Antibody determination in the diagnosis of
*Wuchereria bancrofti* infection in man

S. DISSANAYAKE¹ & M. M. ISMAIL²

The levels of IgG and IgE antibodies reacting with somatic antigens of adult *Setaria digitata* and *Wuchereria bancrofti* microfilariae were determined in sera of 90 patients with Bancroftian filariasis and 379 non-filarial subjects. Antibodies reacting with adult antigens and with soluble microfilarial antigens were seen in both microfilaraemic and amicrofilaraemic patients. Antibodies reacting with surface antigens of *W. bancrofti* microfilariae were seen only in amicrofilaraemic subjects. IgE antibodies were detected with the adult antigen only in both microfilaraemic and amicrofilaraemic patients. The absolute levels of IgG antibodies were significantly higher than those of IgE antibodies. It is concluded that the determination of serum antibodies reacting with adult antigens is suitable for the diagnosis of both the microfilaraemic and amicrofilaraemic phases of infection, and the determination of antibody to microfilarial surface antigens is applicable in patients with clinically evident disease.

Determination of circulating antibody to filarial antigens is still the most widely used technique in the immunodiagnosis of Bancroftian filariasis. In order to develop techniques for the determination of circulating antigen, it is necessary to understand the nature of the antibody response in infection. It has been observed that antibodies to microfilarial surface antigens are not found in microfilaraemic patients, but the antigenic specificity of antibodies seen during the different phases of infection is unknown.

Adult antigens (1, 2), microfilarial antigens (3, 4), and larval antigens (5) have been used previously for the determination of serum antibodies in filariasis. We have shown that adult *Setaria digitata* contain antigens that react with serum antibodies in patients with *Wuchereria bancrofti* infection (6, 7). In the present study, we have compared the levels of serum antibodies (both IgG and IgE) reacting with the adult *S. digitata* antigen and with *W. bancrofti* microfilarial surface and soluble antigens in groups of filarial and non-filarial subjects.

**MATERIALS AND METHODS**

**Filarial subjects**

Blood samples were collected from patients attending an antifilaria clinic in Colombo, Sri Lanka, and from microfilaraemic subjects detected during night blood surveys. The clinic patients generally had symptoms such as lymphoedema, lymphangitis, elephantiasis etc., while the microfilaraemic subjects were asymptomatic. The indirect immunofluorescent antibody (IFA) test, with *W. bancrofti* microfilariae as antigen, was used for serological diagnosis. The study groups comprised 42 asymptomatic microfilaraemic subjects, 30 symptomatic microfilaraemic subjects whose sera were positive by the IFA test, and 18 elephantiasis cases with a clinical history of more than 5 years duration.

**Non-filarial subjects**

Three groups of non-filarial subjects were studied. The first group consisted of 33 subjects from the filariasis endemic area who visited the antifilaria clinic for treatment, but who had no serological or parasitological evidence of infection. This group will be described as non-filarial subjects from the endemic zone. The second group came from a non-endemic area and had no evidence of filarial infection. They were selected from about 400 subjects on the basis of having a high level of serum antibodies reacting with a somatic antigen preparation of adult *Ascaris lumbricoides*. The 44 subjects in this group will be referred to as non-filarial *Ascaris* subjects. The third group consisted of 302 non-filarial subjects from the non-endemic area, who had no evidence of *Ascaris* infection.

**Enzyme-linked immunosorbent assay (ELISA) with adult *S. digitata* antigen**

The ELISA was performed using *S. digitata* antigen SD2-4 (6), as described previously (7). Polystyrene

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Counterimmunoelectrophoresis with ELISA with of antigen was as antigen was 20 ml of Tween 20 per litre (PBS ICS Tween). Aliquots of 25 μl of the test serum in 1 ml of PBS ICS Tween were added in duplicate to the antigen-coated tubes, which were then incubated at 37 °C for 1 hour, and washed with PBS ICS Tween. A rabbit antiserum to human IgG Fc/IgE Fc (made specific by absorption against CNBr-Sepharose 4B insolubilized human Fab) was then added to the tubes, at a predetermined concentration of 1 μg of specific antibody per tube. After incubation for 1 hour at 37 °C, the tubes were washed and a goat antibody to rabbit Ig conjugated with horse-radish peroxidase* (absorbed against human and cattle immunoglobulins) was added, and the tubes again incubated and washed. O-phenylene diamine in 3% H₂O₂ was used as substrate, and the incubation was carried out in darkness for 20 minutes at 37 °C. The colour reaction was measured by reading the optical density at 490 nm in an SP 500 spectrophotometer.

**ELISA with soluble antigens of W. bancrofti microfilariae**

W. bancrofti microfilariae (mf) were isolated from the blood of microfilaraemic subjects. The washed mf were homogenized in PBS containing 10 ml of Tween 20 per litre, the soluble material remaining after centrifugation (10 000 g for 30 min at 4 °C) was dialysed exhaustively against PBS. This microfilarial antigen was used in the ELISA in exactly the same way as was S. digitata antigen, at a predetermined concentration of 1 μg per tube.

**IFA test**

The IFA test was performed according to the method of Jayawardene & Wijayaratnam (3). Intact, washed mf were incubated with serially diluted test sera for 2 hours at 37 °C. An appropriately diluted fluorescein-conjugated sheep antibody to human Ig₃ was then added, the mixture was incubated at 37 °C, washed, and the fluorescence read. Sera giving a well defined cuticular fluorescence at a dilution of 1:16 were considered positive.

**Counterimmunoelectrophoresis with A. lumbricoides antigen**

Adult A. lumbricoides worms were collected from the stools of infected children treated with piperazine citrate. The worms were washed in PBS and homogen-ized in PBS containing 10 ml of Tween 20 per litre. The soluble material was obtained by centrifugation (10 000 g for 30 min at 4 °C) and dialysed exhaustively against PBS. This material was then used in counterimmunoelectrophoresis to detect serum antibodies to Ascaris antigens. Counterimmunoelectrophoresis was performed in Veronal buffer (pH 8.6) containing 15 ml of agarose B₃ per litre. Sera were tested at a dilution of 1:5 and the A. lumbricoides antigen was used at a concentration of 100 mg/litre. Aliquots of 25 μl of the antigen solution with 50 μl of the 1:5 diluted test sera were subjected to electrophoresis at 50 V/cm for 2 hours at room temperature. The gel plates were then washed in PBS for 2 days, dried, and stained with Coomassie brilliant blue. Sera giving well-defined precipitation lines were considered to have high levels of antibodies to Ascaris antigens.

**RESULTS**

**Interpretation of ELISA titres**

With antigen SD2-4, the mean ELISA reading for the 302 non-filarial controls was 0.07 ± 0.06, and that for the non-filarial Ascaris subjects was 0.14 ± 0.07. Based on these figures, an ELISA reading greater than 0.2 was taken as significantly positive. With the W. bancrofti microfilarial soluble antigen, the mean reading for the non-filarial Ascaris group was 0.17 ± 0.05 and therefore the cut-off point was taken as 0.22. In the case of the IFA test, an antibody titre of 1:16 was taken as positive.

**Prevalence of antibodies**

**Microfilaraemic subjects** were all negative by the IFA test. However, all of them had serum antibodies reacting with the adult S. digitata antigen SD2-4, and 67% also had antibodies to the soluble microfilarial antigens, as determined in the ELISA (Table 1). In general, the absolute levels of antibodies reacting with the adult antigen were much higher than those reacting with the microfilarial antigen, and there was no correlation between the two levels (Table 2).

**Amicrofilaraemic subjects** were all positive in the IFA test. In addition, 77% had antibodies reacting with the SD2-4 antigen and 75% were positive in the ELISA performed with the soluble microfilarial antigen (Table 1). In general, the levels of anti-adult antigen antibody found were lower than those seen in the microfilaraemic subjects. There was a significant correlation between the levels of antibodies reacting with the adult antigen and with the soluble microfilarial antigen (Table 2).

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* From Cappel Laboratories Inc., Cochraneville, PA 19330, USA.
* From Pharmacia Fine Chemicals AB, Box 175, Uppsala, Sweden.
Table 1. Number of subjects giving positive results in the ELISA and IFA tests

<table>
<thead>
<tr>
<th>Subject group</th>
<th>No. tested</th>
<th>ELISA (IgG antibody)</th>
<th>IFA (Total antibody)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Antigen SD2-4</td>
<td>Mf soluble antigen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.  %</td>
<td>No.  %</td>
</tr>
<tr>
<td>Microfilaraemic, asymptomatic</td>
<td>42</td>
<td>42 100</td>
<td>28 67</td>
</tr>
<tr>
<td>Amicrofilaraemic, symptomatic, including elephantiasis</td>
<td>48</td>
<td>37 77</td>
<td>36 75</td>
</tr>
<tr>
<td>Non-filarial, Ascaris-positive</td>
<td>44</td>
<td>4 9</td>
<td>8 18</td>
</tr>
<tr>
<td>Non-filarial, Ascaris-negative</td>
<td>302</td>
<td>4 1</td>
<td>—</td>
</tr>
</tbody>
</table>

Levels of IgG and IgE antibodies

IgG and IgE antibodies reacting with the adult antigen were present in both microfilaraemic and amicrofilaraemic patients. IgE antibodies were found less frequently than were IgG antibodies, and the levels were usually lower. IgE antibodies to the soluble microfilarial antigen could not be detected in any of the filarial patients studied. In all filarial groups, there was a significant correlation between IgE and IgG antibody levels (Table 2).

Table 2. Comparison of IgG and IgE antibody levels (mean ± SD) as determined in the ELISA. Figures in parentheses give percentage of subjects with a positive result

<table>
<thead>
<tr>
<th>Subject group</th>
<th>ELISA titre</th>
<th>Ratio of IgG antibodies to SD2-4 and mf</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD2-4/IgG</td>
<td>Mf/IgG</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD2-4/IgE</td>
<td>(1)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2)c</td>
</tr>
<tr>
<td>Microfilaraemic</td>
<td>1.1 ± 0.45</td>
<td>0.26 ± 0.09 (67)</td>
<td>0.418c</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td></td>
<td>0.074</td>
</tr>
<tr>
<td>Amicrofilaraemic</td>
<td>0.5 ± 0.3</td>
<td>0.25 ± 0.16 (53)</td>
<td>2.38 ± 2.41</td>
</tr>
<tr>
<td></td>
<td>(85)</td>
<td></td>
<td>0.56c</td>
</tr>
<tr>
<td>Elephantiasis</td>
<td>0.4 ± 0.35</td>
<td>0.24 ± 0.12 (56)</td>
<td>0.94 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>(61)</td>
<td></td>
<td>0.49c</td>
</tr>
<tr>
<td>Non-filarial, Ascaris-positive</td>
<td>0.14 ± 0.07</td>
<td>0.17 ± 0.05 (18)</td>
<td>0.8 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Non-filarial IFA-negative from endemic area</td>
<td>0.33 ± 0.22</td>
<td>0.22 ± 0.13 (30)</td>
<td>1.76 ± 1.55</td>
</tr>
<tr>
<td></td>
<td>(63)</td>
<td></td>
<td>0.67c</td>
</tr>
</tbody>
</table>

a Correlation between levels of IgG and IgE antibodies to SD2-4.

b Correlation between levels of IgG antibody to SD2-4 and microfilariae.
c Significant correlation.

DISCUSSION

The present study has shown that the IFA test, performed with intact *W. bancrofti* microfilarial surface antigens, is of sufficient specificity and sensitivity for the serological diagnosis of amicrofilaraemic symptomatic filarial infection. On the other hand, the ELISA detected antibodies to adult SD2-4 antigen in both microfilaraemic and amicrofilaraemic subjects, and was particularly appropriate...
for microfilaraemic cases, who were found to have very high antibody levels. Therefore, the combined use of microfilarial surface antigens and adult antigens should make it possible to detect all phases of infection.

The presence in microfilaraemic patients of antibodies reacting with the adult antigens is of considerable diagnostic importance. At present, microfilaraemic patients can be detected only by parasitological examination, and in the case of *W. bancrofti*, diagnosis is difficult because of the periodicity of the microfilariae. Also, when the level of microfilaraemia is low, concentration techniques have to be used, which limit the applicability of the method in epidemiological studies. The results presented in this paper clearly demonstrate that the use of adult *S. digitata* antigens in the ELISA provides an alternative method for the diagnosis of asymptomatic microfilaraemic patients.

Our results are in agreement with the findings of Grove & Davies (8), who demonstrated the presence of antibody to *Brugia malayi* adult antigens in all patients with Brugian and Bancroftian filariasis, and the presence of antibody to microfilarial antigens in patients with chronic lymphatic obstruction. It is therefore concluded that the detection of antibodies to adult antigens is a useful indicator of infection, while the presence of antibodies to microfilarial antigens (particularly surface antigens) is related to the clinical disease.

A high prevalence of IgG antibodies in patients with Bancroftian filariasis has been seen previously (S. Dissanayake, unpublished data, 1980). It has also been observed that the level of IgG antibodies gave better discrimination than did those of IgM or total antibody. These observations are in general agreement with those of Bryceson et al. (9).

The ratio of the levels of IgG antibodies reacting with adult antigens and soluble antigens of microfilaria was highest in the microfilaraemic patients and lowest in the chronic elephantiasis cases (Table 2). This suggests that, in the microfilaraemic subjects, the predominant antibody response is to adult antigens, a hitherto unpublished observation. Furthermore, in the amicrofilaraemic symptomatic patients, the level of IgG antibody to adult antigens was correlated with the level of antibody to soluble microfilarial antigens, but there was no such correlation in the microfilaraemic subjects.

Contrary to expectations, IgE antibodies were detected only with the adult antigen and the levels were too low for any meaningful interpretation. It may be that the ELISA was not sensitive enough for the determination of IgE antibodies and we are presently comparing the ELISA with radiimmunoassay techniques. It is also possible that not all antigens are suitable for IgE antibody determination, and this question is also under investigation.

The group of supposed non-filarial subjects from the filariasis-endemic area need special consideration. Although they were considered as non-filarial on the basis of history and IFA results, the diagnosis may have been incorrect. In the ELISA with the adult antigen, about 63% of them had significant levels of antifilarial antibodies. It is possible that these positive subjects had just passed the microfilaraemic phase, but had not yet developed antimicrofilarial antibodies. Fifteen of these ELISA-positive subjects were tested again after 3 months and it was found that one patient had developed antimicrofilarial antibodies, as shown by the IFA test. At this stage we do not rule out the possibility that this resulted from exposure to cross-reacting animal filariae. This group of subjects is still being investigated to clarify their infective status.

ACKNOWLEDGEMENTS

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RÉSUMÉ

**DÉTERMINATION DES ANTICORPS POUR LE DIAGNOSTIC DE L'INFECTION A WUCHERERIA BANCROFTI CHEZ L'HOMME**

On a déterminé par la méthode ELISA et par immuno-fluorescence indirecte, dans le sérum de malades atteints de filariose à *Wuchereria bancrofti*, les taux des anticorps IgG et IgE réagissant avec un antigène somatique partiellement purifié de *Setaria digitata* adulte, avec des antigènes solubles de microfilaires de *W. bancrofti* et des antigènes superficiels de ces mêmes microfilaires. On a étudié 42 malades microfilarémiques, 30 sujets symptomatiques amicrofilarémiques, 18 cas d'éléphantiase et 379 sujets indemnes de filariose. Les anticorps réagissant avec les antigènes de surface des microfilaires de *W. bancrofti* ont été observés uniquement chez les malades amicrofilarémiques.
En revanche, les anticorps réagissant avec les antigènes solubles des microfilaires de *W. bancrofti* et avec des antigènes de *S. digitata* adultes étaient présents chez les malades microfilaremiques comme chez les sujets amicrofilaremiques. Chez les premiers, les titres des anticorps réagissant avec les antigènes adultes étaient notablement élevés. Les anticorps IgE n'ont été décelés qu'avec l'antigène de vers adultes et ils étaient présents chez les sujets microfilaremiques comme chez les sujets amicrofilaremiques. Les titres absolus d'anticorps IgG étaient bien plus élevés que ceux des anticorps IgE. On en conclut que la détermination des anticorps sériques réagissant avec les antigènes d'adultes se prête au diagnostic de la phase microfilaremique comme de la phase amicrofilaremique de l'infection et que la détermination de l'anticorps contre les antigènes de surface des microfilaires est utile chez les malades présentant une maladie cliniquement patente.

**REFERENCES**