Introduction
INTRODUCTION

Lymphatic filariasis (LF), a deforming and debilitating disease transmitted by mosquitoes, causes elephantiasis and male genital damage and is a major social and economic scourge in the tropics and subtropics of Africa, Asia, the Western Pacific and parts of the Americas, affecting over 120 million people in 73 countries. More than 1.1 billion people (20% of the world's population) live in areas where there is a risk of infection (WHO 1998). It has been identified as the second leading cause of permanent and long-term disability (WHO 1995), but the true amount of disability it causes is only beginning to be quantified accurately (Evans et al. 1993; Ramu et al. 1996). Defining and quantifying the psychological burden of this deforming, mutilating disease of the limbs and genitalia is still an extremely important issue that requires much greater attention than it has received to date (Ottesen et al. 1997).

India accounts for 40% of the global prevalence of lymphatic filarial infection. LF is second only to malaria as the most important vector-borne disease in India. The disease is endemic in 18 states or Union territories, including the populous states of Uttar Pradesh and Bihar. Approximately 420 million people reside in endemic areas and 48.11 million are infected. Bancroftian filariasis, caused by *Wuchereria bancrofti* and transmitted by the tropical house mosquito *Culex quinquefasciatus*, accounts for 95% of the total lymphatic filariasis cases in India (Ramaiah et al. 2000).

THE PARASITES

Of the eight species of filarial worms that infect humans, six are generally recognized as pathogens. These may be classified into three groups based on the location of the adult worms: i) the lymphatic filarial parasites including *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori*; ii) the cutaneous filariids including *Onchocerca volvulus*, *Mansonella perstans*, and *Dipetalonema streptocerca*; and iii) the body cavity parasites including *Mansonella ozzardi*. 
Fig 1 Distribution, by WHO region, of the 120 million persons currently infected with lymphatic filarial parasites (*Wuchereria bancrofti, Brugia malayi, Brugia timori*)
Adopted from Ottesen et al., 1997.
Table 1: COUNTRIES WITH LYMPHATIC FILARIASIS

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<th>African Region</th>
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*a Windward Islands, Leeward Islands, Tuamotu Archipelago, Austral or Tabual Islands, and Marquesas Islands

Wuchereria bancrofti, Brugia malayi, or Brugia timori, the causative agents of lymphatic filariasis, account for most human filarial disease and pose a major public health problem (Chandrasekhar 1997). Wuchereria bancrofti accounts for 90% of the global burden of lymphatic filariasis while Brugia malayi and Brugia timori are responsible for the rest 10% cases (Nicolas 1997).

Filarial parasites provide a challenging experimental system because of the complexities of their life cycle and their requirement for a mosquito vector for transmission. The infective form of the mammalian host is the third-stage larvae (L3). Infection is initiated by the L3 via the bite of an infected mosquito vector. The L3 then migrate to the lymphatics and develop through two further molts to become adult worms (commonly named 'macrofilariae', take 3-15 months to mature); following mating, the adult female produces an abundance of microfilariae (MF or L1) which circulate in the blood stream of the infected host. When ingested by a susceptible mosquito, the MF migrate to the thoracic muscles and develop through further two molts to the L3, which are transmitted to a new host when the infected mosquito next takes a blood meal (Devaney et al. 1996, Osborne et al. 1996) (Fig 1).

One of the interesting features of the filarial life cycle is the block in development undergone by the MF in the mammalian host and the L3 in the mosquito vector. For both life cycle stages, the block marks the end of the developmental phase in either mammal (MF) or mosquito (L3) and is released only upon exposure to the environment of the new host, suggesting the intriguing possibility that filarial nematodes may utilize host-derived signals as a means of regulating development.

Filarial nematodes are obligate parasites, characterized by their ability to persist in the infected host for many years (adult life span 5-12 years) producing millions of MF. A striking feature of the filarial infection is the protracted and stable relationship reached between the host and the parasite. This balance serves to limit mortality of the host directly caused by filarial worms but fails to prevent substantial morbidity and disfigurement in many
Fig 2. *Brugia malayi* Adult Parasites
(F - Females, M - Males)
Fig 3: Life cycle of Lymphatic filarial parasites.
individuals. The parasite survives inside the lymphatics which is at the heart of the immune system for extended periods of time. The persistence of infection is related to the ability of the worm to down regulate the immune response of the infected individual (Ottesen 1992). Because filarial worms do not replicate in the human host, their long-term survival requires sophisticated mechanisms of immune evasion and modulation (Maizels et al. 1993; Devaney et al. 1996).

An important feature in the filarial life cycle is the presence of *Wolbachia*, a group of intracellular bacteria. *Wolbachia* have been detected in majority of filarial parasites, including the major human filarial parasites *W. bancrofti, B. malayi* and *O. volvulus*, however, the rodent filaria *A. vitae* and the deer parasite *O. flexuosa* are devoid of these bacteria. These bacteria are widespread in female worms than males and the principal mode of transmission is via the eggs of females. The phylogenetic analysis suggest a long coevolutionary history and reciprocal coadaptation between filarial worms and their *Wolbachia* endosymbionts. *Wolbachia* seem to play an important role in the development, viability and fertility of filarial nematodes, however, the molecular basis of these interactions is unknown (Taylor and Hoerauf 1999).

Much of the information regarding stage-specific antigens of filarial parasites has been obtained through immunological studies. This indicates that different life cycle stages induce distinct immune responses in the infected human. The L3/post-infective L3 stage of the parasite are the likely target of protective immune responses in humans and it has been suggested that while L3-specific antigens are crucial for the development of immunity, the antigens derived from MF and adult stages are involved in generating profound T-cell hyporesponsiveness and pathogenesis/tolerance, respectively (Lawrence 1996; Devaney et al. 1996). For such reasons, of late, the genomics (the identification of trait genes) and proteomics (the identification and characterization of all the proteins expressed by a genome or tissue, and understanding how these proteins function, both by themselves and in concert,
within an organism) approach are being applied for thorough understanding of this disease.

THE HOSTS

Filarial nematodes infect humans, domestic animals, and a range of wild vertebrates. The filarial parasites require a two-host life cycle: a definitive host (human) and an intermediate host (the hematophagous arthropod vector i.e. mosquito). Of the human filarial parasites causing lymphatic filariasis, *W. bancrofti* is remarkably host-specific, in that natural infections occur only in humans. *Brugia* parasites, in contrast, are more cosmopolitan and include zoonotic species (Denham and McGreevy 1977).

Animal Model(s)

The animal species that have been used to study filarial infections include cats, dogs, ferrets, and gerbils, rats and mice. In general, carnivores are highly permissive to infection and display lymphatic pathology (Crandell et al. 1987; Miller et al. 1990). Where as work on animal model systems has greatly assisted research into the basic pathophysiology of a number of human diseases, filariasis suffers from the lack of a suitable animal model.

The most widely investigated animal model is that of Ash and Riley (1970), who demonstrated that a substrain of *B. malayi* would grow in a rodent called gerbil or Mongolian jird (*Meriones unguiculatus*). The jirds are highly susceptible to infection, lymphatic pathology is prominent, and chronic infections induce a state of immunologic and inflammatory hyporesponsiveness (Rao et al. 1996; Nasarre et al. 1998). The major deficiency in this animal model is that the infection in the jird does not mimic the human disease in the anatomical localization of the adult worms, in the symptomatology, in the immune effector mechanisms that may be involved. Furthermore, reagents for identification and characterization of immunoglobulins or lymphoid cells and
their subsets in the jird are not readily available. This species do not have genetically defined strains.

Given the extensive knowledge of immunogenetics, the mouse would be the ideal animal for any investigation of the role of the immune system in the immunopathogenesis of a disease and thus have increasingly been used as subjects in studies of lymphatic filariasis (Lawrence 1996). *W. Bancrofti* is responsible for 90% of the world’s lymphatic filariasis but unfortunately will not survive either in intact mice or in the immunocompromised mice tested so far. Although intact mice are refractory to the full developmental cycle of *Brugia sp.*, each stage can survive for limited periods of time. Adult male and female worms can be implanted in the peritoneal cavity and survive for around 90 days. The female worms continue to produce MF under these conditions. In addition, MF alone injected by i.p. route will survive for at least 28 days and if injected by i.v. route, they will circulate in the blood stream for approximately 65 days. Although L3 of *B. malayi* (injected either by i.p. route or by s.c. route) will not normally survive for longer than 10 days, athymic and severe combined immunodeficiency (SCID) mice are susceptible to full infection with the development of MF-producing adults following L3 injection by either route. Adult worms implanted into athymic mice will also survive longer than in intact mice. L3 of Brugia species inoculated into normal mice, however, die before reproducing and patency does not occur. The utility of mouse model is thus diminished by the interrupted parasite life cycle and any demonstrable pathology in the host, leaving the model with few measurable indicators for comparison with Brugian filariasis in humans (Lawrence 1996).

Animal model, like cat, have provided the strongest evidence that protective immunity can be induced by attenuated larvae and by repeated infection (Oothuman *et al.* 1979; Denham *et al.* 1983). Cats, dogs, and ferrets infected with *B. pahangi* also display a range of associated pathological symptoms. Cats that kill adult worms, in contrast to those that tolerate the parasites have good IgE responses to filarial worms.
Rats are immunologically better understood and have inbred strains. They have been used as experimental models for brugian filariasis. In them, full development of the life cycle, from L3 to MF occurs with long-term patency of the latter. The features of the infectivity/latency patterns in rats are compared with recognized patterns observed in human populations. Thus, rats provide a valuable and may be underutilized model for the experimental analysis of filarial infections (Bell et al. 1999).

**EPIDEMIOLOGY**

Lymphatic filariasis is known to occur in 73 countries in the world out of which 38 are in the African Region, 7 are in the Region of the Americas, 4 are in the Eastern Mediterranean Region, 8 are in the Southeast Asia Region, and 16 are in the Western Pacific Region (Table 1). The highest number of infected persons are found in the Southeast Asia Region, with India alone accounting for 45.5 million (~38% of the global burden) (Ottesen et al. 1997) and about one third of the Indian population is exposed to the risk of Bancroftian filariasis (WHO 1992).

Bancroftian filariasis is a major cause of clinical morbidity and social handicap in many countries in Asia, East Africa, Latin America, the Middle East, the Pacific Rim, and the Pacific islands. Brugian filariasis is restricted to Asia and parts of the South Pacific (Ottesen et al. 1997; Addiss 1998), it is most highly endemic in India and China (32% and 20%, respectively, of the global burden); it is also prevalent in Indonesia, Thailand, Malaysia, Philippines, Viet Nam and Republic of Korea (Ottesen et al. 1997).

While there is no direct evidence demonstrating the existence of protective immunity to *W. bancrofti* infection in humans, the presence of a large number of individuals, with no clinical evidence of past or current infection despite appreciable exposure to the infective larvae, in areas where infection is endemic suggests that protective immunity to filarial parasites may...
Fig. 4 The relationship of the host immune response and the consequences of infection (King and Nutman 1991)
occur naturally (Freedman et al. 1989; Raghavan et al. 1994). Human filariasis is a spectral disease: one represents MF carriers (microfilaraemic patients, with high parasite numbers and down-regulated cell-mediated responses) and the other represents patients with elephantiasis and other chronic lesions (who typically have few parasites and vigorous specific immune reaction).

Clinical Manifestations

Lymphatic filariasis is manifested by a spectrum of symptoms that range from MF carrier state to chronic state with gross immunopathology. The variations in the clinical symptoms seen in this disease have been explained by heterogeneity in host responses (Bundy et al. 1991). The different clinical spectra of lymphatic filariasis are described below:

Asymptomatic microfilaraemia: In all endemic areas a proportion of the population does not show microfilaraemia or clinical manifestations of the disease although these individuals have the same degree of exposure to infective larvae as those who become infected. These individuals are termed as 'endemic normals' (EN). Now a days with the help of diagnostic aids like Og4C3 ELISA (More and Copeman 1990) / AD12 ELISA (Weil et al. 1987) it is seen that some of the EN are positive for the presence of CFA. Thus, these individuals are again subdivided into two categories: the 'true' endemic normals (MF -ve, CFA -ve) and the antigen positive endemic normals (MF -ve, CFA +ve). So for all the immunological studies the 'true' endemic normals are considered as Endemic normals (EN).

Asymptomatic Microfilaraemia: These groups of individuals are those with microfilaraemia but without clinical manifestations of filariasis. A considerable proportion of these persons remain microfilaraemic and asymptomatic for years- in some instances for life. Some individuals in this group spontaneously become microfilaraemic, especially when they move to areas that are not endemic for filariasis. In recent times, modern diagnostic techniques like
lymphoscintigraphy and ultrasonography have revealed that almost all the infected asymptomatic individuals had marked, abnormal, dilated lymphatics with dramatically abnormal patterns of lymph flow. Even though majorities of the infected persons are asymptomatic, but virtually all have subclinical lymphatic damage and approximately 40% have renal involvement (proteinuria and hematuria) ((Dreyer et al. 1992; Amaral et al. 1994; Suresh et al. 1997). It has thus become clear that infections are by no means benign, and that patients should be treated as early as possible to prevent or limit irreversible damage to the lymphatic system (WHO 1995; Suresh et al. 1997).

**Acute manifestations:** The acute clinical manifestations of lymphatic filariasis are characterized by episodic attacks of lymphadenitis and lymphangitis associated with fever and malaise. Sometimes the fever precedes the adenolymphangitis by a few days.

Acute bancroftian filariasis may commence with malaise and fever, followed by lymphadenitis in the groin or armpit and a typical retrograde lymphangitis. In most endemic areas, such as: Africa, India, Indonesia, and the Pacific area, the lymphatics of the male genitalia are frequently affected, leading to funiculitis, epididymitis, and orchitis. The spermatic cord becomes thickened and rope-like, and is painful on palpation. The acute attacks of adenolymphangitis, etc. may last for 3-15 days, and may occur several times a year in the same individual.

In acute brugian filariasis, lymphadenitis occurs at intervals, with fever, chills, and other constitutional symptoms. The patient may be incapacitated for a few days or may remain ambulatory. With rest the symptoms often subside spontaneously. At times, Lymphadenitis is followed by a characteristic retrograde lymphangitis. There is an induration around the affected lymphatics, which may spread to the surrounding tissues and occasionally involves the whole thigh or the entire lower limb. The affected lymph vessel feels cord-like and tender. At this stage, there is often lymphoedema of the foot and ankle. In most cases the lymphadenitis occurs in the inguinal region on one side, and
there is lymphangitis on the medial side of the limb and foot on the same side. The frequency of episodic lymphadenitis varies from 1-2 attacks per year to several attacks per month. The acute clinical course, including complications, may last from 3 weeks to 3 months.

**Chronic manifestations:** The chronic stage of filariasis usually develops in 10-15 years from the onset of the first acute attack. Hydrocoele, elephantiasis, and chyluria are the main characteristic features of the chronic bancroftian filariasis. Elephantiasis begins as lymphoedema. The leg(s), scrotum, arm(s), penis, vulva, and breast(s) are affected usually in that order of decreasing frequency. In most countries males are more often affected than the females. In brugian filariasis the characteristic sites for elephantiasis are the leg(s) below the knee(s), or less frequently the arm(s) below the elbow(s), although the lymph nodes affected during the acute stage are usually located in the inguinal or axillary regions. In most cases only the foot and the distal part of the lower leg are affected. Genital involvement and chyluria have not been reported, except in areas where brugian filariasis occurs together with bancroftian filariasis

**Tropical Pulmonary Eosinophilia (TPE):** TPE is a severe form of allergic asthma caused by the host inflammatory response to filarial helminths (acute hypersensitivity reaction to MF of *W. bancrofti* and *B. malayi*) in the long microvasculature (Neva and Ottesen 1978; Pinkston et al. 1987). TPE is a syndrome that occurs in both adults and children and is more common in males. This syndrome is characterized by nocturnal paroxysmal cough, hypereosinophilia, elevated erythrocyte sedimentation rate (ESR), radiological evidence of diffuse miliary lesions or increased bronchovascular markings (especially at the bases of the lungs), extremely high titers of filarial antibody (including IgE), and a good therapeutic response to DEC. As the name suggests, the clinical features mainly affect the respiratory system. In many cases the lung function is impaired with a reduction in the vital capacity, total lung capacity, and residual volume. Although spontaneous remission occurs,
TPE if untreated tends to relapse and progress to a condition of chronic pulmonary fibrosis. Chronic pulmonary fibrosis during which the lung gets badly scarred, resulting in loss of lung function with destruction of the alveolar epithelium (Udwadia 1993). The interaction of filarial parasitic protein with the lung epithelium affects the proliferative potential of the lung epithelial cells in vitro, and causes a drastic change in morphology (Maya et al. 1995). The direct interaction of filarial proteins with lung epithelial cells causes necrosis via an early induction of proto-oncogene c-H-ras and TNF-α expression (Maya et al. 1997).

**Diagnostic Options For Filariasis**

Diagnosis of filariasis is important for the identification of infected individuals and for studies on epidemiology, protective immunity immunopathology, and control. Efficient diagnosis of *Wuchereria bancrofti* infection is especially important as control programs move towards the new strategy of community diagnosis and repeated annual mass therapy with single-dose or combination regimens. Diagnosis requires highly specific tests based on detection of circulating MF, circulating filarial antigen (CFA), and/or antibodies. Several methods are available for diagnosis of bancroftian filariasis (Table 2) and have been reviewed (Chandrasekhar 1997; Nicolas 1997; Weil et al. 1997).

**Microfilariae detection**

Detection of MF in blood, apart from clinical examination, remains the only diagnostic test available for filariasis in most endemic areas, apart from clinical examination. However, for this blood needs to be sampled at night when MF is present in the peripheral vascular system (nocturnal periodicity). This problem is not encountered in regions where *W. bancrofti* is sub-periodic.

Two methods, based on microscopic examination of blood samples, are routinely used: (1) the sampling of 20-60μl of capillary blood from finger-prick, followed by smearing into a glass slide (thick-film or blood smear method); and
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<th>Sensitivity</th>
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*NB. Semiquantitative ratings/opinions are based on the personal experiences of the authors, with '+' lowest and '++++' highest. Cost ratings are based on the cost of materials and requirements for expensive laboratory equipment.

bNo score assigned; highly variable.

cMf detection requires night blood samples in areas where Mf exhibit nocturnal periodicity. Additional testing is needed to determine the sensitivity of PCR for detection of parasite DNA in blood collected by day in areas with nocturnally periodic Mf. Preliminary results have shown that PCR can detect parasite DNA in some day blood samples, but signals are stronger with night blood.

(2) the sampling of venous blood, followed by filtration of 1ml of blood using a Nucleopore membrane and microscopic counting (membrane filtration method). Often, MF prevalence surveys are conducted using the finger-prick method, which is less invasive than venous blood sampling. However, the filtration method gives more reproducible results than the finger-prick tests, and has been proved to be more sensitive as well, especially for low-MF carriers, because of the higher volume of the blood sample used.

Monitoring adult worm populations
An important breakthrough in the monitoring of the presence of adult worms was the development of two monoclonal antibodies (mAbs), AD12 (Weil et al. 1987) and Og4C3 (More and Copeman 1990), which bind to W. bancrofti circulating filarial antigen(s) (CFA) in enzyme-linked immunosorbent assays (ELISAs). Ad12 recognizes a 200kd antigen, which originates from the adult worm (Weil et al. 1996), in sera of filarial patients (Weil and Liftis 1987).

Indirect evidence suggests that the antigen recognized by Og4C3 is also of adult worm origin (Chanteau et al. 1994). The Og4C3 antigen assay has been commercially available for a few years now as an ELISA kit that is sufficiently simple to be used in most endemic countries. The AD12 antigen assay is still under development. Beside, individual immunochromatographic cards (ICT) coated with AD12 or Og4C3 mAbs, have been developed recently, to detect W. bancrofti CFA. These could be convenient tools for direct assessment of parasitological burden:

PATHOLOGY
The pattern of filarial infections in human populations is typically spread over a wide spectrum ranging from individuals who appear free from infection or disease, through asymptomatic carriers, to patients with varying degrees of acute or chronic manifestations. There is good (albeit circumstantial) evidence that the pathology has an immunological origin and that in disease the immune system becomes hyper-reactive to filarial antigens (Ottesen 1980, 1984;
Partono 1982). The paradox of filariasis has always been the inverse association between parasite density, in terms of circulating MF in the blood, and severe pathology (Maizels and Lawrence 1991).

The pathogenesis of filarial disease is characterized by acute and chronic inflammation. Inflammatory responses are thought to be generated either by the parasite, the immune response or opportunistic infection. The presence of large number of *Wolbachia* bacteria in the parasite tissues in a variety of developmental stages suggests that, either on the death of the parasite or through a variety of secretory/excretory mechanisms, the release of bacteria and/or their products might contribute to the inflammatory pathology associated with human filarial disease. The most direct situation in which death of parasites leads to inflammatory responses is the adverse reaction to chemotherapy. The increase in proinflammatory cytokines and inflammatory mediators following treatment and the clinical presentation of fever, headache, lethargy, hypotension and so on, bear many similarities to responses induced by bacterial inflammatory mediators (Ravindran *et al.* 1997; Taylor and Hoerauf 1999; Hoerauf *et al.* 2000).

Invasion of man by the infective-third stage larvae (L3) usually occurs without causing any symptoms. Only after moultng to L4 and young adults (1-3 months later) do these lymphatic-dwelling filariae begin to induce local inflammatory reactions. While most of the pathology associated with infection occurs around adult worms in the lymph nodes and afferent lymphatic vessels, some pathology may develop elsewhere, either around MF cleared from the blood or around adults located in ectopic sites. Such pathological changes have cell-mediated, humoral, and foreign-body components, but little is known about the factors that modulate the sequence and intensity of these reactions since very few direct observations have been made on human tissue. In general, the pathology for all three species in man is similar except that genital and renal lymphatic involvement is limited almost exclusively to *W. bancrofti* infections.
Specific Pathological Lesions (WHO, 1984)

Lymphadenopathy: The lymph nodes which are usually affected are epitrochlear, cervical, suprachlavicular, axillary, antecubital, inguinal, pelvic, and abdominal lymph nodes. The nodes enlarge, become tender or painful, and are discrete or matted, but not attached to skin. In man the earliest stages of infection have not been studied, but in cat following inoculation, it has been shown that L3 penetrate a lymphatic vessel, migrate to the nearest node and provoke both cell-mediated and humoral responses. The larvae then descend through afferent lymphatic vessels where they mature to adults and produce MF. Nodes with or without worms have distended sinuses, and sinuses may be enlarged to the point of forming 'lake-like' expansions containing proteinaceous fluid.

Lymphangitis: Inflamed, dilated, and varicose lymphatics have long been recognized as a feature of filariasis. Degenerating worms in the lymphatics provoke a necrotizing granulomatous response identical to that in lymph nodes.

Genital Lesions (funiculitis, epididymo-orchitis, and hydrocoele): Filarial funiculitis is filarial lymphangitis of the spermatic cord with surrounding inflammation of adjacent connective tissue. Varicocoeles commonly develop after attacks of funiculitis.

Epididymitis usually accompanies funiculitis and is also primarily lymphangitis. The epididymis becomes large, smooth, soft, and tender. Degenerating worms are common in the fibromuscular tissue of the cord and in the lymphatics of the tunica vaginalis.

Filarial orchitis is characterized by a boggy oedematous testis- probably from oedema and inflammation of the tunica and adventitia rather than inflammation of the testis itself.

Hydrocoele is the most common genital manifestation of chronic bancroftian filariasis. Pathologically, it is characterized by a distended, generally thickened tunica vaginalis with hyalinization and fibrosis of the subserosal...
layer, disorganization of the muscle layers, lymphoid and foreign-body giant cell infiltration, in extreme cases, calcification.

**Lymphoedema and Elephantiasis:** As a result of the associated lymphatic obstruction, lymphoedema (which initially is usually transient) and elephantiasis develop progressively. Affected tissues first become edematous and then characteristically develop proliferative dermal changes with subsequent dermal and subcutaneous fibrosis. Increased numbers of mast cells have also been described in this elephantiasis tissue. The external genitalia may be affected and may have verrucous changes of the epidermis.

**Tropical Pulmonary Eosinophilia (TPE):** TPE is a rare manifestation of filariasis and is characterized by clinical and immunological hyperresponsiveness. Histopathological findings during the early weeks of clinical symptoms show histiocytic infiltration of the alveolar spaces and interstitium, followed by bronchopneumonia and eosinophilic abscesses. The final, irreversible phase of the pulmonary pathology in this condition is a chronic interstitial fibrosis.

**Other Pathology:** Chyluria may develop in patients with bancroftian filariasis following obstruction of renal or abdominal lymphatic vessels. Such blockage results in the drainage of lymph into the urinary collecting system usually at the level of the renal pelvis or bladder.

It has been recently reported that bacterial lipopolysaccharides (LPS), present in the soluble extracts of human filarial parasites B. malayi induce potent inflammatory response, including TNF-α, IL-1β, and nitric oxide from macrophages (Taylor *et al.* 2000). Experimental evidences suggest that the source of the bacterial LPS in the extracts of B. malayi is the *Wolbachia* endosymbionts. Thus, the presence of symbiotic *Wolbachia* in all of the major pathogenic filariae of humans (Taylor and Hoerauf 1999) and the apparently exclusive induction of proinflammatory responses by bacterial LPS in B. malayi extracts suggest that *Wolbachia* LPS may be one of the major mediators of inflammatory pathogenesis in filarial nematode disease (Taylor *et al.* 2000).
PREVENTIVE AND CONTROL MEASURES

Chemotherapy: Diethylcarbamazine (DEC) is the drug of choice and has been available for treatment of filarial disease for almost 50 years and is still used today. It destroys larval worms and even some of the adult worms but has drawbacks if used in heavy infections, when the destruction of large numbers of worms may cause severe side effects. TDR-sponsored studies have shown that a single dose of DEC (6mg/kg/day x 1) achieves the same result as the long-recommended two-week course (6mg/kg/day x 14).

Other drugs effective against lymphatic filariasis are ivermectin (IVM) and albendazole (Ottesen et al. 1999, 2000). Ivermectin is a safe and effective drug for the treatment of lymphatic filariasis infection (Chodakewitz 1995). The single dose treatment, especially when given at a dosage of 200μg kg⁻¹ or higher, results in a drastic and sustained decline in levels of microfilariae (Cao et al. 1997). In recently designed strategies, it is proposed that ivermectin be co-administrated with either DEC or albendazole, mainly to enhance the effect on the adult parasite (Ottesen et al. 1997, 1999). For such combination regimens, it is anticipated that annual treatment will lead to elimination of LF within four to six years. This expectation is based on the assumption that the fecund lifespan of the adult worms is four to six years and that community treatment will lead to interruption of transmission (Plaisier et al. 2000).

Chemotherapy schedules for bancroftian filariasis have become much more convenient to use and WHO therefore currently emphasizes chemotherapy for elimination of this disease, with vector control playing only a supplementary role, if any (Ottesen et al. 1997). In a recent study on ‘can vector control play a useful supplementary role against bancroftian filariasis?’ Maxwell et al. (1999) reported that the cost and effort of achieving the reduction in mosquito population could hardly be justified for its impact on filariasis alone, but its noticeable impact on biting nuisance might help to gain community support for an integrated programme. The advent of new and easy-
to-implement control strategies for filariasis, such as annual, single-dose mass chemotherapy with antifilarial drugs, and the recent World Health Assembly resolution to eliminate LF as a public health problem, have generated renewed hopes for the control of this disease.

**Wolbachia as a target for chemotherapy**

The present antifilarials like DEC, IVM and albendazole are targeted at mature MF and have low macrofilaricidal activity (Ottesen et al. 1977; Ottesen et al. 1999; Plaisier et al. 2000). Many of the adult parasites, therefore, survive after the chemotherapy and lead to reappearance of MF several months after treatment. Thus, there has been a pressing need for new antifilarial drugs that have macrofilaricidal efficacy and/or that show total and long-lasting suppression of embryo production to complement currently available microfilaricides. The *Wolbachia* endosymbionts in the filarial parasites have been found to provide a novel target for antibiotic-based chemotherapy. Evidence from work on animals have showed that tetracycline treatment leads to degeneration and sterility of adult filarial worms. Tetracycline treatment had no effect, however, on the development and fertility of *A. vitae*, which are free from *Wolbachia* suggesting that the antibiotic has no direct action on the nematodes (Bosshardt et al. 1993; Bandi et al. 1999; Hoerauf et al. 1999). Recently, effectiveness of targeting *Wolbachia* in human onchocerciasis with respect to worm fertility and survival have been investigated (Hoerauf et al. 2000). With doxycycline (Vibramycin, Pfizer; 100mg orally daily) for 6 months, it has been shown that this antibiotic lead to sterility of adult worms (*O. volvulus*) to an extent not seen with drugs used against onchocerciasis. It has been found that doxycycline totally suppresses normal embryonic development during the early stage, i.e. the oocyte/morula stage. It targets metabolic pathways unique to *Wolbachia* and therefore causes little harm to the mammalian hosts (Hoerauf et al. 2000).
Penicillin, gentamycin, erythromycin, azithromycin, ciprofloxacin and chloramphenicol were found to be ineffective against *Wolbachia* in the *L. sigmodontis* model and did not display antifilarial activity. However, rifampicin showed clear prophylactic activity in *L. sigmodontis* and *B. pahangi* infection and onchocerciasis. The antifilarial properties of rifampicin merit further investigation and the efficacy of a combination treatment (tetracycline/rifampicin) and antibiotics plus antifilarial treatment needs to be evaluated, as the recent information on *Wolbachia* offers a different view of filariasis- as a disease caused by both nematode and bacterial parasites (Taylor *et al.* 1999, 2000).

**IMMUNOLOGY**

In the zones of high endemicity of lymphatic filariasis, the population can be broadly grouped into 3 categories on the basis of their clinical and parasitological status viz. endemic normal (EN), asymptomatic microfilarial carrier (ASM) and chronic filarial patients (CH). One major approach therefore has been to contrast the immune status of the patients with each other, and against the set of individuals exposed to transmission but displaying no outward sign of infection, the so-called EN. A better understanding of the pathogenic mechanisms of lymphatic filariasis could form the basis of new therapeutic interventions to prevent the debilitating consequences of this disease.

The principal difficulty in analyzing filariasis is that of defining who is actively infected with living adult worms. The MF carriers can be distinguished from EN (or normals harboring cryptic infections) (Day 1991) by screening for circulating antigen (in case of Bancroftian filariasis) (Forsyth *et al.* 1985) or IgG4-specific ELISA antibody test (in case of Brugian filariasis) (Kurniawan *et al.* 1993). High levels of antiparasite IgE (Hussain *et al.* 1981) and IgG4 antibodies (Ottesen *et al.* 1985) are produced in filarial patients, generally
accompanied by eosinophilia. Since IgG4 and IgE are often directed at the same determinants, the ratio between the two isotypes will be a major factor in determining whether antigen triggers an IgE-mediated hypersensitivity response or whether or not excess IgG4 can act as an antiallergic blocking antibody (Maizels et al. 1995).

Infections with parasites are often characterized by polarized T-cell responses that control the development of either immunity or pathology. T cells (Thymus derived peripheral blood mononuclear cells, PBMC) play a central role in orchestrating cell-mediated and humoral immune responses to viral, microbial and parasite antigens through release of specific cytokines. CD4+ T cells (T helper, Th) appear to act on cells mostly via the production of secreted cytokines (Aebischer and Stadler 1996). Th1 cells produce interferon-gamma (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor-β (TNF-β) and they are responsible for both humoral and cell-mediated immune responses (antibody production of the IgG2a class (in mouse), macrophage activation, antibody dependent cell cytotoxicity and delayed-type hypersensitivity- DTH). Th2 cells produce IL-4, IL-5, IL-6 and IL-13, and they provide optimal help for humoral immune responses. These include IgE and IgG1 (in mouse) or IgG4 (in man) isotype switching and mucosal immunity through production of mast cell and eosinophil growth and differentiation, facilitation to IgA synthesis (Mosmann and Coffman 1989). Cytokines regulate both the initiation and maintenance of the immune response and they select the type of immune response and the effector mechanisms that mediate resistance to pathogens. The Cytokine milieu present during the priming of naive T cells determines the ultimate fate of differentiation path that is followed. The early presence of IL-4 at an early stage is the most potent stimulus for Th2 differentiation; conversely IL12 and/or IFN-γ favour Th1 development (Nakamura et al. 1997).

Like other helminth parasites, human filarial infection is associated with Th2-cell immune response: eosinophilia, immunoglobulins IgE, IgG4 and IL-4 production (Maizels et al. 1995). The absence of circulating filarial antigens in
TABLE 3: SOURCES OF TYPE 1 AND TYPE 2 CYTOKINES

<table>
<thead>
<tr>
<th>Sources</th>
<th>Type 1 Cytokines</th>
<th>Type 2 Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T lymphocytes</td>
<td>IL-2, IFN-γ, IL-12, TNF-β</td>
<td>IL-4, IL-5, IL-6, IL-10, IL-13</td>
</tr>
<tr>
<td>CD8+ T lymphocytes</td>
<td>IL-2, IFN-γ</td>
<td>IL-4, IL-5, IL-10</td>
</tr>
<tr>
<td>NK cells</td>
<td>IFN-γ, TNF-β</td>
<td></td>
</tr>
<tr>
<td>Monocytes/Macrophages</td>
<td>IL-12</td>
<td>IL-6, IL-10</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>IL-12, TNF-β</td>
<td>IL-6, IL-10</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>IL-12</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td></td>
<td>IL-4, IL-5, IL-6</td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td>IL-4, IL-5, IL-6</td>
</tr>
</tbody>
</table>

Fig. 5 Source: Beckman Coulter, Inc., 1998.
Bancroftian filariasis has been shown to be associated with a Th-1 type response (production of IL-2 and IFN-γ cytokines), while individuals with antigenemia have high levels of specific IgG4 antibody (WHO 1997).

**FILARIAL GENOME**

A special project, named the Filarial Genome Project (FGP) has been initiated, in 1994, after a call from the WHO for genomics approaches to this disease. The project is core funded by the WHO Tropical Disease Research (TDR), and has attracted support from many other agencies. Filarial nematodes infect humans, domestic animals, and a range of wild vertebrates. All the filarial nematodes are closely related (Xie et al. 1994, Raj et al. 1996, Dalai et al. 1998), and so intensive study of the genome of one tractable species should serve as a model for the others. *Brugia malayi* can undergo its entire life cycle in the laboratory and, therefore has been chosen as the model for such study (Williams 1999; Williams et al. 2000).

The FGP has four main goals:

1) To identify a large number of novel genes from new cDNA libraries from all life cycle stages of *B. malayi* parasite,

2) To construct genomic libraries and map the *Brugia* genome;

3) To devise and implement globally accessible databases for display, analysis and dissemination of filarial genome data; and

4) To establish and foster partnerships between laboratories in non-endemic and endemic countries to aid in the training of scientists from endemic nations in DNA sequencing, mapping and all aspects of genomics.

The FGP has to contend with three genomes: the nuclear, mitochondrial, and that of a bacterial endosymbiont, *Wolbachia* (Taylor and Hoerauf 1999). The nuclear genome of *B. malayi* is organized as five pairs of chromosomes: four autosomal and one XY sex determination pair (Sakaguchi et al. 1982). The genome size is about 100 million base pairs (100Mb), about the same as that
of Caenorhabditis elegans. The FGP is building a physical genome map for B. malayi to assist in identification of genes and comparison with the genome of C. elegans. Sequence and mapping data provided by the FGP is being utilized by the nematode research community to develop a better understanding of the biology of filarial parasites and to identify new vaccine candidates and drug targets to get the elimination of human filariasis (Williams et al. 2000).

OBJECTIVE

Infection with W. bancrofti provides a range of immunologic responses in the human host, which manifests in a clinical spectrum. In this spectrum, the EN and ASM are two interesting categories; both these categories are asymptomatic, but contrastingly the ASM are microfilaraemic unlike the EN. Longitudinal observation of these two categories makes one to assume that there might be a component of protective immunity in the EN individuals as they do not harbour (or rather clear the infection) and get rid of the parasite; and experimental evidences support this concept of protective immunity, even though it is not clearly established.

Non-availability of well-characterized parasite antigens is a major handicap in understanding the differential immune response in LF. In a study of leprosy patients Yamamura et al. (1991) have suggested that protective immune response to the pathogens can be defined by measuring the cytokine profile in different clinical groups. In a study by Dimock et al. (1994) using SDS-PAGE fractionated antigens of B. pahangi adults it was found that antigens of different molecular weights induce differential proliferation and secretion of IL-10 in lymphocytes of individuals with chronic lymphatic dysfunction. Dalai et al. (1998) also have shown that Setaria digitata SDS-PAGE fractionated adult soluble antigens induce differential Th1/Th2 response in lymphocytes of ASM and EN individuals. In all these studies, the antigens from the animal parasites have been used. These studies prompted us to dissect the mechanism of
differential immune response (or the protective immune response in EN individuals) by using antigens from the human filarial parasites. Out of the different life cycle stages the adults spend the longest period of time in the human body, and the adults (and the MF they produce) modulate the immune response of the host to a great extent. Among the human filarial parasites, the \textit{B. malayi} parasites are unmatched for a steady supply of antigenic materials. Keeping the above facts in view, we have used the \textit{B. malayi} adult soluble antigens (fractionated and partially characterized) to dissect the mechanism of differential immune response in ASM and EN individuals. The present study was carried out with the following objectives:

1. To identify the antigens, from soluble antigen preparations from adult \textit{B. malayi} parasites (BmA), those are differentially recognized by EN/ASM sera and subsequently, to fractionate the BmA. These fractionated antigens, SDS-PAGE-separated and NCP-bound, would be used to test their ability to induce a differential Th1/Th2 cytokine response in lymphocytes of the EN/ASM individuals.

2. To study the induction of secretory cytokines, internal cytokines and expression of cell surface molecules/receptors in the PBMC culture of EN/ASM individuals, stimulated with fractionated BmA.