SEROLOGICAL DIAGNOSIS OF BANCROFTIAN AND MALAYAN FILARIAISIS*

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Abstract. A study was undertaken to define the sensitivity and specificity of serological tests in bancroftian and malayan filariasis and correlate the findings with clinical disease. Sera were collected from subjects on three different islands in the Philippines: one endemic for bancroftian filariasis, another endemic for malayan filariasis and the third without endemic filariasis. Antibodies were measured, using Brugia malayi as the source of antigen. Antibodies against adult worms measured by indirect immunofluorescence were found at a titer of 1:8 or greater in all patients with bancroftian or malayan filariasis but not in the control subjects. There was no relationship of antibody titer to clinical status. Antibodies against microfilariae were measured by indirect immunofluorescence and by microfilarial agglutination. A high correlation was observed between the two methods. These antibodies were found in only one quarter, approximately, of patients with filariasis. Microfilarial antibodies were found more commonly in those patients with chronic lymphatic obstruction. It is concluded that measurement of antibodies to adult worms is a useful indicator of infection while microfilarial antibodies are correlated with disease.

The diagnosis of bancroftian and malayan filariasis may be difficult in patients with early inflammatory filariasis or in those with advanced obstructive disease as microfilariae are often absent from the blood.1–4 This has stimulated attempts to make the diagnosis indirectly, using immunological methods. The large number of skin tests and serological assays which have been developed have been reviewed by Kagan5 and Ambroise-Thomas.6 The serological techniques used have included complement fixation, passive hemagglutination, double gel diffusion, immunoelectrophoresis and indirect fluorescent antibody tests. A variety of related worms such as Dirofilaria immitis, Onchocerca volvulus, Loa loa, Litomosoides carinii, Wuchereria bancrofti, Dipetalonema species and Setaria species have been used as sources of antigen.5,6 Many problems have remained, however, particularly lack of sensitivity, cross-reactivity with other nematodes and the poor correlation of serological responses with intensity of infection.

The development of an animal model in which subperiodic Brugia malayi is maintained in jirds7 has enabled us to use this human parasite as a source of antigen. Antibodies have been measured by immunofluorescence against two of the major stages in the filarial life cycle in man, i.e., the lymphatic-dwelling adult worms and the microfilariae which circulate in the bloodstream. The studies were controlled by collecting sera from subjects on three different islands in the Republic of the Philippines, one endemic for Wuchereria bancrofti, another endemic for B. malayi and the third island with essentially no filariasis.

MATERIALS AND METHODS

Sera were collected from 135 patients with bancroftian filariasis and 44 patients with malayan filariasis. These patients were all from areas highly endemic for either W. bancrofti or B. malayi and had microfilaraemia, clinical features typical of gross lymphatic obstruction such as hydrocele and elephantiasis, or both. In addition, sera were obtained from 56 Filipinos who were living in an area not endemic for filariasis. The locations of these subjects and their clinical and parasitological details have been described previously.8 Eggs of Ascaris lumbricoides and Trichurus trichiura were found in the feces of 59% and 27%, respectively, of the Filipino control.

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subjects and in a similar proportion of patients with bancroftian filariasis. Furthermore, sera from Americans who had not been overseas were examined. Each serum was tested for the presence of fluorescent antibodies to Brugia malayi adult worms and microfilariae and for antibodies which would agglutinate Brugia malayi microfilariae.

**Fluorescent adult worm antibodies**

The technique employed was similar to that used by Ponnudurai et al. Standard grease-free microscope slides were marked with a crayon pencil to delineate three areas on which separate dilutions of serum could be placed. Living B. malayi adult worms were obtained from the peritoneal cavity of infected gerbils at autopsy, washed in phosphate buffered saline (PBS) and mounted in Tissue-TEK II O.C.T. embedding medium (Lab-Tek Products, Naperville, Ill.). Six-micron sections were cut on a Cryostat and mounted on microscope slides which were then stored at −20°C until required. Before use, the slides were transferred to a desiccator and allowed to warm to room temperature for 1 h, then fixed by immersion in acetone for 1–2 s. One hundredth of a milliliter samples of the test sera were serially diluted in PBS in complement fixation trays (Dispso trays, Limbro Chemical Co., New Haven, Conn.). One drop of each dilution was placed on each section. The slides were placed in humidity chambers, incubated at 37°C for 1 h, then washed several times in cold PBS. The fluorescein-conjugated IgG fraction of goat antihuman immunoglobulin (Cappel Laboratories, Downington, Pa.) was diluted 1:30 with PBS, then one drop was added to each section and the slides were incubated and washed as before. After washing, the sections were mounted under a coverslip with 90% buffered glycerol. Negative and positive control sera were included in each test.

An Olympus® reflecting fluorescent microscope with a 200-watt super-pressure mercury vapor lamp was used with a B exciter filter, BG12 exciter mount and B and 0515 barrier filters. A positive result was recorded when the cuticles of the sectioned worms were seen to fluoresce distinctly on 400× magnification. When a range is given for the antibody titers, it represents statistically-derived confidence limits using logarithms to the base2, viz. antilogarithm (logarithmic mean ± 1.96 × logarithmic standard deviation).

**Fluorescent microfilarial antibodies**

Microfilariae of B. malayi were obtained from the peritoneal cavity of infected gerbils as previously described. After they were washed in PBS, smears of 100–200 living microfilariae were made on marked microscope slides with a bacteriological loop, allowed to dry and then stored at −20°C until required. Serum microfilarial antibodies were measured as described for the adult worm antibodies. A positive result was recorded when the sheaths of the microfilariae were seen to fluoresce distinctly on 400× magnification.

**Agglutinating microfilarial antibodies**

The technique used was similar to that employed by Wong, who used Dirofilaria immitis and B. pahangi microfilariae. Microfilariae were obtained as before, washed and suspended in 50% PBS 50% Dulbecco’s modified Eagles medium (KC Biological, Kansas). After 0.1 ml of each test serum was serially diluted in complement fixation trays, 0.1 ml of suspension containing 1,000 microfilariae was added and the trays were incubated at 37°C for 2–3 h. Results were read with an Olympus® inverted tissue culture microscope. Negative and positive control sera were included in each test. A positive result was recorded when at least one clump of a dozen or more microfilariae was seen.

**RESULTS**

**Adult worm antibodies**

Fluorescent adult worm antibody titers are shown in Figure 1. No antibody was detectable in a majority (65%) of the Filipino control subjects and none had antibody with a titer greater than 1:4. All the sera from 17 Americans were negative except for one subject in whom the serum was positive only when undiluted. Antibody titers in the 135 patients with bancroftian filariasis followed an approximately normal distribution slightly skewed to the left with titers ranging from 1:8 to 1:1,024. Similar results were seen in patients with malayan filariasis. There was no overlap in antibody titers between patients and control subjects (P < 0.001, “r” test).

The relationship between clinical status and serological response was analyzed in the 135 patients with bancroftian filariasis. The geometric
mean antibody titer in patients with microfilaremia but without obstructive disease was 1:36 (range 4–330). This was not significantly different from that found in the 76 patients with obstructive filariasis in whom the geometric mean titer was 1:41 (range 6–280).

The relationship between the level of microfilaremia and antibody titer was measured in the same group of patients. The geometric mean antibody titer in the 52 patients without microfilaremia was 1:41 (range 7–230), in the 47 patients with 1–100 microfilariae per ml was 1:34 (range 4–330) and in the 36 patients with more than 100 microfilariae per ml was 1:43 (range 4–470). There were no significant differences between any of these groups.

**Microfilarial antibodies**

The relationship between the measurement of microfilarial antibodies by fluorescence and by agglutination is shown in Figure 2. There was a highly significant correlation between the two methods ($r = 0.6819$, $P < 0.001$). Fluorescent microfilarial antibody levels are shown in Figure 3. No antibody was detected in a majority (64%)...
Figure 4. Serum agglutinating microfilarial antibody titers in patients with bancroftian filariasis, malayan filariasis, and in Filipino control subjects without filariasis. The percentage of subjects is plotted against the reciprocal of the final serum dilution giving a positive reaction. Undiluted serum cannot be assayed by this technique. A value of 1 represents no reaction with serum diluted 1:2, while a value of 2 represents a positive reaction with serum diluted 1:2.

of the Filipino control subjects and none had an antibody titer greater than 1:2. A similar profile was seen in patients with malayan filariasis. On the other hand, 26 of the 135 patients with bancroftian filariasis (19%) had antibody titers of 1:4 or greater. Similar results were seen when microfilarial agglutinating antibodies were measured (Fig. 4). Forty-nine of the 135 patients (36%) had antibody titers of 1:4 or more.

The relationship between clinical status and serological response was analyzed in the 135 patients with bancroftian filariasis. Microfilarial antibodies were more commonly found in patients with obstructive disease. Microfilarial agglutinating titers of 1:4 or greater were found in 46% of 76 patients with obstructive filariasis compared with 22% of 59 patients with asymptomatic microfilariaemia. This difference was statistically significant ($P = 0.0021$, Fisher’s exact test).

The relationship between the level of microfilariaemia and antibody titer was measured in the same group of patients. Microfilarial agglutinating antibody titers of 1:4 or more were found in 40% of the 52 patients without microfilaraemia, in 34% of the 47 patients with 1–100 microfilariae per ml and in 20% of the 36 patients with more than 100 microfilariae per ml. Although there was a tendency for an inverse relationship between the level of microfilaraemia and the presence of microfilarial antibodies, this was not statistically significant ($P < 0.10$, $\chi^2$, Brandt and Snedecor’s formula).

**DISCUSSION**

This study of serological responses in bancroftian and malayan filariasis has attempted to define the sensitivity and specificity of serological tests using a human filarial parasite, *B. malayi*, as an antigen and correlate these findings with the clinical and parasitological status. Large numbers of sera from patients with bancroftian or malayan filariasis have been compared with sera from subjects on another island in the archipelago who have a similar lifestyle but live in an area in which filariasis is not endemic.

The measurement of antibodies against adult worms appears to be a useful index of exposure to filarial worms. All the patients tested gave a positive reaction by immunofluorescence yet there was no overlap with control subjects from a non-endemic area. This contrasts with earlier studies in which neither *W. bancrofti* nor *B. malayi* but related filarial worms were used as sources of antigen. In those studies, both false positive reactions and false negative reactions were seen in a large proportion of patients. Patients with bancroftian filariasis reacted to *B. malayi* antigen as well as did those with malayan filariasis. This cross-reactivity was noted previously when immediate hypersensitivity skin reactions were measured in the same group of patients. The high prevalence of antibodies against adult worms is similar to other observations in animals and man. Ponnudurai et al. found that sera from all of 33 cats infected with *B. pahangi* had adult worm antibodies. Wong and Guest observed that the majority of patients with elephantiasis or microfilaraemia had antibodies against infective larvae of *B. malayi*. Ambroise-Thomas and Kien Truong detected fluorescent antibodies against *W. bancrofti* adults in 94% of
patients with filariasis. Although adult worm antibody titers indicated exposure to filarial worms, they did not reflect the clinical status of the patients. There was no difference between patients with asymptomatic microfilaremia and those with gross elephantiasis, nor was there any relationship between the level of microfilaremia and adult worm antibody titer.

In contrast to the high prevalence of adult worm antibodies, only a small number of patients had antibodies against microfilariae. Most investigators have found that unless microfilariae are damaged mechanically, or by papain digestion, microfilarial antigens are of little use in detecting antibodies during the microfilaremic phase of bancroftian filariasis. Capron et al. found that antibodies were present on immunoelectrophoresis against *D. vitae* antigens in only 30% of patients with a microfilaria but in all patients without circulating microfilariae. Jayewardene and Wijayaratnam observed that only 23% of patients had fluorescent reactions with *W. bancrofti* microfilariae and that most of these patients had no circulating organisms. Wong and Guest noted that microfilarial antibodies were present in all patients with elephantiasis but in none of the patients with circulating microfilariae. Ponnu durai et al. found that microfilarial antibodies could not be detected in cats during the microfilaremic phase but were present in the postmicrofilaria stage.

We did not find such a clearcut relationship between microfilarial antibodies and the absence of microfilariae in the bloodstream. There was a tendency towards an inverse relationship between the level of microfilaremia and antibody titer but this did not quite reach a statistically significant level. Significantly more patients with obstructive disease, however, had microfilarial antibodies than those without such disease. Smithers has suggested that living microfilariae are relatively inert antigenically and do not stimulate the production of antibodies. Alternatively, microfilarial antibody may be present but undetected because it is absorbed by the microfilariae or combines with soluble metabolic antigens produced by microfilariae. It may be that the detection of microfilarial antibodies depends upon the relative release of microfilariae into the bloodstream and the production of antibodies against those microfilariae.

In conclusion, *B. malayi* appears to be a reliable and easily available source of antigen for the immunological diagnosis of both bancroftian and malayan filariasis. The measurement of antibodies against adult worms is an excellent indication of infection with filarial worms. As such, it may be a useful epidemiological tool to indicate the prevalence of infection in a community. The measurement of microfilarial antibodies is not of immunodiagnostic significance but these antibodies are more commonly found in patients with obstructive disease. Further elucidation of the relationships between microfilaremia and microfilarial antibodies may shed some light on the factors controlling the circulation of microfilariae in the bloodstream.

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**REFERENCES**


