### 1.6 TREATMENT

Diethylcarbamazine (DEC), the chemical name being 1-diethylcarbamyl-4-methyl piperazine is one is the best drug of choice available at present in treating human lymphatic filariasis caused by *W. bancrofti* and *B. malayi*. (Duke, 1980). DEC is used in the form of the dihydrogen citrate under the names Hetrazan, Banocide, and Notezine (Ottesen, 1985b). It is extremely stable and is not affected either by autoclaving and or by cooking (Gelband 1994). DEC causes the rapid clearance of microfilariae of *W. bancrofti* and *B. malayi* from the blood of humans. DEC activates muscle cholinergic receptors in the worm causing depolarisation and muscle paralysis possibly due to the hyperpolarizing effect of the piperazine moiety and causes the dislocation of the parasite from the normal habitats in the host (Lahgahm and Kramer, 1980). It has also been reported that the drug produces alterations in the microfilarial surface membranes, thereby rendering them more susceptible to destruction by host defense mechanisms (Hawking, 1979; Mackenzie and Kron, 1985). Recent works by Mukhopadhyay
and Ravindran (1997) have shown that DEC is known to mediate *in vivo* microfilaricidal activity in conjunction with the host immune system. There is presumptive evidence that DEC kills adult worms of *W. bancrofti* and *B. malayi* but the mechanism of adulticidal action is not known (Hawking, 1979). Because of the apparent macrofilaricidal effect it has been suggested that therapy with this agent may interrupt the progression of lymphatic disease (Ottesen 1985a). In fact Dreyer et al., (1995) have demonstrated, the adulticidal activity of DEC by ultrasonography.

It has been reported by Ottesen (1985a) that the adverse reactions following DEC treatment are directly proportional to the microfilarial density. But these adverse effects are not long lasting in the symptomatic and asymptomatic individuals. It usually consists of fever, oedema, and intense itching, swelling, tenderness of the lymph nodes and headache. Zheng et al (1991a) have shown that most of the toxic reactions which follow DEC treatment are related to the release of inflammatory mediators by host cells or manifestations resulting from the release of products liberated by the dead or dying worms. The drug may also effect specific immune and inflammatory responses in the host by as yet undefined mechanisms. Current studies have shown that DEC-mediated salt induces cessation or long term suppression of mf in most people and the side effects of this type of treatment are minimal.

Ivermectin has a broad-spectrum of antiparasitic activity and has been used for several years in veterinary medicine as an intestinal antihelminthic drug. It binds to glutamate-gated chloride channels in nematodes, interrupting neuromuscular activity. Unlike DEC, which requires the participation of the host factors to exert a microfilaricidal effect this drug kills the microfilariae directly. Extensive clinical trials have demonstrated its utility in treating Onchocerciasis and its efficacy and safety has been reviewed by Chodakewitz (1995). After its success in Onchocerciasis it has been tried in other filarial diseases of humans including *Wuchereria bancrofti* and *Brugia malayi*. It has been shown to be effective at a single dose of 400 μg per kg
of body weight (Moulia-pelat et al., 1995). In recent times studies have proved that Ivermectin and DEC combination has an accumulative effect on clearance of microfilariae without increasing the intensity of adverse reactions (Moulia-pelat et al., 1994). Shenoy et al., (1992) have shown that single dose of 20-200μg/kg body wt of ivermectin cleared mf in B. malayi infected patients but the clearance was not complete. But this treatment produced minimal side effects.

Albendazole is the drug of choice for most enteric nematodes (Enterbius, trichuris, Ascaris and hookworm). This drug disrupts microtubule formation affecting the functioning of the helminth's gut cells and the ability of the parasite to obtain nutrients. Thus currently albendazole is being tried to investigate its macrofilaricidal activity in combination with Ivermectin. Klion et al., (1993) have assessed the filaricidal activity and clinical safety of albendazole, in human loiasis, in an endemic area in Benin, Africa. It was observed that the microfilarial levels began to fall by day 14 after treatment and by 6 months had fallen to 20% of pretreatment levels. Anti-filarial IgG and IgG4 and blood eosinophil levels also fell significantly in response to albendazole. Most interestingly there were no clinical adverse effects and hence this study suggests that albendazole has a primary (possibly embryotoxic) effect on the adult parasite, resulting in a slow decrease in microfilaremia. Studies have been done with the adult and developing stages of Molinema dessetae in the rodent Proechimys oris (Duarte et al., 1994). It was observed that drug in combination with ivermectin or albendazole alone substantially reduced the number of adult and preadult worms, thus indicating that albendazole-Ivermectin combination at a low dose has prophylactic effect and suggests a possible macrofilaricidal activity. The mode of action of these drugs and their immunological response has not been studied systematically.

1.6.1. Dosage

Ottesen and Ramachandran (1995) have reported that a single dose of DEC (6 mg/kg) is essentially equivalent to a full 12 days of treatment with the drug.
Further Kimura et al., (1996) have shown that a single annual repeated dose of DEC to be effective in reducing the mf prevalence and density in large scale longterm field trials.

The recommended regimes for mass treatment would be one of the following:

(i) DEC-fortified salt (0.2-0.4% w/w) for a period of 9-12 months.
(ii) Single annual or semi-annual mass administration of DEC (6 mg/kg body weight).
(iii) Ivermectin (400 µg/kg) once yearly.
(iv) Ivermectin (400 µg/kg) plus DEC (6 mg/kg) given once yearly.

1.7 HUMORAL IMMUNE RESPONSE IN HELMINTHS

The prime candidates for effective immune mechanisms against helminths are the humoral and cellular components of the immune system. IgG is the predominant immunoglobulin, present in serum. IgG subclasses 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. Except IgG4, all the other subclasses can fix complement. IgGl, IgG2 and IgG4 subclasses can bind to staphylococcal protein A, but IgG3 does not bind. IgG1 is the major isotype antibody among the IgG subclasses. In human, IgG antibody responses to polysaccharides are mainly of IgG2 subclass, whereas protein antigens usually induce more prominent IgG1 and IgG3 antibody response (Janeway and Travers, 1994). In Onchocerciasis IgG3 has been associated with “Sowda”, a chronic hyper-reactive oncho-dermatitis (Cabera et al., 1985). It seems likely that antibody and humoral mechanisms initiated by these isotypes may contribute to the pathology, through antibody dependent cell mediated cytotoxicity (ADCC) or immune complex formation. For example IgG3 is a major mediator of Type III hypersensitivity, and is the isotype for which the human Fc receptors on monocytes, macrophages and granulocytes have the highest affinity.
It has been well documented that IgG4 is a monovalent antibody (Vanderr zee et al., 1986) and is not capable of fixing complement by the classical pathway and hence it may lower complement mediated parasite clearance in MF (Ottesen et al., 1985b). IgG4 antibodies are described as ‘blocking antibody’ since it competitively inhibits the reaction between the IgE antibody and the allergens and hence prevent the IgE mediated allergic reactions and by inhibiting the release of vasoactive amines. Hussian and Ottesen (1986) have shown that IgG4 and IgE antibodies recognized same antigens when crude *Brugia malayi* adult worms (*BmA*) antigens were immunoblotted and probed with MF and TPE sera. So it can be explained that mechanism underlying the blocking activity appears to be a competitive inhibition i.e., any antibody with specificity similar to that of IgE could effectively block the immediate hypersensitivity (IH) reactivity triggered by allergen. But in patients with chronic antigenic (allergenic) stimulation as in filariasis, this antibody is most likely of the IgG4 subclass since its regulation is under control similar or identical to those of the IgE antibody response (Hussian et al., 1992). Thus the blocking antibodies (IgG4) may perhaps prevent the unwanted allergic reactivity caused by the helminthic parasites and whether it helps the parasite from elimination or the host from the immunopathology remains to be determined. Simonsen et al (1996) have studied the differences in antibody patterns in microfilaremic and amicrofilaremic adults. A decrease in IgG1 and a increase in IgG4 in microfilaremic individuals was observed when compared with amicrofilaremic indicating that the IgG1 levels were more related to mf status than to infection status, whereas the IgG4 levels were more related to infection status than to mf status.

In MF the ratio of IgG4 : IgE is high as compared to that in CP, suggesting either that IgE is a protective antibody, and/or that high IgE is involved in the pathogenic pathway. IgE occurs only in mammals and is one of five classes of antibodies recognized in man and released during allergic reaction. The role of IgE in defence against parasites was first established by work on the cell mediated killing of schistosomes *in vitro*, as well as by epidemiological studies in areas
endemic to schistosomiasis and other parasitic diseases (Capron and Dessaint 1985). IgE-secreting B cells are abundant in the skin, lungs and gut, which are the main sites of parasitic invasion. IgE elicits a range of cellular responses to parasite antigens resulting in anatomical and physiological changes, like inflammation, itching, coughing, lacrimation, bronchoconstriction, mucus secretion, vomiting, and diarrhoea, that are common symptoms of allergic disorders (Shutton and Gould, 1993).

1.7.1. Cellular Immune response in Filariasis
1.7.1.1 Th1 and Th2- type cytokines

Upon antigenic stimulation, CD4+ T helper (Th) cells differentiate into two distinct sub populations, the Th1 or Th2 cells, each producing unique cytokines and mediating separate effector functions (Mossmann and Coffman 1989). Th1 cells produce interleukin-2 (IL-2), tumour 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, eosinophils, and IgG1 and IgE antibodies and are involved in promoting humoral immunity, possibly suppressing the cellular immune response. Each sub population regulates the other through their different cytokine profile.

1.7.1.2 Parasites and Th1/Th2 cytokine

The major immunologic features of helminth infection are eosinophilia, elevated serum IgE levels, and in the case of certain parasites, mastocytosis. Importantly, each of these responses is stimulated by cytokines or cytokine combinations characteristic of Th2 cells (Modlin and Nutman 1993) like, IgE by IL-4, eosinophilia by IL-5 and mastocytosis by IL-3, IL-4 and IL-10. The microfilarial stage of the parasite plays a central role in the induction and maintenance of hyporesponsive state of the host (Maizels et al., 1993). Recently, human T lymphocyte clones or populations with a characteristic Th2 cytokine production profile can be derived from parasitized hosts by stimulation with worm
antigens or mitogen. This evidences increasingly supports a protective function for Th2 responses in certain helminth infections. Mahanty and Nutman (1995) have studied the parasite specific hyporesponsiveness seen in MF patients and have observed that these patients have greater quantities of IL-10 secretion spontaneously and in response to parasite antigens. Further Matanty et al., (1996) have studied the parasite stage-specific T cell responses in patients with lymphatic filariasis manifesting as elephantiasis and asymptomatic microfilaremia in India, using antigens derived from the microfilarial, adult male only, and mixed adult male and female worms. They have reported that the MF individuals had impaired proliferative responses compared to individuals with CP to microfilarial and mixed (male-female adult worm) antigens whereas the proliferative response was same with the adult male-derived antigens in both groups. Moreover the antigen-driven cytokine secretion by peripheral blood mononuclear cells revealed significantly lower IL-2 and IFN-gamma production by MF individuals in response to microfilarial and mixed antigens (but not to adult male antigen). Thus emphasizing that the MF individuals exhibit preferentially impaired Th1-type responses to microfilarial antigens and that microfilarial-induced IL-10 may be critical in the downregulation of specific Th1 responses.

This is supported by the studies done by various group (Urban et al., 1991, Urban et al., 1992) that IL-10 is involved in the downregulation of antigen responsiveness in parasitic infections. Ravichandran et al., (1997) have demonstrated, in human lymphatic filariasis that Th2 cytokines and relatively low lymphocyte proliferative responses to filarial antigens are found in MF individuals. The mechanism of IL-10 suppression of lymphocyte proliferation may occur either through the ability of IL-10 to inhibit expression of MHC class II molecules on antigen presenting cells (de Waal Malefyt et al., 1991) or by inhibiting the expression of certain costimulatory molecules. Moreover (Pearlman et al., 1993) have studied the change in the cytokines levels following addition of neutralizing anti-IL-10 antibodies to antigen driven lymphocyte cultures. They have observed an enhanced T cell proliferation and subsequent Th1 cytokine production. Most
recent studies by Steel and Nutman (1998) with CD45RA+ CD4+ cells from normal individuals were stimulated with soluble microfilarial antigens (mf Ag) \textit{in vitro} in the presence of APC and it was observed that the (mf Ag) by itself induced proliferation and IFN-γ and IL-5 production, suggesting that the filarial antigen by themselves can prime the CD45RA+ CD4+ cells \textit{in vitro} and thus deviate the immune responses either to the type I or type II cytokines production.

It has been well documented that the filarial antigen-driven IgE production is upregulated by IL-4 and downregulated by IFN-γ, suggesting that amount of IgE production depends on the relative quantity of IL-4 and IFN-γ generated by filarial antigen specific T cells (King et al., 1990). The effect of interleukin-12 (IL-12) on IgG4 and IgE production was examined with cells derived from filarial patients and European controls. Studies were done to determine the role IL-12 in Ag-driven polyclonal IgE production using rIL-12, anti IL-12 and endogenous IL-12. Recombinant IL-12 inhibits IgE synthesis by IL-4-stimulated lymphocytes from healthy persons and influenced the development of Th subset selection involved in IgG isotype selection. It was demonstrated that IL-12 modulates helminth Ag-driven IgE production, in part, by regulating the relative quantities of IFN-γ and IL-4 generated by Ag-specific lymphocytes (King et al., 1995).

1.7.2 Parasite defense strategies

The unique biochemical makeup of the host and vector requires the parasite to tailor the tools it uses for nutrition, migration and for its long-term survival. Since filarial worms do not replicate in the human host, thus for long term persistence they have sophisticated mechanisms of immune evasion and modulation. The nature of these evasive mechanisms and the differences between protective and pathogenic immune responses are the central questions now being addressed in filariasis. Recent advances in the field of immunology and parasitology have highlighted a range of mechanisms by which the parasite actively modulates the immune response to allow survival. It has been demonstrated that the parasites have
developed a range of mechanisms to annihilate the host response, which induce chronic infections that persist over long periods of time in the host.

Atleast two ways are proposed to explain the longevity of helminth infections: (i) Immuno-evasion (ii) Immunomodulation.

1.7.2.1 Immuno-evasion

This is an escape mechanism in which the parasites is surrounded by a non-immunogenic host protein coat and disguise itself as host molecules which then mask the crucial antigens and fail to stimulate an immune response. The parasite reacts to the immune response by altering the immunogenicity of their antigens. The trypanosomes have developed an elaborate method of antigenic variation, mainly through transcriptional regulation of surface glycoprotein. Secretion of hapten, phosphorylcholine (PC) by the parasite may be a diversionary tactic (Maizels et al., 1993). Schistosomes absorb host MHC, decay-accelerating factor which gives protection against the complement mediated attack (Pearce et al., 1990), contrapsin (an antithrombotic serum serine protease inhibitor (Modha et al., 1988) and LDL, possibly causing the progressive loss of antibody binding sites and evades the immune responses (Chiang and Caulfield, 1989). In these instances, uptake of host molecule is quintessentially parasitic, to subvert host functions for the well being of the parasite.

An investigation carried out by (Bright and Raj 1994) on the surface antigens of S. digitata by in situ localization revealed the occurrence of shared antigens in the egg, embryo, mf and adult stages. It has been proved that the egg and embryo have exposed surface epitopes whereas the microfilariae and adults did not have any such epitopes. "The surface epitope hiding", is an immunoevasive strategy of the filarial parasite which explains why the naturally shed surface antigens evoke antifilarial immune response in the host even though the system could not recognize the microfilariae or adult parasite due to lack of exposed
surface epitopes. Antigen processing generally requires cestinyl and aspartyl proteases such as cathepsin B and D (Diment 1990). Filarial worms release a cystatin like molecule, which may block cathepsin B. Interference with antigen presentation may not only block the response, but also induce a state of immunological unresponsiveness, or tolerance. A key aspect of parasite persistence appears to be down-regulation of the immune system resulting in tolerance or anergy. Moreover, production of protease is considered important for the conversion of the host tissues to nutrients and also for invasion and dissemination through host tissues to establish a suitable environment for the parasite to survive. Parasite can directly or indirectly block the effects of antibody with surface or released protease capable of degrading host immunoglobulin molecules (Auriault et al., 1981). Filarial parasite use endogenous and exogenous arachidonic acid to produce and release prostanoids (prostacyclin and PGE2), which in addition to the anti-inflammatory property may also inhibit T-cell proliferation (Liu et al., 1990). Helminths expresses surface or secreted antioxidant enzymes such as superoxide dismutase (Simurda et al., 1988), glutathione peroxidase(GPX) (Cookson. et al., 1992; Williams et al., 1992.) and Glutathione S-transferase (GST) (Smith et al., 1986), to counteract the oxidative burst of activated host leukocytes. Hence helminth parasites combine specific molecular strategies to combat the threat of immediate immune attack by the host.

1.7.2.2 Immunomodulation

This is seen as a more active interaction with the immune system to reduce the impact of the response against the parasite. These factors can directly suppress the function of certain immune response as well as stimulating other cell populations, which have suppressive activity like interfere with the balancing the type 1 - type 2 response balance, general suppression of the B and T cell responses and mimicry of the host proteins that directs the immune response (Riffkin et al., 1996).
The mechanisms underlying this parasite-specific anergy may involve adherent suppressor cells, serum suppressive factors or suppressor T lymphocytes (Piessens et al., 1980). The suppressor cells may be induced in vitro using filarial antigens and patients with patent microfilariae had either the greatest number of suppressor cells or the greatest functional activity of these cells. Alternatively, the anergic state may result from a diminished number of parasite antigen-responsive lymphocytes (Nutman et al., 1987b) as a consequence of prenatal sensitization or toleration to the parasite (Steel et al., 1994). Removal of the suppressor T cells, which were activated both in vivo and in vitro, restored the lymphocyte reactivity (Piessens et al., 1982). Similarly lymphocytes from microfilaremic patients who are hyporeactive to filarial antigens exhibited increased proliferative ability after the same patients were treated with DEC (Suba 1997). The immunosuppressive effects of BmA antigen on phytohemagglutinin (PHA) driven T cell proliferation were studied in filariasis patients (Lal et al., 1990). They have demonstrated that the suppressive agent appeared to be the phosphocholine containing antigens that were present in abundance in the filarial parasites and in the circulating filarial antigens. King et al (1992) have studied the diminished parasite specific T and B cells in MF individuals by measuring the proliferative responses of T and B cell precursor (CD3+) to the parasite antigen and a mitogen. They have observed that the frequency of parasite-specific CD3+ T cells was significantly lower in MF patients than in the CP patients in contrast the frequency of mitogen or non parasite-specific CD3+ T cells were same in CP and MF individuals.

1.7.3 Protective Immunity

Protective immunity in filariasis is indicated by the existence of putative immune individuals (PI), who do not develop patent infections or clinical symptoms after living in endemic areas for extended periods of time. Hence to gain insight into the nature of the humoral response of PI and the nature of antigen present in them several groups have performed studies and the initial results are promising. Earlier studies by Freedman et al (1989) have shown distinct recognition pattern of larval stage antigens by putatively immune individuals when compared with the MF
individuals by immunoblot analysis. Similar studies in onchocerciasis have shown that the PI individuals preferentially recognised an 45-50kDa tripet and a 22kDa L3 antigen when compared with individuals with active infection. But there was no difference in the antigen recognition pattern in both the groups while using *Onchocerca volvulus* adult antigens (Nutman *et al*., 1991). Thus suggesting the efficient form of protective immunity would be that directed against the infective or the early developing larval form of the parasite. Another candidate antigen namely paramyosin (62kDa) was seen to enhanced clearance of L3 from the peritoneal cavities of BALB/c mice (Li *et al*., 1991). Studies done by (Eisenbeiss *et al*., 1994) have reported that repeated low dose infections of the jird *Meriones unguiculatus*, with the filarial parasite *Acanthocheilonema viteae* caused a substantial increase in the humoral immune response that specifically affects larvae during the third molt but not with the inactivated L3 or live L4 stage larvae. This indicated that the protective antigens produced by the infective-stage larvae of filarial parasites during molting period (precisely during their molt from the L3 to the L4 stage) are potentially important targets for a protective immune response. Further the above studies by different group of researchers support the validity of the assumption that a unique status of immunity develops in certain individuals living in areas where filariasis is endemic.

1.7.4 Importance of Recombinant DNA technology in studying parasites

The major difficulty to study the protective role of various antigens from infective larvae is the lack of parasite material. With recent advances in molecular biology techniques, this problem has been circumvented by employing a reverse transcription, PCR-based strategy which exploits the presence of a conserved 22-nucleotide spliced leader sequence present at the 5' end of a proportion of nematode transcripts. The cDNAs were amplified from the late-vector-stage larvae of the filarial nematode *Brugia malayi*. One of the PCR cDNA clones (Bmserpin) encoding the first member of the serine proteinase inhibitor (serpin) superfamily has been reported. The reverse transcription PCR analysis of RNA from different stages of the parasite demonstrated that infective-stage larvae contained 10- to 16-fold-
more Bmserpin than adults or microfilariae and Bmserpin was immunogenic in gerbils (Yenbutr and Scott., 1995). Identification of antigens produced during molting L3 using recombinant DNA technology will emphasize the central importance of this larval stage for future efforts aimed toward the development of diagnosis and a vaccine.

1.8. SCIENTIFIC ADVANCES IN FILARIAISIS

With the advances in medical imaging technology we can locate the live filarial parasite by ultrasonography and the damages caused by the parasite can be imaged by lymphoscintigraphy.

1.8.1 Ultrasonography

Ultrasonography is an inexpensive, rapid and non-invasive imaging technique that has no adverse side effects. In addition it allows the direct localization of the adult *W. bancrofti* parasites in the scrotal lymphatics of asymptomatic microfilaraemic men (Amaral *et al.*, 1994). They have demonstrated that the parasites are vigorously motile (exhibiting filarial 'dance') and clustered together at definite sites, where they seemingly attach to the lymphatic endothelium. Interestingly they have shown that for several centimeters on either side of the parasite the lymphatic are abnormally dilated, though there was no evidence of an active inflammatory response around these living worm (Freedman *et al.*, 1995). Thus this technique can be used to study the pathogenesis of the filarial disease and the effects of different chemotherapeutic agents can be assessed.

1.8.2 Lymphoscintigraphy

Lymphangioscintigraphy (LAS) is the currently available non invasive isotopic technique to assess the lymphatic function in patients with filariasis (Freedman *et al.*, 1994). Technetium 99m (Tc99m) labeled human albumin or
dextran is injected intradermally or subcutaneously, into a web space on the dorsum of the foot or hand and is traced by a gamma camera. The colloid collects in the lymphatics and join the lymph flow, thereby giving functional and anatomical information about the state of the lymphatics. Most intriguing finding from such studies is the presence of profound lymphatic abnormalities seen in individuals who are asymptomatic and manifest the filarial infection only by the presence of circulating microfilariae. Their lymphatics are markedly dilated, with many collateral channels, and unexpectedly, the lymph flow is more rapid than normal lymphatics. Patients with elephantiasis, in contrast, show lymph stasis and significantly altered lymphatics, with vessel dilatation, tortuosity, dermal back flow and obstruction.

1.9 NEW CONTROL INITIATIVES

There is a major need to replace night blood surveys as the primary method. This is to identify potential transmission or established human infection and to study the effectiveness of control programs. Currently evaluation of antigenemia in daytime finger prick blood specimens has proven to be a workable alternative to the night blood survey.

The geographical information system (Michael et al., 1996) has facilitated the development of a rapid epidemiological mapping tool for filariasis. This will help us to study the systematic geographical covariation of the infection and disease patterns with specific etiological factors.

Nicolas (1997) has proposed that monitoring parasite presence in mosquitoes by PCR poolscreening technique and circulating filarial antigen in humans is essential to evaluate the transmission rate of the parasite and to study the efficacy of the control programs. Hence it appears that it is essential to develop a reliable early diagnostic method for intervening active filarial infection by mass chemotherapy. In this thesis an attempt was made to develop recombinant antigen
based assays for diagnosis and for the chemotherapeutic follow up of individuals having active filarial infection.