SENSITIVITY AND SPECIFICITY OF SKIN REACTIVITY
TO BRUGIA MALAYI AND DIROFILARIA IMMITIS ANTIGENS
IN BANCROFTIAN AND MALAYAN FILARIASIS
IN THE PHILIPPINES*

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Abstract. Saline antigen extracts of microfilariae, adult worms and third-stage larvae of
subperiodic Brugia malayi maintained in gerbils were prepared for use as skin test reagents.
Patients were studied on three different islands in the Philippines, one endemic for Bancroftian
filariasis (Sorsogon, Luzon), another endemic for Malayan filariasis (Palawan) and the third
without endemic filariasis (Cebu). A dose-response curve was established initially in patients
with Bancroftian filariasis: thereafter 1.0 mg of the B. malayi antigens and 0.05 mg of Diro-
filaria immitis FST antigen (obtained from Dr. T. Sawada) were used. Sizes of reactions were
measured by recording the diameters of wheals at 20 minutes, 24 and 48 hours. There
was a very high correlation in immediate hypersensitivity reactions among the three B. malayi
antigens. Reaction sizes followed a normal distribution. When an area of an antigen-induced
wheal 3X that of the saline control was considered a positive reaction, 99% of 150 patients
with Bancroftian filariasis and 96% of 45 subjects with Malayan filariasis reacted to B. malayi
larval antigen. Only 68% of patients with Bancroftian filariasis but 90% of those with Ma-
layan filariasis reacted to D. immitis FST antigen. There was no relationship between skin
reactivity and age, sex, microfilaremia or severity of clinical disease. Approximately half of
50 patients who lived in an endemic area for W. bancrofti but had neither patent infection
nor clinical disease reacted to B. malayi antigens. A maximum of 7% of 120 age- and sex-
matched controls from Cebu gave false positive reactions with any of the antigens. Only a
small proportion of patients gave 24- and 48-hour reactions. It is concluded that the use of
antigens prepared from a human parasite, subperiodic B. malayi, which is easily maintained
in a laboratory animal host, improves the ability to diagnose filarial infections by immuno-
logical means.

The definitive diagnosis of Bancroftian or
Malayan filariasis is based on the demonstration of microfilariae circulating in the blood. Patients
with the lymphadenitis, lymphangitis, or painful
genital swelling of early, inflammatory filariasis
do not usually have a microfilaremia,1,2 nor is it
present in most patients with advanced, obstruc-
tive filarial disease.3,4 Furthermore, very light
microfilaremias are difficult to detect without
using concentration methods such as the Nucle-
pore filter technique.5 In view of these problems,

many investigators have used immunological
methods to aid in the diagnosis of filariasis since
Fairley and Liston, in 1924,6 prepared an antigen
from Dracunculus medinensis.

By the time of the major review by Kagan in
1963,7 there had been 79 reports of the use of the
skin test, 42 investigations with a complement
fixation test, and 12 studies with the precipitin
test. A variety of related worms, D. medinensis,
Litomosoides carinii, Loa loa, Onchocerca vol-
vulus, and Setaria were used as sources of antigen
but, because of its availability, Dirofilaria immitis
antigens were most frequently utilized. These
tests were generally unsatisfactory as they were
not sufficiently sensitive or specific, false negative
and false positive reactions both being common. Attempts were made, therefore, to improve the sensitivity and specificity of *D. immitis* extracts by fractionation of antigens. These preparations were still insensitive and non-specific, however, as only 70% to 80% of patients gave positive reactions, while 10% to 30% of subjects who had never been exposed to filarial worms gave positive reactions.

An alternative approach to the fractionation of a dog filaria is to prepare antigens from the filarial species which infect man in the hope that the use of such antigens might improve both sensitivity and specificity. It has been difficult to obtain sufficient material however, and only scattered reports have appeared of the use of antigens prepared from microfilariae or third-stage larvae of *Wuchereria bancrofti*. While these studies involved relatively small numbers of patients, they have suggested that homologous antigens may be valuable in the diagnosis of filariasis.

In addition, the responses of the hosts to these antigens may help to elucidate the role of immunological factors in the development of filarial disease and the transmission of infection. The granulomatous lymphadenitis and lymphangitis of the inflammatory stages of the disease reportedly involve cell- and antibody-mediated reactions. Serum antibodies may be important in determining levels of microfilariae, thereby influencing the availability of the parasite to mosquito vectors. Analysis of immediate (reaginic antibody), Arthus (precipitating antibody), and delayed (cell-mediated) skin test reactions to antigens may therefore add to our understanding of the immunological processes in filarial disease.

The recent development of an animal model in which the human parasite, subperiodic *Brugia malayi*, is maintained in jirds has enabled us in the present study to prepare antigens from three of the major stages in the life cycle: microfilariae, adult worms and third-stage infective larvae. We have compared these antigens with the Savade FST antigen of *D. immitis* in patients with filariasis in the Republic of the Philippines. Both filarial parasites of man, *W. bancrofti* and *B. malayi*, are found in different locations in the islands. Controlled studies of a large number of patients were carried out at six sites on three different islands, one being endemic for *W. bancrofti* (Sorsogon), another endemic for *B. malayi* (Palawan) and the third with essentially no filariasis (Cebu).

**Materials and Methods**

**Preparation of antigens**

Jirds (*Meriones unguiculatus*) infected with a subperiodic strain of *Brugia malayi* were obtained from Dr. J. McCall of the University of Georgia, Athens, Georgia (provided by the U.S.-Japan Cooperative Medical Science Program-NIAID). Four months after inoculation with 300 infective third-stage larvae, six jirds were anesthetized with sodium pentobarbital, their abdomens shaven and sterile 18 gauge angiocaths (Deseret Pharmaceutical Co., Sandy, Utah) inserted into the midline. The peritoneal cavities were washed twice with 15 ml sterile phosphate buffered saline. Contaminating white cells were removed as described by Ah et al. and a pure concentrate of 10 million microfilariae was obtained which was then stored at -20°C. The procedure was repeated on the same jirds 3 weeks later and 6 million microfilariae were obtained. The pooled microfilariae were lyophilized, pulverized with a pestle and mortar and the powder was extracted in 5 ml of phosphate buffered saline overnight at 4°C. The sediment was spun down in a Spinco centrifuge (Beckman Instruments, Inc., Southfield, Michigan) at 50,000 × g for 30 minutes at 4°C. The supernatant was passed through a 22 μm Millipore disposable filter unit (Millipore Corporation, Bedford, Massachusetts) and the protein concentration was determined by the method of Lowry et al.

Approximately 100 adult worms were removed from the peritoneal cavity of one jird, washed by sedimenting several times in phosphate buffered saline, ground in a Ten Broeck grinder, extracted, sterilized and the protein concentration determined as described above.

Female *Aedes aegypti* mosquitoes (Liverpool strain) were fed on jirds with moderate microfilaraemias. Twelve days later they were crushed on a block of ice, placed on four layers of Kimwipes in a Baermann apparatus and the larvae were sedimented and collected. The larvae were washed several times in phosphate buffered saline, homogenized in a Ten Broeck grinder, extracted, sterilized and the protein concentration was determined as described above.
The microfilarial, adult and third-stage larval antigen preparations were adjusted to a concentration of 200 μg/ml, 1:10,000 merthiolate was added and they were stored at −20°C.

The lyophilized Sawada FST3-1 antigen preparation of Dirofilaria immitis was kindly supplied by Professor T. Sawada, Maebashi, Japan.

Locations and subjects

The studies were carried out in three different areas of the Philippines, one locality highly endemic for Wuchereria bancrofti, another highly endemic for Brugia malayi and the third with a very low prevalence of filariasis (Fig. 1).

Infection with periodic W. bancrofti is highly endemic in Sorsogon province of the island of Luzon. In one extensive survey, thick blood films were positive in 6,525 (8.4%) of 77,650 subjects. In addition, many patients have evidence of chronic obstructive filariasis such as hydrocele and elephantiasis of the legs. Filarial transmission is facilitated by the widespread cultivation of abaca (Musa textilis) for the production of Manila hemp, as larvae of the principal vector (Aedes poecilus) breed in the leaf axils of the plant. One hundred and fifty subjects in the coastal municipalities of Castilla, Bacon, Casiguran, and Juban were studied. They had previously been shown in surveys by the filariasis control unit of the Department of Health to have either a microfilaraemia or clinical filariasis (enlargement of the scrotum or legs). Most microfilaremic patients had been treated with diethylcarbamazine between 5 and 10 years previously. The mean age was 45 ± 14 years, the age distribution being shown in Table 3: 77% of the patients were males and 23% were females. In addition, 50 subjects (local controls) from the municipality of Sorsogon who had neither microfilaraemia nor clinical evidence of filariasis were studied. The age range was 38 ± 10 years: 56% of the subjects were males.

Infection with subperiodic B. malayi is highly endemic in parts of the municipality of Quezon on the island of Palawan. Filariasis is found amongst those who live close to the fresh water swamps, the breeding place of the vector, Mansonella bonneae. Forty-five patients from the sitios (small villages) of Gungnan, Marirong, Kambing, and Tagpis of Quezon municipality were studied. They had been found to have either a microfilaraemia or clinical filariasis in previous surveys.

Many microfilaremic patients had been treated with diethylcarbamazine 10 years previously. The age range was 35 ± 16 years: 71% of the patients were males.

Filarial infection is rare in the province of Cebu. Only one patient with microfilaraemia was found in the 3,766 persons studied in the survey by Estrada and Basio. One hundred and twenty subjects (distant controls) from the municipality of Cordova on the island of Mactan in the province of Cebu, who were age- and sex-matched for the patients with bancroftian filariasis, were tested as a control group. None of these subjects had migrated from areas where filariasis is endemic.

Each patient was examined and a clinical history was taken using protocol B recommended for filariasis surveys by the World Health Organization. Blood samples were taken by venipuncture in the evening. One milliliter of anticoagulated blood was passed through a Nuclepore 5 μm pore filter, 25 mm in diameter in a Swin-Lok holder (Nuclepore Corporation, Pleasanton, California), washed twice with water, fixed with methanol, mounted on a glass slide and stained with Giemsa. Patients were classified arbitrarily into the following groups: asymptomatic microfilaraemia, microfilaraemia with a history of lymphadenitis or lymphangitis, scrotal enlargement up to 10 cm across, scrotal enlargement greater than 10 cm, and edema of the legs. The number of patients with bancroftian or Malayan filariasis in each group is shown in Table 4.

Fecal samples were collected from 50 patients.
with bancroftian filariasis in Sorsogon and the eggs of intestinal helminths were counted using a modification of the Kato technique. No eggs were found in 16% of subjects, eggs of *Ascaris lumbricoides* in 68%, and *Trichuris trichiura* eggs in 56%. A similar distribution of intestinal helminthiasis was seen in 50 control subjects from Cebu: 24% were negative, eggs of *A. lumbricoides* were found in 59% and *T. trichiura* eggs in 27%.

**Skin tests**

Preparations of *B. malayi* microfilarial, adult and third-stage larval antigens and *D. immitis* FST (Sawada) antigen were thawed and diluted before use with sterile physiological saline containing 1:10,000 merthiolate. Skin tests were performed by injecting 0.1 ml of each antigen solution intradermally into the volar aspect of the left forearm. 0.1 ml of physiological saline containing 1:10,000 merthiolate was injected as a control. Reactions were read at 20 minutes, 6, 24 and 48 hours after injection.

**Twenty-minute reactions.** The diameter in millimeters of the wheals 20 minutes after injection was measured with a ruler. If the wheal was oval, the average of the length plus breadth was recorded. Results have been expressed in two ways: 1) ratio of area of wheal induced by antigen to area of wheal induced by saline control. The numbers of subjects with a reaction twice, three times and four times that of the saline control were recorded. Comparisons were made using Fisher's exact test; 2) diameter of wheal induced by antigen minus diameter of wheal induced by saline control. Results have been shown both in diagrammatic form and as mean ± standard deviation. Statistical comparisons were made using Student’s "t" test. In order to determine the most appropriate dose of *B. malayi* antigen, the first 10 patients with bancroftian filariasis, eight of whom had a microfilaraemia and two with clinical disease, were injected with 0.3, 1.0 and 3.0 µg protein of the microfilarial, adult and third stage larval preparations. Wheals three times the area of the control were produced by 0.3 µg protein in 9 of 10 patients with the microfilarial and third-stage larval antigens and in all 10 subjects with the adult antigen. All patients reacted to the three antigens when given in doses of 1.0 or 3.0 µg protein. Testing was subsequently carried out using 1.0 µg of each of the *B. malayi* antigens and 0.05 µg *D. immitis* FST antigen (the recommended amount) in 0.1 ml saline.

**Six-hour reactions.** It was hoped that the measurement of Arthus reactions 6 hours after injection might give an indication of circulating precipitating antibody. Measurements were taken for the first 100 patients with bancroftian filariasis, but the residua of the large immediate hypersensitivity reactions made such observations difficult and unreliable. These data are not presented.

**Twenty-four and 48-hour reactions.** Delayed skin test reactions were measured 24 and 48 hours after injection. Induration 6 mm or more in diameter was considered a positive reaction.

**RESULTS**

**Twenty-minute reactions**

Reactions to the saline control with wheals of 9 mm or more in diameter were seen in 6 of the 375 subjects studied (2 patients with bancroftian filariasis, 2 local control subjects and 2 distant control subjects); these individuals were excluded from analysis.

**Patients with bancroftian filariasis (Sorsogon).** The proportions of patients with bancroftian filariasis who reacted to each antigen with wheal areas 2×, 3× and 4× that of the saline control are shown in Table 1. Responses were maximal to third stage larval antigen with 99% of patients having reactions 3× the area of the saline control. There was a high correlation among the three *Brugia* antigens with 91% of patients reacting with 3× the area for all three antigens (Table 2). The mean diameter of the saline control reactions was 4.5 ± 1.2 mm. The mean increases in wheal diameter above that of the saline controls for microfilarial, adult and third-stage larval antigens were 5.6 ± 2.6, 5.8 ± 2.7 and 7.4 ± 2.9 mm respectively. The reactions to third-stage larval antigen were significantly greater than to microfilarial and adult antigens (P < 0.001, Student’s "t" test).

When compared with any of the *Brugia* antigens, the reactions to Sawada FST antigen were significantly smaller (P < 0.001, Fisher's exact test) with only 68% of patients reacting with 3× the area (Table 1, Fig. 2). The mean increase in wheal diameter above that of the saline control was 3.4 ± 2.8 mm. Reactions to all four antigens were highly significant when compared with those
remia. Similar results were found in each case so only the values for microfilarial antigen are given. There were no significant sex differences, the mean increase in diameter above the saline control being $5.7 \pm 2.6$ mm for males and $5.5 \pm 2.7$ mm for females. Size of the wheals was not related to the age (Table 3) or the clinical status of the patient (Table 4). When the results were further analysed individually for the presence or absence of lymphadenitis/angitis, chyluria, scrotal enlargement or leg edema, no significant differences were apparent. Similarly, the size of the wheals was not related to the presence or intensity of microfilaria: the mean increase in diameter of wheals above that of the saline control was $5.5 \pm 3.0$ mm for patients without circulating microfilariae, $6.2 \pm 3.0$ mm for those with 1 to 50 microfilariae per ml and $5.4 \pm 2.6$ mm for those with more than 50 microfilariae per ml.

Patients with Malayan filariasis (Palawan). The proportions of patients with Malayan filariasis who reacted to each antigen with areas $2\times$, $3\times$ and $4\times$ that of the saline control are shown in Table 1. Most reactions occurred to third-stage larval antigen with 96% of patients having reactions $3\times$ the area of the saline control. There was a high correlation among the three *B. malayi* antigens with 90% of patients having reactions $3\times$ the area of the control for all three antigens (Table 2). The mean diameter of the saline control reactions was $5.3 \pm 0.1$ mm. The mean increases of wheal diameter above the saline control for microfilarial, adult and third-stage larval antigens were $4.4 \pm 1.9$, $4.1 \pm 1.6$ and $4.8 \pm 2.1$ mm, respectively, there being no significant differences among the antigens.

In contrast to the findings in bancroftian filari-

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**Table 1**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>2× area</th>
<th>3× area</th>
<th>4× area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microfilarial</td>
<td>94</td>
<td>96</td>
<td>48</td>
</tr>
<tr>
<td>Adult</td>
<td>95</td>
<td>96</td>
<td>56</td>
</tr>
<tr>
<td>Third stage</td>
<td>99</td>
<td>98</td>
<td>60</td>
</tr>
<tr>
<td>FST</td>
<td>71</td>
<td>96</td>
<td>20</td>
</tr>
</tbody>
</table>

* W.b., Bancroftian filariasis; B.m., Malayan filariasis.
† L.c., local controls; D.c., distant controls.
The reactions to Sawada FST antigen were not significantly different from those to the Brugia antigens: 90% of patients had reactions 3X the area of the saline control (Table 1), the mean increase in wheal diameter above that of the saline control being 4.2 ± 1.4 mm (Fig. 2).

There were no significant differences in reactions to any of the antigens with respect to sex, age, microfilaraemia or clinical classification (Table 4).

Local control subjects (Sorsogon). The proportions of subjects who lived in an area endemic for W. bancrofti (Sorsogon) but did not have microfilaraemia or clinical signs of filariasis and who reacted to each antigen with areas 2X, 3X and 4X that of the saline control are shown in Table 1. Approximately 50% of subjects reacted to the Brugia antigens but only 20% reacted to Sawada FST antigen; this difference was statistically significant (P < 0.001, Fisher's exact test). Sixty-seven percent of subjects reacted to at least one of the three Brugia antigens, but a large proportion reacted to only one of the antigens (Table 2). The mean diameter of the saline control reactions was 4.5 ± 0.9 mm. The mean increases in wheal diameter above that of the saline controls for microfilarial, adult, third-stage larval and Sawada FST antigens were 2.7 ± 2.7, 2.8 ± 2.9, 3.3 ± 2.9 and 0.4 ± 1.5 mm, respectively (Fig. 2). They were all significantly less than in patients with bancroftian filariasis (P < 0.001, Student's "t" test) but significantly more than in distant controls (P < 0.001, Student's "t" test) except for the very small reactions to Sawada FST antigen.

Distant control subjects (Cebu). The proportions of age- and sex-matched patients who lived in an area not significantly endemic for filariasis (Cebu) and who reacted to each antigen with areas 2X, 3X and 4X that of the saline control are shown in Table 1. Seven percent of subjects reacted to one or more of the Brugia or Sawada FST antigens (Table 2).

The mean diameter of the saline control reactions was 5.0 ± 1.1 mm. The mean increases in wheal diameter above that of the saline controls for microfilarial, adult, third-stage larval and Sawada FST antigens were 0.4 ± 1.4, 0.6 ± 1.5, 0.8 ± 1.5 and 0.2 ± 1.0 mm, respectively (Fig. 2). There were no significant differences between sexes or among ages.

Twenty-four hour reactions

Delayed reactions, 24 hours after injection, occurred in only a small proportion of patients with Bancroftian filariasis (Table 5). Although these reactions were definite, they were capricious in distribution. Thirty-six percent of patients with bancroftian filariasis reacted to at least one of the three Brugia or the Sawada FST antigen, most subjects (62%) reacting to only one antigen and only 8% of them reacting to all four antigens. There was no relation between microfilaraemia or clinical classification and the number of reactions. The reaction rate in local controls in the endemic area was similar except that no subjects reacted to the Sawada FST antigen. The reverse occurred in patients with Malayan filariasis, however, for 11% reacted to Sawada FST antigen but there were almost no reactions to the Brugia antigens.
### Table 4

Mean increase in diameter of wheals induced by B. malayi microfilarial antigen 20 minutes after intradermal injection minus the diameter of wheals induced by the saline control in patients with Bancroftian or Malayan filariasis when analysed according to the clinical status of the patients.

<table>
<thead>
<tr>
<th>Patients with</th>
<th>Bancroftian filariasis</th>
<th>Malayan filariasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean ± S.D. (diameter in mm)</td>
</tr>
<tr>
<td>Asymptomatic microfilaremia</td>
<td>23</td>
<td>4.9 ± 1.9</td>
</tr>
<tr>
<td>Microfilaremia and history of lymphadenitis/angitis</td>
<td>40</td>
<td>5.9 ± 2.4</td>
</tr>
<tr>
<td>Scrotal enlargement ≤10 cm</td>
<td>34</td>
<td>5.7 ± 3.0</td>
</tr>
<tr>
<td>Scrotal enlargement &gt;10 cm</td>
<td>26</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td>Leg edema</td>
<td>25</td>
<td>5.6 ± 2.9</td>
</tr>
</tbody>
</table>

Very few reactions were seen in distant controls (Table 5).

**Forty-eight hour reactions**

Delayed reactions, 48 hours after injection, occurred in an even smaller number of patients with bancroftian filariasis (Table 5). Fifty-six percent of the reactions which were positive at 24 hours were negative by 48 hours. Conversely, 23% of the reactions which were positive at 48 hours had been negative at 24 hours.

**DISCUSSION**

The definitive diagnosis of filariasis depends upon finding microfilariae in the blood. The procedure is both time-consuming and unreliable since it fails to detect many early and late infections as well as those with very light microfilaremias. The development of reliable immunological tests would therefore greatly facilitate both epidemiological and clinical studies. The immunodiagnostic tests previously reported have depended largely on antigens prepared from the easily available dog filaria, *D. immitis*, and have been relatively insensitive and nonspecific. While there is no generally available animal host for *W. bancrofti*, the ability to maintain the life cycle of human subperiodic *Brugia malayi* in gerbils has provided an opportunity to prepare antigens from that human parasite in quantities sufficient for clinical evaluation.

The Republic of the Philippines was an ideal location in which to determine the efficacy of these preparations as it is an archipelago in which filariasis has a patchy distribution. Investigations in two separate provinces, Sorsogon, where bancroftian filariasis is highly prevalent, and Cebu, where filariasis is not endemic, allowed the comparison of skin test reactions in communities which are similar in regard to such factors as coastal location, housing, food and intestinal helminths. Furthermore, although *W. bancrofti* is the predominant parasite in the Philippines, scattered foci of *B. malayi* occur. Subjects were examined on the island of Palawan in an area where *B. malayi* is the only prevalent filaria.

These investigations have shown that antigens prepared from the human parasite, subperiodic *B. malayi*, are much more effective in the diagnosis of bancroftian filariasis than is the commonly used FST preparation of *D. immitis* antigen. It is apparent that there is considerable cross-reactivity between *B. malayi* and *W. bancrofti*, for 99% of patients with bancroftian filariasis had immediate skin reactions to *B. malayi* third-stage larval antigen. Furthermore, there is a very high correlation between the *Brugia*
microfilarial, adult worm and third-stage larval antigens, with 91% of bancroftian patients reacting to all three antigens. Although the percentage of reactors to third-stage larval antigen was only slightly greater than that to microfilarial and adult antigens, the size of the wheels of the former was significantly greater. It is possible that this stage, the one to which the body is first exposed, may be more immunogenic.

Wheal areas two, three and four times those of the saline controls were classified as indicating positive reactions. When wheal areas three times that of the control were compared with those twice the control, the number of false positives is halved, whereas the sensitivity in known patients is reduced by only 1%. When a wheal area four times that of the control was analysed, the number of false negative reactions increased markedly whilst having only a small effect on the false positive rate.

The rate of positive immediate skin reactions in Cebu control subjects was 7%. Microfilaraemia, although rare, has been recorded in Cebu, so some subjects may have been exposed to *W. bancrofti* even though they did not develop a patent infection or clinical disease. Others may have been bitten by vectors of *D. immitis* which is widespread in the Philippines. Although this parasite is not able to maintain its life cycle in the human host, it might induce an immunological response. Furthermore, it has been suggested that there may be some cross-reactivity between filariae and intestinal helminths. Both the Sorsogon and Cebu populations had comparable prevalences of intestinal worms; these parasites may have contributed to the false positive reactions seen in a few Cebu control subjects.

*D. immitis* FST antigen was much less sensitive than the *Brugia* antigens in bancroftian filariasis. Only slightly more than two-thirds of the patients gave positive reactions. *D. immitis* antigen was no more specific, however, as the number of false positive reactions was only slightly less. Our results are similar to those seen by others who have used *Dirofilaria* antigens.

This divergence between *Brugia* and *D. immitis* antigens was also seen in control subjects from an endemic area for *W. bancrofti* who were without patent infections or clinical disease. More than half of these subjects reacted to *Brugia* antigens while only 20% reacted to *D. immitis* FST antigen. The number of subjects who reacted to *Brugia* antigens was intermediate between patients with Bancroftian filariasis and control subjects from a non-endemic area in Cebu. It is of interest that many of these subjects reacted to only one of the *Brugia* antigens. This group had probably been exposed to *W. bancrofti* thus eliciting an immunological response, but the worms may not have developed to maturity, fertilization may not have occurred, or previously fertile worms may have died. These observations are consistent with the conclusions on epidemiological grounds of other investigators that a high proportion of the population of an endemic area must be carrying parasites that are either dead or immature. A positive reaction, therefore, appears to indicate exposure to filarial worms, whether or not patent infection or clinical disease develops.

Patients with Malayan filariasis reacted to *Brugia malayi* antigens virtually as well as did those with bancroftian filariasis. In contrast to the latter group however, reactions to *D. immitis* FST antigen were similar to those observed with the *Brugia* antigens. *W. bancrofti* is a purely human parasite, subperiodic *B. malayi* infects both man and animals, while *D. immitis* is essentially an animal parasite. If immunological cross-reactivity also follows such a spectrum, then *D. immitis* may be antigenically closer to subperiodic *B. malayi* than to *W. bancrofti*, thus accounting for an increased frequency of positive reactions to *D. immitis* antigen in patients with the former infection.

Twenty-four hour or delayed skin test reactions have been noted by some authors but not by others. Only 36% of our patients reacted to at least one antigen at 24 hours and an even smaller proportion had reactions at 48 hours. Reactions bore no relationship to age, sex, microfilaraemia or clinical status. Furthermore, many patients reacted to only one of the four antigens. Immediate hypersensitivity reactions are mediated by reaginic antibodies and it is not surprising therefore that raised serum IgE levels have been found in patients with filariasis. Wong and Ponnudurai et al. have suggested from animal studies that serum antibodies may eliminate circulating microfilariae. If reaginic antibodies were important in preventing microfilaraemia, it might be expected that there would be greater immediate
hypersensitivity reactions in exposed subjects without microfilaremia than in those with microfilaremia. These were indeed the findings of Franks et al., who used *D. immitis* microfilarial antigen to test 320 Okinawese. In contrast, Sawada et al. thought that there was less reactivity in subjects without microfilaremia. Our observations, however, are in agreement with most investigators who have found no differences between infected subjects with or without a microfilaremia.

There is little information on the relationship between skin test reactions and the different clinical manifestations of filariasis. Previous studies using *D. immitis* antigen have given conflicting results. Acton and Rao claimed, but provided little evidence, that patients with lymphangitis and elephantiasis had greater immediate hypersensitivity reactions than asymptomatic carriers. On the other hand, Franks et al. and Taliaferro and Hoffman suggested there were more negative reactions in their few patients with elephantiasis. Sawada et al. thought that 20 symptomatic patients without microfilaremia had less reactivity than did microfilaria carriers. Wharton found that 26 of 29 patients with elephantiasis had immediate hypersensitivity reactions. This ratio appears to be little different from that found in asymptomatic carriers by other authors. Smith et al. found no differences between asymptomatic carriers and those with clinical filariasis. Our studies, using human parasite antigens in large numbers of patients with obstructive disease, microfilaremia with a history of lymphatic inflammation, or asymptomatic microfilaremia, showed no significant relationship with either presence or absence of microfilaremia and clinical status.

In conclusion, we have demonstrated that antigens prepared from microfilariae, adult worms and third-stage larvae of subperiodic *Brugia malayi* are of value in the diagnosis of patients with prepatent infections with both *W. bancrofti* and *B. malayi* and in those patients with chronic obstructive disease but without microfilaremia. While they are more sensitive than *D. immitis* FST antigen, they do not have a significantly higher number of false positive reactions. Preparation of *B. malayi* antigens is much easier now that the life cycle of a human filarial parasite can be easily maintained in the laboratory in an inexpensive animal host.

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