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Diagnostic utility of monoclonal antibodies raised against microfilarial excretory-secretory antigens in bancroftian filariasis

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Abstract. Two monoclonal antibodies *Wuchereria bancrofti* E 33 and *Wuchereria bancrofli* E 34 raised against *Wuchereria bancrofti* microfilarial excretory-secretory antigens were studied for their diagnostic utility. *Wuchereria bancrofti* E 34 monoclonal antibody was found to be relatively specific and sensitive in detection of circulating filarial antigen. When *Wuchereria bancrofti* E 34 monoclonal antibody was used alongwith immunoglobulin G fraction of human filarial serum immunoglobulins in double antibody sandwich enzyme linked immunosorbent assay. 68% of microfilaraemic sera (26 out of 38). 12% of clinical filarial sera (3 out of 25), 13% endemic normal sera (2 out of 15) and none of the 20 non-endemic normal sera showed the presence of filarial antigen. The filarial antigen detected by *Wuchereria bancrofti* E 34 monoclonal antibody in double antibody sandwich enzyme linked immunosorbent assay is possibly associated with the active stage (microfilaraemia) of infection.

Keywords. *Wuchereria bancrofti;* bancroftian filariasis; excretory-secretory antigens; monoclonal antibodies; enzyme linked immunosorbent assay; circulating filarial antigen

Introduction

In immunoparasitology, recent studies have attempted to develop diagnostic methods based on the detection of parasite antigen. Compared to conventional methods based on antibody detection, these tests are more informative in detecting the active infection and in assessment of effectiveness of chemotherapy. Filarial antigen has been demonstrated in the sera and urine samples of infected humans and animals (Dasgupta and Shukal Bala, 1978; Desowitz and Una, 1976; Dissanayake *et al.,* 1982; Hamilton *et al.,* 1984; Kaliraj *et al.,* 1979; Reddy *et al.,* 1984a) using antisera raised against heterologous and homologous filarial antigens. The utility of immunoglobulin fraction of clinical filarial serum was explored for the detection of circulating filarial antigen (Kaliraj *et al.,* 1981; Reddy *et al,* 1984b). Hybridoma derived monoclonal antibody is another reagent with great potential

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Abbreviations used: IRMA, Immunoradiometric assay: *Wb* mf ES Ag, *Wuchereria bancrofti* micro-filarial excretory-secretory antigens; mf, microfileraemia; FSIgG, immunoglobulin G fraction of human filarial serum immunoglobulins; ELISA, enzyme linked immunosorbent assay; PVC, polyvinylchloride; BSA, bovine serum albumin; PBS/T, phosphate buffered saline containing tween 20.

for detection of specific antigen of interest. Dissanayake *et al.* (1984) and Forsyth *et al.* (1985) have explored the utility of one of the monoclonal antibodies *(Gib* 13) raised against *Onchocerca gibsoni* in detection of filarial antigen in sera and urine samples using immunoradiometric assay (IRMA). This communication reports the detection of circulating antigen in sera of filarial patients employing the monoclonalantibodies raised against *Wuchereria bancrofti* microfilarial excretory-secretory antigens (*Wb* mf ES Ag).

Materials and methods

Sera

Human sera (98 samples) belonging to different groups *viz.,* normal subjects from endemic and non-endemic regions and filarial (microfilaraemia and clinical filariasis) were screened in this study. Filarial blood samples were collected from Sevagram and surrounding villages which are endemic for nocturnally periodic form of *W. bancrofti.* Endemic normal samples were from healthy individuals living in Sevagram and its surrounding villages and having neither microfileraemia (mf) in their blood nor any clinical symptoms. The presence or absence of mf was confirmed by night blood (wet smear) examination. Non-endemic normal blood samples were collected from students of this Institute coming from places like Chandigarh, Kashmir etc. where there is no filariasis. Sera were separated from blood samples and stored at -20°C after addition of sodium azide as preservative.

Wb mf ES Ag

Wb mf Es Ag was prepared as described by Kharat *et al.* (1982) and concentrated 200 fold by ultrafiltration and freezedrying.

Preparation of monoclonal antibodies against Wb mf ES Ag

Monoclonal antibodies were raised against *Wb* mf ES Ag as described earlier (Reddy *et al.,* 1984c). The monoclonal antibodies *Wb* E 33 and *Wb* E 34 which were relatively more reactive with *Wb* mf ES Ag were selected for evaluating their diagnostic utility in filariasis. The monoclonal antibodies were concentrated from culture supernatants by 50% saturation with ammonium sulphate. The conjugation of concentrated monoclonal antibody (20 mg) and 1000 units of enzyme penicilli nase (Sigma Chemical Co., USA) was achieved by the single step method of Avrameas (1969) using glutaraldehyde.

Immunoglobulin G fraction of human filarial serum immunoglobulins

Immunoglobulin G fraction of human filarial serum immunoglobulins (FSIgG) was prepared from pooled clinical filarial sera showing anti-microfilarial soluble antigen antibody by ammonium sulphate precipitation followed by DEAE-cellulose (Whatman DE 52) chromatography as described by Reddy *et al.* (1984b). Twenty mg of FSIgG was conjugated to 1000 units of enzyme penicillinase (Sigma Chemical Co., USA) by the method of Avrameas (1969).

Sandwich enzyme linked immunosorbent assay

Sandwich enzyme linked immunosorbent assay (ELISA) was carried out as described by Reddy *et al.* (1984b). The optimal dilutions of all the reagents were determined by chequer board titration. The wells of the Polyvinylchloride (PVC) microtitre plates (Dynatech Laboratory Inc., Singapore) were sensitized with optimal concentration of monoclonal antibody (10 μg/ml) in carbonate buffer (0·06 M, pH 9·6) at 37°C for 3 h and then drained. The plate was further incubated with 200 μI of 3% (w/v) bovine serum albumin (BSA) in carbonate buffer at 37°C for 2 h. After washing the plate 5 times with phosphate buffer saline (0·01 M, pH 7·2) containing 0·05% (v/v) tween 20 (PBS/T), 100 ,µl of optimally diluted test sera (1:300) in PBS/T were added and incubated at 37°C for 3 h. After washing the plate again, 100 μI of penicillinase conjugate of the same monoclonal antibody (used for sensitizing the plate) was added and incubated at 37°C for 3 h. Following the final washing, the immune reaction was observed by incubating the wells with 100 μI of substrate solution at 37°C for 30 min. The substrate consisted of soluble starch (150 mg) in 27·5 ml of 0·25 M sodium phosphate buffer (pH 7·2) containing 10·6 mg penicillin V, and 65 μl of 0·08 M iodine in 3·2 M potassium iodide solution. The substrate was prepared fresh before use.

The enzyme reaction was terminated by adding 25 μl of 5 N HCl, and the results were evaluated visually. The complete decolorization or decolorization with a slight tinge of substrate colour denoted a positive reaction, while negative reaction was confirmed by persistance of blue colour.

Similarly sandwich ELISA was also done using FSIgG and FSIgG penicillinase combination.

Double antibody sandwich ELISA

Double antibody sandwich ELISA using FSIgG and *Wb* E 34 monoclonal antibody penicillinase conjugates was done with modification in incubation steps as described for two site IRMA by Dissanayake *et al.* (1984) and Forsyth *et al.* (1985).

The wells in the PVC microtitre plate were incubated with 100 μl of optimal concentration of FSIgG (25 μg/ml) in carbonate buffer (0·06 M, pH 9·6) at 4°C for overnight and then drained. The wells were further incubated with 200 μl of 3% BSA in carbonate buffer at 37°C for 2 h, followed by washing with PBS/T. While the BSA incubation was going on, optimally diluted test sera (1:150) in PBS/T were pre-incubated with equal volumes of optimally diluted *Wb* E 34 monoclonal antibody penicillinase conjugate (1:200) in PBS/T in small plastic vials at 37°C for 3 h. The reaction mixture (100 ,µl) was then transferred to FSIgG coated wells and incubated at 37°C for 3 h followed by washing with PBS/T. The immune reaction was then observed by incubating with 100 μl of substrate and the results were evaluated as described above for sandwich ELISA.

Results

A total number of 64 sera belonging to different groups were screened in sandwich ELISA using the same monoclonal antibody on either side and the results are summarized in table 1. When *Wb* E 34 monoclonal antibody was used, none of the

Table 1. Detection of circulating filarial antigen using the same monoclonal antibody on both the sides in sandwich ELISA.

Sera	No. exam.	No. showing positive reaction* using the monoclonal antibody	
		Wb E 33	*Wb* E 34
Nonendemic normal (mf — ve)	12	3	0
Endemic normal (mf — ve)	20	2	2
Microfilaraemia (mf + ve)	20	10	8
Clinical filariasis (mf — ye)	12	4	

* Sera showing positive reaction for filarial antigen at a dilution of 1:300.

12 nonendemic normal sera, only 2 out of 20 endemic normal sera (10%), 8 out of 20 microfilaraemic sera (40%) and 2 out of 12 clinical filarial sera (17%) were positive for filarial antigen. Though the *Wb* E 33 monoclonal antibody detected ilarial antigen in higher number of microfilaraemic (10 out of 20) and clinical filarial sera (4 out of 12), it showed nonspecific reactions with .3 out of 12 nonendemic normal sera. *Wb* E 34 monoclonal antibody was used together with FSIgG in double antibody sandwich ELISA and a total of 98 sera were analysed (table 2). Filarial antigen was detected in none of the 20 nonendemic normal sera, 2 out of 15 endemic normal sera (13%), 26 out of 38 microfilaraemic sera (68%) and 3 out of 25 clinical filarial sera (12%). The same 98 sera were also analysed in

Table 2. Detection of circulating filarial antigen in double antibody sandwich ELISA and FSIgG sandwich ELISA.

Sera	No. exam.	Sera showing positive reaction*			
		Double antibody sandwich ELISA using FSIgG and *Wb* E 34 monoclonal antibody		FSIgG sandwich ELISA	
		No.	Per cent	No.	Per cent
Nonendemic normal (mf – ve)	20	0	0	0	0
Endemic normal (mf – ve)	15	2	13	3	20
Microfilaraemia (mf + ve)	38	26	68	28	74
Clinical filariasis (mf – ve)	25	3	12	13	52

*Sera showing positive reaction for filarial antigen at a dilution of 1:150 in double antibody sandwich ELISA and 1:300 in FSIgG sandwich ELISA.

sandwich ELISA using FSIgG and FSIgG penicillinase and the results are compared with double antibody sandwich ELISA (table 2). In both the assay systems, none of the 20 non-endemic normal sera were positive for filarial antigen. While the percentage positivity in microfilaraemic and endemic normal sera was more or less same in both the assay systems, only 12% clinical filarial sera were positive in double antibody sandwich ELISA, compared to 52% positivity in FSIgG sandwich ELISA.

Discussion

Out of the 2 monoclonal antibodies (*Wb* E 33 and *Wb* E 34) which were more reactive to *Wb* mf ES Ag, when evaluated in sandwich ELISA using the same monoclonal antibody on either side, *Wb* E 34 was found to be relatively specific than *Wb* E 33 and showed no cross reactions with any of the 20 nonendemic normal sera screened. However, using *Wb* E 34 monoclonal antibody, the sensitivity was found to be limited as only 40% microfilaraemic sera and 17% clinical filarial sera were positive for filarial antigen. Earlier studies from our laboratory (Kaliraj *et al.* , 1981; Reddy *et al.,* 1984b) have shown the FSIgG, a polyclonal antibody isolated from clinical filarial serum to be useful in detection of circulating filarial antigen. Hence *Wb* E 34 monoclonal antibody was used together with FSIgG in double antibody sandwich ELISA. With the inclusion of FSIgG, the number of microfilaraemic sera positive for filarial antigen was considerably increased from 40% in sandwich ELISA to 68% in double antibody sandwich ELISA. However, still in about one third of microfilaraemic sera, the circulating filarial antigen could not be detected, as is the case in most of the antigen assays reported in filariasis (Ouaissi *et al.,* 1981; Desmoutis *et al.,* 1983; Reddy *et al.,* 1984b). This can be explained by the expected interference of the excess of antibodies which keep the antigen in immunecomplex form saturating the antigen sites and possibly leading to its clearance from circulation. Filarial antigen was detected in 13% of endemic normal persons in this assay system. These people may be having prepatent or asymptomatic infection. In contrast, about 50% of this group showed filarial antigen when *Gib* 13 monoclonal antibody raised against heterologous filarial antigen *(O.gibsoni)* was used by Dissanayake *et al.* (1984) and Forsyth *et al.* (1985). The results of FSIgG sandwich ELISA (table 2) agree with our previous observations using the same assay system (Reddy *et al.,* 1984b). However, compared to the results of FSIgG sandwich ELISA, the higher percentage of positivity in microfilaraemic sera (68%) than in clinical filarial sera (12%) in double antibody sandwich ELISA suggests that the antigen recognized by *Wb* E 34 monoclonal antibody is possibly associated with the active stage of infection. Thus the double antibody sandwich ELISA, using the polyclonal antibody FSIgG and *Wb* mf ES Ag monoclonal antibody *Wb* E 34, may be useful in detecting the circulating antigen in active infection.

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