REVIEW

# Immunopathogenesis of lymphatic filarial disease

**Subash Babu · Thomas B. Nutman**

**Abstract** Although two thirds of the 120 million people infected with lymph-dwelling filarial parasites have subclinical infections, ~40 million have lymphedema and/or other pathologic manifestations including hydroceles (and other forms of urogenital disease), episodic adenolymphangitis, tropical pulmonary eosinophilia, lymphedema, and (in its most severe form) elephantiasis. Adult filarial worms reside in the lymphatics and lymph nodes and induce changes that result in dilatation of lymphatics and thickening of the lymphatic vessel walls. Progressive lymphatic damage and pathology results from the summation of the effect of tissue alterations induced by both living and nonliving adult parasites, the host inflammatory response to the parasites and their secreted antigens, the host inflammatory response to the endosymbiont *Wolbachia*, and those seen as a consequence of secondary bacterial or fungal infections. Inflammatory damage induced by filarial parasites appears to be multifactorial, with endogenous parasite products, *Wolbachia*, and host immunity all playing important roles. This review will initially examine the prototypical immune responses engendered by the parasite and delineate the regulatory mechanisms elicited to prevent immune-mediated pathology. This will be followed by a discussion of the proposed mechanisms underlying pathogenesis, with the central theme being that pathogenesis is a two-step process—the first initiated by the parasite and host innate immune system and the second propagated mainly by the host's adaptive immune system and by other factors (including secondary infections).

**Keywords** Filariasis · Pathology · Lymphedema · Hydrocele · Cytokines · Immunity

## Introduction

The term "lymphatic filariasis" encompasses infection with three closely related nematode worms—*Wuchereria bancrofti, Brugia malayi,* and *Brugia timori*. All three parasites are transmitted by the bites of infective mosquitoes and have quite similar life cycles in humans with the adult worms living in the afferent lymphatic vessels while their progeny, the microfilariae, circulate in the peripheral blood and are available to infect mosquito vectors when they feed. Though typically not fatal, lymphatic filarial disease is responsible for considerable suffering, deformity, and disability and is the second leading parasitic cause of disability with disability-adjusted life years estimated to be 5.549 million [1, 2]. Bancroftian filariasis, caused by *W. bancrofti*, is responsible for 90 % of those with lymphatic filariasis and is found throughout the tropics and some sub-tropical areas [3]. The rest are caused by Brugian parasites that have a more restricted geographical distribution. Lymphatic filariasis is a global health problem. At the present time (2012), the World Health Organization estimates that over 1.25 billion people are at risk in 72 countries and territories. Approximately 120 million people already have been infected with lymphatic filariasis and over 40 million are seriously incapacitated or disfigured by the disease. Clinical disease is manifested primarily as acute and chronic

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lymphedema, which may lead to elephantiasis in men and women and to the formation of hydroceles in men.

All human filarial nematodes have a complex life cycle involving an insect vector, with Wuchereria and *Brugia* being transmitted by mosquitoes. Infection begins with the deposition of infectious-stage larvae or L3 larvae in the skin during a mosquito bite. The larvae then crawl in through the puncture wound and enter into the lymphatics and lymph nodes. They undergo a process of molting and development to form L4 larvae and then adult worms. The adult worms reside within the lymphatics and lymph nodes and following mating release live progeny called microfilariae (mf), which circulate in the bloodstream. These microfilariae can then be ingested by a mosquito during a blood meal, where in they undergo development to form L2 and finally L3 larvae and the life cycle continues. The complex life cycle engenders a complicated host immune response, and it is this complexity of the host–parasite interaction that is thought to underlie the varied clinical manifestations of lymphatic filariasis.

**Clinical manifestations**

Lymphatic filariasis can manifest itself in a variety of clinical and subclinical conditions. Traditionally, it has been accepted that people living in an endemic area can be classified into five groups: (1) uninfected but exposed; (2) clinically asymptomatic, infected; (3) those with acute filarial disease with or without microfilaremia; (4) those with longstanding chronic infection associated with pathological conditions; and (5) those with tropical pulmonary eosinophilia (TPE).

Uninfected, but exposed individuals (asymptomatic amicrofilaremia or endemic normals)

In endemic areas, a proportion of the population remains uninfected despite exposure the parasite [3]. This group has been termed endemic normals. The incidence of endemic normals in a population ranges from 0 % to 90 % in different endemic areas [3].

Subclinical (or asymptomatic) patent infection (with or without microfilaremia)

In areas endemic for lymphatic filariasis, many individuals exhibit no symptoms of filarial infection and yet, on routine blood examinations, demonstrate the presence of significant numbers of parasites or the presence of circulating parasite antigen (a surrogate for viable adult worms). These individuals are carriers of infection (and for those that are microfilaria+the reservoir for ongoing transmission). The parasite burdens in these individuals can reach dramatically high

numbers exceeding 10,000 microfilariae in 1 ml of blood. With the availability of imaging techniques (e.g., ultrasound, lymphoscintigraphy, MRI, CT), it has become apparent that virtually all persons with microfilaremia have some degree of subclinical disease. These include marked dilatation and tortuosity of lymph vessels with collateral channeling, increased flow, abnormal patterns of lymph flow [4, 5]; scrotal lymphangiectasia [6, 7]; and microscopic hematuria and/or proteinuria [8]. Thus, while apparently free of overt symptomatology, the subclinical patently infected individuals clearly are subject to subtle pathological changes.

Acute clinical disease

The acute manifestations of lymphatic filariasis are characterized by recurrent attacks of fever associated with the inflammation of lymph nodes (lymphadenitis) and lymphatics (lymphangitis) [9]. In brugian filariasis, episodes of fever, lymphadenitis, and lymphangitis are common, while bancroftian filariasis presents more insidiously with fewer overt acute symptoms [10]. The lymph nodes commonly involved are the inguinal, axillary, and epitrochlear nodes and, in addition, the lymphatic system of the male genitals are frequently affected in *W. bancrofti* infection leading to funiculitis, epididymitis, and/or orchitis [11]. It has been proposed that there are at least two distinct mechanisms involved in the pathogenesis of acute attacks. The more classical is acute filarial adenolymphangitis, which is felt to reflect an immune-mediated inflammatory response to dead or dying adult worms. The striking manifestation is a distinct well-circumscribed nodule or cord along with lymphadenitis and retrograde lymphangitis. Funiculo-epididymoorchitis is the usual presenting feature when the attacks involve the male genitalia. Fever is not usually present, but pain and tenderness at the affected site are common [12]. The other has been termed acute dermatoto-lymphangitis, a process characterized by development of a plaque-like lesion of cutaneous or sub-cutaneous inflammation and accompanied by ascending lymphangitis and regional lymphadenitis. There may or may not be edema of the affected limbs. These pathological features are accompanied by systemic signs of inflammation including fever and chills. This manifestation is thought to result primarily from bacterial and fungal superinfections of the affected limbs [12].

Chronic pathology

The chronic sequelae of lymphatic filariasis develop years after initial infection [9]. In Bancroftian filariasis, the main clinical features are hydrocele, lymphedema, elephantiasis, and chyluria. The manifestations are hydrocele and swelling

of the testis and/or lymphedema of the entire lower limb, the scrotum, the entire arm, the vulva, and the breast [11]. In Brugian filariasis, the leg below the knee and the arm below the elbow are commonly involved but not the genitals. The development of pathology is thought to be dependent on the presence of the adult worm. Histologically, the worm elicits little reaction as long as it is alive; however, upon death of the adult worm, a granulomatous reaction ensues [13, 14]. The granulomas are characterized by macrophages (which develop into giant cells), plasma cells, eosinophils, neutrophils, and lymphocytes. There is endothelial and connective tissue proliferation with tortuosity of the lymphatics and damaged or incompetent lymph valves. This typically results in lymphatic dilatation and subsequently lymphatic dysfunction and compromise, leading to lymphedema. Early pitting edema can give rise to subsequent brawny edema with hardening of tissues and later hyper-pigmentation and hyper-keratosis with wart-like protuberances which, on histological examination, reveal dilated loops of lymphatic vessels within nodular lesions. Very important in the progression of these lesions is the fact that redundant skin folds, cracks, and fissures in the skin provide havens for bacteria and fungi to thrive and intermittently penetrate the epidermis to lead to either local or systemic infections. Sometimes, the skin over the nodules breaks down, causing the dilated lymphatic within to rupture and discharge lymph fluid directly into the environment, at the same time serving as a pathway for entry of microorganisms into the lymphatic [15].

In men, scrotal hydrocele is the most common chronic clinical manifestation of bancroftian filariasis [9, 13]. It is uncommon in childhood but is seen more frequently post-puberty and increases in incidence with age. In some endemic communities, 40–60 % of all adult males have hydroceles. Hydroceles are due to accumulation of edematous fluid in the cavity of the tunica vaginalis testis. Though the mechanism of fluid accumulation is unknown, direct ultrasonographic evidence indicates that in bancroftian filariasis, the scrotal lymphatics are the preferred site of localization of the filarial worms, and their presence may stimulate not only the proliferation of lymphatic endothelium but also a transudation of hydrocele fluid whose chemical composition is not dissimilar to serum. Chronic epididymitis and funiculitis can also occur. Chyloceles can also occur. The prevalence of chyluria (excretion of chyle) is very low.

Tropical pulmonary eosinophilia

Tropical pulmonary eosinophilia (TPE) is a distinct syndrome that develops in some individuals infected with *W. bancrofti* and *B. malayi* [16, 17]. The main clinical features include paroxysmal cough and wheezing that are usually nocturnal (and probably related to the nocturnal periodicity of microfilariae), weight loss, low-grade fever, adenopathy, and pronounced blood eosinophilia (~3,000 eosinophils/ul). Chest X-rays may be normal but generally show increased bronchovascular markings; diffuse miliary lesions or mottled opacities may be present in the middle and lower lung fields. Tests of pulmonary function show restrictive abnormalities in most and obstructive defects in half of the cases. Total serum IgE levels (10,000 to 100,000 ng/mL) and antifilarial antibody titers are characteristically elevated.

Other manifestations

Lymphatic filariasis has been associated with a variety of renal abnormalities including hematuria, proteinuria, nephrotic syndrome, and glomerulonephritis [18]. Circulating immune complexes containing filarial antigens have been implicated in the renal damage. Lymphatic filariasis may also present as a mono-arthritis of the knee or ankle joint [19].

## Prototypical immune response and immunoregulation

The canonical host immune response to filarial parasites is of the T helper 2 (Th2) type and involves the production of cytokines—IL-4, IL-5, IL-9, IL-10, and IL-13; the antibody isotypes—IgG1, IgG4, and IgE, and expanded populations of eosinophils, basophils, mast cells, and alternatively activated macrophages [20]. While the Th2 responses induced by filarial parasites is a stereotypical response of the host, its initiation requires interaction with many different cell types, most notably: (1) stromal cells; (2) dendritic cells and macrophages; (3) eosinophils; (4) mast cells; (5) basophils, and (6) epithelial and innate helper cells [20]. These in turn can induce and culminate in type-2 responses. Over time, with chronic infection, these prototypical type-2 responses are modulated by both adaptive and natural regulatory T cells, alternatively activated macrophages, eosinophils and likely other, heretofore, unidentified cell populations [21]. Pathways of immune clearance mediated by Th2 cells are more clearly defined in intestinal helminth infections than in systemic or tissue invasive helminth infections [20]. In the lymphatics and lymph nodes as well as in the circulation, filarial parasites are open to attack by the full range of host innate effectors, including macrophages, eosinophils, and neutrophils [22]. The ability of these cells to kill the parasites is often dependent on one or more isotypes of specific antibody (often IgE but also IgM) and complement [23–25]. Activated macrophages or granulocytes can release damaging nitrogen intermediates as well nitric oxide onto the surface of the parasites [26, 27], but in vivo killing methods are not yet fully understood. One of the most consistent

findings in filarial infections is the elevated level of IgE that is observed following exposure [28]. Most of the IgE produced is not antigen specific, perhaps representing nonspecific potentiation of IgE producing B cells or deregulation of a normally well-controlled immune response. Interestingly, these IgE antibodies persist many years after the infection has been treated, indicating the presence of long-lived memory B cells or plasma cells in filarial infections [29]. IgE production both in mice and humans is absolutely dependent on IL-4 or IL-13 [30]. Other isotypes that are commonly elevated in chronically filarial-infected humans are IgG4 and IgG1 [31], the former being most dependent on both IL-4 and IL-10.

Another hallmark of filarial infections is their chronic nature, with parasites surviving in the host for decades [32]. Chronic infections certainly reflect an adaptation that leads to "parasitism" in that causing mortality would prevent parasite transmission, if the host were to die before larval release or before egg production could occur. In addition to the long-lived nature of the infection, filarial parasites exist within a balanced host–parasite interface so that relatively asymptomatic carriers are available as reservoirs for ongoing transmission. When this balanced co-existence is interrupted, pathology—exemplified by elephantiasis associated with lymphatic filariasis—can ensue.

Filarial parasites exert profound immunoregulatory effects on the host immune system with both parasite-antigen specific and more generalized levels of immune suppression [33]. Three inter-related states of homeostasis and tolerance have been described to occur in filarial infections [20]. In immunosuppression, effector responses are dampened by immunoregulatory cytokines released by regulatory lymphocytes through different mechanisms. In immunological tolerance, effector Th2 cells enter a state of anergy and fail to develop specific T effector cells that would mediate resistance to infection. In the modified Th2 response, the downstream effects of normal Th2 responses is muted—including switching antibody production to the non-inflammatory isotype IgG4 (in humans) and induction of alternatively activated macrophages. It has been shown that patients with lymphatic filariasis have markedly diminished responses to parasite antigens [34] and in addition, some measurable attenuation in responses to bystander antigens and routine vaccinations [35]. Thus, while host immunosuppression is usually antigen-specific, chronic infection can be associated with some spillover effects. Among the mechanisms utilized by parasites to avoid immune-mediated elimination are those of suppression, regulation, or blockade of immune effector pathways [35].

Among the notable immune-evasion strategies, a key one is the secretion of products that modulate host immune function [36]. Phosphorylcholine (PC) is a small hapten-like moiety present in the excretory/secretory products of

many helminths and one particular PC containing molecule called ES-62 from filarial worms has been shown to have a wide variety of immunomodulatory properties [37]. Thus, ES-62 can inhibit the proliferation of CD4+ T cells and conventional B cells, decrease IL-4 and IFNγ production, can promote proliferation and IL-10 production by B1 B cells, and condition antigen-presenting cells to drive Th2 differentiation with concomitant inhibition of Th1 responses [37]. Similarly, filarial parasites produce cytokine- and chemokine-like molecules to interfere with the function of host innate immune products [TGF-β and macrophage migration inhibitory factors (MIF) homologs] [38].

Among the host factors influencing immunoregulation, the key players are the induction of regulatory T cells, modulation of effector T cells, and antigen-presenting cells and apoptosis of responder cells [33]. Evidence for the involvement of regulatory T cells in helminth-mediated downmodulation of the immune response has been accumulating in recent years [39]. IL-10 and TGF-β, both factors associated with regulatory T cells, are elicited in response to helminth infections and in vitro neutralization of IL-10 and TGF-β, at least partially restores T cell proliferation and cytokine production in lymphatic filariasis [34, 40]. Evidence from mouse models argues for a major role of CD25+ Foxp3+ Treg cells in immunity during filarial infections. In murine filarial infections, parasite survival is linked to Treg activity, and immunity to infection can be restored by Treg depletion [41]. Effector T cell responses can be turned off or modulated through a variety of mechanisms including through CTLA-4 and PD-1 [39]. Interestingly, increased expression of CTLA-4 and PD-1 has been demonstrated in filarial infections, and blocking of CTLA-4 can restore partially a degree of immunological responsiveness in cells from infected individuals [42, 43]. Moreover, T cells have decreased induction of T-bet, the Th1 master transcription factor, indicating a failure at the transcriptional level to differentiate into Th1 cells [44]. Finally, T cells from filarial-infected individuals exhibit classical signs of anergy including diminished T cell proliferation to parasite antigens, lack of IL-2 production, and increased expression of E3 ubiquitin ligases [43].

Filarial parasites induce downregulation of MHC class I and class II as well as cytokines and other genes involved in antigen presentation in dendritic cells, thereby rendering them suboptimal in activation of CD4+ T cells [45, 46]. Filarial parasite interaction with macrophages induces a population of macrophages preferentially expressing arginase instead of nitric oxide due to increased activation of arginase-1 by IL-4 and IL-13 [47]. These macrophages, termed alternatively activated macrophages, are characterized by their ability to upregulate arginase-1, chitinase 3-like proteins 3 and 4 (also known as YM1 and YM2, respectively), and resistin-like molecule-α (RELMα) [48, 49]. These

alternatively activated macrophages are known to be important in wound healing and have been postulated to play a potential role in repairing wound damage that occurs during migration of filarial parasites [50]. By virtue of expressing regulatory molecules such as IL-10, TGF-β, indoleamine 2,3 dioxygenase (IDO), and programmed cell death 1 ligand 2 (PDL2), these macrophages may also have a predominantly regulatory role in filarial infections [20]. Another mechanism of immune evasion is the ability of filarial parasites to induce host cell apoptosis. Apoptosis of CD4+ T cells has been demonstrated in vivo in the spleens of *Brugia*-infected mice [51]. In addition, *Brugia* microfilariae can interact with dendritic cells and NK cells and induce their apoptosis [46, 52]. Thus, host–parasite interactions can lead to a variety of immunological responses, not all of which lead to pathology or resistance to infection (Fig. 1).

## Pathogenesis of lymphedema – a two-step process

The most severe clinical manifestations of lymphatic filariasis are lymphedema and elephantiasis. Although the immune responses to filarial parasites have been well studied with respect to natural history, diagnosis, and treatment, there is a relative paucity of information in terms of the mechanisms underlying development of pathology. The two major independent components of lymphatic filarial disease are lymphangiectasia and inflammatory reactions around the adult worms (Fig. 2). While most infected individuals exhibit lymphangiectasia, clinically apparent lymphedema is not common [5, 13]. It is also clear that with patent infection, lymphangiectasia develops in the vicinity of adult worm nests [13]. Subclinical lymphangiectasia of the lymphatic vessels containing live adult worms have been shown to exhibit distention with no apparent inflammatory reactions in the vessel wall, with little or only a fleeting inflammatory response to living adult parasites [53]. Further, the fact that lymphangiectasia is not restricted to the exact segment of lymphatics where the worms reside [54, 55] suggests that this process is mediated by soluble products excreted or secreted by the parasite that act on the lymphatic endothelial cells. It is also clear that with the advent of adaptive immunity, the host inflammatory response against the dead or dying worm and the subsequent release of parasite products and inflammatory mediators, a stage of irreversible lymphatic dysfunction ensues [14, 56, 57]. This then manifests clinically as progressive lymphedema. In addition, lymphatic dysfunction has been shown to predispose infected individuals to secondary bacterial and fungal infections and trigger inflammatory reactions in the skin and subcutaneous tissue that accelerates the progression of lymphedema and precipitates the development of elephantiasis [58, 59]. This two-step model of pathogenesis is mirrored in chronically infected animals or immune reconstituted—immunodeficient animals with the development of reversible pathology initially and subsequent fibrosis and cellular hyperplasia in lymphatics [60–62].

The first major insight into the role of lymphatic damage in the pathogenesis of lymphatic filarial disease came from studies using Brugian infections of animals. Infection of normal or nude (lacking T cells) mice resulted in lymphangitis and perilymphangitis in both groups of mice with acute and chronic inflammation predominating in the former [61]. Interestingly, since normal mice are not permissive to infection, lymphangiectasia was observed to progress only in nude mice. While infection of nude mice was characterized predominantly by lymphangiectasia, reconstitution of these mice with spleen cells from normal mice (thereby restoring normal adaptive immunity) resulted in progressive fibrosis, obliterative lymph thrombus formation, interstitial infiltrates, and extensive perilympangitis [60]. Similarly, studies using SCID mice (lacking both T and B cells) showed that lymphangitis and lymphangiectasia were classical features of infection in the absence of adaptive immunity, and that reconstitution with spleen cells from normal mice resulted in progressive disease [62]. Finally, experimental infection of susceptible animal models including the Mongolian jird (*Meriones unguiculululatus*) and cats also suggest that early lymphatic pathology is dependent on the presence of live adult worms and that progression to irreversible disease is due to the host immune response to living or dying worms [63, 64]. For example, infection of cats with *Brugia* results in obliteration of afferent lymphatics and fibrous tissue formation in lymph nodes as well as collateral lymphatic
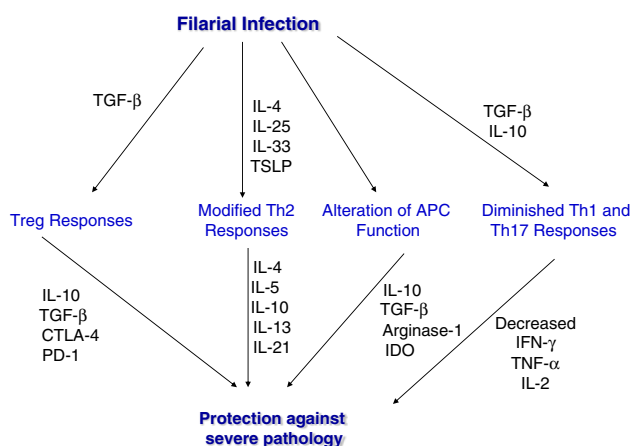
**Fig. 1** Regulation of the immune responses in filarial infections. The complex outcome of the interaction between the filarial parasite and the host immune system determines the immunological outcomes including protection against pathology. The host–parasite interaction involves a variety of cell types, cytokines, and other molecules that interact to influence the development of pathology. *Treg* regulatory T cell; *APC* Antigen-presenting cell; *TSLP* Thymic stromal lymphopoietin; *TGF-β* Transforming growth factor-β; *IDO* Indoleamine 2,3-Dioxygenase

**Fig. 2** Pathogenesis of lymphatic filarial disease. Live filarial parasites and/or their products have a direct effect on lymphatic endothelial cells as well as on the cells of the innate and adaptive immune system. The interplay between inflammatory/ immune mediators, slow attrition of the parasites, *Wolbachia* and other factors contribute to pathogenesis and development of filarial disease. Secondary microbial infections further aggravate the pathology. The clinical manifestations of filarial disease include lymphedema, hydrocele, and elephantiasis
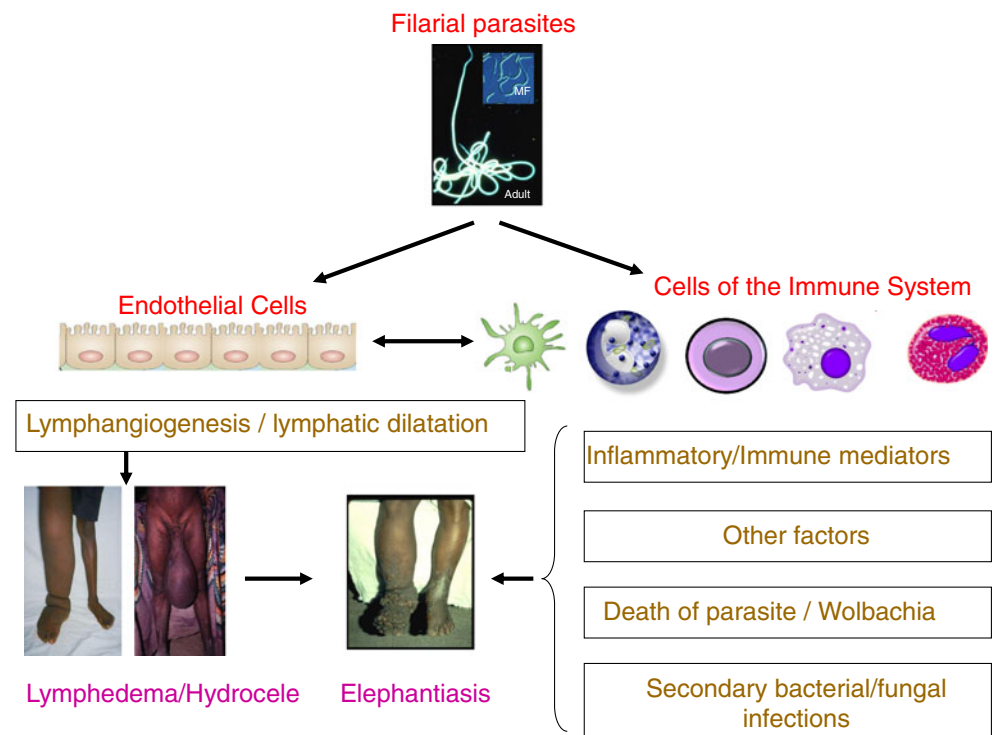
vessel formation [65]. Similarly, infections of dogs with *Brugia* results in limb edema, which is associated with increased spontaneous levels of histamine and prostaglandin E2 and increased filarial antigen-driven TNF-α [66]. Pro-inflammatory cytokines of innate origin also appear to play an important role in brugian infection since infection of nude mice results in elevated levels of IL-1, IL-6, TNF-α, and GM-CSF in lymph fluid [67]. In addition, migrating L3s in Mongolian jirds have been shown to elicit an acute inflammatory response characterized by elevated levels of IL-6 and TNF-α [68]. Therefore, innate cytokines appear to play a prominent role in the initiation of pathology in filarial-infected animal models. Studies in animals also implicate an important role for endothelial cells in pathogenesis of lymphatic dysfunction since these cells exhibit decreased numbers of vesicles (that presumably transport fluid) and increased numbers of vacuoles (that presumably are the result of cellular damage) upon chronic infection [69, 70]. However, more detailed studies on the role of endothelial cells in pathogenesis of filarial disease is lacking in animal models of infection.

**Pathogenesis of lymphedema—parasite products (including *Wolbachia*) and cells of the innate immune response**

The importance of pro-inflammatory cytokines, possibly of innate origin, in the pathogenesis of lymphedema, has been strengthened by a series of studies in humans with chronic

pathology, either in early or late stages or lymphedema. Studies have shown that individuals with chronic lymphatic pathology have elevated levels of C-reactive protein (an acute-phase protein, indicating an acute inflammatory response) [71], pro-inflammatory cytokines such as TNF-α, IL-6, and soluble TNF receptor [72, 73], endothelin-1 and IL-2 [74], as well as IL-8, MIP-1α, MIP-1β, MCP-1, TARC, and IP-10 [75] in the peripheral circulation. Similarly, while patients with both acute and chronic manifestations of LF have elevated circulating levels of IL-6 and IL-8, only those with chronic disease manifestations have elevated levels of sTNF receptors [73]. Very few studies have actually examined the inflammatory milieu within the affected lymphatics; one study has described elevated levels of gamma-globulins, α-1 acid glycoprotein, and IL-1β in the lymph fluid [76]. Monocytes and granulocytes are thought to be the predominant source of most of the above-mentioned pro-inflammatory cytokines. Despite this, very little is known about the regulation of monocytes and granulocyte function in filarial lymphedema.

Monocytes from patients with asymptomatic filarial infection exhibit hallmarks of alternative activation, with diminished expression of Nos2 and enhanced expression of Arg-1, along with increased expression of resistin, mannose receptor C type 1 (MRC-1), macrophage galactose type C lectin (MGL), and chemokine ligand 18 (CCL18) [77]. This is potentially the result of monocytes being primed under a predominantly type 2 cytokine milieu with high levels of IL-4 and IL-13, known to drive differentiation into alternative monocyte activation [78]. Interestingly arginase-1 expression serves not only as a marker for alternative

activation but also has other functions as inhibition of arginase-1 results in significantly diminished expression of the genes encoding resistin, MRC-1, MGL, and CCL18 [77]. However, no study to date has examined the activation phenotype of monocytes in individuals with filarial-induced pathology.

Filarial pathology is characterized by high levels of circulating immune complexes and immune complex-mediated granulocyte activation, including increased production of neutrophil granular proteins and pro-inflammatory cytokines [79]. In terms of other antigen-presenting cells, dendritic cell dysfunction has been shown to be a characteristic feature of exposure to filarial parasites [80]. Live mf have been shown to induce cell death in human dendritic cells, inhibit their ability to make IL-12 and IL-10, and reduce their capacity to activate CD4+ T cells [46]. Similarly, asymptomatic filarial infection is characterized by increased numbers of circulating myeloid dendritic cells (defined as Lineage-, HLA-DR+, CD11c+ cells) [81]. In addition, live L3s have also been shown to cause downregulation of MHC class I and II, IL-8, and multiple genes involved in antigen presentation in skin-resident Langenhans cells [45]. Again, no study has examined the role of dendritic cells in disease manifestations associated with LF. Thus, filarial infection without overt pathology is characterized by profound changes in the antigen-presenting cell compartment with potential to regulate adaptive immune function and protect against development of pathology.

Since the endothelium appears to be closely associated with pathogenesis of lymphatic disease, studies targeting the interaction between endothelial cells (vascular or lymphatic) and filarial parasites have been performed. The anatomical changes in the architecture of lymphatics that range from lymphangiectasia and granulomatous responses to the development of collaterals suggests that active lymphatic remodeling involving endothelial cell growth, migration, and proliferation is an important feature of early disease [82, 83]. Indeed, although earlier studies using blood vascular endothelial cells failed to demonstrate an effect of soluble somatic filarial antigens [84], a more recent study suggests that live filarial parasites (and their excretory/secretory products) induce activation, proliferation, and tube formation in lymphatic endothelial cells [83]. Moreover, only serum from patently infected or diseased individuals was shown to induce significant lymphatic endothelial cell (LEC) proliferation [85]. This pattern of lymphatic remodeling resembles the observations seen in vivo in immunodeficient mice.

Differentiation of LEC into tube-like networks was found to be associated with significantly increased levels of matrix metalloproteinases (MMPs) and inhibition of their endogenous inhibitors—TIMPs (tissue inhibitors of MMPs) [85]. Global gene expression analysis revealed alterations in genes involved in junction adherence pathways that

decreased trans-endothelial transport, implicating parasite-induced alterations in normal physiology of the lymphatic endothelium [85]. Recent studies have also implicated the vascular endothelial growth factor (VEGF) family in lymphangiogenesis [86]. It was recently shown that lymphatic endothelial-specific VEGF-C levels are significantly elevated in individuals with filarial disease [87]. Moreover, increased circulating levels of VEGF-C may not be confined to individuals with overt pathology since filarial-infected individuals with subclinical disease also exhibit elevated levels of this factor [88]. VEGF-C (along with VEGF-D) is a factor that specifically controls lymphangiogenesis by activating the VEGF receptor-3 (VEGF-R3), that is primarily expressed only in the lymphatic endothelium [89, 90]. Over-expression of VEGF in the skin of transgenic mice results in lymphatic endothelial proliferation and dilation of lymph vessels [91], processes resembling the lymphatic changes seen in filarial infections. Therefore, the observation that filarial-infected individuals (especially those with overt disease) have increased circulating levels of VEGF-C and its cognate receptor (VEGF-R3) suggests that VEGF-C/VEGF-R3 interactions are the principal mechanism of lymphangiectasia in filarial infections [87]. The other VEGF family member that has been implicated to play a role in filarial disease is VEGF-A. Elevated levels of VEGF-A and endothelin-1 have been observed in the serum of filarial-infected individuals, and more specifically, VEGF-A has been implicated to play a role in the development of hydrocele due to its ability to induce increased vascular density, enhance leukocyte adhesion, and promote lymphangiogenesis [92]. Thus, excess secretion of pro-inflammatory cytokines and angiogenic factors like VEGF-A could result in extravasation and accumulation of fluids, plasma, and lymph from the blood and lymphatic vessels into the scrotum resulting in the formation of hydrocele. Other angiogenic factors such as angiopoietins-1 and -2 are also found at elevated levels in individuals with filarial-induced pathology [88].

A major factor involved in the initiation of the pro-inflammatory response and the increased production of VEGF-A and C might be the endoymbiont, *Wolbachia*, present in most filarial nematodes (including *W. bancrofti* and the two *Brugia* spp.) [86]. It has been known for several decades that filarial parasites harbor the endosymbiotic bacteria of the order Rickettsiales [93]. These bacteria are found in the hypodermis of male and female adult worms as well as in the oocytes, embryos, and larval stages [94]. Initial studies demonstrated that the inflammatory responses induced by filarial parasites were mainly mediated by LPS-like activity from *Wolbachia* [95]. Thus, interaction of *Wolbachia* and its products with the pattern-recognition receptor TLR4 was thought to be responsible for the production of cytokines such as TNF-α and IL-1β [95]. Later studies

revealed that *Wolbachia* predominantly activated the receptors—TLR2 and TLR6 but not TLR4, which resulted in signaling through the adapter proteins—MyD88 and Mal [96]. More recently, it has been demonstrated that the increased levels of VEGF-C and sVEGF-R3 (observed in lymphedema patients) were reduced following doxycycline treatment (a regimen that eliminates *Wolbachia*) and that there was improvement in lymphedema [87]. Similarly, in patients with hydrocele, targeting *Wolbachia* with doxycycline led to a reduction in circulating levels of VEGF-A, with consequent reduction in the size of the hydrocele [97].

Not all studies, however, favor a role for *Wolbachia* in inducing lymphangiogenesis. For example, diethylcarbamazine (DEC) treatment of patients with bancroftian filariasis failed to alter VEGF levels [98], while another study showed that levels of VEGFs were not affected by treatment with doxycycline [88]. In addition, it has been found that elevated levels of VEGFs have also been observed in infection with *Loa loa*, a filarial parasite that does not harbor *Wolbachia* [88]. Although the exact mechanism remains to be elucidated, it is however clear that the interaction between the filariae and TLR does play an important role in the pathogenesis of filarial disease.

One of the main characteristics of asymptomatic or subclinical filarial infection is the modulation of TLR expression and function in a variety of cell types including B cells, T cells, and monocytes. Either baseline or antigenstimulated expression of TLR 1, 2, 4, and 9 was shown to be diminished in B cells, T cells, and monocytes of infected individuals [99, 100]. Moreover, stimulation of B cells, T cells, and monocytes with TLR ligands resulted in decreased activation/cytokine production, indicating a state of immune tolerance. Furthermore, live filarial parasites have the capacity to downregulate TLR expression (specifically TLR3 and 4) on dendritic cells as well [101]. This is accompanied by an impaired ability of dendritic cells to produce IFN-α, MIP-1α, IL-12, and IL-1α in response to TLR ligands. The diminished expression and function of TLRs on immune cells is thought to be a likely consequence of chronic antigen stimulation and probably serves as a novel mechanism to protect against the development of pathology in filariasis [102]. In contrast to what has been seen in those with subclinical disease, peripheral blood mononuclear cells (PBMC) from filarial lymphedema individuals exhibit elevated levels of TLRs and Nod-like receptors (NLRs), as well as heightened responsiveness to TLR stimulation [103]. Indeed, data from our lab have clearly demonstrated the ability of TLR2, TLR7, and TLR9 agonists to induce enhanced levels of Th1 and other pro-inflammatory cytokines (including IL-17 and IL-23) [104]. While the TLR adaptors are not differentially induced, TLR2 and 9 ligands were shown to induce significantly higher levels of phosphorylated extracellular signal-related kinase 1/2 (ERK 1/2)

and p38 mitogen-activated protein kinases (MAPK) and cause increased activation of NF-κB. In addition, TLR ligands and filarial antigens were shown to induce significantly higher expression/production of VEGF-A, VEGF-C, and angiopoietin-1 in those with chronic lymphatic pathology in a MAPK, NF-κB dependent pathway [105]. These data strongly suggest an important association between pattern-recognition pathway signaling and lymphangiogenesis.

Persistent immune activation is associated with elevations of circulating microbial products, acute-phase proteins, and the so-called microbial translocation molecules [106]. Translocation of microbial products from the lumen of the intestine into the periphery is thought to contribute to induction of inflammation by stimulating immune effector cells directly through their pattern-recognition receptors [106]; however, intra- and peri-lymphatic damage—an underlying feature of filarial disease—might also contribute to the presence of microbial translocation products in the bloodstream. Indeed, we have shown that increased circulating levels of LPS (which serves as a marker for microbial translocation) and decreased levels of LPS-binding protein (LBP) are characteristic features of filarial lymphatic pathology [107]. In addition, the chronic immune activation that often accompanies this process is associated with development of an acute-phase response and the presence of markers of inflammation in plasma—CRP, alpha-2 macroglobulin, serum amyloid protein-A, and haptoglobin [107]. Moreover, increased serum levels of pro-inflammatory cytokines—IL-1β, IL-12, TNF-α, and IL-6 are associated with progressive immune activation in filarial pathology. Since filarial lymphedema is known to be associated with increased bacterial and fungal loads in the lymphatics, our studies reveal that these damaged lymphatics may serve as a potential nidus for bacterial translocation through leaky lymphatic endothelium.

Apart from systemic immune activation, progressive fibrosis and extracellular matrix remodeling is another salient feature of filarial pathology. The turnover of collagen and other ECM proteins is controlled by a large family of proteolytic enzymes called matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases [TIMPs]), produced by a variety of cell types including macrophages, granulocytes, epidermal cells, and fibroblasts [108]. Tissue immunopathology is known to be associated with dysregulation of MMPs and TIMPs in several infections, including viral, bacterial, spirochetal, protozoan, fungal, and parasitic infections [109]. Along the same lines, recent data suggests that an increase in circulating levels of MMPs and TIMPs is characteristic of the filarial disease process and that that altered ratios of MMP/TIMP are an important underlying factor in the pathogenesis of tissue fibrosis in filarial lymphatic disease [110]. In addition, this is correlated with elevated levels of type 2 cytokines known

to be intimately involved in fibrosis—IL-5, IL-13 and TGF-β. Another study has also examined the alterations in pro-fibrotic factors in filarial pathology and revealed that increased levels of basic fibroblast growth factor (bFGF) and placental growth factor (PIGF) can also occur in filarial lymphedema patients [88]. Thus, filarial pathology arises out of a complex early interplay between the parasite and the host's innate responses and its tissue homeostasis.

## Pathogenesis of lymphedema—role of adaptive immunity

The earliest description of a role for adaptive immunity in the development of pathology was the observation that individuals with chronic pathology exhibited significantly lower levels of suppressor T cells (as defined at that time by expression of CD8 on T cells) [111]. Although, the designation of CD8+ T cells as suppressor cells was a misnomer, the early studies implicated a role for T cells in filarial pathology. The first seminal study that identified a direct role for adaptive immunity in pathology was from a study reporting that PBMC from individuals with chronic lymphatic pathology made significantly higher levels of IL-2 and IFNγ in response to parasite antigens compared to the asymptomatic-infected individuals [112]. Another important attribute shown to be markedly different between the two groups was the diminished proliferation of PBMC in response to parasite antigen. Thus, lack of T cell proliferation as well as production of type 1 cytokines was inferred to potentially protect against the development of overt pathology [34]. Since then, a number of studies have utilized the strategy of contrasting immune responses in PBMC of asymptomatic-infected individuals to those with chronic pathology to glean useful information on the components of adaptive immunity (including cellular phenotypes and cytokines) that influence filarial disease. However, since almost all of these studies have been cross-sectional studies providing only a snapshot of information, it has been difficult to unequivocally attribute a causal or etiological role for any T cell subset or cytokine in the development of filarial pathology. In terms of cellular subsets, it was first discovered that individuals with chronic pathology have increased frequencies of activated CD8+ T cells (HLA-DR+, CD8+ T cells) in peripheral blood [113]. Later, it was also shown that the frequency of CD8+ T cells in tissues (including skin and subcutaneous tissues) was increased as well [114]. Indeed, biopsy specimens from affected tissues exhibited increased levels of VCAM-1 [115], and PBMC supernatants from diseased individuals showed the capacity to upregulate both MHC-class I molecules and VCAM-1 on endothelial cell cultures [115, 116]. Moreover, TCRVbeta phenotyping revealed a biased TCR repertoire in the T cells infiltrating the affected tissues in diseased individuals [117]. In addition, examination of chemokine receptor expression on T, B, and NK cells revealed a significant increase in the frequencies of circulating T and B cells expressing CCR9 and a decrease in the frequencies of cells expressing CXCR1 and CXCR3 [75]. These results suggested that chemokine receptors (particularly CCR9) are involved in the pathogenesis of lymphatic filarial disease and that trafficking of particular cellular subsets may influence clinical outcome. Unpublished data utilizing multi-parameter flow cytometry has failed to reveal any significant difference in the frequencies of circulating naïve, effector memory and central memory CD4+ and CD8+ T cells in filarial lymphedema patients compared to those with asymptomatic infection. Thus, alterations in T cell numbers and function, especially at the site of pathology, are probably of major importance in pathogenesis.

As mentioned previously, a major hallmark of longstanding filarial infection (especially of the asymptomatic or subclinical variety) is the downregulation of parasite antigen driven Th1 differentiation. This is manifested by a significantly lower production of IFNγ and IL-2 upon filarial antigen stimulation in asymptomatic-infected compared to diseased individuals [112]. Moreover, using filter-spot or ELISPOT techniques, it was also demonstrated that the frequency of CD4+ T cells expressing IFNγ was significantly lower in asymptomatic-infected individuals [118]. Interestingly, there is considerable discordance in the results concerning the role of Th2 cells. While some studies suggest that individuals with chronic pathology mount equivalent filarial antigen-driven Th2 responses [118], others have shown increased Th2 differentiation in chronic pathology patients [119, 120]. More recent data using multi-color flow cytometry has shown that the frequency of Th1 cells (CD4+ T cells expressing either IFNγ or IL-2 or TNF-α) is significantly enhanced in filarial lymphedema patients, while the frequency of Th2 cells (CD4+ T cells expressing IL-4 or IL-5 or IL-13) is significantly diminished in comparison to asymptomatic, infected individuals both at homeostasis and following parasite antigen stimulation (Babu, S et al., unpublished). Similar to Th1 cells, Th17 cells might also have an important role in the pathogenesis of disease since PBMC from individuals with pathology (but not asymptomatic patients) express significantly higher levels of the Th17 markers—IL-17A, IL-17F, IL-21, and IL-23 as well as the master transcription factor—RORC at the mRNA level [103]. The increase in Th17 cells has also been confirmed by findings that chronic pathology individuals have higher frequencies of CD4+ T cells expressing IL-17 and IL-22 (unpublished observations). Therefore, immunopathology in lymphatic filariasis appears to be mostly associated with poor regulation of effector CD4+ and CD8+ T cells that can unleash pro-inflammatory Th1 and Th17 type immune

responses. How these pro-inflammatory Th1 and Th17 cells interact with innate cells, endothelial cells, and other target cells to initiate and propagate lymphatic damage and tissue fibrosis remains to be elucidated.

The subsets of CD4+ T cells constitute an ever-expanding repertoire, classified by their discrete cytokine profiles and often by expression of prototypical transcription factors and/ or cell surface molecules [121]. One of the major cell types now known to regulate effector CD4+ T cell responses is the subset of regulatory T cells (Tregs), characterized by surface expression of CD25 and the transcription factor FoxP3 [122]. Recently, a number of regulatory factors, including Tregs, IL-10, TGF-β, CTLA-4, and PD-1, have been implicated in the establishment of chronic viral and bacterial infections [123]. An important role for IL-10 in preventing pathology was described several years ago by the finding that significantly increased levels of IL-10 was induced upon filarial antigen stimulation in asymptomatic, infected patients but not in those with chronic pathology [124]. In addition, blockade of IL-10 could partially reverse the impaired proliferation and Th1 differentiation of PBMC in infected individuals [118]. Interestingly, the frequency of CD4+ T cells expressing IL-10 also appear to be significantly elevated in infected individuals in comparison to both uninfected individuals and those with chronic pathology [124, 125]. It has also been clearly demonstrated that the main source of IL-10 in infected individuals are CD4+, CD25− T cells and not the nTregs. [125, 126] Although, nTregs are not the major source of IL-10 in infections, they might still have an important role to play in the prevention of pathology as individuals with filarial lymphedema exhibit an inability to upregulate Foxp3 expression in response to filarial antigens [103]. In addition, nTregs might also contribute by helping turn off exuberant immune responses by their capacity to upregulate CTLA-4 and PD-1 surface expression and to produce TGF-β, a molecule known to be induced by parasite antigen stimulation in infected individuals but not in those with filarial pathology [103, 125].

While most of the immunological studies in filarial infections have focused on filarial antigen induced immune responses, the study of the immune responses engendered by live parasites provides some interesting details. Live parasites cause a significant impairment of both Th1 and Th2 cytokines in response to both L3 and mf stages with diminished production of IFNγ, TNF-α, IL-4, and IL-5 [43]. Examination of the molecular basis of this impaired response reveals three major networks of immune regulation and tolerance. First, impaired induction of T-bet (the master Th1 transcription factor) and GATA-3 (the master Th2 transcription factor) mRNA underlies the Th1/Th2 deficiency in infected individuals. Second, regulatory networks as evidenced by significantly increased expression of Foxp3, TGF-β, CTLA-4, PD-1, ICOS, and IDO play an important role in immune suppression. Third, the compromise of effector T cell function is mediated by the enhanced induction of anergy-inducing factors—cbl-b, c-cbl, Itch, and Nedd4. Finally, blocking CTLA-4 or neutralizing TGF-β restored the ability to mount Th1/Th2 responses and reversed the induction of anergy-inducing factors. Thus, a variety of regulatory factors including IL-10, TGF-β, nTregs (perhaps through PD-1 and CTLA-4) have been implicated in the downmodulation of immune responses in patent filarial infection and might have a potentially vital role in prevention of overt pathology. These data also identify an important role for T cell anergy, in the establishment of chronic, asymptomatic infection and in the prevention of pathology.

Finally, neonatal tolerance might be a major factor that prevents pathology following infection [127]. This became evident from studies that had followed up on non-endemic individuals becoming exposed to filarial infection after adulthood. In a study in the 1940s during World War II, among >38,000 US Naval personnel with exposure to infection in the South Pacific, >10,000 (27 %) had clinical signs of filarial fever and other evidence of acute pathology, while only 20 individuals (0.05 %) actually became microfilaremic. In another study examining long-term exposure among individuals relocating in Indonesia, it was observed that the transmigration of individuals from a non-filarial endemic setting to a filarial-endemic area resulted in high prevalence of clinical disease and low prevalence of microfilaremia [9]. Similarly, follow-up studies of individuals born to infected mothers reveal that such individuals tend to manifest with higher rates of asymptomatic (or subclinical) infection with high parasite loads but significantly less pathology, indicating that neonatal induction of tolerance may be instrumental in the prevention of overt pathology [128, 129].

## Immunogenetics

Host genetics are known to play an important role in susceptibility to infection and disease in a variety of infectious diseases. Similarly, in lymphatic filariasis, the pathogenesis of lymphedema and hydrocele might be influenced by host genetic factors. Epidemiological studies in areas where filariasis is endemic have revealed differential susceptibility to infection, both within entire populations as well as within families [130–132]. Although the cause of differential susceptibility to clinical expression of filarial infection has been only addressed in a few studies, early studies implicated the major histocompatibility complex (MHC) [133, 134]. However, analysis of class II HLA loci, namely, DQA, DQB, and DRB failed to identify an association with filarial infection nor outcomes within the infected group [135]. Two studies in Haiti examining genetic associations within families have suggested a genetic basis for developing pathology in

lymphatic filariasis [136, 137]. These studies found that 42 % of patients with lymphedema in at least one leg had parents with lymphedema. In addition, the incidence of multiple cases of lymphedema was clustered in families.

Studies examining the exact genetic factors that lead to families having a greater incidence of infection or disease have only recently begun utilizing single nucleotide polymorphisms or whole genome associations. Moreover, most of the genetic studies have examined susceptibility to infection rather than development of disease. Thus, chitotriosidase I and mannose-binding lectin 2 (MBL2) polymorphisms were shown to be associated with increased susceptibility to filarial infections in one study [135], although this could not be confirmed in another geographical area [138]. Another study revealed a significant association between MBL genotypes and the presence of infection in Africa [139]. Studies have also implicated TLR2 polymorphisms in susceptibility to infection [140]. A case–control study examining the role of VEGF-A SNPs in hydrocele revealed that a VEGF-A gene polymorphism in −460C/T was significantly associated with higher levels of plasma VEGF-A as well as the development of hydrocele [92]. A recent study has implicated polymorphisms of endothelin-1 and TNFR II with the development of chronic disease [141]. Future studies utilizing genome-wide scans as well as candidate gene approaches to identify loci and genes associated with pathogenesis should shed more light on the role of genetic factors in development of disease.

## Conclusions

A characteristic feature of all parasite infections is that complete elimination of all parasites is rarely achieved, presumably since sterilizing immunity might necessitate host deleterious immune responses. Therefore, immune-mediated pathology is often associated with disease manifestation in many parasitic infections. The optimal host response is one that balances parasite control at levels at which the parasite load can be tolerated and leads to maintenance of immune homeostasis without irreparable tissue damage. Filarial infections are a classical example of host–parasite interactions resulting in an immune system–parasite homeostatic balance, which can fail (albeit rarely). However, in the rare instances of failure, the effects are of a debilitating and devastating nature, in large part due to exuberant host immune responses.

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