

## Immunodiagnosis of bancroftian filariasis—Problems and progress

BHASKAR C. HARINATH

Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha 442 102, India

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**Abstract.** The immunodiagnosis of bancroftian filariasis is a major challenge to the immunoparasitologist. Significant progress is yet to be made in developing convenient laboratory animal model and in *in vitro* cultivation of filarial parasites making it very difficult to obtain required amount of parasite material for research. Parasitological examination techniques are not useful in low microfilaraemia, occult or chronic filarial infections. A precise and accurate immunodiagnostic technique is very much needed for successful filaria control programmes. Such a test will also avoid the need for laborious night blood examination in bancroftian filariasis.

Due to comparatively easy availability, a good amount of work has been done to explore immunodiagnostic potential of heterologous filarial antigens isolated from *Litomosoides carinii*, *Dirofilaria immitis*, *Brugia malayi*, *Setaria digitata*, *Setaria cervi* and number of other filarial species. However, there has been limited or no significant success due to number of false negative and false positive reactions.

Extensive study has also been made with antigens isolated from *Wuchereria bancrofti* microfilariae. Soluble antigens of microfilariae have been used in different immunological techniques such as skin test, counter immuno electrophoresis, indirect haemagglutination test, indirect fluorescent antibody test and enzyme linked immunosorbent assay. Fractionation of *Wuchereria bancrofti* microfilarial soluble antigens yielded mfs3e antigen fraction which was found to be highly reactive in microfilaraemia by enzyme linked immunosorbent assay, but the yield of the purified antigen was not sufficient enough to make it a practical proposition for large scale isolation of antigen.

*Wuchereria bancrofti* microfilarial excretory-secretory antigens were found to be specific and highly sensitive requiring as little as 0.35 ng antigen protein per well in penicillinase enzyme linked immunosorbent assay for detection of filarial antibody. One ml of culture fluid was found to be sufficient for 400,000 tests. Field evaluation of this test showed that it can replace laborious night blood examination.

Assay systems have been developed for detection of filarial antigen in serum, urine, hydrocele fluid and immune complexes using immunoglobulins from chronic filarial sera and antisera to excretory filarial antigens. Further purification of excretory-secretory antigens by affinity chromatography and production of monoclonal antibodies should hopefully give suitable reagents for use in sensitive assays such as enzyme immuno assay and immuno radiometric assay, providing an ideal assay system for detection of active filarial infection in the not too distant future.

**Keywords.** Immunodiagnosis; filarial antibody; filarial antigen; enzyme linked immunosorbent assay; bancroftian filariasis.

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Abbreviations used: GD, Gel diffusion; CIE, counter immuno electrophoresis; IHAT, indirect haemagglutination test; IFAT, indirect fluorescent antibody test; ELISA, enzyme linked immunosorbent assay; ES, excretory-secretory; IRMA, immunoradiometric assay, FSI, filarial serum immunoglobulin; mfs, soluble microfilarial antigen; PEG, polyethylene glycol; CIC, circulating immune complex.

## Introduction

Bancroftian filariasis is an infectious disease produced in man by the filarial parasite *Wuchereria bancrofti*. It is a major public health problem affecting about 250 million persons in tropical countries (Duke, 1978). In India alone 22 million people harbour microfilariae in blood while 16 million suffer with filarial disease manifestations such as hydrocele, lymphoedema and elephantiasis. From 25 millions in 1953, the figure rose to 304 million Indians in 1981 who are exposed to the risk of filarial infection (Sharma *et al.*, 1983). Although almost never directly fatal, chronic infection can lead to disability and disfigurement causing untold pain, misery and impairment of health in the developing world. The clinical course of lymphatic filariasis can be divided into asymptomatic, acute and chronic stages. We know so little about the parasite from the time when it disappears beneath the surface of the skin through infective mosquito bite, until some nine months later it announces its survival by the production of microfilariae. When in health and undisturbed, the parasite seems to be an expert in host's immune evasion and lives in perfect harmony with its host for considerable periods, giving a challenge to the immunologists.

One of the essential requirements for effective filaria control programme is a precise and accurate diagnostic test for detection of filarial infection on mass scale in field surveys. A definite diagnosis of filariasis is still based on the demonstration of microfilariae in peripheral blood collected at night by various parasitological examination techniques such as thick smear, counting chamber procedure, concentration test and nucleopore membrane filtration. These are not useful in low microfilaraemia, occult or chronic infection. Hence there is a need for simple, sensitive and specific immunodiagnostic test which would be of great value in nocturnally periodic *W. bancrofti* infection as a means of avoiding laborious night blood surveys and in serology for early detection.

## Immune responses

Serum immunoglobulins IgG and IgE were found to be at elevated level in bancroftian filariasis compared to controls (Subrahmanyam *et al.*, 1976). However, specific IgE antibody levels were found to be lowered in chronic filariasis when assayed by solid phase radioimmuno assay (Hussain *et al.*, 1981) and enzyme linked immunosorbent assay (ELISA) (Malhotra, *et al.*, 1984b) using *Brugia malayi* and *W. bancrofti* antigens respectively. It is interesting to note that antibodies to microfilarial surfaces (sheath) as measured by immuno fluorescent assay are usually absent in microfilaraemia but present in chronic filariasis. However on sonication cuticular and cytoplasmic antigens of microfilariae are exposed and thus antibody to these antigens could be detected in microfilaraemia sera as well (Wong and Suter, 1979; Hedge and Ridley, 1977; Kaliraj *et al.*, 1979a). Similar observation (Subrahmanyam *et al.*, 1976) was made when skin test was performed using soluble *W. bancrofti* microfilarial antigen. Antibody was found to be absent in microfilaraemia but was present in chronic filariasis. This is possibly due to the neutralization of cuticular or sheath antibodies by excess of antigen present in the circulating blood in microfilaraemia. Microfilarial surfaces were observed to acquire

blood group antigens (Ridley and Hedge, 1977) and serum albumin (Maizels *et al.*, 1984) which possibly help in parasite survival by immune evasion. In filariasis with chronic pathology, usually microfilariae are absent in peripheral blood. Sera from cases of elephantiasis promoted an intense adhesion of peripheral blood leucocytes to *W. bancrofti* microfilariae *in vitro*. The adhesion was complement independent and was associated with the IgG fraction in the human system. Antibody-dependent cellular cytotoxicity studies showed the involvement of neutrophils and macrophages as major cell types (Mehta *et al.*, 1981). In contrast cell mediated immunity was found to be suppressed in chronic filariasis as observed by leucocyte migration inhibition and lymphocyte transformation assays. Though no such suppression was observed in low microfilaraemia, however in filaria cases with high microfilarial density ( $\geq 50$  mf/c.mm), suppression of cell mediated immunity could be observed (Raghunath *et al.*, 1980; Ottesen *et al.*, 1977). Piessens (1981) rightly described lymphatic filariasis in humans as an immunologic maze.

### Immunodiagnostic tests

For an immunodiagnostic test to be acceptable as a tool for diagnostic and epidemiological purposes, it should satisfy certain requirements in sensitivity, specificity, cross reactivity, predictive value and reproducibility. Further it should be simple to perform, economic costwise, acceptable to population and should be adaptable for field study. Immunological methods such as precipitin and immunodiffusion, complement fixation, immuno and counter immuno electrophoresis, indirect haemagglutination and flocculation, immunofluorescence, ELISA, chemiluminescent ELISA radio immunoprecipitation polyethylene glycol (PEG) assay, immunoradiometric assay (IRMA), intradermal test, cell mediated immunity test, passive cutaneous anaphylaxis, *in vitro* histamine release assay and immune adherence have been explored to develop useful test for the diagnosis of different parasitic infections. The different techniques used in parasite serology and their evaluation have been published (Voller and de Savigny, 1981; Houba, 1980). Kagan (1981) classified some of these techniques based on the reactivity. Gel diffusion, complement fixation and latex agglutination are of low reactivity requiring high concentration of antibody and are not useful for detection of antibody within first week of infection. Tests of 'medium reactivity' are the indirect haemagglutination and indirect immunofluorescence techniques which can detect antibody by the second week of infection. Tests of 'high reactivity' are the radio immuno assay and ELISA. They can detect low concentrations of antibody (nano gram or pico gram/ml) on 3rd or 4th day of infection in some cases.

#### *Immunodiagnosis based on the detection of antibody in serum*

The immunodiagnosis of filariasis is one of the major challenges to the immunoparasitologist. The progress in sero diagnosis was reviewed by Kagan (1963) and Ambroise-Thomas (1980).

#### *Heterologous antigens*

The non availability of the human parasite (*W. bancrofti*) in required quantity for

antigen extraction has become an obstacle for the progress in filaria diagnosis. Hence sharing of antigens (cross reactivity) by different filarial parasites has been exploited in various immunological tests for diagnosis of filariasis. The antigens of *B. malayi* (Grove and Davis, 1978), *Setaria digitata* and *Setaria cervi* (Dissanayake and Ismail, 1981; Tandon, *et al.*, 1981), *Litomosoides carinii* (Rao *et al.*, 1980; Das gupta *et al.*, 1980), *Dirofilaria immitis* (Sawada *et al.*, 1968) and number of other species have been explored for their diagnostic potential for bancroftian filariasis. Gidel *et al.* (1969) conducted a trial of two immunological tests namely intradermal test and complement fixation test for diagnosis of filariasis using purified antigens from *D. immitis* by Sawada but without success. Studies on the detection of filarial antibody using heterologous antigens were not useful in development of a specific diagnostic test due to significant number of false positive and false negative reactions. Thus the heterologous antigens have limited or no potential use for immuno diagnosis of filarial infection.

#### *Homologous antigens*

Specificity depends on the quality of the antigen employed and acceptable levels of specificity can only be obtained by using homologous and purified antigens. Though there are some encouraging leads, significant progress is yet to be made in the development of suitable animal model and in *in vitro* cultivation of human filarial parasite. Until then infected mosquitoes and humans are the only sources for *W. bancrofti* infective larvae and microfilariae respectively making it difficult to get required parasite material. Hence studies using homologous (*W. bancrofti*) antigens are scanty.

#### *Somatic antigens (W. bancrofti)*

Studies have been made to explore the utility of *W. bancrofti* microfilarial antigens in gel diffusion (GD), counter immuno electrophoresis (CIE), indirect haemagglutination test (IHAT), indirect fluorescent antibody test (IFAT) and ELISA for detection of filarial antibody in filarial sera. GD was found to be least sensitive and the sensitivity in detection of antibody in maximum number of positive cases increased with each test in the above order and ELISA was observed to be highly sensitive (Kaliraj, 1980). Naidu *et al.* (1984) used *W. bancrofti* microfilarial as well as infective larval antigens in CIE and IHAT and observed that both the antigens were comparable in reactivity in IHAT. *W. bancrofti* infective larval antigen was used in skin test and positive reaction in all proven filarial infection cases as well as in endemic normals was observed (Chandra *et al.*, 1974). However when soluble microfilarial antigen was used in skin test, positive reaction in chronic filariasis and negative reaction in microfilaraemia were observed (Subrahmanyam *et al.*, 1976). Antibodies were detected against microfilariae, larvae and adult worms of *B. malayi* (Grove and Davis, 1978; Wong and Guest, 1969) and microfilariae and larvae of *W. bancrofti* (Kaliraj *et al.*, 1979a; Yong, 1973) in filarial infections by IFAT. From these studies it may be concluded that measurement of antibodies to surface antigens of adult worms is a useful indicator of infection while antibodies to surface antigens of microfilariae are correlated with disease. Using soluble *W. bancrofti* mf antigen, the efficiency of IHAT, IFAT and ELISA tests were compared for the detection of antibody in filarial sera. Filarial antibody could be detected in 93 %, 100 %, 81 % of the microfilaraemics, 75 %, 90 %, 100 % of chronic pathology and none

of the non endemic sera by IHAT, IFAT and ELISA respectively. However, 45–65 % endemic normal sera showed the presence of filarial antibody by these tests. *W. bancrofti* mf antigen showed cross reaction against *Ancylostoma duodenale* sera in IHAT and ELISA when a few non endemic nonfilarial helminth infected sera were tested, necessitating further purification of this antigen to be useful for diagnosis (Kaliraj *et al.*, 1981 b,c).

Soluble antigens (mfS) isolated from *W. bancrofti* microfilariae were fractionated by Sephadex G-150 gel filtration into 3 antigenic fractions (mfS1, 2 and 3). The mfS3 fraction was weakly reactive in IHAT but the same was found to be highly reactive in ELISA. The mfS2 antigen fraction showed cross reaction with non filarial helminth infected sera similar to the crude soluble antigen (mfS). The antigenic fractions (mfS1 and mfS3) were further fractionated by DEAE cellulose chromatography. Analysis by ELISA showed that mfS1b and mfS3e antigen fractions were highly active in the detection of filarial antibody in chronic filariasis (85 %) and microfilaraemia (88 %) sera respectively (Kaliraj *et al.*, 1982). However, processing of microfilariae from 100 ml of blood sample containing  $\geq 50$  mf/20 c.mm, gives antigen fraction (mfS3e) just sufficient for about 2000 tests, not a practical proposition for large scale isolation of antigen for field surveys.

#### *Excretory-secretory antigens*

*W. bancrofti* microfilarial excretory-secretory (ES) antigens were obtained by maintaining *W. bancrofti* microfilariae in medium 199 (3–4 thousand mf/ml of medium) supplemented with organic acids and sugars of Grace's medium but without serum (Kharat *et al.*, 1980). Utility of *W. bancrofti* ES antigen has been explored in IHAT (Kharat *et al.*, 1981), CNBr-Sepharose IFAT (Kharat *et al.*, 1983) and Penicillinase ELISA (Kharat *et al.*, 1982) for detection of filarial antibody. When used in ELISA, ES antigen was found to be highly sensitive and fairly specific compared to somatic antigen. As little as 0.35 ng ES antigen protein per well was found to be sufficient in detecting filarial antibody (Kharat *et al.*, 1982) compared to earlier study (Kaliraj *et al.*, 1982) with soluble microfilarial antigen (1.5  $\mu$ g/well) or fractionated antigen (0.1  $\mu$ g/well). One ml of culture fluid may be diluted to 4.0 liters and thus can be used for 400000 tests by penicillinase ELISA. Antibody isotype analysis revealed the presence of IgM antibody in all micro-filaraemics and IgG antibody in all cases with chronic pathology while some of each group contained both IgM and IgG antibodies. Reciprocal of the antibody titre varied from 1280 to 20 million in filarial sera. The level of specific antibody (IgM or IgG) titre did not show any correlation either between microfilaraemia and clinical filariasis or with microfilariae density (20–120 mf/20 c.mm) or clinical status of filaria patients (Kharat *et al.*, 1982). High ELISA antibody titre (1:1000000) was also observed in clinical toxocariasis with the culture antigen (de Savigny *et al.*, 1979). Fractionation of ES antigens by membrane filtration gave ES4 antigen fraction, which was found to be a glycoprotein in nature and was highly reactive in microfilaraemia sera (Reddy *et al.*, 1984a). *W. bancrofti* mf ES antigen specific IgE antibody was detected in filariasis and tropical eosinophilia by immunofluorescence assay (Kharat *et al.*, 1983) and ELISA (Malhotra *et al.*, 1984b). These studies showed that detection of ES antigen specific IgG + M + A antibodies

will be more useful than specific IgE for the immuno-diagnosis of filariasis. Antigens have been isolated from microfilaraemia sera, hydrocele fluid, immune complexes and urine samples of filarial patients and were found to be useful in detecting filarial antibody, providing other sources for isolation of filarial antigen of diagnostic importance (Reddy, M. V. R., Malhotra, A., Prasad, G. B. K. S. Hamilton R. G. and Harinath, B. C., unpublished observations).

### **Immunodiagnosis based on antigen detection**

In detection of an active filarial infection or in assessment of the effectiveness of chemotherapy, the level of circulating antigen in serum will be more informative than antibody. Filarial antigen has been demonstrated in the sera and urine of infected humans and animals using antisera raised against heterologous filarial antigens from *L. carinii*, *D. immitis*, *B. malayi*, *S. digitata* (Dasgupta and Shukul Bala, 1978; Desowitz and Una, 1976; Tanabe, 1959; Dissanayake *et al.*, 1982; Hamilton *et al.*, 1984). Few attempts have been made to produce antisera against *W. bancrofti* microfilarial antigens. Administration by subcutaneous route was found to be more effective than intravenous injection in eliciting maximum immune response. Rabbit antisera against *W. bancrofti* microfilarial soluble antigens showed 2 specific precipitin bands with the corresponding antigens in agar gel diffusion (Kaliraj *et al.*, 1978; 1981a).

Circulating filarial antigen was concentrated from microfilaraemia plasma by salt precipitation and was identified as an antigen of microfilarial origin using anti rabbit mf sera in CIE (Kaliraj *et al.*, 1979b). Use of immunoglobulin from chronic filarial serum (FSI) was explored for detection of circulating antigen in filarial sera and culture fluid by CIE and IHAT. FSI was found to be more efficient compared to rabbit anti mf serum in detecting circulating antigen in serum and culture fluid (Kaliraj *et al.*, 1981d; Kharat *et al.*, 1981). Use of IgG fraction of FSI (FSI-G) in sandwich ELISA was found to be quite sensitive in detecting circulating antigen in 27 out of 33 microfilaraemia sera and an apparent positive correlation between the microfilarial density and the antigen titre was observed (Reddy *et al.*, 1984b). Filarial antigen was also detected in the neat urine samples of microfilaraemia patients by double antibody sandwich ELISA using FSI-G and anti rabbit urinary filarial antigen immunoglobulin and by IRMA using [<sup>125</sup>I]-rabbit IgG antibodies to *B. malayi* antigen (Reddy, M. V. R., Malhotra, A., Naidu, J. N., Hamilton, R. G. and Harinath, B. C, unpublished observations). Monoclonal antibody is another reagent with great potential for detection of specific antigen of interest. Monoclonal antibodies have been produced to heterologous filarial antigens and *W. bancrofti* mf ES antigens and are being explored for detection of filarial antigen in bancroftian filariasis.

### **Immune complexes**

A study of immune complexes will be of interest to understand the antigens involved and pathogenic mechanisms in disease processes. Immune complexes were determined by 3% PEG precipitation and complement consumption tests. Significant elevated

levels of circulating immune complexes (CICs) were observed in clinical filariasis compared to microfilaraemia and endemic normals. Immunofluorescence assay revealed the presence of mostly IgG and IgM immunoglobulins in the immune complexes (Prasad *et al.*, 1983a; Gajanana *et al.*, 1982). Specific filarial immune complexes have been determined by ELISA using anti C3 and filarial serum immunoglobulin-G. No correlation could be observed between the levels of CICs and clinical manifestations of the patient (Prasad and Harinath, 1984). The presence of filarial antigen in immune complex was detected by direct ELISA and the involvement of *W. bancrofti* mf ES antigen was demonstrated by competitive ELISA (Prasad *et al.*, 1983b). Antimicrofilarial ES antigen-antibody was also demonstrated in immune complexes in bancroftian filariasis by enzyme immuno assay (Prasad, 1983c).

### Field evaluation

Blood samples collected and dried on filter paper have been shown to be useful in seroepidemiological studies for detection of parasitic diseases. These filter paper blood samples are even more relevant and will be of advantage in nocturnally periodic bancroftian filariasis, where it is difficult to collect night blood smears in field studies. Malhotra *et al.* (1982) has successfully used filter paper blood samples in immunodiagnosis of bancroftian filariasis by indirect ELISA using *W. bancrofti* mf ES antigen. *W. bancrofti* mf ES antigen antibody by indirect ELISA and ES antigen by inhibition ELISA were monitored in microfilaraemia patients during diethylcarbamazine therapy. *W. bancrofti* mf ES antigen specific IgM antibody levels showed a gradual decrease in reciprocal antibody titre from pretreatment mean levels of about 15000 to 4000 at the end of the treatment (Malhotra *et al.*, 1983). However, the antigen titres during DEC therapy showed an initial increase followed by a gradual decrease during DEC treatment (Malhotra and Harinath, 1984a).

Field evaluation of ELISA using *W. bancrofti* mf ES antigen was done by screening filter paper blood samples of 462 persons residing in area endemic for bancroftian filariasis. This assay system when compared with night wet blood smear examination for microfilariae, gave a relative sensitivity of 98 % and specificity of 86 %. Day time blood sample can also be used in this test and thus can replace tedious night blood examination in field surveys in endemic areas (Harinath *et al.*, 1984).

Indirect ELISA using *W. bancrofti* mf ES antigen has been found to be quite useful in detection of filarial infection and in better coverage of large population in endemic areas by collecting filter paper blood samples at any time of the day in field surveys. Sensitivity and specificity of the test may be increased by including additional tests for detection of antigen or immune complexes. However, this test can not distinguish an active infection from chronic filariasis. Purification of *W. bancrofti* larval and microfilarial ES antigens by affinity chromatography and production of polyclonal and monoclonal antibodies to specific antigens should hopefully give suitable reagents for use in sensitive assays such as enzyme immunoassay and immuno-radiometric assay, providing an ideal assay system for detection of active filarial infection in the not too distant future.

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