CHAPTER-1

1 INTRODUCTION

Filariasis, a debilitating disease, is the second leading cause of permanent and long-term disability worldwide (Molyneux, 2009). Lymphatic filariasis (LF) commonly known by the name *elephantiasis*, one of the oldest neglected tropical disease and major causes of chronic disability in the developing countries, produces a considerable economic burden. It is caused by *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. Globally, around 120 million people in 73 countries are currently infected, and an estimated 1.393 billion people live in areas endemic for lymphatic filariasis where mass drug administration (MDA) is required. LF is the second leading cause of chronic disability worldwide due to its stigmatizing and disabling clinical manifestations, which include 15 million people with lymphoedema (elephantiasis) and 25 million men with urogenital swelling, principally scrotal hydrocele (WHO, 2012), http://www.who.int/wer. In India, more than 553 million people are exposed to infection with 31 million people showing circulating mf and another 23 million people suffering from chronic manifestations of the disease (Raju *et al.*, 2010). The most debilitating manifestations are the grotesque deformities of limbs (elephantiasis), breast and genitalia, particularly the scrotal sac where it causes 'hydrocoele', which are largely irreversible and cause huge loss of useful man-hours 5.5 million Disability Adjusted Life Years (DALYs). Around 50% of the infected persons suffer from episodic adenolymphangitis (ADL), which causes acute suffering and incapacitation (Pani *et al.*, 1995; Ramaiah *et al.*, 1997). A million individuals have cryptic infections resulting in conditions such as tropical pulmonary eosinophilia (TPE). The other health problem due to filariasis include renal disease, arthritis, endomyocardial fibrosis, etc (Das & Pani, 2000; Pani & Lall, 1998). The mosquito vectors involved in filariasis transmission include different species of Culex, Aedes, Mansonia and Anopheles in the world. *W. bancrofti* is transmitted by ubiquitous mosquito, *Culex quinquefasciatus* (Sasa, 1976) and *B. malayi* is transmitted by *Manson*ia mosquitoes (Sabesan *et al.*, 1991).

The filarial adult worms live for about six years or more in the lymphatic system of human, where female worms release millions of microfilariae (1st stage larva) that circulate in the blood and are picked up by mosquitoes during a blood meal. In the mosquito, the mf undergoes two molts.
to become 3\textsuperscript{rd} stage infective larvae (L\textsubscript{3}) which then enter human host during a blood meal of the vector. L\textsubscript{3} secretes certain proteases and other enzymes that facilitate their penetration through local connective tissue and migrate to local lymphatic vessels where they take 2 to 12 months to develop into adult worms through two molts. Sexually mature male and female adult worms residing in afferent lymphatic vessels copulate, female worms subsequently release the mf which then enter the bloodstream and are picked-up by mosquitoes; and the infection cycle continues.

Filarial parasites having a diverse array of antigens elicit a broad spectrum of immune and inflammatory responses in their hosts. Many of these responses (perhaps the majority) are irrelevant in terms of protective value and only relatively few contribute directly to resistance to infection. Several studies have been carried out to attempt to identify the responses, and their target antigens, that correlate well with immunity but other than in a small number of experimental systems, such correlations remain elusive. Further, many host responses, both non-protective and protective, have the potential to cause pathological changes both locally and systemically. It is therefore necessary to precisely identify the parasite molecules and delineate the immune mediated pathways activated by the parasite molecules.

Studies carried out in this laboratory have revealed that BmAFII (Sephadex G-200 eluted fraction of adult \textit{B. malayi}) with molecules ranging from 21 to 84 kDa could induce host-protective immunity and conferred significant level of protection against \textit{B. malayi} parasite challenge in \textit{M. coucha}. BmAFII stimulate the release of pro and anti-inflammatory cytokines \textit{in vitro} (Dixit \textit{et al.}, 2004; Dixit \textit{et al.}, 2006) and cross protect from \textit{Leishmania donovani} infection in hamsters (Murthy \textit{et al.}, 2008) by stimulating predominantly pro-inflammatory responses. Further, 4 of the 15 SDS-PAGE fractions within this molecular weight range of BmAFII have revealed inflammation modulating molecules (Dixit \textit{et al.}, 2004). MALDI-TOF analysis of the 4 fractions showed 11 proteins, among which 6 proteins were identified to be immunomodulatory. These are: Heat Shock Protein 60 (HSP60), Cytoplasmic intermediate filament (CIF), Elongation factor 2 (EF2), dTDP-D-glucose 4, 6-dehydratase, Disorganize muscle protein 1 (DIM-1), small HSP (p27) (Sahoo \textit{et al.}, 2009). Thus these findings have opened up avenues for studying precisely the role of these protein molecules in host-parasite interactions. DIM-1 (Acc. No.- 14972.m07771, Database search- \textit{B. malayi} CDS). has shown similarity with As37 antigen of \textit{Ascaris suum} (Sahoo \textit{et al.}, 2009). Tsuji \textit{et al.} (2002) reported that As37 has significant similarity with \textit{Caenorhabditis elegans} DIM-1 protein and has low similarity to part of the multi-repeat Ig domain from nematode
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Twitchin and mammalian skeleton muscle titin, and to members of the IgSF at the amino acid sequence level. The investigators’ study further revealed that antibodies to recombinant As37 reacted with muscle cells and hypodermis. In preliminary vaccine trials, rAs37 was found to be protective against A. suum in BALB/c mice. Further, the sera of mice, rabbits and pigs immunized with A. suum infective eggs reacted with rAs37 in immunoblot analyses suggesting that rAs37 was immunogenic (Tsuji et al., 2002). Besides, vaccination of goats with DNA vaccine encoding DIM-1 induced partial protection against Haemonchus contortus (Yan et al., 2013).

Encouraged with the findings on prophylactic potential DIM-1 of A. suum, the present investigation was aimed at cloning, expression and purification of an immunomodulatory protein, DIM-1 of human filarial parasite, B. malayi and investigating its responses to know the role in protection of the host from parasite/parasite protection and or infection-induced inflammatory reaction in the host. Aso the role of the protein in pathogenesis of lymphatic filariasis is not clear. This information would help in evolving new and effective control and treatment strategies for human lymphatic filariasis. The objectives of the present study were:

Objectives:

1. Cloning of disorganize muscle protein 1 (DIM-1) of B. malayi adult worms which was identified through proteomic analysis.
2. Over expression and purification of the recombinant protein.
3. Testing of immunomodulatory potential of the recombinant (r) protein using splenocytes/macrophages of balb/c or swiss mouse or mouse macrophage cell line (RAW 264.7)/human monocyte-macrophage cell line (THP-1).
4. Responses of rodent hosts (BALB/c or Mastomy coucha) to the r-protein.