Update/Le point

Lymphatic filariasis: diagnosis and pathogenesis*

WHO Expert Committee on Filariasis†

In the past few years, knowledge of many aspects of lymphatic filariasis, a debilitating disease with serious economic and social consequences, has increased. This article presents the sections on diagnosis, pathogenesis, immunopathology and protective immunity from the recently published Expert Committee’s report.

Diagnosis

Parasitological diagnosis

Detection of parasites. The parasitological methods available for detection of microfilariae, such as the use of the thick blood film, counting chamber, Knott’s concentration technique, membrane filtration techniques and the DEC (diethylcarbamazine) provocative test are described in detail in the WHO manual Control of lymphatic filariasis (1) and in the fourth report of the WHO Expert Committee on Filariasis (2). In addition, a test for detection of microfilariae in preserved blood using membrane filters has recently been developed (3); the preservation of blood samples in a mixture of formalin and anionic detergent makes it possible to utilize the convenience and sensitivity of membrane filtration while eliminating the need to perform tests immediately after blood is collected.

Differentiation of filarial species and stages (4–6). New methods have been developed using DNA probes and monoclonal antibodies to identify filarial larvae in body fluids (5) and in mosquito vectors (6), and these can be used to differentiate between larvae of filarial species that infect humans and those that parasitize animals (e.g., between Brugia malayi and B. pahangi).

Virtually all species-specific DNA probes developed so far detect DNA sequences that are highly repeated in the filarial genome, and they are theoretically sensitive enough to detect DNA from a single filarial larva. However, the release of sufficient DNA from the larva and its detection within extracted or crushed mosquitos have proved difficult, so that an intermediary step to amplify parasite DNA by the polymerase chain reaction (PCR) technique is now being investigated. In the case of B. malayi, B. pahangi and Wuchereria bancrofti, the sensitivity of the species-specific DNA probes compares favourably with microscopic techniques to identify infected mosquitos. However, the usefulness of DNA probes to monitor infectivity rates in natural vectors remains to be determined, and probes useful for reliable detection of filarial species variants (if these exist) have yet to be developed.

One shortcoming of current DNA probes is that, because they react with all developmental stages of a given filarial species, they cannot discriminate between infected and infective mosquitos.

A monoclonal antibody that specifically reacts with B. malayi L₃ larvae and distinguishes them from

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Reprint No. 5365
other stages and species of filarial larvae in mosquitoes has been developed and successfully field-tested (6). No equivalent stage-specific monoclonal antibody for the identification of *W. bancrofti* L₃ in mosquitoes is available yet.

**Lymphatic imaging**

Contrast lymphangiography, while widely used to visualize the morphology of the lymphatic vessels, carries the potential risk of lymphatic damage. The unpredictable consequences of such studies have hampered the early evaluation of the lymphatics of asymptomatic individuals. To overcome these difficulties lymphoscintigraphy using radiolabelled albumin or dextran has been developed. This technique can be performed and repeated safely so that serial studies of individuals are possible. Preliminary studies with this technique have demonstrated the presence of lymphatic abnormalities in asymptomatic microfilaremics with no evidence of oedema. With lymphoscintigraphy it should now become possible to make a clear and precise analysis of the lymphatic system function in patients at risk. This technique could be used for the examination of infected but asymptomatic individuals to determine whether they have morphological or functional lymphatic abnormalities and how these alterations could be changed, especially by chemotherapy. It could also provide a new epidemiological tool for detailed studies of morbidity due to endemic filariasis.

**Immunodiagnosis**

Expectations that the availability of monoclonal antibodies selected for stage- and species-specificity and molecularly defined filarial antigens would permit the rapid development of new serodiagnostic assays for lymphatic filariasis have proved too optimistic. A number of these reagents have already shown considerable value in studies on the basic biology of filarial worms and the infections they cause, but their full diagnostic potential remains to be realized.

Some of the factors that have hampered the development of new serodiagnostic tests for lymphatic filariasis have now been partially overcome. The scarcity of parasite materials from species that infect humans has been alleviated somewhat by the ability to maintain complete life-cycles of several *Brugia* species in small rodents and, more recently, by the development of genomic and cDNA libraries from different stages of several filarial species that parasitize humans. In consequence, a number of recombinant filarial antigens have now become available for testing.

The specificity of newer serodiagnostic tests for lymphatic filariasis has been substantially improved, either through the use of excretory-secretory (ES) antigens (which appear to be subsets of whole-worm somatic extracts) or through the use of specific reagents to detect antibody isotypes or subclasses. For example, tests measuring antifilarial IgE or IgG4 antibodies appear to be much more specific than those measuring total antibody responses. Perhaps the most important conceptual advance stems from the realization that much of the cross-reactivity among nematodes is related to the immunodominance of antigenic determinants containing phosphorylcholine (PC), which apparently do not stimulate production of IgG4 antibodies. Thus, assessment of antibody responses to non-PC determinants greatly improves the specificity of serodiagnostic tests for lymphatic filariasis.

Other problems still exist that impede the rational development of serodiagnostic tests for lymphatic filariasis. Foremost is the lack of clear-cut criteria that can be used to define the appropriate “negative” controls within endemic populations and the significance of a positive test result in an microfilaraemic, asymptomatic resident of an endemic filariasis area. Some of these issues may be resolvable by studies in experimental animal models.

A second problem is the minimal amount of information available on the kinetics of antibody responses to defined filarial antigens during the natural course of infection and on the effect of treatment or other control measures on the rate of acquisition and loss of different types of antibodies to such antigens. This information is essential for the rational development of serodiagnostic tests to confirm, for example, the filarial nature of acute adenolymphangitis. Our ignorance of the relationship between antigen recognition patterns and clinical outcomes of infection is an additional obstacle to the development of diagnostic tests with predictive or prognostic value.

The notion that a single “universal” diagnostic test could provide the factual information needed for the management of individual cases and for population-based control programmes is probably unrealistic and should be abandoned. However, it appears possible to develop a relatively small number of “targeted” serodiagnostic tests that have performance characteristics tailored to provide correct answers to specific clinical and epidemiological questions. Some such tests are available; others are being developed.

Two conceptually different techniques for the diagnosis of lymphatic filariasis have been, and are still being, developed: antigen-detection assays and antibody-detection assays.

**Antigen-detection assays.** Several laboratories have developed monoclonal antibody-based assays to
detect and quantify filarial antigens in sera, urine and other body fluids of persons with lymphatic filariasis. While the sensitivity and specificity (for bancroftian or brugian filariasis) of individual tests differ, these tests are generally capable of detecting filarial antigens in the majority of persons with microfilaraemic bancroftian or brugian infections. With some tests, filarial antigens are also detected in variable proportions of sera from microfilaraemic individuals with or without clinical manifestations of infection (7). As seropositivity in these assays presumably reflects the presence of filarial worms, antigen-detection assays should be useful for detecting all persons with “active” infections in an endemic population. Studies in animal models support this concept. It appears that some microfilaraemic and asymptomatic individuals (called “endemic normals” in some reports) are, in fact, not normal but carry subclinical infections that are not detectable by traditional diagnostic techniques.

Though studies in animal models suggest that filarial antigen levels in sera correlate with worm burdens (either adult worms or microfilariae, depending on the specificity of the monoclonal antibody used to detect the antigen), the assays are not yet precise enough to permit accurate quantification of the worms present in individual animals. Both in animal models and in humans, antigen levels decline after treatment, but antigen clearance appears to be slow. Such antigen assays should be useful to monitor the effect of chemotherapy on filarial worm burdens, but the validity of this concept remains to be confirmed by additional studies in animals and humans.

**Antibody-detection assays.** Even though suppression of filaria-specific immune responses is a common feature of patent lymphatic filariasis, virtually all adult residents of endemic areas who have been exposed to filarial parasites develop detectable levels of IgG antibodies to crude worm extracts. Seropositivity in this type of assay merely indicates that the individual has been sensitized to parasite antigens and should not be interpreted as evidence of current infection. Such diagnostic tests are of little practical value in endemic areas except for the diagnosis of tropical pulmonary eosinophilia, but they may be useful in expatriates or temporary visitors in endemic areas in whom a positive test result suggests possible filarial infection.

It has been proposed that an IgG4 response to somatic extracts of filarial worms is indicative of active, chronic filarial infection, even in the absence of other clinical or parasitological evidence, and that specific IgG3 responses correlate with the presence of chronic lymphatic pathology (see below, “Factors predisposing...”). Both these suggestions need to be confirmed by additional studies.

**Role of immunodiagnostic tests in the control of lymphatic filariasis.** Most residents of endemic areas give a positive reaction in many serodiagnostic assays that utilize complex mixtures of filarial antigens, such as whole-worm homogenates or crude ES products. These diagnostic tests therefore cannot be used to confirm the filarial origin of clinical conditions that have been associated with but not causally linked to lymphatic filariasis (e.g., acute arthritis). Hence, in the absence of other indications, seropositivity alone is not a sufficient reason to institute antifilarial therapy in residents of endemic areas. Pilot studies suggest that newer diagnostic tests may be useful for diagnosing acute filarial disease, but these are not yet widely available.

Serological tests that would permit the identification of microfilaraemic individuals within populations in endemic areas without the need for night-blood collection, tests that would detect all individuals with current active infections, or tests that could be used to quantify adult worm burdens following chemotherapy would greatly facilitate filariasis surveys and constitute invaluable tools to monitor the impact of control programmes. Such tests are currently being evaluated in multicentre trials supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and may become available in the near future.

**Suggestions for further study**

- Diagnostic assays need to be developed and validated that will:
  - (a) detect active infection (both cryptic and microfilaraemic);
  - (b) replace night-blood examination for detection of microfilaraemia in areas where nocturnally periodic filariasis exists;
  - (c) distinguish filarial from non-filarial adenolymphangitis;
  - (d) identify to species (and, where appropriate, to subspecies) parasites in the mosquito vectors; and
  - (e) quantify worm burdens in infected persons.

Such assays will be important for improving the management of individual patients, for providing tools that can be used for epidemiological research, and for monitoring filariasis control programmes.

- Age-related changes in immunological parameters of the host response to filarial parasites should be determined in areas of different endemicity, as
should the effect of treatment on the immune response. This is necessary for the development of diagnostic assays to monitor filariasis control programmes.

Pathogenesis, immunopathology, and protective immunity

Lymphatic pathology

During the past decade several important conceptual advances have been made relating to the pathogenesis of lymphatic lesions in bancroftian and brugian filariasis. These concepts are based primarily on observations in experimental animal models that mimic aspects of filarial disease in human beings (such as Brugia infections in ferrets, dogs, cats, and immunodeficient mice) and on the results of studies attempting to correlate antiparasite immune responses in humans with various clinical outcomes of infection.

Distinction between parasite-induced and immune-system-induced pathology. Experimental model systems have provided clear evidence that while much of the pathology resulting from infection with brugian parasites results from the host’s immune response to these parasites, a portion is also derived from the direct action on the lymphatic tissue of the parasites themselves or the molecules they release. The most convincing demonstration of this is the finding that immunodeficient mice infected with brugian parasites develop marked endothelial cell proliferation and lymphatic dilatation, with resultant lymphoedema and elephantiasis, in the absence of any appreciable immune response to the parasite. Reconstitution of these immunodeficient mice by immunocompetent cells from filaria-sensitized normal mice results in inflammatory reactions around the parasites, with local granuloma formation and obstruction of the lymphatics, that again leads to lymphoedema and elephantiasis. Thus, there appear to be two distinct forces acting to damage the lymphatic function of such infected animals, one involving the immune system and the other independent of it.

The findings in these animal models parallel those described previously in affected humans (i.e., lymphatic proliferation, dilatation, and oedema formation in the presence of living worms, but obstructive, obliterative reactions in the lymphatics around dead parasites). Furthermore, they are consistent with previous observations that microfilaraemic persons, who appear “hypo-responsive” to parasite antigens (see below, “Immuno regulation”), are often asymptomatic, whereas those with past or amicrofilaraemic infections are the ones with heightened immunological responsiveness and the ones who often have obstructive lymphatic pathology.

Lymphatic histopathology reflecting the immune responsiveness of the host. Many of the generalizations about the lymphatic histopathology of bancroftian filariasis have been inferred from tissues removed from soldiers who acquired filariasis after serving in endemic regions of the Pacific during the Second World War. The pathological reactions described were, for the most part, inflammatory, with an abundance of eosinophils and mononuclear cells infiltrating the lymphatic and perilymphatic spaces. It is now recognized, however, that the clinical and pathological responses of such expatriates entering endemic regions and acquiring infection are very different from those of individuals living their whole lives in endemic regions.

A recent study of lymphatic pathology in patients from a region where bancroftian filariasis is endemic (Recife, Brazil) clearly showed, rather than the exuberant inflammatory reactions seen in the expatriate cases, a generally much more subdued condition, with little or no inflammatory activity around adult worms and microfilariae, in asymptomatic patients whose tissue specimens were obtained for reasons other than the suspicion of filariasis (i.e., were not taken because of an acute inflammatory syndrome similar to that described in the infected soldiers) (8). These findings indicate that the immunological “hypo-responsiveness” identified from studies of peripheral blood lymphocytes in such patients is an accurate reflection of the local lymph-node immunopathology (or lack thereof) observed in the majority of infected patients who remain asymptomatic.

Factors predisposing to the development of lymphatic lesions

A recent review of the literature indicates that both the prevalence of lymphatic filariasis and the degree of microfilaraemia are lower in women than in men (9). Clinical disease is also less common in women, and pathology has a later age of onset and rise to peak prevalence than in males.

The notion that prenatal conditioning could influence subsequent immunological (and thus, pathogenic) responses to filarial infection has received confirmation from recent studies in Haiti that have shown that maternal microfilaraemia predisposes to microfilaraemia in the offspring, an observation consistent with those made previously in jirds and dogs. This may be very important in explaining the differences in the clinical manifestations of lymphatic filariasis both among residents in endemic
areas and between these persons and those who migrate to such endemic areas.

The manner in which an infected individual reacts to (i.e., immunologically “processes”) filarial antigens may determine whether or not lymphatic pathology will develop. Data from humans showing that IgG3 antibody responses to filarial antigens are made almost exclusively by patients who develop lymphatic pathology whereas production of IgG4 antibodies predominates in those who remain asymptomatic but microfilaraemic are compatible with such a hypothesis, though they do not prove it. If the hypothesis is correct, however, it will have profound implications for the development of antifilarial vaccines, as the “right” type of immune response might be protective, whereas the “wrong” type of immune response to the same vaccine might lead to filarial disease. (A different working hypothesis — namely, that resistance and pathology are caused by immune reactions to different antigens — is discussed below, “Protective immunity”).

Though studies in animal models indicate that bacterial infections can aggravate lymphatic pathology caused by filarial worms, and though antibiotics are routinely used in addition to specific antifilarial chemotherapy in many endemic areas, whether or not bacterial (or fungal) infections contribute to the pathogenesis of acute or chronic filarial disease in humans remains a matter of speculation because of insufficient information.

Pathogenesis of tropical pulmonary eosinophilia

The immunological hyperresponsiveness of patients with tropical pulmonary eosinophilia has been well defined previously through studies of eosinophils and filaria-specific antibodies in the blood. Recent investigations employing bronchoalveolar lavage have increased our understanding of the pathogenesis of this syndrome by showing that patients with acute tropical pulmonary eosinophilia have markedly increased numbers of inflammatory cells infiltrating their lungs, the majority of these (60–80%) being eosinophils with the characteristic morphology of activated cells. Also, direct assessments have identified a preferential accumulation (“compartmentalization”) of eosinophils in the lung compared to the blood and similar compartmentalization of filaria-specific IgG, IgM, IgA, and IgE antibodies in the lung. Functionally, the extent of this lung eosinophilia has been recently shown to correlate with the degree of compromise in the lung’s ability to oxygenate the blood.

Pathogenesis of renal lesions

Though the pathogenesis of the recently described renal abnormalities in microfilaraemic individuals with bronchofilariaisis has not yet been investigated, it is probably a form of nephritis related to parasite-antigen-specific immune complexes and their deposition beneath the basement membrane of the renal glomeruli.

Immunoregulation

Correlation with pathology. It has been recognized for years that, except for individuals with tropical pulmonary eosinophilia, patients with lymphatic filariasis (especially those with microfilaraemia) appear to respond poorly to filarial antigens. This “hyporesponsiveness” is not to be confused with broad-spectrum immunodeficiency but, rather, appears limited almost exclusively to the response to parasite antigens. Recent studies in animal models present direct evidence that the “parasite-specific immunosuppression” induced by filarial infection leads both to decreased pathology in the host and to decreased local immunological responses to filarial antigens. These experimental observations support the correlation made repeatedly between the finding of little or no pathological response to the parasite and a “diminished” immunological responsiveness to parasite antigen in microfilaraemic patients.

Mechanisms underlying the antigen-specific immunosuppression (10). It has previously been hypothesized that a variety of suppressive elements (cells and humoral factors) are responsible for the parasite-specific “hyporesponsiveness” identified in infected patients. A major recent advance has been the precise definition of some of the specific molecules involved.

One of these “down-regulating” molecules actually appears to be specific antibody of the IgG4 subclass, which is made in relatively large amounts by microfilaraemic patients (generally considered as the most hyporesponsive of the filariasis patients) and which has been implicated as the “blocking antibody” responsible for controlling IgE-mediated allergic responsiveness to the parasite. Thus, microfilaraemic individuals who have appeared for years to be the least responsive to parasite antigens in conventional assays are now recognized to be, in fact, vigorous responders to such antigens, but their responses involve the production of inhibitory, not stimulatory, molecules (both antibodies and cytokines).

In addition to such host-produced products, molecules derived directly from the parasites themselves have recently been shown to inhibit human
lymphocyte function in vitro and, by inference, might play a similar modulating role in vivo, since some of these same molecules have also been found circulating in the blood during active infection.

Protective immunity (10)

It is generally hypothesized that in an area of endemic filariasis the “endemic normals” (or asymptomatic microfileraemics) will include not only a proportion of individuals harbouring subclinical infections (below the threshold of detection) but also individuals with true protective immunity. It is perhaps less widely appreciated that it is possible that those with high microfilaria loads may also possess an effective immune response that protects them from superinfection in the face of continuing transmission of infective larvae. Recent analyses of age-stratified populations in Papua New Guinea and India give support to this proposition. In Papua New Guinea, worm burdens were quantified by a circulating antigen assay performed at 1-year intervals. Parasite loads were seen to increase in children and adolescents, while those in adults (>20 years) remained stable. A similar conclusion was drawn from a large-scale study of infection dynamics in Pondicherry, India, in which the rate of gain of infection, measured by microfilaremia, levelled off after adulthood was reached.

In the Papua New Guinea study, a further important finding was that the “immune” adults all possessed antibody to the surface of third-stage larvae (L₃), while the “non-immune” children mostly did not. These findings considered together have the following important implications: first, L₃ stage-specific antigens may act as effective targets for a protective immune response in both infected and uninfected individuals; secondly, protective immunity takes many years to develop, a finding in conformity with known epidemiological characteristics; and thirdly, protective immunity may be induced to antigens (on the infective larval stages) that are not involved in the development of immunopathology, thus making the development of safe (i.e. non-pathogenic) vaccines a more feasible prospect. Whether such “natural” immunity can be artificially induced by vaccination strategies remains to be determined. The hypothesis of concomitant immunity, which implies that protective and pathogenic immune responses are directed against distinct life-cycle stages of filarial worms, can be used to guide selection of candidate antigens for potential future vaccines. By analysing differences in antigen recognition patterns between “immune” and non-immune populations, several filarial antigens that might be involved in protective immunity have been identified. Attempts to confirm the protective potential of these antigens in animal models are only just beginning. Thus, it is not likely that a vaccine to prevent lymphatic filariasis in humans will become available in the near future.

Suggestions for further study

- Studies are needed to define the prenatal and perinatal influences of maternal filarial infection on subsequent outcomes (clinical, parasitological, and immunological) of exposure to filarial infections.
- The role of bacterial, viral and fungal infections in the pathogenesis of the lymphatic lesions in patients with filariasis should be determined.
- Research is required to identify differences in immune responses between individuals and populations with distinct clinical manifestations of lymphatic filariasis in order to develop predictive markers of disease development and to define the mechanisms and dynamics of the disease process. Such information could be used to develop rational strategies for disease control and prevention by immunological interventions.
- Methods are required by which to generate large numbers of infective larvae of W. bancrofti and B. malayi for use as an antigen source in differential screening studies to define potential protective immunogens and as source material for making cDNA libraries expressing genes that encode potentially protective protein immunogens and drug targets.

References


