Antibody Analysis in Human Filarial Sera by ELISA Using Wuchereria bancrofti Microfilariae Culture Antigen*

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A total of 120 human sera were screened in enzyme linked immunosorbent assay for detection of filarial antibodies using W. bancrofti microfilariae culture antigen. As little as 0.35 ng antigen protein per well was found to be sufficient in detection of filarial antibody. Four out of the 30 endemic normal, 28 out of the 30 microfilaraemia, all the 30 clinical filarial and none of the 30 non endemic normal sera showed the presence of filarial antibody (1:320). The identification of filarial antibody was further studied using class specific anti-immunoglobulin conjugates which revealed the presence of IgM antibody in microfilaraemia and IgG antibody in clinical filarial sera whereas some of the sera contained both IgM and IgG antibodies.

The diagnostic utility of exoantigens in various parasitic diseases such as chagas' disease, schistosomiasis, toxocariasis, and filariasis employs various immunological tests showed that the exoantigen collected from the living parasites could be used in immunodiagnostic test because of its high specificity and sensitivity. Toxocara larval secretions and excretions collected from in vitro culture were used as antigen in passive haemagglutination and soluble antigen fluorescent antibody tests for the diagnosis of visceral larva migrans in experimental animals and man. The enzyme immunoassay was adapted to toxocariasis by using Toxocara canis secretory antigen obtained from in vitro culture. The exoantigen obtained from culture fluid of Wuchereria bancrofti mf was used in indirect haemagglutination for the diagnosis of bancroftian filariasis. This study reports the detection and identification of filarial antibody against Wuchereria bancrofti microfilariae culture fluid antigen by enzyme linked immunosorbent assay.

Materials and Methods

Culture antigen—W. bancrofti microfilariae were separated from microfilaraemia blood samples by nuleopore membrane filtration and maintained for 15 days in Medium 199 (3-4 thousand mf/ml of medium) supplemented with organic acids and sugars of Grace’s medium. The medium was changed every 24 hr. The culture fluid (120 ml) was centrifuged at 13,000 g for 15 min and the supernatant was stored at -20°C until use.

This experiment was repeated thrice. The protein in the culture fluid (140-220 μg/ml) was estimated by Lowry’s method.

Sera—A total of 120 sera belonging to different groups namely healthy normals (endemic and nonendemic, having no parasitic infection), filarial (microfilaraemia and clinical filarial cases with clinical manifestations such as elephantiasis and hydrocele) were screened in this study. Filarial blood samples were collected from Sevagram and its surrounding villages endemic for filariasis. Nonendemic sera were collected from Chandigarh and other regions in India where there is no filariasis. Microfilaraemia was confirmed by blood film method.

Enzyme linked immunosorbent assay—Conjugation of anti-human immunoglobulins (anti-human immunoglobulin IgG + M+ A and individual anti-human IgG, IgM and IgA were obtained from Immunodiagnostics, New Delhi) and penicillinase (purified penicillinase EC 3.5.2.6, specific activity 60,000 units/mg protein was kindly supplied by Dr U Joshi, Institute for Research in Reproduction, Bombay) was achieved by the method of Avrameas using glutaraldehyde.

Substrate in ELISA consisted of soluble starch (150 mg) in 27.5 ml of sodium phosphate buffer (0.2 M, pH 7.0) containing 10.64 mg of penicillin ‘V’ (Hindustan Antibiotics, Pimpri, Pune) and 65 μl of 0.08 M iodine in 3.2 M potassium iodide solution. The substrate was prepared fresh before use.

The procedure followed for micro-ELISA was that of Walls et al., excepting for a modification that 3% bovine serum albumin (Sigma) was used to avoid nonspecific reaction. The test was carried out in rigid
polystyrene microtitre U plates (Cooke engineering Co., Dynatech) using 100 μl volumes of optimum diluted culture antigen, serum, penicillinase labelled conjugate and substrate. The optimum working dilutions in each case were determined by checker board titration. Each serum sample was examined in duplicate with suitable control.

Diluted culture antigen (3.5 ng/ml) in carbonate buffer (0.06 M, pH 9.6) was dispensed into each well of microtitre plate and the plate was incubated at 37°C for 3 hr and stored at 4°C until use. The antigen coated plate was incubated for 2 hr with 200 μl of 3% BSA in carbonate buffer (0.06 M, pH 9.6). The plate was then washed 5 times with PBS (pH 7.2, 0.01 M) containing 0.05% Tween 20 (PBS/T) by flooding it and then draining, inverting and vigorously shaking to remove excess of antigen, each wash lasting about 5 min. The plate was further incubated with test serum (1:320) diluted in PBS/T (pH 7.2, 0.01 M) for 3 hr at 37°C or overnight at 4°C. The plate was then washed as before and was incubated for 1 hr with 3% BSA in PBS/T, washed and incubated for 3 hr with penicillinase conjugate with anti-IgG + M + A (1:1600) in PBS/T. After a thorough washing, 100 μl of substrate was added and the plate was allowed to remain for 30 min at room temp. (30°C). The reaction was then terminated by adding 25 μl of 5 N HCl and the results were evaluated visually. The disappearance of the color denoted the positive reaction while negative reaction was confirmed by the persistance of blue color. The end titre point was the last serum dilution showing 3+ decolourization (decolorisation with slight tinge of substrate color) of the substrate mixture.

Additional studies were carried out with penicillinase labelled with anti-human IgG (1:800), IgM (1:400) or IgA (1:200) conjugates individually for detection of class specific antibody in microfilaraemia and clinical filarial sera.

Results

A total number of 120 human sera belonging to different groups were screened for filarial antibody by ELISA using penicillinase conjugate with anti-IgG + M + A and the results are summarised in Table 1. Three out of 30 nonendemic normal sera showed positive reaction at 1:160 and hence 1:320 titre was considered as +ve reaction for filarial antibody when culture antigen was used in ELISA. Four out of 30 endemic normal, (people living in filaria endemic area but do not harbour filaria parasite), 28 out of 30 microfilaraemia (mf + ve) sera, all the 30 clinical filarial (with different clinical manifestations such as elephantiasis of leg, hydrocele, lymph varix and filarial edema) and none of the nonendemic normal sera (healthy individuals living in nonendemic area and having no

| Table 1—Analysis of Human Filarial Sera by ELISA Using Microfilaria Culture Antigen |
|----------------|-----------|------------|-------------|
| Sera                        | No. Exam | No. showing +ve reaction | % of +ve reaction |
| Non endemic normal (mf -ve) | 30        | 0              | 0            |
| Endemic normal (mf -ve)     | 30        | 4              | 13           |
| Microfilariaemia (mf + ve)  | 30        | 28             | 93           |
| Clinical filariasis (mf + ve)| 30        | 30             | 100          |

*Serum showing an antibody titre of 1:320 when penicillinase conjugate with anti-IgG + M + A was used.

| Table 2—Detection of IgG, IgM and IgA Antibodies in Human Filarial Sera by ELISA to Microfilaria Culture Antigen |
|----------------|-----------|-------------|
| Sera                        | No. Exam | No. showing +ve reaction* |
| Microfilariaemia (mf + ve)  | 28        | 11           | 10 | 7 |
| Clinical filariasis (mf - ve)| 30        | 14           | 16 |

*Serum showing an antibody titre of 1:320 when penicillinase conjugate with respective anti-IgG, anti IgM and anti IgA was used.

parasite infection) showed positive reaction while employing culture antigen in ELISA. Table 2 shows the detection of IgG, IgM and IgA antibodies in human filarial sera by ELISA when penicillinase conjugate labelled individually with anti-human IgG, IgM or IgA was used. Out of the 28 positive microfilaraemia sera examined, 10 sera showed the presence of IgG and IgM antibodies, 7 sera showed IgA and IgM antibodies and 11 sera showed only IgM antibody. Similarly, out of 30 clinical filarial sera, 16 sera showed the presence of IgG and IgM antibodies while 14 sera showed only IgG antibody. The distribution of antibody titre in filarial sera is shown in Table 3. Serum samples from microfilariaemia (11) showing only IgM antibody and clinical filariasis (12) showing only IgG antibody were analysed to get the end titre of specific antibodies. Reciprocal of IgM antibody titre in microfilariaemia varied from 1280-20 million whereas that of IgG antibody in clinical filariasis from 5120-5 million.

Discussion

The enzyme linked immunosorbent assay using microfilariae culture antigen found to be highly sensitive and as little as 0.35 ng antigen protein per well was found to be sufficient in detection of filarial antibody compared to our earlier study with soluble microfilarial antigen (10 μg/well) or fractionated
antigen\textsuperscript{11} (0.1 \mu g/well) (in communication). Soluble \textit{W. bancrofti} mf antigen in ELISA showed a reciprocal antibody titre of 2560 in filarial sera\textsuperscript{10} whereas in the present study with culture antigen, reciprocal titre of IgM antibody in microfilaraemia varied from 1280 to 20 million and IgG antibody in clinical filarial sera varied from 5120-5 million. This does confirm the high sensitivity of culture antigen in detection of antibody. Similar observation regarding the high ELISA antibody titre (1:1,000000) was made in clinical toxocariasis with the culture antigen\textsuperscript{a}. In the present study, IgM antibody was found to be present in all microfilaraemia sera and some contained IgG as well. Similarly all clinical filarial sera showed the presence of IgG antibody and some of them also had IgM antibody. The development of IgM and IgG antibodies during larval infection of \textit{Litomosoides carinii} in cotton rats was studied by Tanaka \textit{et al.}\textsuperscript{12} They observed that IgM antibody in early infection was replaced by IgG antibody in late infection. To study whether there is similar correlation if any between the IgM and IgG levels in active and chronic infection, 10 samples each from microfilaraemia and clinical filarial sera showing IgM as well as IgG antibodies were analyzed for distribution of antibody titre. The reciprocal antibody titre of IgM and IgG antibodies in microfilaraemia sera ranged from 320 to 20,480 whereas in clinical filariasis, from 1280 to 20 million. The level of specific antibody (IgM or IgG) titre did not show any correlation either between microfilaraemia and clinical filariosis or with microfilariae density (20-120 mf/mm\textsuperscript{3}) or clinical status of the filarial patients. However this study does reveal that the culture antigen will be highly sensitive compared to somatic antigen in detection of filarial antibody. Further fractionation of culture antigen in detection of class specific antibody may be helpful in detection of active infection.

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