DETECTION OF CIRCULATING ANTIGENS OF *PARASTRONGYLUS CANTONENSIS* IN HUMAN SERA BY SANDWICH ELISA WITH SPECIFIC MONOCLONAL ANTIBODY

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Abstract. A specific monoclonal antibody (AW-3C2) as revealed by ELISA was produced against the adult worm antigens of *Parastrongylus cantonensis* and used in a sandwich ELISA for the detection of circulating antigens in the sera of parastrongyliasis patients and those with other parasitic diseases. A total of 60 sera was used in this study. Of these, 10 each were from patients with parastrongyliasis, filariasis, gnathostomiasis, malaria and toxocariasis. The control group consisted of 53 serum samples from normal healthy Thais and Malaysians. The mean ± optical density (OD) values for the normal Thai and Malaysian groups were 0.126 ± 0.028 and 0.124 ± 0.029, respectively. Mean OD values of parastrongyliasis patient group differed significantly from that of the narmal groups as well as those of other parasitic infections. Using a cut-off point of mean OD ± 3SD of the normal control groups as indicating a positive reading, the specificity of the assay with this monoclonal antibody was 100% while the sensitivity was 50%.

INTRODUCTION

The diagnosis of human parastrongyliasis is based on both clinical features as well as laboratory tests. Definitive diagnosis is through the finding of larva or juvenile worms in the CSF of the infected individuals. However, immunological tests are useful in the absence of definitive diagnosis as *Parastrongylus cantonensis* worms are seldom found in the limited volume of cerebrospinal fluid (CSF).

Although significant progress has been made in improving and developing procedures for diagnosis of parasitic infections, it is generally accepted that the specificity and sensitivity of immunodiagnostic procedures for the detection of specific antibodies depend on the purity and specificity of the antigens and the type of serological test used. An alternative to antibody detection is antigen detection assays which unequivocally demonstrate presence of parasites in the host. Serological methods for the detection of circulating antigens present in the sera of current or a recent past infection would therefore be valuable in individual diagnosis as well as in epidemiological studies.

The usefulness of conventional antibody preparations as probes for parasite antigen detection, however, has been limited by the variability of the immune response to various antigens and by the heterogeneity of antibody affinity and avidity (Hague *et al*, 1981). Chen *et al* (1973) used the IHA for the detection of circulating antigens in the CSF of *P. cantonensis* infected monkeys but the antigen titers were relatively low and the results in four experimental animals were variable. Monoclonal antibodies (MAbs) with defined specificities could theoretically be used as immunodiagnostic reagents for the detection of *P. cantonensis* antigens. Such MAbs have been produced and evaluated for their usefulness in detecting *P. cantonensis* antigen in the sera and CSF of patients with clinical symptoms of eosinophilic meningoencephalitis by means of an eazyme-linked fluorescent assay (Shih and Chen, 1991).

In the present study, a specific MAB AW-3C2 produced against the antigens of *P. cantonensis* adult worms was evaluated by a sandwich ELISA to determine its usefulness for the detection of *P. cantonensis* antigen in the sera of patients with parastrongyliasis.

MATERIALS AND METHODS

Sera of patients with parastrongyliasis

Serum samples were obtained from two patients with parasitologically confirmed parastrongyliasis (one with cerebral parastrongyliasis in whom a fourth stage larva was recovered from the CSF, and the other with ocular parastrongyliasis where an immature male was recovered from the anterior chamber of eye) and 8 patients with presumptive parastrongyliasis. The latter group was diagnosed as parastrongyliasis based upon clinical symptoms, a previous history of residing in endemic areas as well as high OD values in an ELISA for parastrongyliasis.

Sera of patients with other parasitic infections

Sera were collected from 10 gnathostomiasis patients. One confirmed gnathostomiasis serum was from a Thai patient in whom a *G. spinigerum* larva was recovered from the skin, while 9 presumptive gnathostomiasis sera were from patients with intermittent cutaneous migratory swelling and positive serology for the infection.

Sera were also collected from 10 Thai patients diagnosed pathologically as having subcutaneous *Taenia solium* cysticerci.

Ten serum samples each were collected from Malaysian patients with filariasis, toxocariasis and malaria. All of these patients with filariasis and malaria were diagnosed parasitologically, while the patients with toxocariasis were confirmed serologically.

The normal control group of sera consisted of 53 serum samples obtained from 28 healthy Malaysians residing in Kuala Lumpur and 25 healthy Thais residing in Bangkok.

Rabbit polyclonal antibodies

Polyclonal antibodies against *P. cantonensis* parasite were produced in a laboratory-bred rabit immunized with 3 doses of *P. cantonensis* adult worm antigens subcutaneously at 2 weekly intervals. Each immunizing dose consisted of 1.0 ml soluble antigens (500 µg protein) emulsified in 1.0 ml Freund's complete adjuvant. The antiserum used in this study was that collected 2 weeks after the last immunizing dose.

Monoclonal antibody

An IgM specific MAb (AW-3C2) against *P. cantonensis* adult worm antigens as revealed by ELISA was selected for this study. Spent culture supernatant fluid was used in a sandwich ELISA system for the detection of circulating antigens in the sera of patients with parastrongyliasis, gnathostomiasis, cysticercosis, toxocariasis and malaria.

Sandwich ELISA

The ELISA system used in this study was a modification of the sandwich ELISA described by Huijun *et al* (1987). Wells of ELISA plates (Immulon II, Dynatech) were coated with 50 µl of rabbit anti-*P. cantonensis* serum diluted in PBS-Tween 20, pH 7.4 (1 in 2,000 dilution) overnight at 4 °C. After washing four times with PBS-Tween, 200 µl of 1% bovine serum albumin in PBS-Tween were added and the plates incubated at room temperature for 1 hour. The plates were then washed again four times with PBS-Tween and 50 µl of human patient serum (diluted 1 in 5 with PBS-Tween) were added. The plates were incubated for 2 hours at room temperature followed by washing with PBS-Tween. Fifty microliters of undiluted hybridoma culture supernatant were added and the plates incubated for three hours at room temperature. After washing with PBS-Tween, 200 µl of goat serum diluted 1:100 with PBS-Tween were added and the plates incubated for another hour at room temperature. After washing with PBS-Tween, 50 µl of the enzyme conjugate, peroxidase goat anti-mouse immunoglobulins (Cappel Laboratories, USA) diluted 1 in 5,000 with PBS-Tween were added. The plates were then incubated for 1 hour, washed with PBS-Tween and 50 µl of the enzyme substrate (20 mg orthophenylene-diamine in 50 ml buffer, pH 5 consisting of 12.15 ml of 0.1 M citric acid, 12.85 ml 0.1 M disodium hydrogen orthophosphate and 25 ml distilled water, and 20 µl of 30% hydrogen peroxide were added. The plates were then incubated for 30 minutes

in the dark at room temperature and the reaction stopped by adding 25 µl of 2.5 M sulphuric acid. The absorption values at 492 nm (OD 492 nm) were read using a Dynatech ELISA reader.

The optimal dilutions of rabbit immune serum, patient's serum, and conjugate were determined using a chequerboard titration. For each test, a negative, a positive and a PBS-Tween controls were included. Each serum sample was tested in duplicates and the mean OD value calculated.

The test result was considered positive if the mean OD value exceeded the mean OD + 3SD of the values obtained with the 53 negative sera as described by Huijun *et al* (1987).

RESULTS

The mean OD values and positivity rates of the sandwich ELISA for detecting circulating antigens in various groups of individual sera by MAb AW-3C2 are summarized in Table 1. The mean ELISA values (OD ± SD) for the sera of the normal Thai and Malaysian groups were 0.126 ± 0.028 and 0.124 ± 0.029, respectively. There was no significant difference in the mean OD values between the sera of normal Thais and Malaysians ($p > 0.05$). For the parastrongyliasis patient group, the mean OD value differed sigificantly from those of the normal Thai and Malaysian controls as well as the cysticercosis, filarissis, gnathostomiasis, malaria and toxocariasis groups ($p < 0.05$) using the Least Significant Difference test.

Table 1

Positive rates (%) and mean OD values (492 nm) in the sandwich ELISA for the detection of circulating antigens in various groups of sera using monoclonal antibody AW-3C2.

Serum group	Sample size	No. positive / No. tested (%)	Mean OD ± SD
Parastrongyliasis	10	5/10 (50)[a]	0.200 ± 0.044
		5/10 (50)[c]	
Cysticercosis	10	0 10 (0)[a]	0.115 ± 0.023
		0/10 (0)[c]	
Filariasis	10	0/10 (0)[b]	0.108 ± 0.035
		0/10 (0)[c]	
Gnathostomiasis	10	0/10 (0)[a]	0.152 ± 0.031
		o/10 (0)[c]	
Malaria	10	0/10 (0)[b]	0.153 ± 0.021
		0/10 (0)[c]	
Toxocariasis	10	0/10 (0)[b]	0/130 ± 0.028
		0/10 (0)[c]	
Normal Malaysian control	28	0/28 (0)[b]	0.124 ± 0.029
		0/28 (0)[c]	
Normal Thai control	25	0/25 (0)[a]	0.126 ± 0.028
		0/25 (0)[c]	
All normal control	53	0/53 (0)[c]	0.125 ± 0.029

Positive cut-off values calculated from normal Thai[a], Malaysian[b] and combined[c] sera readings were 0.210, 0.211 and 0.212 respectively.

Using the cut-off point of the mean OD + 3SD of either the normal Thai, Malaysian or combined normal control group, OD values above 0.210, 0.211 and 0.212 were considered positive in the sandwich ELISA, respectively. Thus, AW-3C2 was able to detect circulating antigens in 5 out of the 10 parastrongyliasis sera, giving a sensitivity of 50%. None of the sera from normal controls and other parasitic infections was positive with the MAb AW-3C2; thus the specificity of this ELISA was 100%.

DISCUSSION

The application of MAbs in immunodiagnosis offers several advantages including decreased false-positive reactions, decreased cross-reactivity, increased reproducibility and standardization of the test (Felice and Siracusano, 1987). MAbs employed in an ELISA system may also provide a useful tool for antigen detection since ELISA is relatively simple and can be automated. In the present study, the MAb AW-3C2 produced against the antigens of *P. cantonensis* adult worms was able to detect soluble antigens in the sera of patients with parastrongyliasis. The sensitivity of the test was 50%. As the sensitivity of the sandwich ELISA developed in this study was relatively low, a further purification of both the rabbit hyperimmune antiserum and the spent culture supernatant may improve the sensitivity of the test.

It could also be speculated that an antigen detection assay using a combination of specific MAbs would have higher sensitivity than that using only one MAb. This approach was attempted by Shin and Chen (1991) using a combination of TD2 and 3A5 MAbs produced against the somatic antigens of *P.cantonensis* third-stage larvae in an enzyme-linked fluorescent assay for the detection of circulating antigens in the CSF and sera of 35 patients with eosinophilic meningoencephalitis. All the CSF samples (100%) and most of the serum samples (88%) showed positive reactions. Since the somatic and metabolic antigens of *P. cantonensis* are more abundant in human CSF than in the serum, it is possible that the detection rate of the parasite' s antigens in the CSF could be greater with the use of the MAb AW-3C2. However, spinal puncture is a cumbersome technique which requires great skill and CSF is not as convenient to use as serum for immunodiagnosis purposes.

The specificity of the sandwich ELISA with MAb AW-3C2 in the present study was 100% since sera from patients with heterologous parasitic infections and normal controls did not produce a positive reaction in the assay. This is not surprising as earlier studies (unpublished data) have shown that this MAb used in the system did not cross-react with various other helminth antigens in the ELISA, thus indicating the absence of common or similar antigenic epitope among these helminth parasites.

The present study shows that the specific MAb AW-3C2 is potentially useful for the specific diagnosis of human parastrongyliasis. However, further studies need to be carried out as this study was based on a small number of serum samples from confirmed parastrongyliasis patients. In addition, attempts to develop a more sensitive and suitable antigen detection system, such as a dot-blot ELISA using nitrocellulose paper which permits the detection of less abundant antigens in the samples and obviates the need for a spectrophotometer should also be persued and evaluated.

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