Published in final edited form as:

Microcirculation. 2013 July ; 20(5): 349–364. doi:10.1111/micc.12031.

Lymphatic filariasis: Perspectives on lymphatic remodeling and contractile dysfunction in filarial disease pathogenesis

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Abstract

Lymphatic filariasis, one of the most debilitating diseases associated with the lymphatic system, affects over a hundred million people worldwide and manifests itself in a variety of severe clinical pathologies. The filarial parasites specifically target the lymphatics and impair lymph flow, which is critical for the normal functions of the lymphatic system in maintenance of body fluid balance and physiological interstitial fluid transport. The resultant contractile dysfunction of the lymphatics causes fluid accumulation and lymphedema, one of the major pathologies associated with filarial infection. In this review, we take a closer look at the contractile mechanisms of the lymphatics, its altered functions and remodeling during an inflammatory state and how it relates to the severe pathogenesis underlying a filarial infection. We further elaborate on the complex host parasite interactions, and molecular mechanisms contributing to the disease pathogenesis. The overall emphasis is on elucidating some of the emerging concepts and new directions that aim to harness the process of lymphangiogenesis or enhance contractility in a dysfunctional lymphatics, thereby restoring the fluid imbalance and mitigating the pathological conditions of lymphatic filariasis.

Introduction

Lymphatic filariasis is a debilitating disease involving the lymphatic system with over 120 million affected individuals worldwide annually and 1.2 billion people with the risk of infection [22, 89, 125]. The lymphatics play a vital role in maintaining fluid homeostasis, intake of dietary lipids and transport of immune cells, which is dependent on proper lymph flow [129, 130]. Malfunctioning or obstruction of the lymphatic system results in lymphedema and impairment of its key physiological functions. The removal of large macromolecules (like extravasated plasma proteins) and particulate matter from the interstitial space is a critical function of the lymphatic system, given that the blood vessels are generally not well suited for that task. If these macromolecules were left in the interstitium, they would lead to increases in tissue oncotic pressures, resulting in an imbalance of the transvascular exchange and edema formation and compromise immune function [75, 105, 113, 128]. The present review is focused on bringing together the various facets of the filarial disease and how an increased understanding of lymphatic contractile dysfunction can lead to the development of new therapeutic strategies that can effectively manage the pathological conditions associated with this disease. We summarize the current

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understanding of the mechanisms of lymphatic vessel contractility, its altered contractile patterns and the significant remodeling that the lymphatic vessels undergo during an inflammatory state as is characteristic of chronic filariasis. We also discuss how those mechanisms may be manipulated to inhibit the progress of lymphedema and other pathological conditions associated with the onset and progression of filariasis.

Development of filariasis

Among the various parasitic filarial nematodes that infect humans, *Wuchereria bancrofti* (90%), and *Brugia malayi* and *Brugia timori* (10%) are responsible for lymphatic filariasis, causing considerable morbidity, primarily because of their effects on the lymphatics [18], [22, 86]. The sexually dimorphic adult filarial parasites reside in the lymphatic vessels and produce thousands of first-stage larvae for up to 8 years. Mosquitoes of the genera *Aedes*, *Anopheles*, *Culex* or *Mansonia* are required for development of the larvae into the human infective stage, and for transmission to the human hosts. The vectors ingest microfilariae during blood meals. Within the mosquito, the larvae develop into infective larvae (L3), which are deposited on the skin of the humans during subsequent blood meals. The infective larvae penetrate the skin at the site of the bite and migrate to the lymphatic system and undergo two more molts to develop into adult worms, completing the cycle [22, 89]. *W. bancrofti* adults are typically found in the lymphatic vessels of the lower extremities in females and the lymphatic vessels of the spermatic cord and juxtatesticular regions in males [39]. Adult worms live in the afferent lymphatics or sinuses of the lymph nodes. Females release hundreds to thousands of fully formed, sheathed microfilariae per day into the lymphatic circulation of the host. From the lymph, they transit into the peripheral circulation [73]. Infected patients may exhibit asymptomatic infection, acute, or chronic manifestations [22, 86].

Clinical manifestations and Disease management

It is now well documented that the earliest damage to lymphatics is by vessel dilation by mediators released by adult parasites and in course of disease progression results in gradual impairment of lymphatic contractility. Once this lymphatic damage progresses, stasis of lymph tends to occur in the dilated vessels due to incompetence of the unidirectional valves [81]. The damage is further advanced by bacterial or secondary infections causing dermatolymphangio-adenitis. Frequent attacks of lymphangio-adenitis causes lymphedema that later evolves into elephantiasis and hydrocele [109]. Although about two thirds of the affected individuals exhibit subclinical symptoms or are asymptomatic, approximately 40 million people have other pathologic manifestations including hydroceles (and other forms of urogenital disease), episodic adenolymphangitis, tropical pulmonary eosinophilia, lymphedema, and in its most severe form elephantiasis [6] (extensively reviewed in [29, 41]. Four factors are currently thought to be central to the onset and progression of the pathogenesis of lymphatic filariasis: a) the living adult worm, b) inflammatory responses caused by death of the adult worm, c) secondary bacterial infections and d) microfilariae. It has been suggested that the lymphatic dilatation could be attributed to the parasite and its related products, whereas the more severe lymphatic obstruction may well be a consequence of immunologically mediated inflammation during a chronic infective state [18]. The various clinical stages of lymphatic filariasis, the disease manifestation and its effect on lymphatics are described in Table 1.

Several methods are used in the detection of lymphatic filariasis including antigen detection, x-ray detection, ultrasound, immunochromatographic test, lymphoscintigraphy, DNA probes etc., (reviewed extensively in [69],[86]). A global strategy to eliminate the disease has been through mass drug administration (MDA) that has shown marked success in reducing levels

of microfilariae [22, 49]. Several drugs that are effective at reducing the levels of microfilariae or adult worms include DEC, ivermectin and albendazole (extensively reviewed in [84]). Once lymphedema sets in, filariasis has no known pharmacological intervention and treatments are limited to mainly providing relief or prevention of further swelling. These include the use of elastocrepe bandages, special stockings, application of manual massage to facilitate lymph movement towards larger vessels, pneumatic compression of the affected limb using single or multi-cell jackets and heat therapy. Prolonged treatment with oral or topical coumarin and flavonoids is considered to be effective in reducing the lymphedema [109]. The other option is surgical procedures that mainly include lymph nodo-venous shunts (LNVS), omentoplasty, excisional surgery and skin grafting, or Charles operation. Surgical interventions to recanalize the lymphatic vessels and consequently improving lymph flow have been reasonably successful in reducing the pathogenesis of early stages of filariasis [31, 35].

Lymphatic system and lymph pump

The lymphatic system consists of a network of lymphatic vessels and interconnected lymph nodes distributed throughout most of the body, which plays a vital role in the controlled transport of immune cells, antigens, lipids, macromolecules, fluid, and particulate matter in the form of lymph. The transportation of lymph along the lymphatic network is directed from the parenchymal interstitial spaces into the nodes via the afferent lymphatic trunks. It exits the nodes through the efferent lymphatics and travels between the nodes, and eventually the lymph exits the lymphatic system, emptying into the blood in the subclavian vein of the neck. Anatomically, the initial lymphatics are composed of a layer of endothelial cells that are loosely placed but with overlapping edges. These resultant gaps are thought to function as “primary valves” that provide unidirectional fluid flux into lymphatics [62, 68, 75, 129]. The predominant mechanism driving lymph formation and flow appears to be the development of transient fluid pressure gradients between the interstitium, the initial lymphatic, and downstream collecting lymphatics. These gradients occur during variations in the local interstitial fluid pressures due to tissue movement and/or compression [75]. The lymphatic system uses lymph pumps (extrinsic and intrinsic) to provide the energy necessary to overcome the steady state opposing pressure gradients and propels lymph along the lymphatic network [50, 67]. Despite the myogenic origin of these rhythmical lymphatic contractions, many factors have been found to modulate the rate of spontaneous intrinsic pumping. The major lymphatic contraction modulators are physical (e.g. temperature, lymph flow, vessel distension) and chemical stimuli (e.g. endothelium-derived factors, circulating hormones and neurotransmitters) [47, 75, 121, 129]. However, spontaneous contractions can still occur in the absence of these factors. The local physical factors such as stretch/pressure and shear/flow also modulate lymphatic tone and function [130].

Uniqueness of lymphatic contractile apparatus

The lymphatic capillaries are blind-ended vessels with a single layer of overlapping endothelial cells, that are uniquely tethered to the surrounding basement membrane through characteristic anchoring filaments [120]. The collecting lymphatic vessels contain a layer of endothelial cells surrounded by muscular walls capable of both phasic and tonic contractions, which both generate and regulate lymph flow [26, 130]. The lymphatic vessels function as both regulated conduits and regulated pumps, and thus they have functional characteristics of both blood vessels and hearts [45, 93]. In the intrinsic lymph pump, flow through a lymphatic bed is generated by coordinated contractions of the lymphatic muscle cells [70, 71].

Despite the preponderance of information on both smooth and striated muscle regulatory mechanisms, very little is known about the molecular basis of lymphatic muscle contraction.

In fact, our previous studies provided the first evidence that lymphatic muscle contractile apparatus consists of both striated and smooth muscle contractile elements [74]. Furthermore, we have previously demonstrated that regulatory contractile mechanisms that modulate the tonic and phasic contractions of the lymphatics are due to intrinsic differences in both the contractile function and contractile machinery, that exist between blood vessels and lymphatics as well as among lymphatics from different body regions [45, 77, 123]. Davis et al. have demonstrated that myogenic responses occur in mesenteric lymphatics in response to changes in intraluminal pressures and lymphatic muscle exhibits rate-sensitive contractile responses to stretch [32]. Thus the unique mechanical and contractile characteristics of lymphatic muscle is central to the maintenance of its essential functions for protection against formation of edematous and other pathological conditions [120].

Effect of lymph flow on lymphatic contraction

Distension of the lymphatic wall by intraluminal/transmural pressure is an important factor to regulate the phasic contractile activity in lymphatics. In the muscular collecting lymphatics, distension of the lymphatic wall activates the lymphatic contraction. Lymph flow is the result of a complicated combination of lymph formation, intrinsic and extrinsic forces and pumps. Thus the lymph flow patterns can be extremely variable and bidirectional [45]. During the active contractions of lymphangions, the lymphatic muscle cells create an increase in intra-lymphatic pressure and form a local positive pressure gradient to propel lymph [67]. A flow-dependent inhibition of the active lymph pump was found in mesenteric lymphatics and thoracic duct [46] as well as femoral and cervical lymphatic vessels [45]. Pumping or resistive activity of the lymphatics is regulated by flow or shear stress in order to adapt to the local needs to transport lymph through a continuous modulation of the extrinsic and intrinsic flows. Thus, flow through the lymphatic in turn modulates its tonic and phasic contractile activity. This in turn also alters its ability to generate and regulate lymph flow [47]. This is evidenced by the relatively high values of resting intra-lymphatic pressures and peak pressure fluctuations in the leg lymphatic net in humans compared to other lymphatic beds, reflecting the much higher outflow resistance for leg and the physiological demand for local lymphangions to develop much stronger contractions than are seen in other animals [44].

Increased lymph pressure/stretch generally activates the intrinsic lymph pump up to a point, beyond which the lymph pump begins to fail [47]. This supports the fact that in the leg (where most of the problems in filariasis-induced lymphedema occurs), the external (passive) compression of the initial and contractile lymphatics must play a major role. In one study comprised of patients with varying stages of post-inflammatory obstructive lymphedema, intrinsic leg movements and calf muscle contractions were found to increase intralymphatic pressures or lymphatic contractions, both of which led to an increased flow of lymph. Further, in advanced lymphedema, changing from horizontal to upright position altered intralymphatic mean pressure from around 7mmHg, with no lymph flow to about 18 mmHg with increased lymph flow. However, in the same study it was observed that in chronic lymphedema with sclerotic changes in skin and subcutaneous tissue, muscular contractions had no effect on flow [80, 81]. In secondary lymphedema condition with dermal backflow, it has also been shown that lowered lymphatic pump force due to impaired contractility directly correlates with fluid accumulation [72]. Further lymphatic valves help to minimize lymph backflow when the pressure gradients are not conducive to central lymph flow. They also help reduce the gravitational influence on lymph pressure by breaking up the hydrostatic lymph column [130]. It is thus evident that an intricate regulatory mechanism controls lymphatic pumping and fluid flow, disruption of which causes lymphedema.

Lymphatic function and inflammation

The single largest source of secondary lymphedema worldwide is lymphatic filariasis [59, 99]. Impairment of the lymphatic vessels and insufficient lymphatic function cause interstitial accumulation of fluid, leading to chronic swelling of the limbs, or lymphedema. In addition to swelling, the protein-rich interstitial fluid induces an inflammatory reaction, leading to fibrosis, accumulation of adipose and connective tissue. This will consequently cause impaired immune responses and wound healing, which will increase susceptibility of the patients to secondary infections [59, 99]. In this section we will review the importance of lymphatics in inflammatory processes and how that promotes the disease progression.

Role of the lymphatics in mediating an inflammatory response

An important feature of inflammation is infiltration of immune cells such as neutrophils, eosinophils, and macrophages into the inflamed tissues. Lymphatic vessels serve as a route for the transport of dendritic cells, memory T cells, macrophages, and antigens from the periphery to lymph nodes and therefore play an important role in initiating immune responses [82, 96]. The lymphatic vasculature provides an exclusive environment where immune cells can respond to foreign antigens, and the means for circulating lymphocytes to traffic to the lymph nodes and return to the bloodstream. Lymph flow is generally enhanced during an inflammatory condition because of higher microvascular permeability and increased interstitial fluid load, causing an increase in lymphatic fluid pressure. The resulting stretch of the lymphatic vessel wall determines the ability of the vessels to propel lymph and the immune cells, and inflammatory mediators that gain access to the lymph during this time directly influence lymphatic contractile function [19, 20, 67, 127]. The involvement of lymphatic vessels in edema resolution, immune cell trafficking and their sensitivity to inflammatory mediators make them pivotal players of the inflammation process. Inflammatory mediators have been closely associated with alterations in lymphatic pumping and drainage [3, 55, 65] [54, 120, 126]. We have shown that Substance P (SP), a neuropeptide that is major mediator of inflammatory response alters lymphatic contractility and pumping efficiency. SP also activates both contractile and inflammatory pathways in the lymphatic muscle cells [1, 28, 33, 77]. In the inflammatory 2,4,6-trinitrobenzene sulfonic acid (TNBS) model of guinea pig ileitis, a significant inhibition of lymphatic contractile function has been observed along with a marked dilation of lymphatic vessels. It is suggested that a possible cause for the dilated lymphatic vessels could be downstream lymphatic obstruction due to the inflammatory conditions [127].

Exposure to an inflammatory stimulus and recognition of pathogen-associated molecular patterns causes dendritic cells to capture antigens in peripheral tissues and migrate through afferent lymphatic vessels into lymph nodes [4, 91]. Podgrabinska et al., [91] provide evidence to show a direct role of lymphatic endothelial cells (LECs) in modulating the inflammatory immune response by suppressing maturation of dendritic cells (DCs) in response to TNF- α . These effects were only elicited in the absence of pathogen-derived signals such as LPS-induced activated TLR signaling. In the presence of pathogen-derived signals, these suppressive effects were abolished and the DCs were much less responsive to LECs. Lymphatic endothelium also responds to LPS with the production of NO through activation of inducible nitric oxide synthase (52). It could be that the signals induced by pathogen-associated molecular patterns (like LPS), in DCs, override regulation by LECs, because in the presence of a pathogen, the full immunogenic potential is required and down-regulation of DC maturation is therefore not desirable.

Generally, accumulation of protein-rich interstitial fluid in tissues is associated with an increased cellularity resulting from a massive infiltration of leukocytes including neutrophils, macrophages, and DCs [111]. The poor lymphatic drainage of interstitial fluid

leads to impaired transport of immune cells, pathogens and macromolecules, potentially promoting infection and delaying the immune response [122, 127]. Indeed, as a consequence of lymph drainage failure as observed during chronic pathologies, it is possible that cytokines and chemokines that are usually cleared from the interstitium remain in the tissue and thus promote the recruitment of leukocytes from the blood leading to an ongoing inflammatory response in the tissue. It is recognized that lymphedema is associated with altered immunity. This can be clearly seen, as patients with lymphedema are prone to developing chronic bacterial, fungal, and viral infection in the lymph edematous limb. This is possibly due to an inefficient clearance of the pathogen via the lymph in addition to impaired adaptive immunity [3, 65]. In general, failure of DCs to migrate to the lymph nodes normally may exacerbate local immune and inflammatory responses. Indeed, DCs left in the tissues can promote the recruitment of further leukocytes by secreting cytokines and chemokines to sustain local inflammation [3, 4, 65]. In mouse models of acute cutaneous inflammation, activated lymphatic vessels undergo remodeling, and increase in both size and numbers to significantly limit edema formation by increased lymph flow. Further, these lymphatic vessels are shown to become dysfunctional during the establishment of chronic inflammatory skin lesions and that lymphatic function can be restored by genetically overexpressing VEGF-C [52]. Thus, the role of lymphatic vessels in acute and chronic inflammation vary as lymphatic vessels may help promote or resolve inflammatory cells from site of inflammation depending on the lymph flow and drainage patterns [52].

Lymphatic remodeling during filariasis: Response to an inflammatory disease state

Filarial lymphedema is associated with characteristic alterations of the lymphatic system, including the dilation of lymphatics with extensive collateral formation, loss of functional valves and retrograde lymph flow [27]. In animals models it has been shown that host immune response is believed to play an important role in establishment of chronic filariasis and secondary bacterial infections are shown to aggravate preexisting filariasis [23, 40]. Factors contributing to either the death or survival of filarial worms play a pivotal role in the pathogenesis of bancroftian filariasis. No direct evidence exists that the immune system kills adult *W. bancrofti* in vivo in long-term residents of endemic areas. However, the inflammatory host response that either causes the death of worms or is induced by the worms has emerged as a major risk factor for the development of some types of chronic filariasis syndromes, such as hydrocele, chylocele and chyluria [40].

Lymphatic vessel dilation is believed to be an early event following antigenic stimulation that occurs when the adult worms are still alive and the offspring larvae are released. Moreover, lymphoscintigraphy studies demonstrate that even patients with subclinical manifestations of the disease exhibit considerable structural anomalies and aberrant patterns of lymph flow [42]. To determine the extent to which lymphatic dilatation occurs in the presence of living adult *W. bancrofti*, Dreyer G et al., performed longitudinal ultrasonographic measurements in 80 men (mean age 24 years) in Brazil who had a total of 107 *W. bancrofti* nests detectable by ultrasound. Initial mean lymphatic vessel diameter at the site of the worms was 3.4 mm, with it being around 3.9mm in men with 2 or more nests compared to 3mm in those with only one nest. During the study period (2–35 months, mean, 13.7), lymphatic vessel diameter increased at the site of 92 (86.0%) adult worm nests. Mean rate of increase of lymphatic vessel diameter was 1.2 mm per person-year (range, 0–0.93 mm per month) [39]. In another longitudinal study of patients infected with *Wuchereria*, Dissayanake S et al., [36] showed that clearance of microfilaraemia by DEC therapy does not appear to reverse the lymphatic architectural changes induced during microfilaraemia [36]. These findings corroborated in nude and SCID mouse models of filariasis suggest that asymptomatic lymphatic dilation may be caused by the direct effect of

filarial 'toxins' on lymphatic endothelia [76, 118], whereas clinical lymphedema results from host immune responses to adult filarial worms [83].

In chronic infection, death of the worm or parasite related toxin further exacerbates the inflammatory condition in an already dilated poorly functioning lymphatic vessel and renders it completely obstructed and non-functional [40]. Phagocytic uptake of degenerate filarial larvae triggers the innate immune system and causes production of inflammatory cytokines and lymphangiogenic molecules [89]. In the lower limbs, the enlarged lymphatic vessels become very inefficient at transporting lymph from the periphery and against gravity. The condition is exacerbated as the poorly draining lymphatics become vulnerable to secondary infections through minor bruises and these infections trigger further inflammation as well as lymphangiogenesis, leading to acute dermatolymphangioadenitis. Insufficient fluid transport leads to fluid extravasation, particularly in the lower limbs, and eventually to lymphedema [89].

The sequential alterations in the architecture of the lymphatics during the course of lymphatic filarial infections occur along a continuum from lymphangiectasia to granulomatous responses to development of collaterals indicating active lymphatic remodeling [18]. A characteristic feature of long-term filarial infection in humans and animals is the fibrosis and cellular hyperplasia in and around the lymphatic walls. Infection with the parasites for long periods results in the fibrosis of the infected lymph nodes, which eventually become non-functional and are bypassed by new lymphatic vessels [100]. In experimental animal models, irregular large vacuoles, often containing degenerating organelles, have been commonly found in endothelial cells lining *Brugia*-infected lymphatic vessels. These studies suggest that damage of cells by living or dead worms or worm products may have a direct effect on the endothelial lining of lymphatic vessels and may compromise the efficiency of vessels that collect and transport edematous fluid in affected limbs [101, 102]. The injury possibly makes the lymphatics less effective in transporting edematous fluid and thereby contributes to the edema and collagen accumulation [17]. Studies on the morphology of infected lymphatics in animal models show that the endothelium appears activated with associated adherent mononuclear cells. On histological analysis, infiltration of the infected lymphatic vessels with various immune cells eosinophils, macrophages and plasma cells are seen [79].

Local surgical pathological records in endemic areas show that *Wuchereria bancrofti* induced lymphatic filariasis encompasses distinct histological features determined by the presence of either dead or live worms harbored within the lymphatics. It has been well documented that the nearer the parasite is to the lymph node the greater the level of lymphoid hyperplasia, characterized by large follicles with germinal centres, hypercellularity of the paracortical areas and sinus histiocytosis [41]. The most commonly observed change in vessels with live worms is the lymphangiectasia where in the walls of the affected vessels, areas of fibrosis alternate with scant and isolated smooth muscle cells, and areas with muscle-cell hyperplasia in the walls of the affected vessels. [41, 56, 57]. When coupled with pre-existing lymphatic dilatations, these lesions may lead to damage of the lymphatic valves that in turn induce lymphatic backflow, stagnation and lymphedema [17].

In permissive hosts, lymphangiectasia is present in all individuals who harbor living adult worms, which can remain subclinical for undetermined periods of time, or evolve into chronic disease. [40]. Parasitic larvae can mature and migrate into lymphatics not closely associated with the host lymph nodes. It is well established that the larvae do not move passively but rather do so in response to various yet undefined stimuli that enables them to choose between superficial or deep routes of travel [104]. Clinical and histopathological findings are consistent with the hypothesis that the 'definitive' habitat of adult worms in

bancroftian filariasis shifts from lymph nodes to lymphatic vessels and trunks [40]. It is conceivable that this shift is triggered or facilitated at the time of puberty at least in males [78]. It has been documented that lymphatic vessels of spermatic chords in men which are less than 1 mm in diameter may reach up to 20 mm or more during the course of infection [39]. Viable adult worms cause lymphangiectasia in animals via mechanisms that do not involve lymphatic obstruction and that are independent of specific host anti-parasite immune responses [40]. Further, it is notable that while adults are generally restricted to a single anatomical site during their life span, the lymphangiectasia is not restricted entirely to the exact segment of lymphatics, indicating that this process is mediated by soluble products excreted or secreted by the parasite that act on the lymphatics. Thus, it is proposed that lymphangiectasia and inflammatory reactions are two independent components of lymphatic pathology that are triggered by 'toxins' of living adult worms and by host reactions to damaged or dead worms, respectively [40]. Progressive lymphatic damage and pathology potentially 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 *Wolbachia*, and those seen as a consequence of secondary bacterial or fungal infections [41, 113]. Thus, inflammatory damage induced by filarial parasites appears to be multifactorial, with endogenous parasite products, *Wolbachia*, and host immune responses all playing important roles [16]. The sequential alterations seen in lymphatic vessel architecture during the course of filariasis, starting from the onset of subclinical lymphangiectasia, to the development of a chronic inflammatory response and eventually to tissue fibrosis or scarring, appear to involve significant lymphatic remodeling events as proposed in Figure 1.

The association of lymphangiectasia with the presence of active filarial infection suggests that soluble parasite factors may be mediating the inflammatory effect *in vivo*. It is hypothesized that the excretory/secretory (ES) products of the worms activate the lymphatic endothelium; however there have been contradicting evidence with regards to its exact role. In order to model the intimate interaction between worm ES products and the LECs in filarial infection, Weinkoff et al., established an *in vitro* model system in which LECs were exposed to filarial ES products released by the parasite. However, no evidence of LEC proliferation or activation was found [124]. The authors hypothesize that the LECs maybe indirectly activated by filarial ES products through the production of lymphangiogenic mediators or cytokines from various activated immune cells [124]. In fact, Bennuru et al., 2009 [17] have previously shown that serum from infected individuals induce LEC proliferation. It is speculated that a complicated network is established between the parasite and the host, and the LECs may be indirectly activated through a host accessory cell or its mediators [124].

Host-parasite interaction plays a key role in disease pathogenesis

It is generally believed that the host provides some development cues to the parasite [94]. The dependency of parasite development on host environmental cues appears to be most pronounced in the infective stage of the parasite [21, 110]. Babu et al., have shown that growth of *B. Malayi* within mammalian host is dependent on Natural Killer (NK) cells function that either down-regulates the innate host immune response or provides a trophic factor that enhances parasite growth [13]. It has been shown that live infective-stage larvae (L3) or live microfilariae (Mf) of *B. malayi*, rapidly induce activation of NK cells, alter co-stimulatory and natural cytotoxicity receptor expression, and induce type 1 and type 2 cytokine production, with L3 inducing IFN- γ and TNF- α , and Mf additionally inducing IL-4 and IL-5. Upon prolonged stimulation with live L3, NK cells undergo caspase-dependent apoptosis, indicating a complex interplay between host and the parasite as well as modulation of the inflammatory milieu by NK cells [11]. Babayan et al., 2010 have shown

that in the filarial parasite *Litomosoides sigmodontis*, variations in filarial larval size and stage are a plastic response to an early, local, and transient predictor of their host's immune response. They demonstrate that IL-5 and eosinophils, necessary for immune clearance of filarial infections, in fact act as a developmental cue causing accelerated moulting and growth in the larva [5]. This shows that in response to increased immune surveillance nematodes alter their reproductive rates considerably in order to maximize offspring. IL-4 and IL-5 are also important in the regulation of worm fertility, as infected IL-4 KO and IL-5 KO mice produce more microfilariae over a longer period of time [5, 119]. In the case of filarial parasites, the adults reside in the lymphatics, while the microfilariae are present in the peripheral circulation and hence different stages of the parasite need to modulate their host environment accordingly for survival [11]. As the parasite rapidly adapts itself between drastically changing host environments of the mosquito and the human lymphatics with wide variations in oxygen tension, pH, temperature, and substrate availability, it is evident there are metabolic switches that enable it to adapt to its rapidly changing environment [14]. It is conceivable that the lymphatic endothelial or muscle cells release a chemotactic agent or express receptors that recognize parasitic excretory and secretory molecules and may aid in their migration into the lymphatics. However, no study till date has clearly addressed this in the context of the lymphatic cells. It has been recently shown that abrogation of the chemokine CXCL12/CXCR4 axis that is involved in immune surveillance, favors *L. sigmodontis* infection. Paradoxically, the CXCL12/CXCR4 axis appears to have a dual effect on its life cycle acting as a host-cell restriction factor for infection, and as a growth factor for worms [24]. This is especially relevant for filarial pathology as chronically infected but asymptomatic individuals express lower CXCR4 on T cells as compared to both uninfected and resistant individuals and lymphatic endothelial cells express CXCL12 [9, 115]. Also, higher levels of T and B cells expressing CCR-9, a marker for lymphocyte homing has been observed in patients with lymphedema.

While several studies have focused on the inflammatory or immune responses activated by the various stages of this parasite, relatively little is known about its excretory or secretory (ES) products and its effects on lymphatics. To survive in a hostile environment, filarial nematodes have adopted a number of strategies to evade, modify or neutralize the hosts' defense mechanisms. Increasing evidence shows that a parasite's enzymatic pathway plays an important role in coordinating these mechanisms [106]. High throughput gene expression studies have revealed that the transition of the various stages of *Brugia malayi* from the mosquito host to the mammalian host is accompanied by genes that encode proteins with various functions such as invasion and immune evasion in the infective stage L3i (proteases, pathogenesis related, immunogenic and cuticular proteins) and growth and development in later stages (ribosomal proteins, cell cycle, phagocytosis and fiber growth) [61]. Hewitson JP et al., collected *B. malayi* ES products from adult parasites and identified over 80 identified genes that may play an important role in parasite localization homing or reproduction and may also provide attractive drug targets [51]. Several other investigations also identified novel ES products that provided information on stage, gender and strain-specificity [17, 73]. A list of the filarial enzymatic products or ES toxins and their mode of action in filarial pathogenesis has been summarized in Table 2.

Molecular mechanisms underlying lymphatic filariasis progression

Filariasis remains one of the most immunologically complex diseases in humans and the occurrence of filarial parasite associated modulation of the immune response in microfilaremic patients is supported by a large body of clinical evidence as well as animal studies [60, 79, 85, 90, 103]. Filarial patients are known to exhibit strong proinflammatory immune responses and this inflammatory milieu is thought to promote the development of physiologic abnormalities of the lymphatic vasculature [16]. Figure 2 outlines the complex

synergetic roles of various signaling molecules and pathways, and activation or suppression of specific immune cells and cytokines, which contribute to the disease progression.

Studies in animal models of filarial infection and cellular studies in humans underline the key role played by Wolbachia derived molecules from filarial parasites in inducing proinflammatory cytokines and thereby initiating an inflammatory response [8]. This inflammatory response to Wolbachia is mediated primarily through Toll like receptors (TLRs) [25]. To elucidate the role of TLRs, Babu S et al., examined cytokine responses to different Toll ligands in patients with lymphatic pathology, infected patients with subclinical pathology, and uninfected, normal individuals [6]. Their results suggest an important role for TLR2 and TLR9 mediated pro-inflammatory cytokine induction such as IFN- γ , TNF- α , IL-12 and IL-1 β . In addition, activation of both the MAPK and NF- κ B pathways are associated in the development of pathology in human lymphatic filariasis [6]. There is also increased expression of the NLR family (Nod like receptor) family of Nod1 and Nod2 in lymphedema patients compared to asymptomatic carriers. These are cytosolic proteins that play a role in NF- κ B mediated regulation of proinflammatory pathways are involved in the inflammatory cascades associated with tissue damage in the lymphatics [8, 58]. In addition, individuals with lymphedema also have significantly higher concentrations of interleukin-8, macrophage inflammatory protein -1 α , MIP-1 β , monocyte chemotactic protein 1, thymus-and-activation-regulated chemokine, and interferon-inducible protein 10 in their serum than did uninfected individuals [9].

Innate immune responses that are triggered by the filarial antigen ultimately result in the activation of vascular endothelial growth factors (VEGF), thus promoting lymph vessel hyperplasia as a first step to lymphedema development. The presence of elevated levels of lymphangiogenic factors is associated with the severity of lymphatic pathology [15]. Plasma levels of VEGF-A, VEGF-C and VEGF-R3 are increased with disease progression in filariasis and in clinical cases of lymphedema, hydrocoele, and chyluria [89]. VEGF-C induces lymphatic endothelial proliferation and dilation and hyperplasia of the lymphatic vasculature in transgenic mouse models similar to what is observed in the lymphatics of filarial patients [34, 53, 114]. Wolbachia stimulates pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6, and nitric oxide in human patients; these cytokines are known to augment the expression of VEGF-C/VEGFR3, presumably by the lymphatic endothelial cells of host lymphatics [89, 117]. In doxycycline-treated patients a significant decrease in serum levels of VEGFc and sVEGFR3 is shown, providing an association between reductions in pro-lymphangiogenic factors and amelioration in LF disease pathology [34, 116]. It has been shown that TLR2-mediated enhancement of angiogenic growth factor production in patients with lymphatic pathology is dependent on mitogen-activated protein kinase (MAPK) and NF- κ B signaling. Pharmacological inhibition of either extracellular signal-regulated kinase 1/2 (ERK1/2), p38 MAPK, or NF- κ B signaling resulted in significantly diminished production of VEGF-A and Ang-1 [7]. Overexpression of lymphangiogenic marker, VEGF-A may cause extravasation and accumulation of fluids, plasma and lymph from blood and lymphatic vessels into the scrotal regions, resulting in the development of hydrocele, chylocele and lymphocele [89]. It is therefore conceivable that any therapeutic intervention that causes the reduction of lymphangiogenic factors may also reduce lymphatic vessel dilation [89].

The presence of circulating immune complexes (ICs), aggregates of antigens, immunoglobulin and complement components is a characteristic feature of human lymphatic filariasis and has been evaluated as a potential non-invasive way of assessing renal damage [37, 38]. Senbagavalli et al., have shown that high levels of circulating antigen, in combination with antigen-specific antibodies, activate the complement system in asymptomatic persons, whereas the reduced status of complement activity in patients with

chronic pathologic changes may aggravate disease morbidity [107]. Studies in murine models suggest the involvement of both the Th1 and Th2 arms of immunity in resistance to filarial parasites [10, 95]. Hence, a compromise in Th1/Th2 effector functions could play a pivotal role in establishment and maintenance of chronic, high- density filarial infections without triggering exuberant host immune responses as is evident in a majority of asymptomatic cases with circulating microfilariae. Studies in endemic populations suggest that truly endemic normal individuals mount a Th1-like antifilarial immune response, thus remaining infection free. Another intriguing hypothesis suggests that the induction and release of Th1 cytokines assist growth and development of filarial larvae and a study shows that live L3 elicit a Th1-like inflammatory response from host cells [12]. Moreover, different life cycle stages of the parasite have been shown to elicit different immune responses. In one such study, Babu et al., have shown that the cytokine profile of a cohort of filaria-infected and uninfected individuals in response to live infective-stage larvae or microfilariae of *Brugia malayi*, showed significant impairment of both Th1 and Th2 cytokines [8]. Three major networks of immune-regulation and tolerance involving impaired induction of TGF- β and GATA-3 mRNA, were found to be mediating this depressed Th1/Th2 response [10]. Thus it is clearly evident that a complex interplay of parasite products, host immune response and an inflammatory milieu determine the degree of infection establishment and filariasis pathogenesis.

Future directions

Filariasis continues to be one of the most incapacitating diseases in the world despite the WHO's efforts to administer mass drug administration and considerable success in reducing microfilaremia. In lymphatic filariasis, evidence suggests that once severe lymphatic damage has been established, the disease state is irreversible. Hence there is a need for control strategies that interrupt transmission so that early, difficult to detect, and perhaps irreversible damage to the lymphatic system can be avoided [43]. Further understanding of the molecular mechanisms underlying the pathogenesis of this disease and delineation of the quantitative and qualitative immune response patterns differentiating patients with overt or subclinical manifestations is very important. This will enable the design of effective pharmacological inhibitors and pathogenesis specific interventions aimed at the early stage of the disease before major lymphatic functions have been chronically affected. In spite of a surge of recent research in this area, several important questions that remain unanswered are: "what are the key molecular elements of filarial pathogenesis, including the mechanisms which attract the worms to the lymphatics, how the parasitic toxins produce vessel dilation or what cues promote worm nests?" Although recent proteomics and secretome studies on specific stages of the parasite have provided valuable information, it is not clear which specific parasitic antigens initiate the pathology and subsequent lymphatic remodeling. Furthermore, no information is available regarding the specific receptors for most of these parasitic antigens on the lymphatic endothelial and muscle cells that maybe promoting the disease state. Identification of such molecules could be very useful as leads for drug development for filariasis.

Dysfunctional lymphatics underlie the majority of the clinical and pathological manifestations of this disease as well as causes significant morbidity and life style changes in affected patients. Thus an increased understanding of the structure and function of the lymphatic architecture must definitely go hand in hand with efforts aimed at eradicating the parasite and its vector. Despite their cardinal function to adapt their contractile activity to changes in fluid load, lymphatic vessels are often considered as passive participants in inflammatory pathologies that are characterized by edema. Currently, there are very few efficacious therapies, and a complete lack of medical treatment options for lymphatic dysfunction [99]. When evaluating the lymphatic system it is crucial to remember that its

principal purpose is the transport of lymph and it is by this regulated transport that all of the body's homeostatic functions that the lymphatic system participates in are served [130]. Although several research has focused on the role of lymphatic endothelial cells in mediating the immune and inflammatory response for progression of filariasis, insights into the role of the lymphatic muscle cells in disease pathogenesis is greatly lacking. While emphasis on lymphangiogenesis and mechanisms that can foster the formation of new functional lymphatic vessels to bypass the damaged ones are important, it must be re-emphasized that coordinated actions of the lymphatic muscle cells are directly responsible for pumping and are the main driving force behind the normal generation and regulation of lymph flow, which in turn enables the lymphatic vessels to carry out its vital physiological functions. There is no information to date as to how the muscle cells are remodeled during chronic filarial infection and contribute to the impaired lymphatic transport and drainage that is a hallmark of the disease.

Acknowledgments

This work was supported by AHA 09POST2280005 to SC, RO1HL70308 to DCZ and NIH RO1 HL80526 and KO2 HL86650 to MM.

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A. Normal lymphatic drainage in absence of parasitic infection

B. Slight impairment of lymphatic drainage in acute infection

C. Impaired lymphatic drainage and vessel remodeling during chronic infection/ elephantiasis

Figure 1.

Different stages of lymphatic vessel remodeling and modulation of lymphatic flow during progression of filarial infection. **A.** Normal lymphatic collecting vessel showing normal flow patterns and lymphatic drainage regulated by the unidirectional valves in the absence of filarial parasitic infection. **B.** Onset and progression of acute filarial infection with microfilariae and adult worms lodged within the vessel. Normal host immune response is initiated. Slight hypertrophy of the lymphatic muscle cell layers is observed with a partial impairment of lymph flow. **C.** Chronic filarial infection results in a major host immune response due to toxins released by dead or live parasites. Various immune cells are observed at the site of infection leading to a strong inflammatory reaction. Secondary infections with bacteria harboring *Wolbachia* exacerbates the condition leading to a chronic infection state or elephantiasis. The lymphatic vessels exhibit largely dysfunctional valves, vessel dilation, impaired lymphatic muscle contractility and insufficient drainage. The resultant fluid accumulation and retrograde lymph flow associated with severe lymphedema. Activation and remodeling of lymphatic endothelial cells during this chronic stage could potentially result in either endothelial dysfunction or promote lymphangiogenesis.

Figure 2.

Schematic showing the synergetic roles of a plethora of signaling molecules and pathways that contribute to the onset and progression of filariasis. **A.** Complex interplay of cytokines and chemokines and associated immune cells triggered by different stages of the worm maintain the balance between a filaricidal or antifilaricidal host response. **B.** Specific parasitic toxins or excretory secretory products act through yet unidentified receptors on lymphatic muscle and endothelial cells to impair lymphatic contractile function.

Table 1

Stages of lymphatic filariasis and their effects on lymphatic vessels

Filarial Disease State	Effect on lymphatics structure and function	Causative agent
Acute manifestations (Asymptomatic Stages)		Microfilariae, adult worm
Lymphangiectasia	Vessel dilation and distension Fibrosis and smooth muscle hyperplasia [41]	Microfilariae Live adult worms
Acute filarial adenolymphangitis	Local inflammation around dead parasite. Intense granulomatous reaction Large accumulation of immune cells and inflammation of lymphatic vessel wall [41]	Death of adult worm Possible role of Wolbachia
Chronic manifestations		Dead and decalcifying worms
Lymphedema	Increasing dilation and tortuosity of the lymphatics. Endothelial cell proliferation Obstructive changes and impaired lymph transport, Extravasation of lymphatic fluid Valvular thickening and incompetence Retrograde lymph flow. Swelling of extremities. New lymphatic channels formed Reversible at very early stage [89, 109].	Adult worm nests obstruct lymphatic vessels Secondary infections
Elephantiasis	Obstructed lymphatic vessels Backpressure causes dilation of superficial vessel. No discrete symmetrical lymphatic drainage trunks Extensive network of collaterals Severe swelling of the scrotum, vulva, and breasts. An irreversible pathological state [69, 109]	Adult worm nests obstruct lymphatic vessels Secondary infections
Chyluria	Ruptured dilated lymphatics in urinary tract Chyle present in urine	No role of bacteria
Hydrocoele	Lymphatic vessel enlargement in scrotum. Fluid accumulation and impaired lymphatic function. [89]	No role of bacteria

Table 2

Filarial toxins and their effects on host

Filarial toxin or excretory secretory products	Stage of parasite expressed	Mode of action and effects on host cells	References
Cysteine protease inhibitor	All stages	Inhibits lysosomal cysteine proteases, interferes with antigen presentation, ablates immune function	[66]
Abundant larval transcript -1 and -2	L3 larvae	Unknown biological function	[64]
Human cytokine homolog, macrophage migration inhibitory factor-1	All stages	Immunomodulatory cytokine, inhibits class II MHC restricted antigen processing, activates macrophage polarization	[87, 108]
Triose-phosphate isomerase	Early stage of adult female, L3 molting to L4	Maybe involved in host parasite interaction	[51]
Cuticular glutathione peroxidase	All stages Higher in adults than microfilariae	Removal of hydrogen peroxide generated by host cells; inhibition of lipid peroxidation; Protects from cellular toxicity; Adult survival	[30]
Thioredoxin peroxidases	All stages	Proliferation of PBMCs; Neutralizes endogenous and exogenous sources of oxygen radicals; defense against host immune cell attack	[63]
Transforming growth factor homolog-2	Blood stage microfilariae	Binding for host TGF- β receptors, immune evasion molecule	[48]
Superoxide dismutase	All stages	Neutralizes superoxide generated by activated host leukocytes anti-inflammatory factor	[112]
Acetylcholinesterase	Microfilariae have 10 times more than adults	Maybe involved in host parasite interaction	[98]
Glutathione S-transferase	All stages	Metabolism of host toxins; favors larval migration	[97]
Serine protease inhibitor-Serpin	Secreted by blood stage microfilariae	Elicit an early Th1-type response; stimulates IFN- γ and IgG1; inhibits neutrophil-derived serine proteinases, cathepsin G and elastase for host immune evasion	[88]
Leucyl aminopeptidase	L3 molting to L4	Immune modulation of host cells; Decreased lymphocyte proliferation; Th2 response by DCs	[92]
Vespid venom allergen	All stages Higher in L3	Establishing infection and pathogenesis	[2]