Update on Lymphatic Filarial Infections

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Filarial infections remain significant causes of disability in tropical areas worldwide. However, insights into the developmental and molecular biology of the parasite and the immunobiology of the host response to infection have advanced our understanding, even as progress is being made towards implementing eradication programs. This article summarizes some of the recent advances in the understanding of filarial biology and parasite immune evasion mechanisms, and reviews those newer aspects of diagnosis and treatment most relevant to clinicians.

Introduction

Lymphatic filariasis, caused by infection with the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, is estimated to infect over 129 million people in tropical and subtropical areas worldwide [1]. Most of the filaria-infected individuals have a subclinical condition associated with patent infection, and acute manifestations (filarial adenolymphangitis, acute dermatolymphangioadenitis, and tropical pulmonary eosinophilia) are rarely life threatening. However, chronic manifestations, such as lymphedema (elephantiasis) and hydrocele, are debilitating and are estimated by the World Health Organization to account for nearly five million disability-adjusted life years. Among parasitic diseases, only malaria ranks as being more debilitating [1]. Both host inflammatory response to adult worms and lymphatic dysfunction (perhaps resulting in secondary bacterial infections) have been implicated as mechanisms for the pathology associated with lymphatic filariasis [2]. Efforts to eliminate the burden of disease, therefore, must necessarily focus on preventing infection, early treatment of infected individuals, and controlling or stabilizing the morbid complications of infection.

A number of important advances have been made in recent months in our understanding of parasite biology and the host-parasite relationship. Our appreciation of the magnitude of clinical disease is deepening, even as diagnostic and therapeutic advances bring us closer to the elimination of lymphatic filariasis as a public health problem.

Parasite Biology

Parasite development

The causative agents of lymphatic filariasis are all transmitted by blood-feeding arthropods. Infective stage larvae (L3) penetrate the host through an arthropod-induced break in the skin. Larvae subsequently migrate to the afferent lymphatics and develop into adults. Adult females produce microfilariae (L1 or mf) that migrate to the bloodstream where they can be picked up by their mosquito vector. Most of the microfilariae display a periodicity in their bloodstream appearance, with the highest concentrations coinciding with the peak feeding time of the local vector.

Understanding the processes involved in life cycle progression may help identify areas amenable to intervention. Unfortunately, many aspects of the developmental biology of filarial parasites await elucidation. This lack of insight has stemmed, in part, from the lack of consistent in vitro methods for culturing the early life cycle stages of the parasite [3]. A serum-free system has been developed that supports the development of L3 into viable fourth stage larvae, providing the opportunity to examine the minimum requirements for early parasite development in the mammalian host. In the absence of human serum, molting to the L4 stage has been shown to require the addition of arachidonic acid and coculture with the yeast *Rhodotorula minuta* [3]. Recently, inhibitors of the lipoxygenase pathway of arachidonic acid metabolism have been shown to block molting in this system [4]. If life cycle progression could be similarly blocked in vivo, readily available pharmacologic agents could be of use in preventing the establishment of infection. Even if molting to L4 is only delayed, the prolonged exposure of L3 antigens to the host immune system could potentially allow for the development of a protective immune response similar to that induced by radiation-attenuated larvae.

Control efforts might be further aided by an understanding of life cycle progression in the insect vector. For example, an appreciation of the seasonal variation in transmission intensity in some areas, as determined by ambient temperature variation [5], could allow limited vector control resources to be focused on those time periods when they are likely to have the greatest impact.

Parasite genetics and molecular biology

Genomic research theoretically could provide the basis for identification of each protein expressed by filarial nematodes during their life cycle, and may accelerate the identification of diagnostic and vaccine candidates; in addition, it should provide insight into the molecular basis of immune

evasion [6]. Sequencing the estimated 100-Mb genome of *B. malayi* is underway (http://helios.bto.cd.ac.uk/mbx/fgn/filgen.html) [7]. Similar to *Taenia* species, *Entamoeba histolytica*, and *Plasmodium falciparum*, filarial nematode genomes are adenine and thymine rich, a bias that persists in the preferential use of amino acids coded by AT-rich codons [8]. Two other genomes, the mitochondrial genome and the genome of the *Wolbachia* bacterial endosymbiont, are also present within the filariae. The Filarial Genome Project, established in 1994, focused on gene discovery using an expressed sequence tag (EST) approach. Thus far, more than 22,200 ESTs have been completed, and more than 7000 gene clusters have been identified [9]. A *Wolbachia* Genome Consortium is also in place to sequence the entire genome of the *B. malayi* endosymbiont [9].

The expanding database of DNA sequences and the improved reproducibility of two-dimensional gel electrophoresis have allowed protein expression under different conditions to be compared. Individual proteins can be fingerprinted using mass spectrometry techniques, and database comparisons can be used to make identifications [10]. Additionally, filarial parasite microarrays are under construction and will be used to understand the patterns of gene expression under varying conditions of biologic relevance.

A genomic approach has already been used to focus attention on individual molecules that may be targets for chemotherapy [11] and/or vaccine development [12••]. Moreover, parasite molecules have been characterized that may modulate host immune functions such as T-cell cytokine production or antigen presentation, induction of apoptosis, or those parasite transcription factors that may be regulated by host growth factors [6].

Wolbachia endosymbiont

Wuchereria bancrofti, *B. malayi*, and perhaps *B. timori* all contain endosymbiotic rickettsial bacteria of the genus *Wolbachia*. Although the existence of these *Wolbachiae* endosymbionts has been known of since the 1970s, they have attracted increased attention recently because they have been shown to trigger immunologic responses when they are released from their nematode hosts, either as a result of natural parasite death or in response to anthelmintic therapy.

By following antibody levels to filarial antigen and *Wolbachia* surface protein in *Brugia*-infected rhesus monkeys [13], increased antibody levels to *Wolbachia* surface protein and filarial antigen were shown to coincide with the onset of lymphedema in amicrofilaremic monkeys, suggesting that an immune response to the bacterial endosymbiont is a factor in transient filarial edema. Moreover, the data suggest that recurrent boosting with bacterial products could lead to the chronic lymphedema characteristic of lymphatic filarial infection.

Evidence of a role for *Wolbachiae* in post-treatment reactions has been accumulating as well. It has been shown that lipopolysaccharide binding protein levels increase following treatment of brugian filariasis [14]. In addition, using a quantitative polyermase chain reaction technique for the measurement of the *Onchocerca volvulus Wolbachia*, it has recently been shown that *Wolbachia* DNA can be detected in serum following treatment of onchocerciasis, and that peak levels correlate with reaction severity in ivermectin-treated patients [15].

What makes these findings of particular importance is the demonstration that these bacterial endosymbionts can be eradicated by antibiotic therapy. Such treatment has the potential to reduce the pathology associated with infection and diminish the severity of post-treatment reactions. Furthermore, loss of the endosymbiont may disrupt filarial homeostasis and lead to decreased microfilaria production, if not achieve the elusive goal of killing the adult stage. Regimens of 6 weeks of doxycycline have been used clinically for onchocerciasis [16], but the development of an in vitro system for testing antibiotic susceptibility, using an insect cell line infected with the *Wolbachia* endosymbiont of arthropods [17], may be used to identify more convenient regimens.

Clinical Manifestations

Although many of the manifestations of lymphatic filariasis have been known for centuries, some much-needed attention has recently been paid to several important clinical aspects, including the sexual dysfunction associated with genital disease [18] and the age of acquisition of filarial infection [19•]. With the ability to assess infection status based on the presence of sensitive assays for circulating parasite antigen, the age of acquisition of patent filarial infection is now being recognized to occur much earlier than previously thought [19•]. Moreover, qualitatively collected data on school-aged children in a filarial endemic region of India suggest that hydroceles and adenolymphangitis play major roles in poor school performance, absenteeism, and dropout [20]. Recognizing that lymphatic filariasis with its clinical manifestations commences in childhood has major implications for prevention of disease in the individual patient and for broader initiatives to improve child health [19•].

Immunology

Filarial nematodes are capable of living years in their vertebrate hosts. The mechanisms by which they are able to subvert host immune responses for such extended periods is a subject of ongoing study and promises to give insight into immunologic mechanisms with ramifications beyond treating parasitic infections.

Recent studies of filarial infection using mouse strains with targeted gene deletions have confirmed the importance of interleukin (IL)-4 [21,22], IL-5 [23], and B cells [24] in controlling filarial infection and determining the clinical manifestations of infection [25]. Marked variations in the results are obtained, however, when using different strains of mice [22,24] or filarial species [21], underscoring the complexity of these rodent models.

Another approach to defining the intricacies of host-parasite interactions is to observe the influence parasites, parasite extracts, or recombinant parasite proteins have on host immune effector cells. Filarial antigens have recently been shown to decrease activation [26] and cytokine expression [27] of monocytes and dendritic cells and to desensitize B cells [28]. Individual filarial proteins have been shown to promote recruitment of eosinophils by inducing a Th2 response [29], or through direct cytokinetic activity [30•]. How precisely this promotes parasite survival or shapes the clinical features of filarial infection have yet to be determined.

The immunologic basis for protective immunity in humans has been notoriously difficult to define, in part because diagnostic tools have not been sufficiently sensitive to determine whether an individual is truly free of infection, and in part because longitudinally collected information on exposure and infection status has generally been unavailable for at-risk populations. In a cohort of subjects studied longitudinally over a 17-year period in the Cook Islands, endemic normal individuals—those who remained free of clinical and laboratory evidence of infection throughout the study period—maintained an increased cellular and humoral response to parasite antigens compared with initially microfilaremic individuals, whether or not those individuals cleared their infection in the interim [31•]. This suggests that infection-free asymptomatic individuals in endemic areas fall into two groups: 1) those who have been infected but cleared their infection, retaining the immune hyporesponsiveness characteristic of patency; and 2) those who are resistant and who maintain immunologic responses to parasite antigens. The relative numbers of individuals in each category is likely dependent on transmission, and therefore re-infection, intensity. This finding has clear ramifications for the development of vaccines based on the strategy of eliciting the immunologic profile of those deemed putatively immune. Toward this goal, in two separate studies examining the humoral responses of endemic normal individuals, this resistant phenotype was found to be associated with both anticarbohydrate antibodies of the IgG2 and IgG3 isotypes [32], and antibodies directed against L3 surface antigens [33]. Polysaccharide vaccines have not yet been widely evaluated for evidence of protective efficacy in filarial infections.

It has long been appreciated that the clinical manifestations of lymphatic filariasis differ between those living in an endemic area and those who acquired infection after a short-term exposure. Recent work seems to suggest that intensity of transmission in a given area can alter the immune response. Comparing two villages in Papua New Guinea in which the parasite, vector, and language of the inhabitants (which, in Papua New Guinea, implies genetic similarity if not familial relationship) were the same but transmission intensity differed by more than 60-fold, it was found that residents of the high transmission village had decreased proliferation and interferon-γ responses to filarial and nonfilarial antigens compared with their counterparts in the low transmission village, even when stratified for intensity of infection (*ie*, microfilarial level). The implication is that ongoing intense exposure to infective parasite stages modifies the immunologic profile and perhaps also the associated pathology [34••]. Along with epidemiologic data, this suggests that decreasing transmission intensity will disproportionately reduce pathology [35].

Diagnosis

Although the detection of the microfilariae in the blood has been the mainstay of the diagnosis of lymphatic filariasis, for *W. bancrofti* infection this method has been largely supplanted by detection of circulating filarial antigen. There are currently two commercially available tests, one in an enzyme-linked immunosorbent assay format [36], and the other a rapid-format immunochromatographic card test, each of which has a sensitivity that ranges from 96% to 100% and a specificity approaching 100% [37].

There are currently no tests for circulating antigens in brugian filariasis. However, recombinant antigens with specificity for *B. malayi* have recently been identified and preliminary experiments have shown improved diagnostic sensitivity for these infections [38]. A rapid-format test for brugian filariasis is envisaged for the near future.

Polymerase chain reaction-based assays for DNA of *W. bancrofti* and *B. malayi* in blood have been developed with equivalent or greater sensitivity than standard parasitologic methods. The utility of these molecular diagnostics, however, has not been well assessed using blood obtained when microfilariae are not present (*eg*, day blood of nocturnally periodic filariae). Methods for detection of DNA free of intact parasites have been developed for *B. malayi* infections [39], although such techniques have been less successful in bancroftian filariasis [40].

Treatment

Treatment of lymphatic filarial infections must be considered in two contexts: 1) the treatment of an individual patient to relieve or prevent symptoms, and 2) the treatment of an entire population in an endemic area to decrease transmission. Diethylcarbamazine (DEC) remains the anthelmintic of choice because of its partial macrofilaricidal activity, though its use should be avoided where concomitant onchocerciasis cannot be excluded because of the potential for severe post-treatment reactions. Recommendations for the treatment of

individuals with any clinical manifestation of lymphatic filariasis (lymphedema, hydrocele, tropical pulmonary eosinophilia, subclinical (asymptomatic) microfilaremia, adenolymphangitis, and asymptomatic adult worm carriers) have been codified in several recent monographs [41,42].

In support of efforts to assess the impact of treatment on transmission, with an eye toward control and possible elimination of lymphatic filariasis as a public health problem, a number of studies using annual single-dose treatment of DEC, albendazole, or ivermectin alone or in various combinations have been performed in multiple geographic settings [43]. Data from 17 studies conducted around the world, with a cumulative total of more than 90,000 subjects (with primarily *W. bancrofti* infection), suggest that the addition of albendazole to either DEC or ivermectin is safe [44•], and may provide additional antifilarial activity along with salutary effects on intestinal helminth infections [45].

In either context, treatment efforts would be improved if the adult-stage worms could be eradicated, obviating the need for annual retreatment. The macrofilaricidal effects of DEC or albendazole alone or in combination, as assessed directly by ultrasound or indirectly by antigen detection assays, have demonstrated a consistent but partial effect [46]. Regimens of higher dosage, longer duration, and new drug combinations, including the targeting of the bacterial endosymbiont, await evaluation.

Control

The development of a global program to eliminate lymphatic filariasis came following a resolution by the World Health Assembly in 1997 [47]. The principal goals of the program are to interrupt transmission of infection and to minimize the disability caused by the disease [48]. Community-wide distribution of yearly single-dose combination chemotherapy (DEC plus albendazole, or ivermectin plus albendazole) has been made possible by a donation of albendazole from GlaxoSmithKline (Philadelphia, PA) and an additional commitment from Merck & Company (Whitehouse Station, NJ) to expand the donation of ivermectin to African countries in which onchocerciasis and lymphatic filariasis are coendemic [49]. In addition, strategies focusing on hygiene and decreasing secondary bacterial and fungal infections are being implemented to control the morbidity associated with infection. Almost 40 million people in 27 countries are scheduled to receive antifilarial treatment in 2001 [1]. Combining mass treatment campaigns with vector control programs adds considerable expense to the endeavor, but may provide some additional assurance of success, particularly in areas where coverage and compliance are suboptimal [50].

Conclusions

The elimination of lymphatic filariasis as a public health problem is a concept that has emerged on the shoulders of sound basic and clinical research. New research efforts will be required to provide new targets for intervention; improved methods for assessing infection status; the ability to test for the emergence of drug resistance; and operational answers to the problems of drug distribution, cost, and socioeconomic impact. Research into vaccines that can prevent disease or block transmission is also ongoing, and may provide a necessary ally in this challenging campaign.

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