# CHAPTER I

# INTRODUCTION

## 1. Background and Rationale

Lymphatic filariasis, known as elephantiasis, is mainly caused by the filarial nematodes, *Wuchereria bancrofti* and *Brugia malayi* (Ottesen *et al.*, 1997). Over 120 million people in 83 endemic countries, including Thailand are infected, with more than 40 million incapacitated or disfigured by the disease (WHO, 2003; Triteeraprapab and Songtrus, 1999; Triteeraprapab *et al.*, 2001; Nuchprayoon *et al.*, 2001; 2003a; 2003b). One-third of the people infected with the disease live in India, one third are in Africa and most of the remainder are in South Asia, the Pacific and the Americas. In tropical and subtropical areas where lymphatic filariasis is well-established, the prevalence of infection is continuing to increase. Lymphatic filariasis is classified by the World Health Organization (WHO) as the world's second leading cause of permanent and long-term disability, and targeted by the WHO to be eliminated as a public health problem by the year 2020 (WHO, 1995; Behbehani, 1998). In terms of disability-adjusted life years (DALYs: the number of healthy years of life lost due to premature death and disability), lymphatic filariasis is responsible for 5.8 million DALYs lost annually, ranking third among the TDR diseases, after malaria and TB (WHO, 2004). Lymphatic filariasis is a vector-borne disease transmitted by mosquito vectors. The infection is established with adult worms that can dwell in the human lymphatic system, where offsprings of female adults, microfilariae, are released into the host's blood circulation. There are a broad range of clinical and subclinical symptoms of longstanding infection with these filarial parasites, including recurrent adenolymphangitis, hydrocele, lymphedema, elephantiasis, and tropical pulmonary eosinophilia.

## 1.1 Pathogenesis

The pathogenesis of lymphatic filariasis is thought to be caused by adult worms, host immune responses, and the secondary bacterial infections (Ottesen, 1992). The pathology in examined lymphatic vessels consists of distinct histological features related to the existence of both alive and dead parasites (Jungmann *et al.*, 1991; 1992). In asymptomatic patients living in endemic areas, lymphangiectasia* is the most common change observed (Dreyer *et al.*, 2000). The lymphatic vessels are dilated with the presence of intact worms that do not provoke any inflammatory responses. In contrast, an inflammatory pathology is usually detected with the presence of dead worms from natural occurring, or antifilarial-drug (diethylcarbamazine; DEC, or ivermectin) treatment reaction (Figueredo-Silva *et al.*, 1996; Dreyer *et al.*, 1999; 2000). The underlying pathology may be associated without any clinical symptoms, or with the acute lymphagitis. This acute manifestation is characterized by local inflammatory reactions around dead worms, and systemic febrile responses with significantly elevated levels of tumor necrosis factor alpha (TNF-$\alpha$) (Dreyer *et al.*, 2000; Das *et al.*, 1996). Therefore, lymphatic dysfunction can be caused by lymphangiectasia and persistent attacks of acute lymphangitis. These factors together with recurrent opportunistic bacterial infections are major risk for development of the chronic manifestations of lymphatic filariasis (Ottesen, 1992; Dreyer *et al.*, 2000).

The drug-induced adverse reactions are also described in lymphatic filariasis as a pathological event of inflammatory responses to the death of worms (Ottessen, 1985). Following antifilarial treatment of microfilaremic patients, adverse reactions commonly occur in which the severity are correlated with pre-treatment microfilarial density (Ismail *et al.*, 1998; Richards *et al.*, 1991; Addiss *et al.*, 1997; Haarbrink *et al.*,

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* lymphangiectasia: a term of pathology that describes dilation of the lymphatic vessels; lymphangiectasia may be congenital or acquired.

2000). This also suggests that it is less likely to be a result of direct drug toxicity. Treated patients may experience systemic reactions, including headache, fever, chills, and body aches and/or localized reactions, which cause painful inflammatory granulomas (Jayakody *et al.*, 1993; Dreyer *et al.*, 1994; Figueredo-Silva *et al.*, 1996). Symptomatic treatment of these post-treatment reactions (with analgesics, antipyretics, antihypotensive agents *etc*). has been successful, but their prevention has been achieved only with the broadly anti-inflammatory corticosteroids (Ottessen, 1987). This observation is related to the findings that adverse reactions are associated with the increased post-treatment concentrations of proinflammatory cytokines and inflammatory mediators, including tumor necrosis factor (TNF), interleukin (IL)-6, IL-10, lipopolysaccharide-binding protein (LBP), and soluble TNF receptors (sTNF-Rs) (Turner *et al.*, 1994; Haarbrink *et al.*, 1999; 2000). However, IL-6 and LBP plasma levels show the strongest association with the severity of adverse reactions (Haarbrink *et al.*, 2000).

### 1.2 The endosymbiont *Wolbachia*

It has been discovered that filarial nematodes themselves harbor the intracellular bacteria *Wolbachia* (McLaren *et al*, 1975; Kozek, 1977; Kozek and Manoquin, 1977). *Wolbachia* is a genus of the class Alphaproteobacteria belonging to the order Rickettsiales, and family Anaplasmataceae (Dumler *et al.*, 2001). These obligate intracellular gram-negative bacteria are widespread only in arthropods and filarial nematodes, including *B. malayi, W. bancrofti*, and *Onchocerca volvulus*, the major human parasitic filarial nematodes, and *Dirofilaria immitis*, the pathogenic filarial nematode of dog heartworm disease (Sironi *et al.*, 1995; Bandi *et al.*, 1998; Henkle-Duhrsen *et al.*, 1998). Two supergroups of the genus *Wolbachia* are found in filarial nematodes: supergroup D *Wolbachia* are present in most species of the genus *Onchocerca* and *Dirofilaria*, including *O. volvulus* and *D. immitis*, while supergroup C

*Wolbachia* are found in the causative agents of lymphatic filariasis, *W. bancrofti* and *B. malayi* (Bandi *et al.*, 1998; Casiraghi *et al.*, 2001). *Wolbachia* are found in all developmental stages of filarial nematodes, in which the bacteria are restricted in the lateral hypodermal cords of filarial nematodes, and in the reproductive tissues of the females (*i.e.* in the oogonia, oocytes, embryos and microfilariae) (Kozek, 1977; Kozek and Marroquin, 1977; Fenn and Blaxter, 2004a; McGarry *et al.*, 2004) (**Figure 1**). This suggests that *Wolbachia* are vertically transmitted through the cytoplasm of the eggs. While the arthropod *Wolbachia* can be characterized as parasitic, there is evidence to suggest that the association between *Wolbachia* and filarial nematodes is mutualistic (Fenn and Blaxter, 2004b). The phylogeny of the filarial nematode *Wolbachia* is congruent with that of their hosts. The evolutionary aspect suggests long-term co-evolution and co-adaptation, which is usually seen in mutualistic relationships (Baumann *et al.*, 1995).

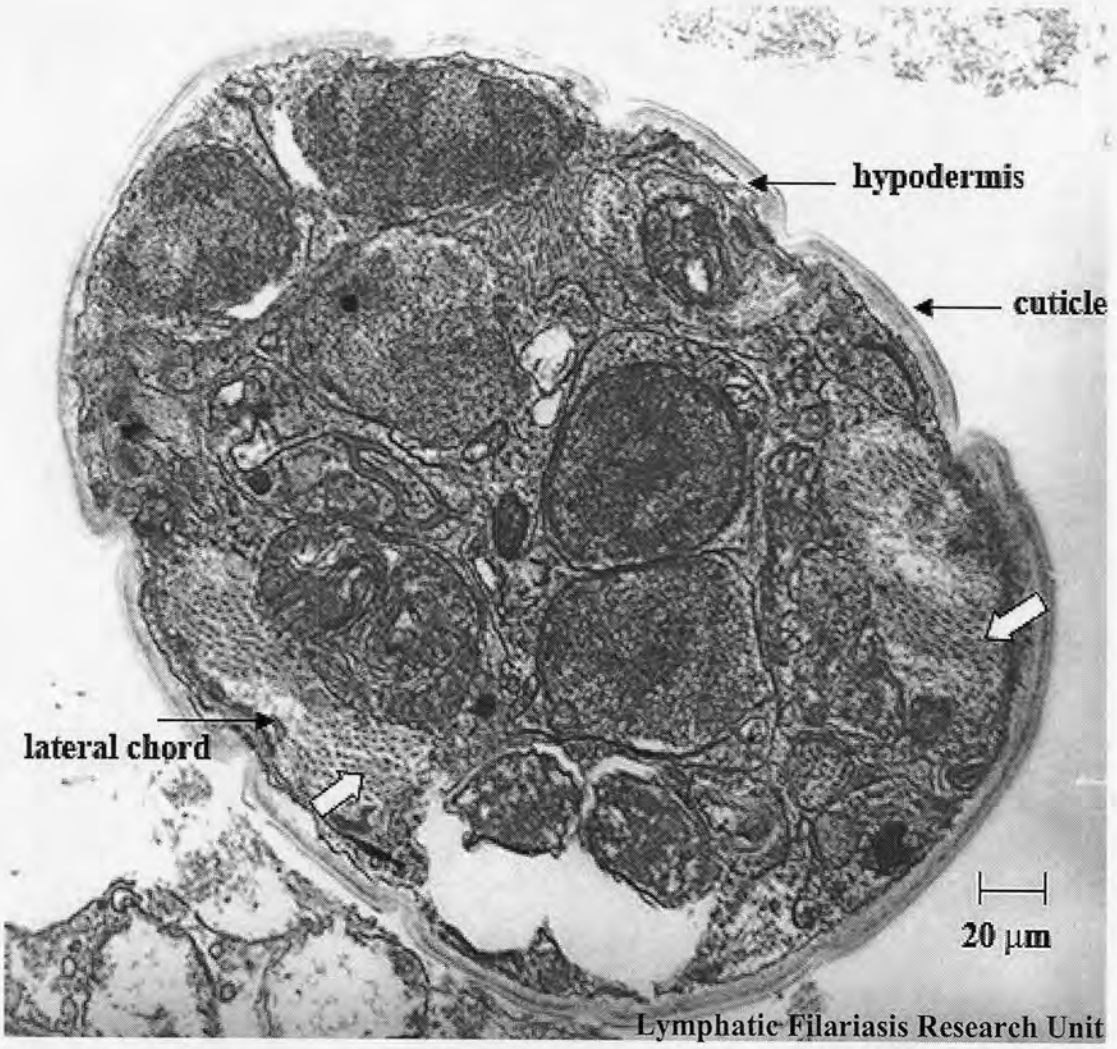



**Figure 1** Electron microscopy of a cross-section of a *Dirofilaria immitis* microfilaria (mf) show *Wolbachia* (⇨) in the lateral chords of the hypodermis. (X 500).

### 1.3 Filarial-specific antibody

To characterize the patterns of immunoglobulin G (IgG) subclass and IgE reactivity during the early stages of onchocerciasis, sera were collected from children residing in a region in endemic, and these samples were tested by ELISA for their reactivity to adult antigens (OvAg) and against four *O. volvulus* recombinant antigens.

Almost of the samples contained detectable levels of anti-OvAg IgG. In samples from microfilaria (mf)-positive children, IgG4 responses were significantly elevated and constituted of the total IgG responses. For mf-negative individuals, the mean contributions of IgG4 to the total IgG response were 11%. OvAg-specific IgE was detectable in the sera from both mf-negative and mf-positive individuals. Nearly 50% of the mf-negative children and almost of the mf-positive children had detectable levels of IgG against at least one of the recombinant antigens (Gbakima *et al.*, 1996).

In lymphatic filariasis, quantitation of serum immunoglobulin M, G, A, D and E levels was carried out in Malaysians with *Brugia malayi* infections. Results showed highly elevated levels of IgM and IgE as well as moderately elevated levels of IgG. These were most significant in patients with tropical pulmonary eosinophilia or elephantiasis. Serum IgE levels were extremely high in microfilaraemic patients (Sim *et al*, 1983).

The recent studies, which suggested that individuals with chronic disease produce higher amounts of specific anti-filarial IgG1, IgG2, IgG3 and IgE and lower amounts of IgG4 than asymptomatic individuals, which was assumed to reflect a role forimmunity in the development of chronic filarial disease.

In contrast to microfilaria positive, patients with chronic pathologic conditions such as elephantiasis exhibit a difference type of immune response. They display IgG1, IgG2 and IgG3 antibody responses to parasite antigens (Maizels et al., 1987) The study patterns of specific antibody responses in Bancroftian filariasis was assessed by analyzing specific IgG1, IgG2, IgG3, IgG4, and IgE profiles among adults from two communities with high and low *Wuchereria bancrofti* endemicity. In the high endemicity community, intensities of the measured antibodies were significantly associated with infection status. IgG1, IgG2, and IgE were negatively associated with mf status, IgG3 was negatively associated with circulating filarial antigen (CFA)

status, and IgG4 was positively associated with CFA status. None of the associations were significantly influenced by chronic lymphatic disease status. In contrast, IgG1, IgG2, and IgG4 responses were less vigorous in the low endemicity community and, except for IgG4, did not show any significant associations with mf or CFA status. The IgG3 responses were considerably more vigorous in the low endemicity community than in the high endemicity one. Only IgG4 responses exhibited a rather similar pattern in the two communities, being significantly positively associated with CFA status in both communities. The IgG4:IgE ratios were higher in infection-positive individuals than in infection-negative ones, and higher in the high endemicity community than in the low endemicity one. Overall, the results indicate that specific antibody responses in Bancroftian filariasis are more related to infection status than to chronic lymphatic disease status (Jaoko *et al.*,2006).

The regulation of the human immune response to filarial parasites is complex with effects on both innate and adaptive immunity.

### 1.4 Roles of *Wolbachia* in host immune responses

In addition to the possible role of *Wolbachia* as a chemotherapy target, evidences suggest that *Wolbachia* antigens can stimulate host immune responses that may be associated with the development of filarial disease. In a laboratory model of onchocerciasis, *Wolbachia* endotoxin has been shown to mediate neutrophil infiltration and stromal haze, when a worm extract including *Wolbachia* antigens was injected into the eyes of mice. Furthermore, *B. malayi*-infected rhesus monkeys mount antibody responses to *Wolbachia* surface protein (WSP) that are temporally associated with the death filarial worms and lymphedema development. Although these evidences suggest that *Wolbachia* may be important in understanding human disease caused by filarial

worms, no studies to date have reported *Wolbachia*-specific immune responses among human populations with lymphatic filariasis.

## 2. Research Questions

What types of anti-*Wolbachia* surface protein (WSP) antibody is (are) associated with various clinical manifeatations in lymphatic filariasis?

## 3. Objectives of the Research

To study types of and levels IgG subclasses humoral immune response against *Wolbachia* surface protein (WSP) in bancroftian filariasis patients with various clinical manifestations.

## 4. Keywords

*Wuchereria bancrofti*

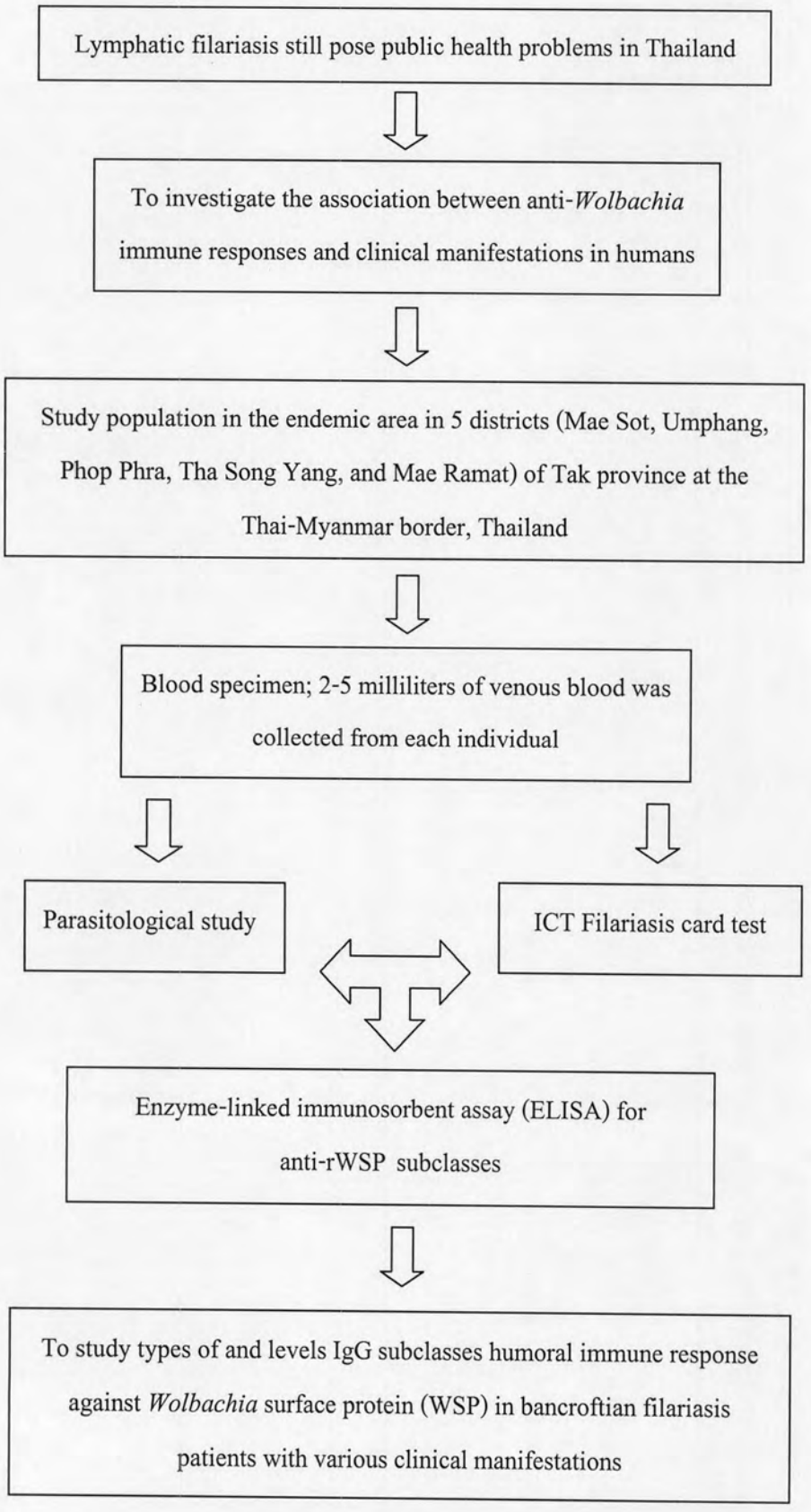
Bancroftian filariasis

*Wolbachia* surface protein

Anti-WSP antibodies

Enzyme-Linked Immunosorbent Assay (ELISA)

## 5. Conceptual Framework



## 6. Expected Benefits Applications

1. The knowledge of association between anti-WSP IgG subclass antibodies and clinical manifestations, as well as microfilaremic status in patients with lymphatic filariasis.
2. Markers associated with chronic pathology in lymphatic filariasis.