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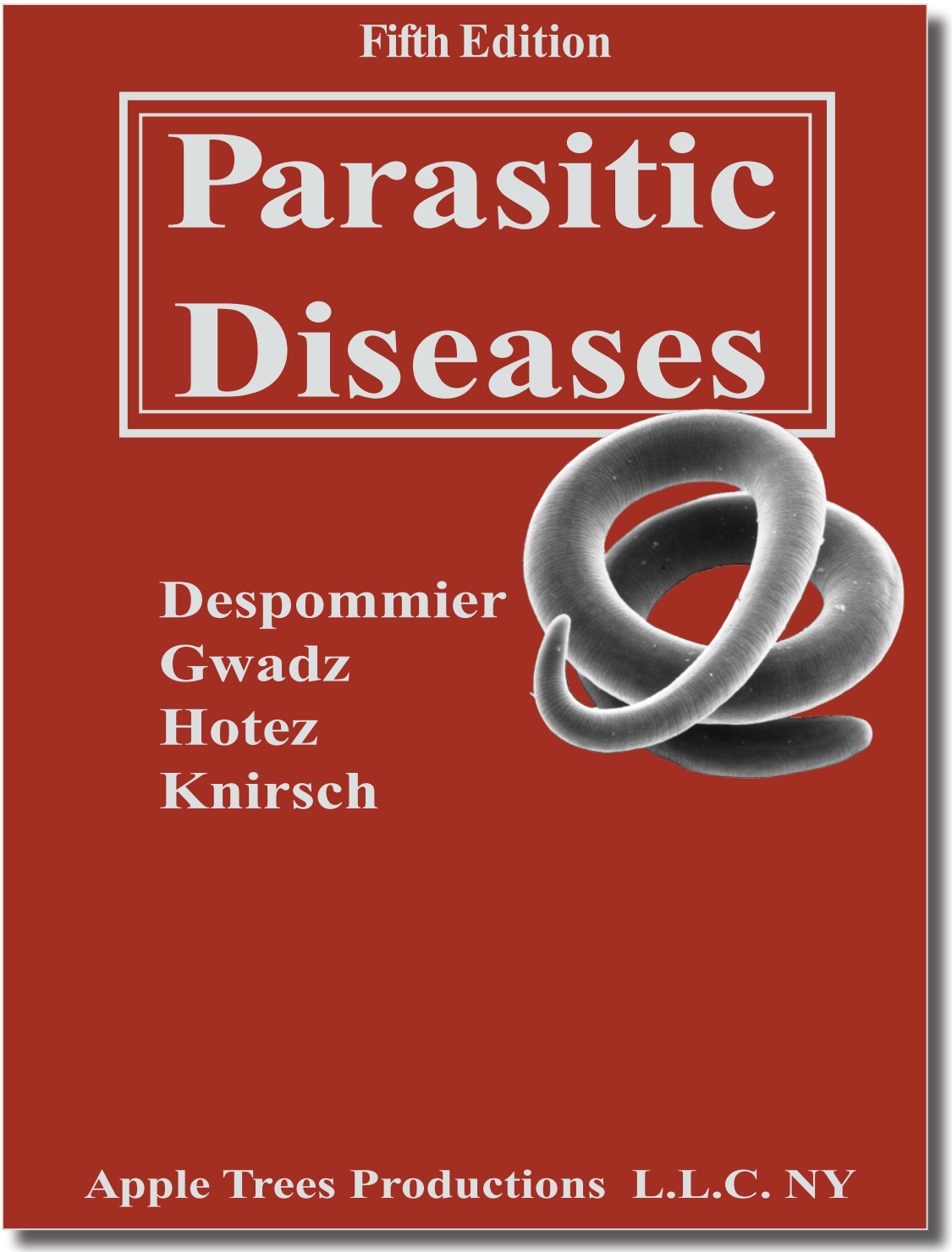


22. Lymphatic Filariae

Wuchereria bancrofti
(Cobbold 1877)

Brugia malayi
(Brug 1927)

Introduction

Wuchereria bancrofti and *Brugia malayi* are thread-like nematodes, and the adults live within the lumen of lymphatic vessels.[1] Approximately 120 million people in 83 countries are infected with some form of filariasis, of which the vast majority of cases occur as a result of *W. bancrofti* infection. Of these, approximately 40 million suffer from clinical disease. Only about 10 to 20 million people are infected with *B. malayi. B. timori* is a minor filarial parasite restricted to southeastern Indonesia. Elephantiasis, a disfiguring disease caused by blockage of the lymphatic vessels, affects large numbers of individuals living in endemic areas. The worms are ovoviviparous, and their larvae are called microfilariae. Lymphatic filariasis (LF) is transmitted by mosquitoes that take up microfilariae in a blood meal.

For *W. bancrofti*, humans are the exclusive host. The infection is widely distributed in the tropics, especially in South Asia, Africa (including Egypt) and tropical regions of the Americas. The major vectors are culicine mosquitoes in most urban and semi-urban areas, anophelines in rural areas of Africa and elsewhere, and Aedes species in the Pacific islands. With the exception of strains in the South Pacific, most of the *W. bancrofti* strains are nocturnal, referring to the periodicity with which the microfilariae appear in the peripheral circulation.

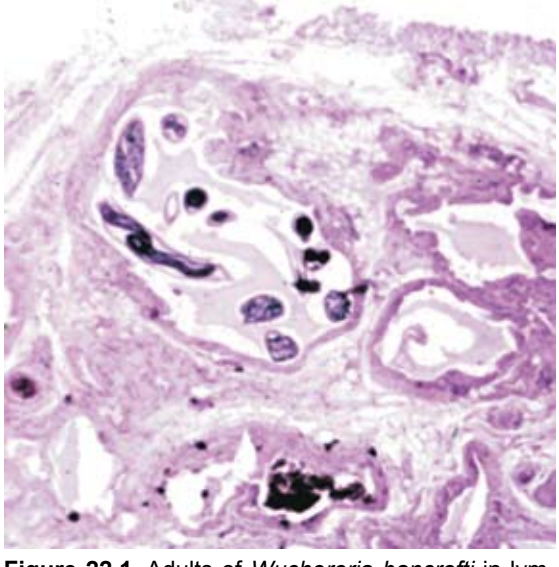


Figure 22.1. Adults of *Wuchereria bancrofti* in lymphatic vessels.

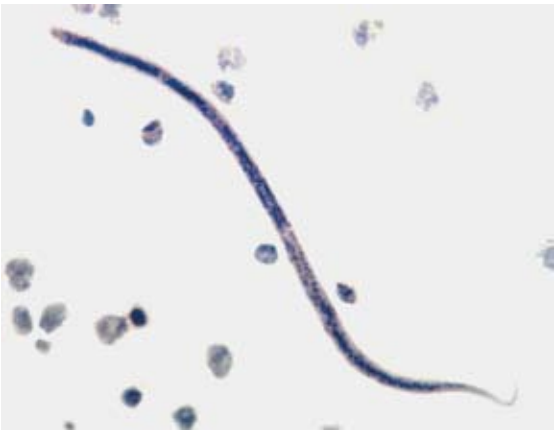


Figure 22.2. Microfilaria of *W. bancrofti.* 250 μm

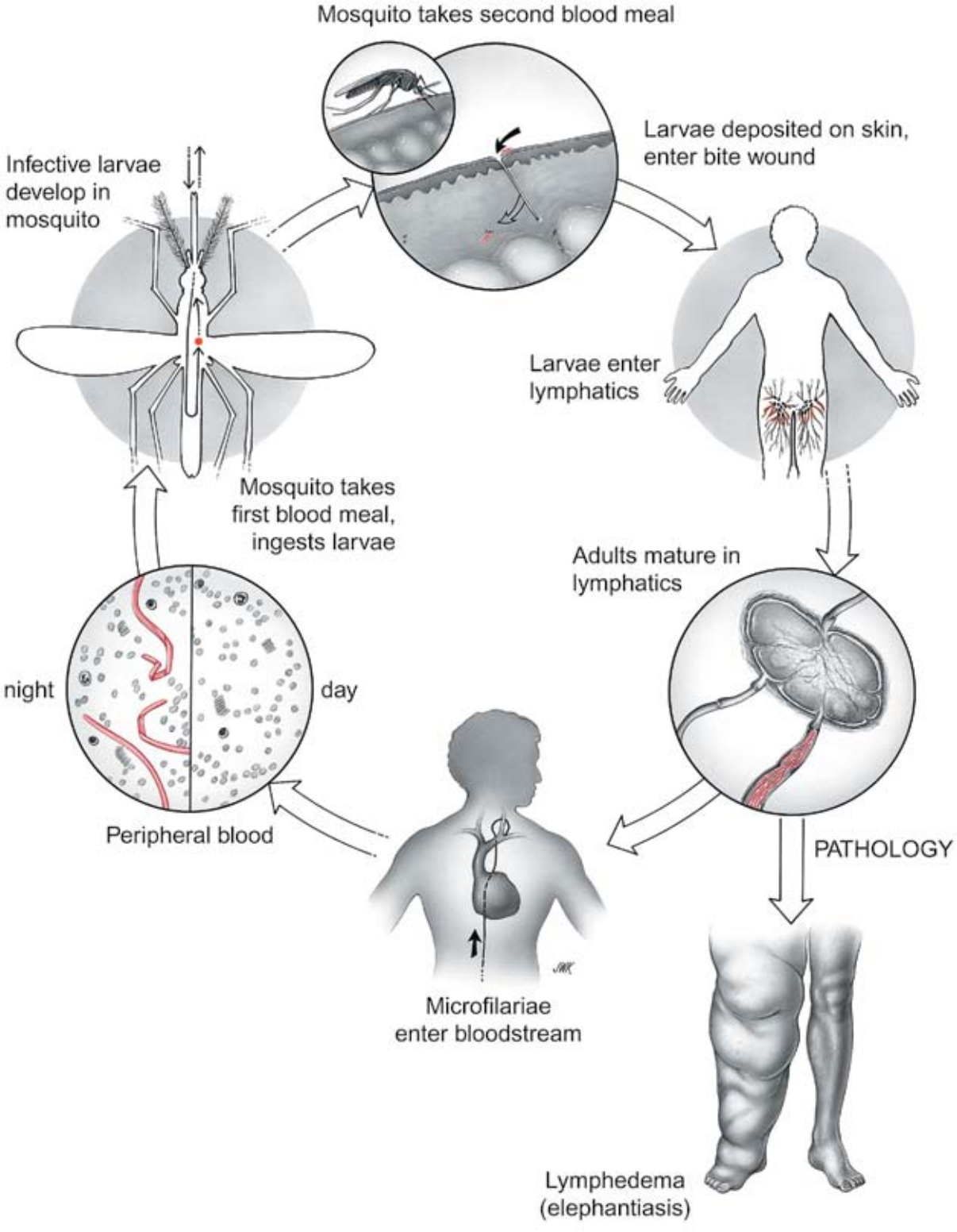
B. malayi infection, on the other hand, is a zoonosis, with both feline and monkey reservoirs. *Mansonia spp.* serve as the major vector, although anopheline mosquitoes are also sometimes involved in transmission. *B. malayi* infection is found in India, Malaysia, and other parts of Southeast Asia. There are other minor members of the genus Brugia that cause disease in humans,[2] including *B. timori* on the Indonesian islands of Timor and Flores, and accidental zoonotic Brugia infections (e.g., *B. beaveri* and *B. lepori*) that occur sporadically in the United States.[3]

A major global effort is underway to eliminate LF over the next 10-20 years.[4, 5] The term 'elimination' refers to the reduction of disease incidence to zero or close to zero, with a requirement for ongoing control efforts.[6] The strategy for LF elimination relies on interrupting mosquito transmission by mass administration of combination therapy in endemic regions in order to reduce the number of microfilariae circulating in the bloodstream of infected individuals.

Historical Information

Demarquay, in 1863,[7] described microfilariae of Wuchereria, while Cobbold, in 1877,[8] wrote a description of the adult worm. Lewis[9] did the same in India that same year. In 1878, Manson[10] completed the description of the cycle while working in Amoy (now called Xiamen) along the Chinese coast in Fujian Province. Today, lymphatic filariasis has been largely eradicated from China. Manson first demonstrated that mosquitoes were intermediate hosts for the parasite. For two decades, Manson maintained that infection was acquired when individuals drank water contaminated with larvae released from dead or dying mosquitoes. Eventually, he came to accept the concept that larvae were

Wuchereria bancrofti



transmitted by the bite of mosquitoes. Filariasis may, in fact, be a water-borne disease under some circumstances, since experimental infections can be induced by the oral route.[11]

One of the most important developments in the history of LF control was the discovery by Chinese parasitologists during the 1970s and 1980s that it is possible to dramatically reduce the prevalence through simultaneous administration of the drug diethylcarbamazine (DEC) to infected populations. This was achieved primarily through medication of regional salt supplies with DEC. Ironically, the LF life cycle was discovered in China and LF was first eliminated in China. The accomplishments of the Chinese provided proof-of-principle that it might be possible to eliminate LF worldwide through similar measures.

Life Cycle

Adult worms occupy the lumen of lymphatic vessels (Fig. 22.1), and have been found at all sites within the lymphatic circulation. It is presumed that they also occupy the adjacent subcutaneous tissues. Most commonly, they live in the lymphatics of the lower and upper extremities and male genitalia. Both species are about the same size. The female typically measures 4 to 10 cm in length, and the male 2 to 4 cm. After mating, the female worm can release 10,000 or more offspring per day. Instead of releasing eggs, the worms release first-stage larvae, which are known as microfilariae. Each microfilaria (Fig. 22.2, 22.3) measures approximately 270 µm by 10 µm and contains nuclei that characteristically do not extend to the tip of the tail. Another distinguishing feature is that the microfilaria is encased in a sheath comprised of chitin. The sheath is possibly a remnant of its eggshell.

Microfilariae migrate from the lymphatic circulation into the bloodstream. However they are typically present in large numbers in the peripheral blood only at night (between 10 PM and 6 AM) in most endemic areas of the world. During the day, the microfilariae aggregate in the capillaries of the lungs when activity of the host is increased (i.e., during strenuous exercise). Nocturnal periodicity can be a result of the microfilaria's penchant for low oxygen tension, at which time they are found in the peripheral blood stream,[12] or it may reflect subtle pH changes in the pulmonary venous circulation during sleep. Experiments in which sleep habits of infected volunteers were reversed also reversed the periodicity of microfilariae. The diurnal periodicity pattern characteristic of the South Pacific strain has not been satisfactorily explained. A less-frequently occurring sub-periodic filariasis caused by *W. bancrofti* occurs in certain regions of the world. Microfilariae live for about 1.5 years, and must be ingested by a mosquito to continue their life cycle.

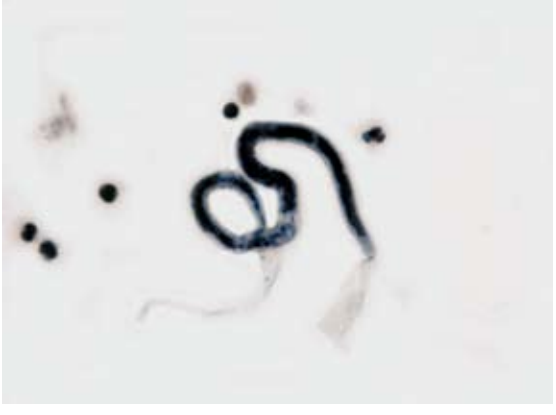


Figure 22.3. Microfilaria of *Brugia malayi*. 220 µm

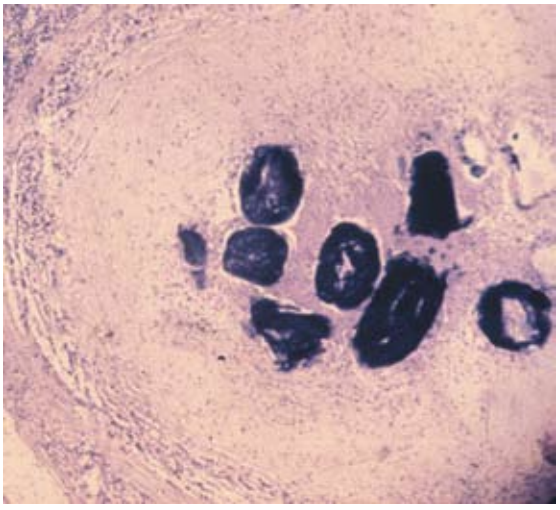


Figure 22.4. Calcified adults of *W. bancrofti* in blocked lymphatic vessel.

W. bancrofti is transmitted by a wide variety of mosquito genera and species, the most important being *Culex pipiens quinquefasciatus, Culex pipiens pipiens, Anopheles gambiae,* and *A. polynesiensis. Aedes aegypti*, the yellow fever mosquito, can also transmit the infection in some of the Pacific Islands. Ingested microfilariae penetrate the stomach wall of the female mosquito and locate to the thoracic flight muscles. There, they undergo three molts, developing into third-stage larvae and become infective after 10-20 days of growth and development in the insect muscle tissue.

Infective larvae locate to the biting mouthparts, and are deposited onto the skin adjacent to the bite wound during consumption of a subsequent blood meal. When the mosquito withdraws her mouthparts, larvae crawl into the open wound. Immature worms migrate through the subcutaneous tissues to the lymphatic vessels. The worms slowly develop into mature adults in about 1 year, and soon after copulation, begin shedding microfilariae. The longevity of adults, measured

by the continuous production of microfilariae, is estimated at 5-8 years. Infections lasting 40 years have been reported.[13]

The adult and larval stages of *B. malayi* resemble those of *W. bancrofti*. The life cycles of the two species of filariae are similar, although animal reservoirs occur for some members of the genus Brugia. The sub-periodic strain of *B. malayi* is a zoonosis acquired from forest monkeys and other wild animals, and transmitted through the bite of *Mansonia spp*. mosquitoes.[2]

Cellular and Molecular Pathogenesis

Pathogenesis of lymphangitis leading to elephantiasis has not been fully explained. It may result from a sequence of host-mediated immunopathologic events that occur in response to dead and dying adults within the lymphatics (Fig. 22.4). In contrast, living adult worms or the microfilariae are believed to suppress these responses. Hence, the processes associated with lymphangitis and elephantiasis can take years to develop and therefore are not commonly seen in children. Exactly how living worms and microfilariae suppress the host inflammatory response is unknown, except for the observations that microfilariae produce prostaglandin E2, a modulatory agent for leukocytes,[14]

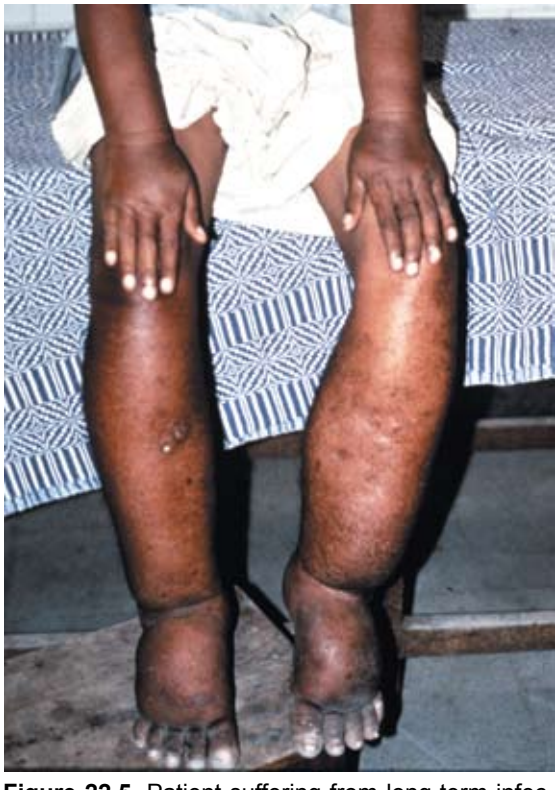


Figure 22.5. Patient suffering from long-term infection with *W. bancrofti.* Most adult worms have died and calcified, blocking all lymphatic drainage from the groin. The result is elephantiasis of both legs.

and adult worms secrete anti-mitotic and immunosuppressive substances.

When dead and dying adult worms relinquish control of the host's defense mechanisms, the result is a series of inflammatory reactions causing alterations of the walls of the lymphatics. After an intense lymphocytic infiltration, the lumen of the vessel eventually closes, and the remnants of the adult worms calcify. The blockage of lymphatic circulation continues in heavily infected individuals until most major lymph channels are occluded, causing lymphedema in the affected region of the body. In addition, hypertrophy of smooth muscle tissue occurs in the area immediately surrounding the site of involvement.

As already implied, the process of lymphatic blockage is a protracted one and results from repeated infections. Consequently, individuals visiting endemic areas for short periods usually do not develop lymphedema, even though they often have microfilaremia.

Not all patients with chronic exposure of infective larvae of *W. bancrofti* develop overt clinical disease. There is an intense clinical investigative effort underway at several laboratories to understand why, despite relatively equal levels of exposure, some infected residents remain largely asymptomatic but with evidence of microfilaremia, whereas other individuals progress to advanced clinical disease comprised of lymphangitis and elephantiasis. Frequently, patients with advanced clinical disease do not have evidence of circulating microfilariae. Differences in host cytokine patterns have been noted among these different groups of patients, and it has been suggested that different populations are prone to either Th2 or Th1 biases in their cellular inflammatory responses.[15-17]

Two major observations within the last several years have challenged the conventional thinking about how the pathologic sequence of events leading to lymphangitis, lymphedema and elephantiasis occurs.

First, there is evidence from ultrasound studies conducted in LF-endemic areas that the living adult filarial worms induce important pathologic changes, including lymphatic dilatation, which may lead to subsequent chronic lymphatic changes. This observation has challenged the notion that only dead and dying worms initiate the pathologic sequence. Adding to the complexity is an ultrasound observation that one part of the adult worm can die and calcify while another can remain alive and moving.

Second, there is evidence that secondary bacterial and fungal infections contribute significantly to the chronic pathology of elephantiasis. It has been further established that adult *W. bancrofti* worms harbor bacterial symbionts of the genus Wolbachia. Adult *W. bancrofti* depend on these symbionts for their survival, and antibiotics that target them exhibit an anthelmintic effect. Further, Wolbachia contain endotoxin-like

molecules and it is believe that these molecules may contribute to the inflammatory responses seen to dead and dying worms. Therefore, there is an emerging picture that Wolbachia contributes a major role to the pathogenesis of filarial disease.[18]

Clinical Disease

There is a spectrum of clinical manifestations resulting from *W. bancrofti* or *B. malayi* infections, ranging from asymptomatic infection to advanced elephantiasis.

Asymptomatic Infection (Lymphatic Dilatation)

The majority of residents living in an endemic area do not manifest strong inflammatory responses to their filarial parasite load. They are noted to be asymptomatic even though they harbor circulating microfilariae. Recently some of these so-called asymptomatic patients have been observed to exhibit subtle pathology when examined more closely by ultrasound or radionuclide studies.[19] It is currently thought that the central event in the pathogenesis of more advanced disease may begin at this stage when dilatation of the lymphatic vessels begins to occur. This dilatation initiates a subsequent series of events that results in the chronic clinical manifestations of LF, including lymphedema and hydrocele.[20] In some cases, the dilated vessels rupture to produce chyluria and chylocele.

Acute Lymphadenitis and Filarial Fevers

Death of the adult worm causes the next step in the progression of disease by producing an acute inflammatory response that is manifested as acute lymphadenitis. In endemic areas, this occurs frequently during the adolescent years and is manifested with fevers and painful swellings over the lymph nodes.[21] This typically occurs in the inguinal area. Episodes of painful swellings can last up to a week and commonly recur. Secondary bacterial infections may also result. Acute filarial lymphadenitis is exacerbated by secondary bacterial infections.

Some short-term travelers to endemic areas can also develop acute lymphadenitis, but the pathogenesis of this process occurs by a poorly understood process. This phenomenon was described in the 1940s among American troops returning from war in the Pacific theatre.

Elephantiasis

A subset of patients with acute lymphangitis and filarial fevers will go on to develop lymphedema of the arms, legs, breasts and genitalia leading to elephantiasis (Figure 22.5.). During these inflammatory processes, the skin becomes doughy and exhibits some degree of pitting, though it is rather firm. As the inflammatory reaction continues, the area becomes firmer still, and pitting disappears. There is substantial encroachment on the subcutaneous tissue and consequent loss of elasticity of the overlying skin. Characteristically, and in contrast to cellulitis caused by some bacteria, filarial cellulitis shows no demarcation line between the affected and the healthy skin. In Bancroftian filariasis, the legs are more likely to be involved than are the upper extremities, and the lower portions of the legs are more involved than the upper ones. The scrotum is frequently affected in the form of hydroceles and may become gigantic, weighing up to 10 kg; much larger scrotums have been described in rare cases.

Tropical Pulmonary Eosinophilia (TPE)

TPE develops in some individuals with filarial infections. This syndrome, which occurs frequently in southern India, particularly in young adult men, is characterized by high levels of serum immunoglobulin E (IgE), nocturnal asthma with interstitial infiltrates on chest radiographs, fatigue, weight loss, and eosinophilia.[22] Left untreated, TPE can progress to chronic restrictive lung disease. Diethylcarbamazine is highly effective in these patients. The pathogenesis of this syndrome is related to local immune responses to microfilariae in the pulmonary vasculature, and results in eosinophil accumulation in the lung with the release of cytotoxic eosinophil products (e.g., major basic protein and eosinophil cationic protein).[22]

Diagnosis

Lymphatic filariasis should be suspected in an individual who resides in an endemic region, is beyond the first decade of life, and has lymphedema in the extremities or genitalia. Definitive diagnosis has traditionally depended upon microscopically observing the characteristic microfilariae in the blood (Figs. 22.2, 22.3; Figs. C.46. – C.53., in Appendix C). Occasionally, infection is so heavy that microfilariae can be observed on a thin blood smear stained with Giemsa. In lighter infections, methods include filtering blood onto a 0.45µm pore sized nucleopore filter, then staining it with Giemsa solution. In the case of very light infection, 1 ml of blood is preserved in 9 ml of 1% formalin and then concentrated by centrifugation (Knott test; see Appendix B). The pellet contains red blood cell ghosts and microfilariae. Stained smears of the pellet are then examined microscopically. Because of the nocturnal periodicity of some strains, it is best to draw blood during the customary hours of sleep (usually between 22:00 and 02:00 hours). It is possible, however, to provoke migration of microfilariae at other times by administering 1 mg of diethylcarbamazine to an adult patient and collecting blood 45-60 minutes later.

Two monoclonal-based ELISAs that detect circulat-

ing *W. bancrofti* antigens have been developed. One of these is available as a rapid format card test. The assay, which recognizes a 200 kDa antigen of adult worm origin, has a sensitivity of 96-100 percent and a specificity of 100 percent.[23] The other assay is marketed as Trop-Ag *W. bancrofti*, which is manufactured by JCU Tropical Biotechnology Pty. Ltd (Townsville, Queensland, Australia). This assay also has a sensitivity of 100 percent in microfilaremic patients. For both assays, the circulating filarial antigen remains diurnally constant, so that blood for diagnosis can be collected during the day. PCR-based tests are also being developed.

Increasingly, ultrasound has provided an important noninvasive modality for monitoring the efficacy of antifilarial drugs.[18] Ultrasound examination of the lymphatic vessels of the spermatic cord of infected men results in a distinctive sign, known as the "filarial dance sign" reflective of nests of live worms in the lymphatics. Adult worm death following treatment with DEC can be subsequently followed.

Treatment

It is recommended that all patients be treated, because even patients with so-called asymptomatic infection may have abnormal lymphatics, and there is increasing evidence that early treatment may prevent subsequent lymphatic damage. Diethylcarbamazine (DEC) has both macrofilaricidal (adult worm) and microfilaricidal properties, and is the treatment of choice for such patients. In many regions it is given in a dose of 6 mg/kg/day for 12 consecutive days for a total of 72 mg/kg body weight.[18, 24] For *W. bancrofti* infections, this results in at least a 90% decrease in microfilaremia within one month. DEC decreases the incidence of filarial lymphangitis, although it is not clear whether this reverses existing lymphatic damage. In men, the efficacy of treatment can be monitored by serial ultrasound examinations (see above), and by serial blood sampling.[18] Since DEC is only partially effective against the adult worm, repeat treatments are often required. This is often done every 6-12 months.[18] Recent data has suggested that single dose treatment with 6 mg/kg of DEC has comparable macrofilaricidal and long-term microfilaricidal therapy. Some clinicians have suggested that single-dose treatment can be repeated every 6-12 months.[18] DEC is associated with fever (probably resulting from disintegration of a few of the adult worms), occasional nausea and vomiting, and fleeting skin rashes. Ivermectin, a drug effective for therapy of onchocerciasis, also kills microfilariae of *W. bancrofti*, but it appears to have no macrofilaricidal properties.

Aside from the use of anthelminthic drugs, there are several treatment modalities that help to improve the chronic sequelae of LF, including lymphedema and elephantiasis. Both conditions, when they occur in the leg, are reversible with a hygienic regimen that includes prevention of secondary bacterial infections by prompt antibiotic treatment of acute bacterial attacks, aggressive treatment of skin lesions including those caused by Candida and other fungi, and physiotherapy.[18] Treatment of secondary bacterial infections has been identified as a critical treatment modality for worsening lymphedema and elephantiasis. Possibly this includes the treatment of Wolbachia symbionts. Hydrocele drainage, while it does provide relief, is often associated with reaccumulation of fluid.[19] For certain affected areas, (e.g., the scrotum) surgical excision and exteriorization of the testes to restore fertility may be required. However, even after surgery the affected area invariably becomes edematous once again. Microsurgery, in which shunting of lymphatic vessels around the area of blockage with stents has been employed, is quite effective in some cases, and surgical teams in India have perfected the procedure. Overall, however, for most clinicians the surgical management of lymphedema is being replaced by the medical management practices outlined above.

As noted in the section on cellular and molecular pathogenesis, a promising new modality of treatment is the use of doxycycline, rifampicin and other antibiotics in order to target the Wolbachia symbionts of *W. bancrofti*.[25] Studies are in progress to determine whether this approach might complement existing DEC treatment strategies.

Prevention and Control

Patent microfilaremia is first detected in children 5 to 10 years old who live in endemic regions.[2] Transplacental immunity and breast-feeding may limit the intensity of infection in younger individuals. The prevalence of microscopically confirmed infection gradually increases up to the age of 30-40 years.

The frequency of exposure to third-stage larvae by vectors is the most important determinant in the community prevalence of filariasis.[26] Prevention depends upon control of mosquito vectors, which, unfortunately, has had limited success because mosquitoes develop resistance to insecticides. Urbanization of vast areas of tropical Asia has resulted in a concomitant rise in the prevalence of both *W. bancrofti* and *B. malayi* varieties of filariasis, carried by mosquitoes that breed in non-sylvatic habitats.

In 1997, the World Health Assembly passed a resolution calling on its member states to undertake a global elimination program for LF. The major strategy for LF elimination is based on two principles: 1) to interrupt transmission of infection and 2) to alleviate and prevent the suffering and disability caused by LF (www.filariasis.org). To interrupt transmission, it is essential

to reduce the levels of microfilariae in the blood for a sustainable period. This is achieved by administering a yearly, single-dose, 2-drug regiment.[27] For most countries, the recommended drugs are DEC (6 mg/kg) and albendazole (400 mg). However, in many parts of Sub-Saharan Africa (and Yemen as well) where there is epidemiological overlap with loiasis and onchocerciasis, the toxicities caused by DEC in people with these conditions necessitate substituting ivermectin (200 mcg/kg). Such populations would receive ivermectin and albendazole. A period of 5 years of annual treatments is currently recommended. To date, the number of serious adverse events from LF control mass chemotherapy has been remarkably low. In some areas, a treatment regimen comprised of daily DEC-fortified salt is used.

To alleviate suffering and decrease the disability caused by LF, the major streategy has been to decrease secondary bacterial and fungal infections of the affected limbs and genitals. This includes meticulous local hygiene, judicious use of antibiotics, physiotherapy and health education.

Finally, there has been great interest in evaluating whether LF control practices that employ albendazole and ivermectin could have an impact on other co-endemic helminth infections including onchocerciasis and soil-transmitted helminth infections. Such integrated pro-poor strategies are attractive because of their economy of scales and cost-effectiveness.[28]

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