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Invited review

Lymphatic filariasis: new insights into an old disease

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Abstract

Lymphatic filariasis has afflicted people in the tropical areas of the world for thousands of years but even up to comparatively recent times it has been poorly understood and its importance under recognised. In the last 2 decades or so there has been a flurry of activity in filariasis research, which has provided new insights into the global problem of filariasis, the pathogenesis of filarial disease, diagnosis and control.

Keywords: Lymphatic filariasis; New insights; Old disease

1. Introduction

Lymphatic filariasis is a mosquito-borne parasitic disease caused by three species of tissue dwelling filaroid nematodes. *Wuchereria bancrofti* is responsible for 90% of cases and is found throughout the tropics and in some sub-tropical areas world-wide. *Brugia malayi* is confined to Southeast and Eastern Asia. *Brugia timori* is found only in Timor and its adjacent islands. Lymphatic filariasis has a long history which reaches back into antiquity (Routh and Bhowmik, 1993). Despite this, lymphatic filariasis has been a disease, which up to recent times, has been poorly understood and largely ignored by health authorities who were struggling to control what were perceived to be more important vector-borne diseases such as malaria and dengue fever. In the last 2 decades or so there has been a flurry of filariasis research, which has provided new insights into global burden filariasis, the pathogenesis of filarial disease, diagnosis, and control.

2. New insights into the global burden of lymphatic filariasis

Lymphatic filariasis has been identified by the World Health Organisation (WHO) as the second leading cause of permanent and long-term disability world-wide (WHO, 1997a,b). In addition to the medical problems, there are severe social and psychological consequences, especially in those who suffer from elephantiasis or hydrocele (Bandyopadhyay, 1996; Dreyer et al., 1997; WHO, 1997a). Lymphatic filariasis also has a huge economic impact upon endemic communities. In addition to the direct costs incurred in medical or surgical treatment, there are the enormous indirect costs resulting from reduced work capacity and labour loss (WHO, 1997a).

The total global burden of lymphatic filariasis is not known and mapping of its endemicity and prevalence is on-going. Endemicity has been confirmed in 76 countries and most recent estimates (Michael et al., 1996a; Michael and Bundy, 1997; Ramachandran, 1997) suggest that filariasis infects 120 million people or 2.0% of the world's population. The WHO estimates that 44 million have overt clinical disease – lymphoedema, elephantiasis, hydrocele, recurrent infections associated with damaged lymphatics, lung disease, chyluria and renal disease. Another 76 million have pre-clinical damage to their lymphatic and renal systems. Millions suffer from debilitating acute attacks of filarial fevers and lymphadenitis (WHO, 1997a,b). The true figures are probably higher than this because most country surveys done in the past were based on the demonstration of microfilariae. Modern surveys using antigen detection (see below) have shown that surveys of microfilaraemia alone can underestimate the prevalence of filariasis by as much as 30% (Turner et al., 1993; Chanteau et al., 1995). There is evidence that the global burden of filariasis is increasing. To quote Piessens and Partono (1980): "Filariasis shares with schistosomiasis the dubious honour of being one of the major diseases whose prevalence and distribution are increasing in the developing world at a time when other tropical diseases are slowly disappearing. This is all the more remarkable in view of the relative inefficiency of

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the filarial transmission, the complexity of the parasite's life cycle and the susceptibility of the worms to the widely available and inexpensive drug diethylcarbamazine, that should render filarial nematodes particularly vulnerable to effectively applied control measures."

There are several reasons for this. Several of the major vector mosquitoes thrive in urban slum areas and the increased urbanisation around the world's major tropical cities is putting an increasing number of people at risk (Hunter, 1992; Albuquerque et al., 1995; Dhanda et al., 1996). Rural–urban migration and uncontrolled urbanisation often lead to overburdening of sewerage and wastewater systems. The resulting pools of stagnant, polluted water provide an ideal breeding ground for *Culex quinquefasiatus*, a major vector of filariasis (Mak, 1986). In contrast to anophelines, culicines can efficiently transmit filariasis in situations where the microfilariae density is low (Webber, 1991).

It has long been recognised that economic development projects can have an adverse impact on health. Irrigation projects in Ghana have been associated with an increase in filariasis where it was found that almost every house in villages bordering a man-made irrigation scheme had a case of filariasis. By contrast, only scattered cases were seen in villages 2 km or more away from the canals (Hunter, 1992). Dzodzomenyo et al. (1999) observed a similar phenomenon in another part of the same country.

3. New insights into filarial disease

Traditionally it has been accepted that three groups of people will be found in a filarial-endemic area (Dasgupta, 1984; Partono, 1987; Ottesen, 1989, 1992, 1993, 1994; Evans et al., 1993; Roberts and Janovy, 1996): (1) those who are exposed, but with no evidence of disease – so called 'endemic normals'; (2) those with 'asymptomatic microfilaraemia'; (3) those with chronic disease such as chronic lymphoedema, hydrocele and elephantiasis. People in all these groups can also suffer episodes of acute filarial disease. In some areas, most notably in Asia, another manifestation of filariasis arises – tropical pulmonary eosinophilia (see below).

In a filarial context 'endemic normal' refers to people who, despite constant exposure to filariasis and circulating anti-filarial antibodies, are amicrofilaraemic and have no clinical evidence of disease (Ottesen, 1989; Weil et al., 1996). Whether these people are truly immune to filariasis is debatable. Kazura et al. (1993a) and Weil et al. (1996) have shown that many of these so called 'endemic normals' have a cryptic infection because they have circulating filarial antigen which is identical to that found in people with microfilaraemia and ultrasound examination has also revealed that many men who are classified as 'endemic normals' have adult worms in their scrotum (Dreyer et al., 1996a; Noroes et al., 1996a,b; Simonsen et al., 1997; Faris et al., 1998; Suresh et al., 1997). A conclusion was arrived at by Day et al. (1991) who stated "if you look carefully enough in any infected population, the endemic normal is a rare individual."

'Asymptomatic microfilaraemia' is often the most common manifestation of filariasis in many endemic populations and has been reported in children as young as 14 months (Lowman, 1944). It is often regarded as a 'non-disease' because the individuals concerned have no inkling that their blood contains large numbers of microfilariae and this situation may persist for decades without any progression to overt clinical disease (Ottesen, 1992). Recent studies, however, have shown that 'asymptomatic microfilaraemia' is not a benign phase, and that considerable occult lymphatic, tissue and organ damage may be occurring (Freedman et al., 1994, 1995; Ottesen, 1994; Dissanayake et al., 1995a). Ultrasound has shown that approximately half of the men with 'asymptomatic microfilaraemia' have nests of motile adult worms in their scrotal lymphatics: the 'filarial dance sign' (Amaral et al., 1994; Noroes et al., 1996a). For several centimetres either side of the worms the lymphatics are abnormally dilated but there is no evidence of an inflammatory response (Amaral et al., 1994). Lymphoscintigraphy has also revealed profound changes in 'asymptomatic microfilaraemics' (Ottesen, 1994; Noroes et al., 1996b). Their lymphatics are markedly dilated with collateral channelling and increased lymph flow. By contrast, patients with elephantiasis (see below) show tortuosity, dermal back-flow, obstruction, stasis and poor regional node visualisation (Witte et al., 1993; Azoubel, 1996). However, Marchetti et al. (1998) observed that these changes are not specific for lymphatic filariasis and can be found in residents of non-filarial-endemic areas as well.

The absence of an inflammatory response in the lymphatics near the adult worm in the asymptomatic stage of the disease suggests that the lymphatic changes are induced by parasite or host-derived molecules acting directly on the lymphatics rather than an inflammatory reaction mounted by the host. Conversely, the changes seen in the tissues of those with chronic pathology are wrought by a mechanism which includes a vigorous inflammatory reaction (Ottesen, 1994). Exactly what triggers the change from one to another is an on-going debate in filarial research, but there is a lot of evidence to suggest that filarial-driven immunological changes and lymphatic obstruction per se, although they undoubtedly play a part, are not as important in the evolution of chronic lymphoedema and elephantiasis as once thought.

Shenoy et al. (1999) and Dreyer et al. (2000a,b) provide convincing evidence that acute episodes of bacterial infection are the most likely cause of worsening lymphoedema and progression to elephantiasis and that active steps to improve hygiene and detect infected lesions, together with exercise and physiotherapy, can slow the onset of elephantiasis, and even reverse it in some cases.

Acute filarial attacks can occur in infants as young as 3

months (Dasgupta, 1984) but they usually start appearing in older children and teenagers and continue throughout life (Nanduri and Kazura, 1989). They can occur in both amicrofilaraemics and microfilaraemics, and are common in people with chronic filarial pathology (Evans et al., 1993; Dreyer et al., 1999a,b). In some cases filarial infection markers such as filarial antigen and anti-filarial IgG4 antibody are absent. A possible explanation in these cases is an inflammatory response against in-coming L3 larvae before the adult worm has become established (Addiss et al., 1994), but as yet there is no experimental evidence to support this hypothesis.

There are at least two distinct mechanisms involved in the pathogenesis of acute attacks. The first is called acute dermatolymphangioadenitis, where there is development of a plaque-like lesion of cutaneous or sub-cutaneous inflammation, which may be accompanied by ascending lymphangitis and regional adenitis. Typical systemic manifestation of bacteraemics such as chills and fevers is usually present. There is often oedema of the affected limb. This may completely or partially regress after the acute attack subsides but as noted above, recurrent acute dermatolymphangioadenitis is an important cause of chronic lymphoedema (Olszewski et al., 1997, 1999; Dreyer et al., 1999a,b). The other syndrome is called acute filarial lymphangitis and is believed to be the result of an immunological reaction to dead or dying adult worms which have either been killed by the immune system or chemotherapy (Dreyer et al., 1999a,b). In contrast to acute dermatolymphangioadenitis, acute filarial lymphangitis presents as a distinct well-circumscribed nodule or cord and there may be local lymphadenitis with lymphangitis spreading in a centrifugal pattern. Fever is not usually present. Both acute dermatolymphangioadenitis and acute filarial lymphangitis can occur in the same patient.

There is good evidence to show that acute filariasis is not confined to the residents of filarial-endemic areas. It is also seen in filarial-infected expatriates, and a large number of cases were seen in heavily-exposed United States servicemen stationed in the Pacific during World War II (Wartman, 1947). Other cases are reported in expatriate workers and travellers from time to time (McQuay, 1967; Bean et al., 1992; Turner and Usurup, 1997; Melrose et al., 2000a).

In recent years the traditional classification of filarial disease has been challenged. The introduction of assays for circulating filarial antigen (see below), the discovery of occult lymphatic pathology and renal disease in 'asymptomatic microfilaraemics' and the recognition of the role of bacterial infection in the pathogenesis of acute and chronic disease suggests that the old classification based on the presence or absence of microfilaraemia and/or chronic pathology is outdated. It is no longer wise to think of individuals as having filarial 'infection' without 'filarial disease' for the same reason – many of the former will have evidence of covert disease if the studies are rigorous enough.

Freedman (1998) proposes a new classification: asymptomatic infected individuals who are antigen negative, individuals who have overt filariasis and active infection (antigen positive) and individuals who have overt filariasis without active infection (antigen negative). Freedman defines overt filariasis as any of the clinical manifestations associated with the known clinical spectrum of filariasis: adenolymphangitis, hydrocele or elephantiasis. On the face of it, this seems to be a very sensible approach and means quite simply that a 'filariasis case' can be defined as anyone who is filarial antigen positive or has microfilaraemia. This classification is by no means perfect, however, and requires some refinement. It lumps together a number of manifestations with differing pathogenesis, and where do the asymptomatic individuals who are antigen positive fit into the classification? At this point in time there is no way of telling (apart from those who have adult worms identified by ultrasound) if antigen positive–microfilariae negative individuals without overt clinical disease have pre-patent, single sex, or post-patent infections.

There is also convincing evidence that prenatal exposure to filarial antigen determines the subsequent character of the immune response to postnatal infection (Ottesen, 1992) and that neonates exposed to parasite antigens in utero develop altered foetal immune responses that influence the development of infection in later life (Malhotra et al., 1997). A child born to (or tolerised by) a microfilaraemic mother is more likely to develop microfilaraemia than a child born to an amicrofilaraemic mother and children born of microfilaraemic mothers are less capable of mounting an immune response to filarial antigens than those children born of amicrofilaraemic mothers. This effect may persist into adulthood (Lammie et al., 1991; Hightower et al., 1993; Steel et al., 1994) but there is no experimental evidence that it impacts upon the development of clinical disease.

There is also evidence that filarial infection may impact upon other diseases. One of the most important is the contribution that filariasis makes to renal disease and haematuria, proteinuria, nephrotic syndrome and glomerulonephritis have all been recorded (Ormerod et al., 1983; Ngu et al., 1985; Langhammer et al., 1997; Barsoum, 1999). Most of the renal damage is blamed on circulating immune complexes containing filarial antigen (Prasad and Harinath, 1988; Lunde et al., 1988; Lutsch et al., 1988). In a study of 20 patients with 'asymptomatic microfilaraemia' Dreyer et al. (1992) found that seven had microscopic haematuria and four had proteinuria but their creatinine clearances were normal. Ten individuals who had not shown haematuria and 10 who had not shown proteinuria developed it after diethylcarbamazine treatment but all patients in the study had normal urinanalysis 2–3 weeks after therapy.

In addition to renal dysfunction, a wide spectrum of other clinical signs and symptoms have been associated with filariasis infection by various investigators. In some of these the connection is somewhat tenuous and a direct causal relationship between filarial infection and the other diseases has not

been established. Those with the strongest link to filarial infection have been reviewed by Dreyer et al. (1999b).

Filariasis can be associated with respiratory signs and symptoms. These are found most notably in tropical pulmonary eosinophilia. Tropical pulmonary eosinophilia is especially common in Southeast Asia, India, and in some parts of China and Africa (Ong and Doyle, 1998). It is caused by a hypersensitivity reaction to allergens being trapped and destroyed in the lungs (for a review of the pathogenesis see Dreyer et al., 1999b). Features of the syndrome are: a paroxysmal cough, wheezing and dyspnoea lung infiltrates visible on X-ray, an absolute eosinophil count above $3{,}000 \times 10^9$/l, an absence of microfilariae in the peripheral blood (but adult worms have been detected; Dreyer et al., 1996b,c), a raised total serum IgE, and a favourable response to diethylcarbamazine (Ong and Doyle, 1998). Raised anti-filarial antibodies are often cited as characteristic of tropical pulmonary eosinophilia. However, Rocha et al. (1995) found that 'anti-filarial' antibodies that are usually considered diagnostic for tropical pulmonary eosinophilia were equally elevated in patients with non-filarial, tropical pulmonary eosinophilia-like syndromes and were diagnostically unhelpful. Some people believe that filariasis may be a trigger for bronchial asthma. de Sylva et al. (1990) found that more than one-third of children with cough and wheeze had filarial infection. Symptoms improved after diethylcarbamazine therapy, probably due both to the elimination of the microfilariae, and the anti-inflammatory effects of the diethylcarbamazine. The authors postulate that atopic individuals have a heightened response to filarial antigen, which may trigger asthma.

Filariasis may present with rheumatic features (Adebajo, 1996) and mono-arthritis of a knee or ankle joint is common in filarial-endemic areas. Chaturvedi et al. (1993) investigated 19 cases of arthritis of unknown origin in children and found that 16 of them had filariasis. Nine of these cases were treated with diethylcarbamazine and the arthritis resolved. Poddar et al. (1994) reported two cases of polymyositis which presented with generalised painful swelling and weakness of the muscles. Serum creatinine kinase was elevated and inflammatory myopathy was present in biopsies. *Wuchereria bancrofti* microfilariae were present in the peripheral blood. The patients responded better to diethylcarbamazine and steroids than to steroids alone and clinical improvement was associated with the clearance of the microfilariae. A patient with acute filarial myositis that responded rapidly to diethylcarbamazine therapy is reported by Sundaray et al. (1992).

Other complications recorded include: cystitis with urethral obstruction (Devasia et al., 1998), fibrosing mediastinitis (Gilbert and Hartman, 1996), tropical vaginal hydroceles (Sivam et al., 1995) and bladder pseudotumours (Gourlay et al., 1999).

All parasites induce a degree of immunosuppression as a means of ensuring their survival in the host and the question arises, what is the impact of this immunosuppression on other infectious agents? Whilst it is commonly believed that cellular immunosuppression induced by filariasis is filarial antigen-specific, there is evidence to the contrary. Grove (1979) found that only one out of 35 patients with filariasis responded to tetanus toxoid (3%) compared with eight out of 31 control subjects (26%) ($P < 0.025$) and that the response to *Salmonella typhi* is effected with only 15 of 26 patients with filariasis (58%) having an antibody titre of 1:40 2 weeks after immunisation compared with 25 of 31 in controls (81%) ($P < 0.05$). Delayed skin hypersensitivity reactions to *Candida*, mumps or streptococcal antigens were significantly reduced in filariasis patients compared with controls ($P < 0.01$). Srivastava et al. (1999) showed that filariasis patients have a significantly impaired antibody response to the H and O antigens of TAB vaccine and reduced skin hypersensitivity to PPD and chlorodinitrobenzine. Malhotra et al. (1997, 1999) have shown that 2–10-year-old children born to mothers who did not have filariasis produced a 10 fold increase in the production of interferon when challenged with PPD than those children born from filarial-infected mothers. This raises two important questions: is the efficacy of BCG vaccine impaired by filarial infection, and does filarial infection influence the course of tuberculosis? Borkow and Bentwich (2000) have already suggested that helminth eradication may be essential for successful vaccination against HIV and tuberculosis. This is clearly an area that requires further research. If it can be shown that filarial infection impacts upon the efficacy of vaccination it is a powerful incentive for global elimination of the parasite. There is also some evidence, albeit limited, that filarial infection may decrease the immunity to other tropical diseases. Sidkey et al. (1987) reported a patient with filariasis who presented with a disseminated *Hymenolepis nana* infection and they postulated that the filarial immunosuppression may have contributed to the spread of the other parasite. The situation was somewhat clouded, however, by the fact that the patient had been treated in the past with corticosteroids. Younis et al. (1997) studied the relationship between filariasis and intestinal parasites in a filarial-endemic area of Egypt. The overall prevalence of parasites was 81% in non-filarial-infected people and 92.4% in filariasis patients. On the basis of these data they concluded that the immune suppression caused by filariasis may increase the host's susceptibility to other parasitic infections. By contrast, however, Mohamed et al. (1983, 1992) could find no evidence of interaction between filariasis and schistosomiasis in an area endemic for both parasites. A study by Schmidt and Esslinger (1981) found that *Plasmodium falciparum* infections in Owl monkeys (*Aotus tri-virgatus griseimembra*) infected with the filarial parasite *Tetrapetalonema barbascalensis* followed a more benign course than in monkeys who were not infected with the filaroid and Yan et al. (1997) showed a down-regulation of murine susceptibility to cerebral malaria by inoculation with third-stage larva of *Brugia pahangi*. There is no evidence at this time, however, that filariasis influences the course of human

malarial infections. Ravindran et al. (1998) studied patients with both filariasis and malaria and found no suggestion of interaction. Again, this is a research area which should be actively explored.

4. New insights into diagnosis

Traditionally, diagnosis of lymphatic filariasis has depended upon the detection of microfilariae in blood collected around midnight in areas where microfilariae exhibit nocturnal periodicity, and around midday where periodicity is diurnal. The simplest method is a thick blood film of capillary blood stained with Giemsa stain (Khamboonruang et al., 1987; Schultz, 1988; Schuurkamp et al., 1990; Sabry, 1992). If a measured amount of blood is used (say 40–60 μl^3) the number of microfilariae per ml can be calculated (Moulia-Pelat et al., 1992). The disadvantage of thick films, like other direct methods, is that they underestimate the prevalence of microfilaraemia if microfilariae densities are low because the theoretical detection limit for such procedures is between 15 and 60 microfilariae per ml (Panicker et al., 1991; Turner et al., 1993; Faris et al., 1998). Another problem is loss of microfilariae from the film during processing especially if anticoagulated blood is used. Denham et al. (1971) observed a loss of between 10 and 40% and Southgate (1973) a loss of up to 51%.

Use of concentration techniques increases the sensitivity but amicrofilaraemic cases will still not be detected. An old but still widely used method is that of Knott (1935). One millilitre of blood is added to 9 ml of a 1% formalin solution in normal saline. After red cell lysis is complete the mixture is centrifuged and the deposit is examined for microfilariae. Because the formalin preserves the microfilariae Knott's tests can be set up in the field and processed when the field worker returns to base. The theoretical detection limit is one microfilariae per ml. The accuracy of the Knott's test and its ease of use suffers when blood is processed from individuals with a large amount of plasma gamma globulin, a common finding in developing country populations. The formalin precipitates the protein and makes the examination of the deposit difficult. The Knott's method has been improved by Melrose et al. (2000b) who add a small amount of Triton X-100 to the diluent which dissolves most of the proteingenous deposit and enhances the visibility of the microfilariae. Another widely used concentration method is the membrane filter technique whereby 1–5 ml of blood which has been diluted in water is passed through a filter fitted with a polycarbonate membrane which traps the microfilariae. The membrane is removed and the microfilariae stained and counted (Bell, 1967; Chulerek and Desowitz, 1970; Desowitz et al., 1973; Nathan et al., 1982; Moulia-Pelat et al., 1992).

In recent years the diagnosis of *W. bancrofti* infection has been revolutionised by the introduction of filarial antigen tests. Because these tests are not dependent upon the presence of microfilariae, blood can be taken at any time, and amicrofilaraemic cases will be detected. The commercial ELISA (Trop Bio Og4C3 Antigen Test, produced by Trop Bio Pty Ltd.) is based on the assay developed by More and Copeman (1990, 1991) and is a very good marker of active filarial infection with adult worms (Chanteau et al., 1994a). Blood samples taken onto filter paper strips can be used for the assay but results are variable. Lalitha et al. (1998) and Itoh et al. (1998) found that the filter paper method and the serum method gave comparable results but Gyapong et al. (1998) found the sensitivity of the filter paper test to be significantly inferior with only 50.3% positive with both tests.

The ICT filarial antigen test (Binax) is a rapid immunochromatographic technique using specific monoclonal and polyclonal antibodies. It utilises capillary or venous blood and is simple enough for field use by people with a minimum amount of training (Weil et al., 1997). Results are available almost immediately and the method is now the one of choice for community surveys and rapid assessment of filarial endemicity. The efficacy of filarial antigen tests has been reviewed by Simonsen and Dunyo (1999) and Phantana et al. (1999) who compared the ICT to thick blood films and membrane filtration and obtained the following results: sensitivity 100%, specificity 96.3%, predictive value positive 70.7%, predictive value negative 100%. Pani et al. (2000), however, found it less sensitive than the filtration test for detecting low-level microfilaraemia citing a 88.3% positive predictive value when compared with the latter test.

Anti-filarial IgG4 antibody is produced in abundance during filarial infections and unlike broad-spectrum IgG antibody shows little cross-reaction with non-filaroid helminths (Lal and Ottesen, 1988; Kwan-Lim et al., 1990; Turner et al., 1993; Haarbrink et al., 1995; Chanteau et al., 1995; Terhell et al., 1996). Mahanty et al. (1994) have shown that it is a good index of the intensity and duration of filarial exposure in endemic populations, and Maizels et al. (1995) found that the level of IgG4 antibody correlates with microfilariae counts. Although IgG4 antibodies are non-specific and will not distinguish between species they are still useful in the diagnosis of brugian infections (Rahmah et al., 1998a; Haarbrink et al., 1999) and a newly-introduced dipstick test shows promise as an epidemiological tool in *Brugia*-endemic areas (Rahmah et al., 2001).

Filarial-specific enzymes have been characterised and show diagnostic potential either as antigens for antibody assays or by the detection of the enzyme itself. Filarial acetylcholinesterase has been identified in the serum of infected people (Misra et al., 1993) and in in vitro culture fluid (Rathaur et al., 1987) and has inter-species cross-reactivity (Sharma et al., 1998). Filarial glutathione binding proteins, glutathione *S*-transferase, proteases and superoxide dismutase, have been detected in the serum of filarial-infected cattle and humans (Beuria et al., 1995; Bal and Das,

1995, 1996, 1999) and the latter has been shown to be strongly antigenic and a possible target for developing an immunoassay.

PCR methods have been successfully used for the detection of *W. bancrofti* DNA in blood, plasma, paraffin-embedded tissue sections (McCarthy et al., 1996) and sputum (Abbasi et al., 1996, 1999), and *B. malayi* DNA in blood (Lizotte et al., 1994; Rahmah et al., 1998b) and urine (Lucena et al., 1998). However, the sensitivity of PCR techniques has been questioned by Dissanayake et al. (2000) who found that the two techniques they evaluated were no more sensitive than the filtration technique for microfilariae and inferior to ultrasonography in the detection of adult worms. PCR is also used for detecting *W. bancrofti* larvae in mosquitoes (Chanteau et al., 1994b; Nicolas et al., 1996; Furtado et al., 1997), but there are many questions about its usefulness and practicality in a field setting. What are the best methods of mosquito collection? Can enough mosquitoes be collected to make the technique worthwhile in areas of low parasite prevalence? PCR is expensive and facilities are often not available in filarial-endemic areas.

As mentioned above, ultrasonography can be used to detect adult worms in the scrotum and breast (the 'filarial dance sign') (Amaral et al., 1994; Simonsen et al., 1997; Faris et al., 1998; Suresh et al., 1997; Dreyer et al., 1996a,b) and has detected viable worms in children (Dreyer et al., 1999a).

5. New insights into control

There are several approaches to controlling vector-borne parasitic diseases: vaccination, vector control, breaking of vector–host contact by use of repellents and bed nets, and chemotherapy. Despite some promising research, a vaccine for any filarial parasite (and indeed any parasite) is not yet a reality (Philip et al., 1988; Selkirk et al., 1992; Chenthamarakshan et al., 1995; Dissanayake et al., 1995b; Grieve et al., 1995; McCarthy and Nutman, 1996). There has been some recent progress in this with cloned filarial antigens, a recombinant chitinase, and a protective epitome SXP1, an antigen found in multiple worm stages, showing promise as vaccine candidates (Cryz, 1991; Wang et al., 1997). Zang et al. (1999) suggests that a serine protease inhibitor expressed by *B. malayi* microfilariae that inhibits neutrophil serine protease and helps the parasite to evade the immune system may be a target for vaccine development.

Vector control does have a part to play in filariasis control and can be very successful in situations where malaria and filariasis have common vectors. In the Solomon Islands, for instance, where both malaria and filariasis are transmitted by *Anopheles farauti* and *Anopheles koliensis*, the prevalence of microfilaraemia was reduced from around 15 to $<2\%$ by vector control carried out during a malaria campaign (Webber, 1991). Vector control, however, does take a long time to become effective. Schuurkamp et al. (1987) estimated that it would take 11 years to reduce the prevalence of microfilaraemia from around 50 to $<2\%$ in the Tabubil area of the Western Province of Papua New Guinea by vector control alone. Such prolonged campaigns are very labour-intensive and costly and the development of insecticide resistance makes them become less and less effective. Both insecticide-treated and untreated bed nets have been shown to reduce mosquito infection rates and are most effective in areas where the most important vectors are nocturnally-biting anophelines (Charlwood and Dagoro, 1987; Burkot et al., 1990).

The mainstay of filariasis control is chemotherapy, and this is likely to remain so for the foreseeable future. There are essentially two regimens used: selective treatment and mass treatment. In selective therapy, individuals are examined for the presence of the parasite and those infected are treated. There are major problems associated with this approach. If microfilaraemia are used as the indicator of infection, many infected people will be overlooked as not all those who have the disease are microfilaraemic. Even if concentration methods are used, people with very low microfilarial densities may not be detected and it has been shown that such people are capable of infecting mosquitoes and causing a resurgence of disease (Lowrie et al., 1989). The use of antigen testing will overcome these problems but you are still left with the logistical problem of screening all members of the community. If selective chemotherapy is used the lost infections are balanced by new infections to produce a dynamic equilibrium and there needs to be continual reassessment of the filarial status of the community to identify the newly infected. By studying the transmission potential of a number of diseases, including helminth parasites, Woolhouse et al. (1997) found that, typically, 20% of the infected host population contributes at least 80% of the net transmission potential and that control programs which fail to reach all of that core group, a problem with selective treatment programs, will be less effective in reducing levels of infection in the group as a whole. Using mass chemotherapy regardless of parasite status excludes the problems of selective therapy. Mass treatment aims to treat all members of the endemic community at the same time and will deal with pre-patent as well as patent infections. In other words the community becomes the focus of the control program rather than the individual (WHO, 1997a,b).

Diethylcarbamazine has been used to treat filariasis since 1947 (Santiago-Stevenson et al., 1947) and still is the most widely used anti-filarial. It is a very effective microfilaricide but how effective it is against adult worms is a contentious issue. MacKenzie and Kron (1985) concluded that it had a limited amount of macrofilaricidal activity and their opinion is supported by histological evidence from Figueredo-Silva et al. (1996) who removed nodules after diethylcarbamazine treatment and found that all contained degenerating adult worms. The degree of activity, however, is still questionable (Ottesen, 1985). Ismail et al. (1996) used fortnightly 10 mg/kg doses of diethylcarbamazine and concluded that there

was some macrofilaricidal activity but the outcomes were inconsistent. The findings of Weil et al. (1988) that filarial antigenaemia persists for up to 12 months after diethylcarbamazine therapy has eliminated circulating microfilariae suggest that diethylcarbamazine is only partly effective against adult filarial parasites. Eberhard et al. (1997) reported that although filarial antigen fell after treatment, in no case did it fall to zero, even in individuals who remained amicrofilaraemic for several years after treatment. This, they suggest, was evidence that adult worms were persisting. Noroes et al. (1996a,b) found that a significant proportion of adult worms were not susceptible to diethylcarbamazine as evidenced by the persistence of the 'filarial dance sign' after treatment. The absence of microfilariae after diethylcarbamazine would suggest, however, that if the adult females are still living, they are prevented from producing microfilariae, or that diethylcarbamazine in some way facilitates the rapid removal of microfilariae from the circulation. In any case, the lack of microfilariae means that the individual is no longer a transmission source.

The standard diethylcarbamazine treatment regimen is 6 mg per kg body weight per day over a 10–20 day period (WHO, 1994). This is suitable for treating isolated cases but it is not a suitable regimen for community-wide mass treatment programs because it is labour-intensive and incurs high costs (Michael et al., 1996b). Other regimens for mass treatment are: monthly doses, 6 monthly doses and annual doses (see below). All these methods are effective but not all are equal when assessed on a cost-effective basis (Michael et al., 1996b).

Common cooking salt medicated with diethylcarbamazine in concentrations ranging from 0.1 to 0.6% (WHO, 1994; Gelbrand, 1994) has been used effectively in mass treatment programs for bancroftian filariasis in China (Jing-yuan et al., 1992; Liu et al., 1992; Shaoquing et al., 1994), India (Rao et al., 1981; Chandrasekharan et al., 1984; Narasimham et al., 1989; Krishnarao et al., 1991) and Tanzania (Michael et al., 1996b; Meyrowitsch et al., 1996) and has shown to be effective in brugian filariasis (Chandrasekharan et al., 1984; Shenoy et al., 1998, 1999). It is still an attractive option where partnerships can be formed with a salt manufacturer and the access to non-medicated salt can be controlled. Other factors come into play as well. When the present author was attempting to introduce diethylcarbamazine-medicated salt onto a Papua New Guinea island as part of a comparative drug trial he was confronted by a local health worker who said "we have been trying for years to get the people to use less salt because of the increasing prevalence of hypertension, now you come along and say that your salt is good for them." Another community agreed to accept the medicated salt but their usage was well below the expected rate for the size of the population. After some time it was found that they only used 'store salt' for curing crocodile skins; salt was added to food by cooking it in seawater!

The corner-stone of global filariasis elimination is annual, mass, community-wide drug administration excluding those under 2 years of age, pregnant and lactating women, and those who are too sick or infirm to be able to take the tablets (WHO, 2000a,b). In areas where there is no co-endemicity with onchocerciasis, diethylcarbamazine at 6 mg/kg and 400 mg of albendazole is used (WHO, 2000a). In some of the control programs in the Pacific and Papua New Guinea, with which this author is involved, colour-coded diethylcarbamazine tablets are used without weighing the patient. A 300 mg tablet is given to adults and a 150 mg tablet is given to children. This simplifies the treatment, increases compliance (which in our experience is often adversely affected by having to swallow a number of small tablets) and does decrease the efficacy of the treatment, or lead to an increase in adverse reactions. In other areas, the size of the tablets would need to be adjusted to suit the average weights of the population.

In areas where there is co-endemicity with onchocerciasis and/or loaisis, diethylcarbamazine is not used because of the possibility of severe reactions. The drug regimen is 400 mg of albendazole plus 150 μg/kg of ivermectin (WHO, 2000b). Mass, community-wide drug administration can be easily accommodated into existing primary health care networks without over-burdening them (Wijers, 1984; Kimura and Mataika, 1996; Taylor and Turner, 1997).

Diethylcarbamazine treatment has been shown to significantly lower the prevalence and density of microfilariae (Forsyth, 1987; Partono et al., 1984, 1989). Panicker et al. (1991) found that there was a reduction in microfilariae prevalence of 74.9% in the annual treatments and 90% in the biannual treatments and that attacks of filarial fever and the incidence of acute oedema cases were also significantly reduced. A trial of diethylcarbamazine treatment in Tanzania achieved microfilariae clearance rates of 92%. The benefits persisted for at least 4 years, and although microfilaraemia did recur in some patients, especially those who had high microfilariae densities before treatment, the level of microfilaraemia did not reach the pre-treatment levels (Meyrowitsch et al., 1996; Meyrowitsch and Simonsen, 1998). A similar result was obtained in Samoa where three annual treatments of diethylcarbamazine at 6 mg/kg achieved an estimated 80% reduction in the prevalence of microfilaraemia and lowered the annual transmission potential from 2.18 to 0.67 (Kimura and Mataika, 1996) and in Fiji where Mataika et al. (1998) obtained similar results with a 5 year annual treatment program. They concluded, however, that the pre-treatment microfilariae density did not influence the treatment efficacy.

Ivermectin has proved to be very effective producing long-term suppression of microfilaraemia in bancroftian lymphatic filariasis in a number of countries (Ottesen et al., 1990; Campbell, 1991; Zheng et al., 1991a,b; Ismail et al., 1991, 1996; Addiss et al., 1993; Kazura et al., 1993b; Coutinho et al., 1994; Ottesen and Campbell, 1994; Nguyen et al., 1994; Chodakewitz, 1995; Moulia-Pelat et al., 1995, 1996; Ottesen and Ramachandran, 1995; Cao et al., 1997)

and is equally effective against brugian filariasis (Shenoy et al., 1993). Nguyen et al. (1994) found that twice yearly doses of 100 μg/kg of ivermectin did not reduce the prevalence of microfilaraemia, but when the dose was increased to 400 μg/kg the prevalence dropped from 21 to 7%, and the microfilariae density to 0.5% of its initial value. Plaisier et al. (1999) found that ivermectin removes 100% of microfilariae regardless of dose and at a dose of 400 μg/kg a single treatment irreversibly reduces the fecundity of the adult female worm by at least 65%. As with diethylcarbamazine, the macrofilaricidal action of ivermectin is debatable. Ismail et al. (1996) found that multiple, high-dose ivermectin treatment (12 fortnightly doses of 400 μg/kg) does have macrofilaricidal activity but the results are neither predictable nor consistent. These findings are based on the reduction in circulating antigen levels, and as the author points out, are contrary to those studies where direct ultrasound observation of adult worms was used (see below). Eberhard et al. (1997) reported that although filarial antigen fell after treatment, in no case did it fall to zero, even in individuals who remained amicrofilaraemic for several years after treatment, suggesting that some adult worms survived. By contrast, Dreyer et al. (1995b, 1996b) could find no evidence of macrofilaricidal activity. In their study 15 men who had living, adult *W. bancrofti* detected by ultrasound were treated with 400 μg/kg body weight of ivermectin at 2 week intervals for 6 months (total dose 4.8 mg/kg). Microfilaraemia was rapidly suppressed but no changes in the motility or location of the adult worm were detected. An important spin off from the use of ivermectin is the potential effect on the vector as there is strong evidence that ivermectin ingested from a treated person during a blood meal decreases mosquito survival and fertility (Tesh and Guzman, 1990; Nasr et al., 1996; Bockarie et al., 1999).

Albendazole has been used for the treatment of intestinal helminths for a number of years but it has only recently been tested as an anti-filarial. The potential for albendazole to be used as a macrofilaricide for the treatment of individual patients is regarded by Ottesen et al. (1999) as one of the most important questions in filarial research.

5.1. Combination therapy

A combination of diethylcarbamazine and ivermectin has shown to be very effective in providing rapid and long-term clearance of microfilariae (Moulia-Pelat et al., 1995, 1996). Nicolas et al. (1997) found that diethylcarbamazine and ivermectin was much more effective than either drug alone for clearing circulating filarial antigen in both amicrofilaraemic and microfilaraemic subjects. Ismail et al. (1998) studied the effects of albendazole, diethylcarbamazine, and ivermectin alone and in combination and concluded that, although all were well tolerated and effective microfilaricides, a single dose of a combination of 600 mg of albendazole and 400 μg/kg of ivermectin was the most effective. Decreasing levels of filarial antigens after treatment suggested that all four regimes also have significant macrofilaricidal activity. The most effective was a combination of 600 mg of albendazole with 6 mg/kg of diethylcarbamazine. With this combination filarial antigen levels decreased by 77% 15 months after therapy. Not all researchers agree on the efficacy of albendazole alone, or on the benefits of using it in combination with ivermectin. Addiss et al. (1997) found that treating children with a combination of ivermectin and albendazole seemed to be more effective than treatment with ivermectin alone in clearing microfilariae, but albendazole alone had no significant effect. There was a similar finding in a double-blind, placebo-controlled study of single-dose treatments with albendazole and ivermectin alone or in combination in Ghana (Dunyo et al., 2000). Albendazole alone had only a minor effect on microfilaraemia and antigenaemia whereas ivermectin alone and in combination with albendazole effectively reduced both antigenaemia and microfilaraemia. There was, however, only a minimal difference in efficacy between ivermectin alone and ivermectin plus albendazole groups. Quite obviously, there needs to be further careful research of this kind to clearly establish the benefits (apart from the effect against intestinal helminths – see below) of using albendazole as an anti-filarial.

Another potential advantage of anti-filarial therapy using albendazole and/or ivermectin is the potent broad-spectrum effects against intestinal helminths. Beach et al. (1999) have shown in a randomised, placebo-controlled study that 6 monthly treatment with albendazole and ivermectin was effective in controlling *W. bancrofti*, *Ascaris lumbricoides*, *Trichuris trichuria* and hook worm in Haitian primary school children with an obvious benefit to their height and weight. A question must be asked however. Is an annual anti-helminthic treatment going to be really effective in areas where the transmission of intestinal helminths is intense, without a big effort being made to prevent re-infection by introducing improved sanitation and active health education?

Another novel approach to filariasis control is to target the endosymbiont bacteria, which live within filaroids, and appear to be essential to healthy growth and development of the parasite. The results obtained to date are very promising. Tetracycline, doxycline and Rifamycin have been shown to inhibit the motility, viability and release of microfilariae and impede their development (Hoerauf et al., 1999, 2000; Taylor and Hoerauf, 1999; Taylor, 2000; Taylor et al., 2000).

6. Conclusion

The last few years have seen a virtual explosion in both basic and applied filariasis research. We now know that opportunistic bacterial infections play an active part in the progression to elephantiasis and that attention to basic hygiene, the use of antibiotics and physiotherapy can

slow, prevent, or in some cases, reverse elephantiasis. There is still much to be learnt however. Some of the basic questions regarding the immunology of infection and disease and the relationship between infection and disease have not yet been adequately answered and more research is required.

The diagnosis of bancroftian filariasis has been revolutionised by the introduction of filarial antigen tests, which do not depend on the presence of microfilariae, removing the need to take night bloods. One of these tests, the ICT test, can be done by finger-stick in the field and the results are available almost immediately. Ultrasound is proving to be a valuable tool for identifying adult worms in situ. Annual mass treatment with diethylcarbamazine either alone or in combination with ivermectin and albendazole has been proven to be very effective in destroying microfilariae, but the ultimate goal of finding a safe, effective and affordable macrofilaricide still eludes us.

In 1997 the World Health Assembly recognized the importance of controlling lymphatic filariasis and passed a resolution (WHA50.29) calling for "the elimination of lymphatic filariasis as a public health problem" and the International Task Force for Disease Eradication labelled filariasis as one of the six diseases that have the potential to be eliminated (Molyneux et al., 2000). A 'global alliance' consisting of the WHO, the World Bank, UNICEF, the Carter Centre, Smith Kline Beecham (now Glaxo Smith Kline), Merck and Co., academic institutions, and other non-government agencies has been formed to help facilitate the task (WHO, 1997a). The generous donation of ivermectin by Merck Sharpe and Dohme and albendazole by Glaxo Smith Kline has meant that the drugs needed to carry out the task are going to be readily available to those countries which need them. Other donor agencies have promised to help with the provision of test kits and diethylcarbamazine. National eradication programs are already underway in several localities in the Pacific countries and elsewhere. Many other endemic countries are preparing national plans of action with a view to undertaking mass drug administration in the near future.

As would be expected, the elimination program does have its critics. Will annual mass, community-wide drug administration really interrupt transmission? Some point to the fact that despite years of diethylcarbamazine treatment in parts of India and the Pacific, transmission continues. 'Elimination criteria' are being debated which will define at what level of infection mass, community-wide drug administration can cease (1% is often quoted). There are anecdotal reports from the Pacific, for instance, where the prevalence of microfilaria fell to well below 1% only to rise rapidly again when mass, community-wide drug administration was stopped. Some believe that the amount of funding promised thus far falls far short of that required for such an ambitious scheme. 'Political will' is a key factor in any disease control project. Many governments and health agencies have pledged their full support to the filariasis control program. Will that commitment be maintained in the face of increasing competition for the health care dollar, personnel and infrastructure from the mounting HIV epidemic, continuing malaria transmission, or outbreaks of diseases such as Ebola. How will poor countries, which are coping with war, civil unrest, famine, economic hardship, and other woes prioritise a filariasis control program?

Those of us who are involved with the elimination program and confront the horrors of lymphatic filariasis on a daily basis, and the millions who suffer because of the ravages of this parasite, fervently hope and pray that the critics are wrong, and that the program will succeed. Perhaps the last word should be left to Millie, a 16-year-old Papua New Guinea girl with early elephantiasis of the left leg, who spoke these poignant words to this author, "What man will marry me now I have this disease?" That was 10 years ago. Today Millie is not married, and she now has elephantiasis of both legs and one arm – a sad, and totally preventable outcome.

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