FILARIASIS DUE TO *BRUGIA MALAYI* IN WEST MALAYSIA

PART II: SKIN TEST ASPECTS

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INTRODUCTION

As explained in Part I of this investigation (Dondero *et al.*, 1971), we wished to study several immunological responses in persons whose manifestations of filariasis due to *Brugia malayi* differed clinically. However, before experimental work could begin, it was necessary, for purposes of later comparison, to document the clinical and parasitological status of the subjects and to check their skin test responses to a standard filarial antigen. This report covers our findings.

Skin tests for diagnosing filarial infections were used as early as 1930 when Taliaferro and Hoffman (1930) used an extract of the dog heartworm *Dirofilaria immitis*, with results showing "considerable promise". Hundreds of later studies with many kinds of antigens followed (Kagan, 1963); their sheer number speaks for their generally limited success. Recurring problems are: lack of standardized antigen, lack of species specificity, and high frequency of false negative and false positive reactions.

Sawada *et al.*, (1965) described a highly purified antigen extract of adult dog heartworm for use in intradermal testing. Although not ideal, the Sawada Filarial Skin Test (F.S.T.) has been experimentally used in various parts of the world (Desowitz *et al.*, 1966; Ata *et al.*, 1967; Higashi *et al.*, 1968; Gidel *et al.*, 1969; Ramachandran *et al.*, 1970). and is currently being evaluated by the World Health Organization (T. Wilson and D.H. Smith, personal communication).

Since the Sawada F.S.T. is the nearest existing approach to a standard method, we used it on our subjects as a basis for later comparison. The interesting results obtained point up certain problems encountered in immunodiagnosis of filariasis.

MATERIALS AND METHODS

The study group comprised 68 persons with and without manifestations of filarial infection, who were selected from a region endemic for nocturnally periodic *Brugia malayi*.

The Sawada F.S.T. was done according to W.H.O. specifications as provided by Professor Wilson and Dr. Smith. Professor Wilson, in connection with a W.H.O. evaluation of the test kindly supplied the diluent and lyophilized antigen, which had been prepared by Professor T. Sawada. The antigen was that portion left over from the W.H.O. study. We injected as precisely as possible 0.02 ml. of freshly reconstituted antigen (2.5 µg. protein per ml.) intradermally on the dry, alcohol-washed volar surface of the forearm. The same amount of the merthiolated 0.9%

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saline diluent served as the control. All injections were made from sterile 0.25 ml. glass syringes with sterile disposable 26-gauge tuberculin needles (Jintan Terumo Co. Ltd., Tokyo). Wheals were outlined with a ball-point pen 20 minutes after injection and the marks were transferred to alcohol-moistened filter paper by pressing the paper on the skin. The areas of the antigen-induced and control wheals were then measured with a transparent grid.

No standard criteria exist for judging positive or negative reactions. Previous workers have used as indicators of a positive reaction such criteria as an antigen wheal diameter greater than 7 mm. (Desowitz *et al.*, 1966; Gidel *et al.*, 1969) or the ratio of antigen wheal area to control area equal to or greater than 1.25 (Ata *et al.*, 1967). We selected the second criterion but took the reciprocal value equal to or less than 0.8 as positive to avoid astronomical values when control areas were minimal.

Statistical analysis was made using chi^2 and student's "t" test as described by Simpson *et al.*, (1960).

RESULTS

Entire group

Among the 68 subjects were 34 with elephantiasis or lymphoedema (one of these with microfilariae), 8 with filarial-type adenolymphangitis without elephantiasis or microfilaremia, 18 with microfilaremia without elephantiasis, and 8 clinically and parasitologically negative for filariasis. We designated these subgroups respectively as elephantiasis, lymphangitis, microfilaremia, and "no infection". Part I of this study (Dondero *et al.*, 1971) described the clinical, laboratory, and parasitological aspects.

Table 1 shows the skin reactions to the Sawada F.S.T. in these 4 clinical-parasitological subgroups. The number of persons with microfilaremia who reacted positively was considerably less than for those with elephantiasis. The difference between the percentages of positive reactors of these 2 subgroups is statistically significant ($chi^2 = 5.20$ for one degree of freedom, $0.025 > P > 0.01$). All the uninfected group reacted positively. Of the 4 elephantiasis patients who reacted negatively 3 had longstanding involvement, but one patient's condition was only 2 years old. There was no statistical difference ($0.2 > P > 0.1$) when the mean values for the duration of elephantiasis of the reactors and non-reactors were compared.

Microfilaremia subgroup

Table 2 presents comparisons of mean age and age range, and Table 3 of microfilarial levels, with skin test reactions in subjects with microfilaremia. Those who reacted negatively were somewhat younger than those who reacted positively. The difference between the mean age values was of border-

Table 1

Sawada Filarial Skin Test results in 4 filarial clinical-parasitological subgroups.

Subgroup	Number of subjects	Skin test positive	% positive reactors	Skin test negative	% negative reactors
Elephantiasis	34	30	88	4	12
Lymphangitis	8	5	62	3	38
Microfilaremia	18	11	61	7	39
No infection	8	8	100	0	0

Table 2

Mean age and age range in 19 microfilaremia subjects and their reactions to the Sawada Filarial Skin Test.

Skin test reaction	Number of subjects	Mean age in years ± standard error	Range (years)
Positive	12	26.1 ± 1.8	17 - 35
Negative	7	20.3 ± 2.9	13 - 31

Table 3

Mean and range of microfilarial levels (per 40 c.mm blood) in 19 microfilarial subjects and their reactions to the Sawada Filarial Skin Test.

Skin test reaction	Number of subjects	Mean level of microfilariae (per 40 c.mm) ± standard error	Range of microfilariae (per 40 c.mm)
Positive	12	110 ± 60.5	1 - 738
Negative	7	217 ± 79.8	8 - 533

Table 4

Intestinal helminthic infestations in 68 subjects given the Sawada Filarial Skin Test.

Skin test reaction	Number of subjects	*Ascaris*	Hookworm	*Strongyloides*	*Trichuris*	One parasite or more
Positive	54	36 (67%)	34 (63%)	1 (2%)	50 (92%)	53 (98%)
Negative	14	10 (72%)	7 (50%)	1 (7%)	12 (86%)	12 (86%)

line statistical significance ($0.1 > P > 0.05$). No statistical difference, however, appeared between the mean values for the microfilarial levels of the reactors and non-reactors ($P = 0.3$).

Five of the 19 persons with microfilaremia experienced or had experienced acute adenolymphangitis. Three of these were skintest negative; two skin-test positive.

Intestinal parasites

The prevalence rates for intestinal helminths were nearly the same for negative and positive reactors to the F.S.T. (Table 4). The positive and negative reactors, particularly in the microfilaremia subgroup, also both had roughly the same distribution of intestinal worms.

Eosinophilia

The mean percentage of eosinophils (± standard error) for the 54 skin-test reactors was 9.1 ± 0.8, that of the 14 non-reactors 7.4 ± 1.1 This difference, however, is not statistically significant ($P = 0.3$). There were wide ranges in the eosinophil levels in both groups (0-27% and 1-14% respectively).

DISCUSSION

These results, although from a relatively small series of patients, reflect some problems that have continually plagued studies on immunodiagnosis of filariasis.

First, about 40% of the microfilariaemic patients reacted negatively to skin tests (moreover all negative results were clear-cut

by our criteria). Desowitz *et al.*, (1966), found 17% of 164 bancroftian microfilaremics to be skin-test negative. However, of the 24 in the study who where 13 years old or less, 67% had negative reactions. Ata *et al.*, (1967) found 30% of 43 microfilaremics *(bancrofti)* skin-test negative. They also noted that many more younger than older filariasis subjects reacted negatively. Among 125 carriers of *W. bancrofti* microfilariae, with or without *Onchocerca volvulus* and *Dipetalonema perstans*, Gidel *et al.*, (1969) found about 25% who were skin-test negative. Ramachandran *et al.*, (1970) obtained almost 30% negative reactions to the F.S.T. in filariasis patients (infected with *B. malayi* and *W. bancrofti*).

A critical discussion of possible reasons why a high proportion of microfilarial carriers do not react to the skin test is beyond the scope of this report. Nevertheless, the observation has often been made, not only with the Sawada F.S.T. but also with other skin tests (Franks *et al.*, 1946; Ciferri *et al.*, 1965) and with various serological reactions (Pandit *et al.*, 1929; Minning and McFadzean, 1956; Duxbury and Sadun, 1967; Wong and Guest, 1969).

Our results reflected the finding of Desowitz *et al.*, (1966) and Ata *et al.*, (1967) that young parasite carriers frequently have negative reactions to the Sawada skin test. We did not have enough observations to allow analysis by age groups and, moreover, we studied no carrier less than 10 years old; however, the mean age of the non-reactors is lower than the mean age of the reactors at borderline statistical significance.

With these limited data we cannot comment on the relationship, if any, between microfilarial level and skin test reaction (Table 3). There seemed to be no correlation between skin-test reactions and the presence or absence of adenolymphangitis.

In our study statistically more microfilaremics than elephantiasis patients proved to be skin-test negative (Table 1). Whether this reflects biological differences between the 2 subgroups, such as lack of immunological sensitization or, contrariwise, antigen excess in some microfilaremics, can be determined only by further investigation.

If skin tests give negative results in appreciably large numbers of young and presumably more recently infected persons, the value of this method of detection when it is used alone is limited; it is obviously not reliable for individual diagnosis. While the skin test might be helpful epidemiologically in a preliminary survey for filariasis in a community, it would probably be worthless in evaluating, for example, the effectiveness of eradication measures, since the search would necessarily be for recent infections among younger members of the community.

Another problem in immunodiagnosis of filariasis is reflected in the 100% positive skin reactions in the "no infection" subgroup. Other workers have observed high rates of skin test positivity among the supposedly non-infected inhabitants in heavily endemic areas (Ata *et al.*, 1967; Ramachandran *et al.*, 1970). Part 1 of this report discusses whether our subjects might actually have been infected (Dondero *et al.*, 1971). They were almost certainly exposed to bites by infected mosquitoes. The findings of Beye *et al.*, (1956) suggest that exposure without apparent infection can lead to positive skin reactions, even after many years away from an endemic area.

Whether exposure to non-human filarial larvae is enough to immunologically sensitize an individual is not yet clear. Filarial antigens have long been recognized to be, at best, group-specific, and sensitization with one filariid, even if non-infective, might logically

allow immunological reactions with the antigens of another. The possibility of exposure to non-human filariids within our group, however, cannot now be evaluated because data are not available on zoonotic infections in the study area.

To postulate that intestinal nematode infestations led to the positive skin reactions in our no-infection subgroup would be unwarranted. As shown in Table 4, subjects with negative reactions carried almost as many and as varied intestinal infections as those with positive reactions. Ciferri *et al.*, (1965), also using a *Dirofilaria immitis* antigen, saw only negative skin reactions in intestinal helminth carriers from non-filarial regions.

Our data showed no correlation between eosinophil level and skin test reactivity. Because the immediate type skin reaction is generally mediated by reaginic antibody (IGE) and blood eosinophilia is associated with the presence of this class of antibody (Zolov and Levine, 1969), a correlation between skin reaction and eosinophil level might have been anticipated. However, the Sawada F.S.T. is highly purified and probably contains only a few antigens. None of these may correspond with the allergen or allergens ultimately responsible for the levels of eosinophilia in our subjects.

SUMMARY

Of the 68 persons examined, 60 in the study variously manifested Malayan filariasis. The Sawada Filarial Skin Test gave positive results for 88% of those with elephantiasis and for 62% of the lymphangitis and 61% of the microfilaremia patients. All 8 of those without parasitologically or clinically detectable filarial infection were skin-test positive.

Statistically fewer microfilarial carriers than elephantiasis patients were skin-test positive. The mean age of microfilaremic subjects with negative reactions was lower than the mean age of those with positive reactions at borderline statistical significance. These findings are discussed and compared with results of other studies in which the same and related antigens were used.

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