Introduction
1. Introduction

Lymphatic filariasis commonly called as elephantiasis is one of the major public health problems in many tropical and subtropical regions of the world. Though the disease is not fatal, it is responsible for considerable morbidity causing social stigma. It affects poor people in both urban and rural areas. Globally around 1100 million people are at risk and 78.6 million showing microfilariae (mf) or overt disease. In India, lymphatic filariasis (LF) is caused by *Wuchereria bancrofti* and *Brugia malayi* and more than 400 million people are at risk of infection, 27 million carry mf in the circulation and nearly 20 million suffer from disease manifestations (WHO 2003). The disease itself is not fatal and is characterized by a wide range of clinical manifestations. The signs and symptoms of the disease often differ from one endemic area to another. In all endemic areas a portion of the population shows no microfilaraemia or clinical manifestations, the reasons for this being that they may either have not been exposed sufficiently to become infected or sufficiently exposed but do not develop infection or have subclinical infection. The population either remains microfilaraemic with no signs and symptoms of the disease throughout life or may develop disease manifestations with or without microfilaraemia at a later age. The most serious manifestations are the acute clinical symptoms characterized by episodic attacks of adenolymphangitis (ADL) associated with fever and malaise. Inflammatory nodules in the breast, scrotum or subcutaneous tissues have also been reported as acute manifestations of the disease. Of the many chronic manifestations, elephantiasis and hydrocoele are the common ones. Chyluria is one of the serious chronic manifestations but its prevalence is low.

Tropical pulmonary eosinophilia (TPE) in filarial infection is well recognized. Patients with this syndrome appear to show hypersensitivity to filarial antigen with asthma type symptoms (Ottesen *et al.* 1979). Thus, though various manifestations of the disease are well described in the literature, the relationship between the complex host-parasite interactions following continuous exposures filarial infections in endemic areas and the appearance of disease manifestations is still obscure.

The acute and painful disease manifestations which affect more than 50% of the exposed subjects are characterized by periodic and self limiting episodes of ADL, fever and
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associated constitutional symptoms, while the chronic disease (which is manifested in less than 5% of exposed cases) includes long lasting grotesque deformities such as elephantiasis, lymphoedema and/or hydrocoele. The recurrent episodic bouts of acute manifestations occurring over a period of time lead to the development of chronic disease manifestations. Fever and malaise often accompanied with lymphangitis and lymphadenitis are the cause of morbidity which adversely affect the country’s economy due to tremendous loss of man-hours. Earlier, the cause of filarial fever was thought to be a co-existing bacterial/ and or fungal infections, but the available evidence is not conclusive. Also no systematic studies were made to find out whether some of these episodes of ADL are triggered or accentuated by bacterial or fungal infections or vice versa.

Our current understanding of the pathogenesis of filarial infection is rather poor. Available information on tissue pathology (Beaver 1970; Ottesen 1980; Yap et al. 1982; Lichtenberg 1987) suggests that though clinical manifestations have their own range of severity, complications and sequelae, they share abnormal lymphatic function. However, the precise mechanism underlying lymphatic pathology is poorly understood. It is believed, almost to certainty, that the clinical manifestations which are largely due to lymphatic dysfunction are the result of overt immunological reactions to the continuous release of parasite product rather than simple mechanical obstruction by the dead worms and/or the dying worm products.

During the past decade several important conceptual advances have been made towards understanding the pathogenesis of lymphatic lesions in bancroftian and brugian filariasis. These concepts are based primarily on the observation in experimental animal models of Brugia sp. in ferrets, dogs, cats and immunodeficient mice. These models mimic some of the aspects of filarial disease in human beings and provided clear evidence that much of the pathology results from the host immune response to the parasites. The Indian leaf monkey (Presbytis entellus) model of B. malayi exhibits characteristics of acute disease manifestations (Murthy et al. 1990; Tyagi et al. 1996). The model showing acute disease manifestations had elevated levels of circulating immune complexes (Murthy et al. 1999). We have also found that sera of these monkeys did not react with some of the filarial antigens that were reactive with sera of monkeys that have never developed the disease
symptoms. These findings suggest that the removal of these antigen molecules from the circulation via immune complex formation and their subsequent deposition in tissues may be involved in the initiation of the characteristic acute pathological manifestations. Murthy et al. (2002) have reported that preadult stage of *B. malayi* may be involved in the development of limb edema in *P. entellus*. Lichtenberg (1987) reviewed the chronic non-specific inflammatory changes and modulation of the parasite-specific immune response in filarial disease. There is evidence that a variety of inflammatory mediators such as arachidonic acid metabolites, prostaglandins, kinins, complement, eosinophilic granules and cytokines like interleukins (IL-1 & 6) are involved in the inflammatory reactions in filarial patients (Ottesen 1987; Turner et al. 1994). Tumor necrosis factor-alpha (TNF-α) has been shown to be an important inflammatory mediator capable of producing symptoms such as fever, chills, myalgia and acute filarial disease episode development (Yazdanbakhsh et al. 1992; Turner et al. 1994; Das et al. 1996). Increased IL-6 and /or TNF-α levels were reported in asymptomatic microfilariae carriers after initiation of antifilarial therapy with DEC (Yazdanbakhsh et al. 1992; Turner et al. 1994). In malaria TNF-α inducing parasite antigens have been reported by Bate et al. (1989). On the contrary IL-4, IL-10 and TGF-β have anti-inflammatory effect (King et al. 1993). At the immune response level, hyporesponsiveness due to active filarial infection is thought to contribute to immunity or pathology in human filariasis (Maizels et al. 1993). IL-12 is reported to play an important role in the production of IgG4 (Bauke et al. 1997). Studies have also shown that a portion of pathology is derived from the direct action of the parasites or their molecules on the lymphatic tissues (Vickery et al. 1983; Vincet et al. 1984; Ottesen 1992). All these findings related to immunological and non-immunological mediated pathology in animals is almost similar to those described previously in affected humans (i.e. lymphatic proliferation, dilatations and edematous reaction in the presence of living worms and obstructive/obliterative reactions in lymphatics around dead parasites). Earlier studies in cats with *B. pahangi* (Schacher and Sahyoun 1967) suggested that events associated with repeated infective exposures or with parasite moulting or maturation may trigger the onset of lymphadenopathy or limb edema. Lawrence and Denham (1993) and Steel et al. (1994) have demonstrated stage specific pattern of cytokine secretion in infected animals and humans towards adults and mf. However none of these studies could correlate quantitatively or qualitatively the immune responses to antigens /and
or antigen molecules with clinical outcome of the infection. Although the immunological basis of clinical manifestations is well known, the identity of antigen(s) evoking inflammatory reaction, and the profile of the inflammatory mediators responsible have not been clearly delineated. Recent studies in *B. malayi*-infected Indian leaf monkey (*P. entellus*), have shown some evidences that the parasite antigens may be responsible for the episodic acute inflammatory (edematous) reaction in the limbs. The precise identity of the antigens and how the antigens participate in the inflammatory cascade remain to be investigated.

Recent studies show that inflammatory cytokines play a central role in the inflammatory reactions in parasitic infections. Tumor necrosis factor-alpha (TNF-α) has been shown to be an important inflammatory mediator capable of producing symptoms such as fever, chills and myalgia in acute filarial disease episode development (Yazdanbakhsh *et al.* 1992; Turner *et al.* 1992, 1994; Das *et al.* 1996). Yazdanbakhsh *et al.* (1992) and Turner *et al.* (1994) demonstrated elevated levels of IL-1β, IL-6 and/or TNF-α levels in asymptomatic microfilariae carriers after initiation of antifilarial therapy with diethylcarbamazine (DEC) which correlated with fever and other reactions after antifilarial therapy in bancroftian patients. These reactions are thought to be initiated by antigens of the parasites which were probably inaccessible in the parasite before the treatment. The close similarity between the reactions provoked by the antifilarial and the profile of the spontaneous acute disease manifestations make it highly likely that inflammatory cytokines play a pivotal role in these reactions. Therefore, the identification of proinflammatory parasite antigen(s) and their effect on the development of inflammatory reaction in the host will be of immense value in understanding the development of disease manifestations.

*Mastomys coucha* and jird (*Meriones unguiculatus*) are the two rodent hosts highly susceptible to *B. malayi* infection. These models have been utilized for various studies in this laboratory and elsewhere. These have shown some resemblances with humans infected with *W. bancrofti* or *B. malayi* with regards to parasitological, immunological and pathological. The models appear to be suitable for identifying proinflammatory parasite antigen(s) with respect to their role in the development of disease manifestations.
**Objectives**

The present study is therefore undertaken to identify and characterize the antigens of pathogenic potential using *B. malayi-M. coucha* as a model.

1. Fractionation and purification of *B. malayi* antigens.

2. Identification of pro-inflammatory antigen(s) of *B. malayi* using mouse/human macrophage cell line assay system.

3. To investigate the effect of identified pro-inflammatory antigen(s) on the course of the infection in the susceptible rodent host, *M. coucha*.

4. To investigate the humoral and cell mediated immune responses of the host exposed to the pro-inflammatory antigen(s).

5. To investigate the *in vitro/in vivo* expression of inflammatory and anti-inflammatory cytokines/mediators in response to the identified antigen(s).