A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis

S. J. More, D. B. Copeman

Graduate School of Tropical Veterinary Science and Agriculture, James Cook University of North Queensland, Townsville, Australia

Abstract

A monoclonal antibody, Og4C3, directed against antigens of *Onchocerca gibsoni* (but not phosphorylcholine) has been used in a sandwich ELISA to detect a circulating antigen of *Wuchereria bancrofti* in human serum. The interfering effect of host antibody was reduced by first boiling one part of serum for 5 min in the presence of three parts of 0.1 M Na_2EDTA, pH 4.0. A total of 119 sera from individuals and 8 pooled sera from clinically and/or parasitologically defined cases of filariasis, plus 8 individual and 1 pooled endemic control sera, all from the filariasis serum bank of the World Health Organisation, as well as 20 non-endemic control sera, were screened with the assay. Circulating antigen was detected in serum from people infected with *W. bancrofti* but not *Brugia malayi, B. timori, O. volvulus* or *Loa loa*, and not in endemic or non-endemic controls. Of the 68 sera from *W. bancrofti*-infected subjects, 55/55 parasitologically confirmed and 12/13 clinically confirmed but amicrofilaraemic cases reacted in the assay. A weak but significant correlation ($r^2 = 0.4016$) was found between numbers of microfilariae in blood and detectable levels of circulating antigen from patients with bancroftian filariasis.

Introduction

The Tropical Disease Research Programme, within the World Health Organisation, has recently re-emphasised the urgent need for tests able to diagnose prepatent, patent and repeat infection with lymphatic filariasis (Anon, 1989). Evidence that such tests may be feasible has been provided by a number of researchers (Lal et al., 1987; Weil and Liftis, 1987; Weil et al., 1987; Zheng et al., 1987; Santhanam et al., 1989) who demonstrated the detection of circulating filarial antigens with monoclonal antibodies in enzyme-linked immunosorbent assays (ELISAs).

This paper describes an antigen detection ELISA for *Wuchereria bancrofti* which uses a monoclonal antibody selected against non-phosphorylcholine antigens of *Onchocerca gibsoni*, and the results obtained from screening a bank of sera from well-defined cases of human filariasis.

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Materials and methods

Preparation of antigen from Onchocerca gibsoni

Male *O. gibsoni* antigen was produced as follows: male *O. gibsoni* were dissected from nodules freshly collected from a local abattoir, washed in phosphate buffered saline (PBS) and homogenised on ice in worm solubilisation buffer (1% (w/v) sodium deoxycholate, 0.14 M NaCl, 0.5% (w/v) sodium azide, 1 mM PMSF in 0.01 M Tris, pH 8.3). After centrifugation (10.000 g) the method of Lowry et al. (1951), using bovine serum albumin as the standard, was used to determine the protein content of the supernatant fluid. This fluid was the preparation known as Og male Ag.

The monoclonal antibody Og4C3

An IgM monoclonal antibody, designated Og4C3, was produced in this laboratory using Og male Ag to prime mice and screen for antibody production by subsequently produced hybridomas. Og4C3 binds to a range of filarial and non-filarial nematodes (including *O. gibsoni, O. volvulus, Dirofilaria immitis, Ancylostoma caninum* and *Toxocara canis*) but not to phosphorylcholine. It recognises antigens of Mr > 130 kDa and 50–60 kDa from Og male Ag, including both protein and carbohydrate moieties. The target antigens of Og4C3, visualised on histological sections of *O. gibsoni* nodules using the immunoperoxidase staining technique, are located at the junction of the cuticle and the hypodermis, in cells of the gut, and in intra-uterine embryos and extra-uterine microfilariae of *O. gibsoni*.

Human sera

A total of 127 individual and 9 pooled human sera were obtained from the filariasis serum bank of the World Health Organisation. They had been collected from people with parasitologically and/or clinically diagnosed infection with *W. bancrofti* (from the Philippines (n = 16), Sri Lanka (23), Papua New Guinea (12), India (6) and Bangladesh (11)); *Brugia malayi* (from Sulawesi (5) and Kalimantan (5)); *B. timori* (from Flores (6)); *O. volvulus* (from the Ivory Coast (7), Sudan (3) and Guatemala (18)); *Loa loa* (from Sudan (5), Central African Republic (1) and Africa (no country specified:1)) as well as endemic (for *B.timori*) non-infected controls (from Flores (8)). In addition bancroftian filariasis serum pools from the Philippines, Sri Lanka, Papua New Guinea and Tahiti; onchocerciasis serum pools from the Ivory Coast and Guatemala and serum pools from people in Flores both with and without *B. timori* infection were tested. A further 20 sera from Townsville residents (non-endemic controls) collected for minor non-parasitological clinical conditions by a local pathology laboratory were also tested for circulating parasite antigen. Sera from the filariasis serum bank were shipped to Townsville on dry ice and stored at −70 °C; locally collected sera were stored at −20 °C until used.

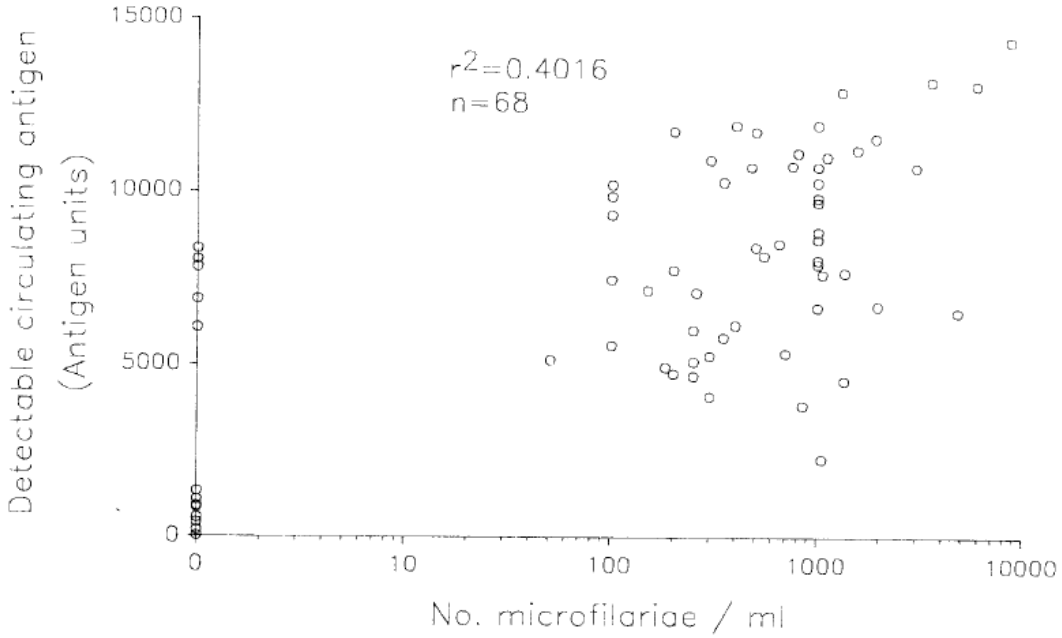

Fig. 1 The relationship between number of microfilariae (number/ml on a $\log_{10}$ scale) and levels of circulating parasite antigen in sera (expressed as antigen units, using the Og4C3-based antigen detection assay) from 68 patients with parasitological and/or clinical evidence of infection with *Wuchereria bancrofti*

Treatment of serum

The method of Weil and Liftis (1987) with slight modifications was used to free parasite antigen in serum from antibody-antigen complexes. One part of serum and 3 parts of 0.1 M Na_2EDTA, pH 4.0 were mixed, boiled for 5 min, and the supernatant fluid recovered after centrifuging at 16,000 g for 7 min.

Antigen detection ELISA

Wells of 96 well (0.25 ml) rigid polystyrene plates were coated overnight at room temperature with 50 µl of Og4C3 (5 µg of protein/ml of ascitic fluid precipitated using saturated ammonium sulphate, and diluted in PBS). After washing with PBS/T (PBS and 0.05% (v/v) Tween 20 (Fisher Scientific Company, Fair Lawn, U.S.A.)), 100 µl of blocking solution PBS/T/cas (PBS/T and 0.5% (w/v) high nitrogen casein (United States Biochemical Corporation, Cleveland, U. S. A.)) was added to each well and incubated in a humid chamber for two hours. After removing the blocking solution, treated serum and the non-reactive and reactive control samples were added (50 µl to each well) and left overnight at room temperature (for a maximum of 18 h). The plates were washed and 50 µl of rabbit hyperimmune serum raised against Og male Ag was added for 1 h. After again washing, 50 µl of a conjugate of horseradish peroxidase and sheep anti-rabbit immunoglobulin (Silenus, Melbourne, Australia) was added to each well. The sample, hyperimmune rabbit serum and conjugate were each diluted in PBS/T/cas. One hour later the plates were washed and 100 µl of TMB substrate solution (0.4 mM 3,3',5,5'-tetramethylbenzidine dihydrochloride and 0.004% (v/v) H_2O_2 in 0.1 M acetate buffer, pH 5.6) was added for 30 min before stopping the reaction with 50 µl of 2 M H_2SO_4. The absorbance was read spectrophotometrically at 450 and 540 nm.

Each plate contained 36 duplicate test serum samples, 6 duplicate non-reactive controls and 6 duplicate reactive samples used to construct a standard curve. Test results were only considered to contain detectable amounts of parasite antigen if the observed optical density exceeded the mean plus 3 standard deviations of the 12 non-reactive control wells. Serum from a Townsville resident with no previous exposure to human filarial infection was used as the non-reactive control. The titration of Og male Ag in the non-reactive control serum was used to produce the positive standard curve on each plate. Quantitation of test results (in antigen units) was achieved following the fitting of a multiple regression line to $\log_{10}$-transformed standard curve data.

Antigen units

The term antigen unit (A. U.) was introduced because the proportion of the Og4C3 target antigen in the total deoxycholate-soluble male *O. gibsoni* is unknown. A sample with 100 A. U. is defined as the amount of the target antigen of Og4C3 found in 100 ng of Og male Ag protein.

Results

Treatment of sera and effect on sensitivity of the assay

The target antigen of Og4C3 was heat stable in buffer and unaltered by the addition of an equal volume of 0.1 M Na_2EDTA, pH 4.0 before boiling for 5 min. The titration of Og male Ag in untreated helminth-free bovine serum resulted in poor sensitivity of the Og4C3-based ELISA in the detection of parasite antigen. Sensitivity was increased sixty fourfold following heat treatment of the serum, and was improved further by prolonging both blocking and incubation steps in the ELISA. Under these conditions, using known amounts of Og male Ag in control human serum, as little as 2.4 A. U. of parasite antigen could be detected with this assay.

Table 1 Results of the Og4C3-based antigen detection ELISA using sera from people with clinically and/or parasitologically defined filarial infection, and non-infected controls. Antigen levels greater than 100 antigen units were considered reactive

Filarial infection	Number tested	ELISA results Re-active	Non-reactive
*Wuchereria bancrofti**	68	67	1
*Brugia malayi**	10	0	10
*Onchocerca volvulus**	28	0	28
*Loa loa**	7	0	7
*Brugia timori**	6	0	6
Endemic control*	8	0	8
Non-endemic control	20	0	20

* from the filariasis serum bank of the World Health Organisation

Table 2 Results of the Og4C3-based antigen detection ELISA of serum pools from the filariasis serum bank of the World Health Organisation. Antigen levels greater than 100 antigen units were considered reactive

Confirmed filarial infection	Country of origin	ELISA result (antigen units)
Wuchereria bancrofti	Philippines	10724.0
	Sri Lanka	10388.0
	Papua New Guinea	5448.0
	Tahiti	10672.0
Onchocerca volvulus	Ivory Coast	0.0
	Guatemala	0.0
Brugia timori	Flores	0.0
Adenolymphangitis (no parasite specified)	Flores	0.0
Endemic controls	Flores	0.0

Table 3 Results of the Og4C3-based antigen detection ELISA using sera from people with clinical signs indicative of infection with *Wuchereria bancrofti*. All sera were obtained from the filariasis serum bank of the World Health Organisation. Antigen levels greater than 100 antigen units were considered reactive

Clinical signs	Number tested	ELISA results Number reactive	Number non-reactive
Asymptomatic/micro-filaraemic	49	49	0
Epididymo-orchitis	4	4	0
Hydrocoele formation	5	5	0
Elephantiais	7	6	1
Adenolymphangitis	1	1	0
Lymphoedema	1	1	0
Hydrocoele/tropical pulmonary eosinophilia	1	1	0

Table 4 Results of the Og4C3-based antigen detection ELISA using sera from people confirmed parasitologically and/or clinically to be infected with *Wuchereria bancrofti*, grouped according to country of origin. Antigen levels greater than 100 antigen units were considered reactive

Country	Number tested	ELISA results Number reactive	Number non-reactive
Philippines	16	16	0
Sri Lanka	23	23	0
Papua New Guinea	12	12	0
India	6	6	0
Bangladesh	11	10	1

ELISA results

Circulating parasite antigen was detected in 67/68 cases of clinically and/or parasitologically confirmed bancroftian filariasis but not in serum from people infected with *B. timori*, *O. volvulus* or *L. loa*, and not from *B. timori*-endemic or Townsville non-endemic controls (Table 1). Parasite antigen (32.7 A. U.) was found in 1 of the 10 samples from *B. malayi*-infected individuals. However, this was less than the arbitrary 100 A. U. chosen as the lower cutoff for a reactive sample. Circulating levels of antigen in sera from people with bancroftian filariasis were very high, with 78% and 91% of the 68 samples containing greater than 5000 and 1000 A. U., respectively. High levels (> 5000 A. U.) of circulating antigen were also detected in the *W. bancrofti* serum pools from the Philippines, Sri Lanka, Papua New Guinea and Tahiti, but no antigen was detected in the *O. volvulus* pools (from the Ivory Coast and Guatemala), the *B. timori* pool (from Flores), a pool from people suffering adenolymphangitis (from Flores) or an endemic control pool from Flores (Table 2).

A number of different clinical forms of bancroftian filariasis were represented in the samples tested. Circulating antigen was found in the sera of 49/49 people who were classified as asymptomatic but microfilaraemic; 4/4 epididymo-orchitis; 5/5 hydrocoele; 6/7 elephantiasis; 1/1 adenolymphangitis; 1/1 lymphoedema; and 1/1 hydrocoele/tropical pulmonary eosinophilia) (Table 3).

A significant but weak correlation ($r^2 = 0.4016$) was measured between level of circulating antigen and number of microfilariae ($t_{(66)} = 6.66$, $P < 0.005$) in microfilaraemic individuals with bancroftian filariasis (Fig. 1). There was no significant correlation between the amount of circulating antigen and the age of the patients infected with *W. bancrofti* ($t_{(66)} = 1.84$, $P = 0.0700$, $r^2 = 0.0489$).

ELISA results from people with *W. bancrofti* grouped according to country of origin are shown in Table 4. Serum from a 14 year old Bangladeshi girl (filariasis serum bank number 253) with no microfilaraemia but clinical signs of elephantiasis contained 30.8 A. U. and was the only 'non-reactive' serum from any patient with bancroftian filariasis.

Discussion

The specificity and sensitivity of the Og4C3-based antigen detection ELISA for *W. bancrofti* is striking. Parasite antigen was detected only in sera from cases of bancroftian filariasis and not from people infected with *B. malayi*, *B. timori*, *O. volvulus* or *L. loa*. This assay would thus be suitable for use in areas where bancroftian filariasis co-exists with other filarial infections.

Sensitivity of the assay was improved by heat pretreatment of sera, a technique used previously with similar success by Weil and Liftis (1987). The assay was then able to detect less than 3 antigen units. However, this level of sensitivity was not needed since very much larger amounts of circulating antigen were detected in serum from most cases of bancroftian filariasis. These findings suggest that modifications of this assay for field use, even if accompanied by up to a hundredfold reduction in sensitivity, would not adversely affect final results.

The Og4C3 target antigen is widely distributed. Og4C3 reacts with antigens of *O. volvulus* as well as a range of canine nematodes, and antibodies able to interfere with the detection of this antigen were present in serum from a calf raised in helminth-free conditions. Functionally, however, in the ELISA with human serum, this monoclonal antibody was highly specific for *W. bancrofti*. Similar results were recorded by Weil and Liftis (1987), however, the reason for this apparent contradiction is not clear.

A major priority for a diagnostic assay for lymphatic filariasis is detection of active infection in the absence of circulating microfilariae (Anon, 1989). Two findings suggest that the antigen detected by Og4C3 is not solely of microfilarial origin and circulates independently of the presence or absence of microfilariae in the blood. Firstly, microfilarial counts and serum levels of parasite antigen from people with bancroftian filariasis were only weakly correlated. Secondly, high levels of circulating parasite antigen were measured in sera from 12/13 amicrofilaraemic patients with clinical signs of chronic bancroftian filariasis. This result is appreciably better than those reported by other workers (Lal et al., 1987; Weil et al., 1987; Zheng et al., 1987; Santhanam et al., 1989) using ELISAs to detect circulating antigens of *W. bancrofti*.

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Dr. S. J. More

Graduate School of Tropical Veterinary Science and Agriculture
James Cook University of North Queensland
Townsville 4811, Australia