DETECTION OF CIRCULATING ANTIGENS OF *PARASTRONGYLUS CANTONENSIS* IN HUMAN SERA BY DOT-BLOT ELISA AND SANDWICH ELISA USING MONOCLONAL ANTIBODY

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Abstract. A dot-blot ELISA was compared with a previously performed sandwich ELISA for the detection of *Parastrongylus cantonensis* antigens in sera from patients. Using the same monoclonal antibody and the same sera, 6 of 10 sera (60%) from parastrongyliasis patients were positive in dot-blot ELISA, whereas with sandwich ELISA, 5 of the same patient sera (50%) were positive. The specificity in both assays was 100% using 50 sera from patients with other parasitic diseases; of these, 10 each were from patients with cysticercosis, filariasis, gnathostomiasis, malaria and toxocariasis. The control group consisted of 53 sera from normal healthy Thais and Malaysians. The sensitivity of the assays was, however, slightly better with dot-blot ELISA and because it is simple, quick and cost-effective, it may be a test of choice for specific diagnosis of human parastrongyliasis.

INTRODUCTION

The application of monoclonal antibodies (MAbs) in antigen detection system circumvents the problem of specificity inherent in many immunodiagnostic assays based on the detection of parasite antibodies. Recently, several diagnostic methods using specific MAbs to detect circulating antigens of *Parastrongylus cantonensis* (previously referred to as *Angiostrongylus cantonensis*) in sera and cerebrospinal fluids (CSF) of patients with eosinophilic meningoencephalitis have been described. These include the sandwich enzyme-linked fluorescent assay (ELFA) and sandwich enzyme-linked immunosorbent assay (ELISA) (Shi and Chen, 1991; Eamsobhana *et al*, 1995). However, the current *Parastrongylus* antigen detection assays are relatively cumbersome and require sophisticated spectrophotometric equipment.

Pappas *et al* (1983, 1984) developed a visually interpreted dot enzyme-linked immunosorbent assay for the rapid serodiagnosis of visceral leishmaniasis. This technique was subsequently used for the detection of many other parasite antigens in samples dotted directly on the nitrocellulose membrane (Brooks *et al*, 1985; Gandhi, 1986; Tellez-Giron *et al*, 1987; Zheng *et al*, 1990; Oprandy and Long, 1990; Allan *et al*, 1993). Nitrocellulose can bind approximately 1,000 times more proteins per surface area than the microtiter plate, and thus allows the detection of less abundant antigens in the samples (Harlow and Land, 1988). Moreover, the dot-blot ELISA has the advantage in that it does not require any expensive instrument and can be applied under field conditions.

The objectives of the present study were to adapt the dot-blot ELISA for the detection of circulating antigens of *Parastrongylus cantonensis* in sera of patients and to compare the specificity and sensitivity of the dot-blot ELISA with the earlier performed sandwich ELISA (Eamsobhana *et al*, 1995).

MATERIALS AND METHODS

Sera

The same groups of sera previously used in a

sandwich ELISA were analyzed (Eamsobhana *et al*, 1995). Of these, 10 sera each were from patients with parastrongyliasis, filariasis, gnathostomiasis, malaria, cysticercosis and toxocariasis. Two of the patients with parastrongyliasis, one with gnathostomiasis and all patients with filariasis and malaria were diagnosed parasitologically. Others showed typical symptoms and were confirmed serologically. The normal control group of sera consisted of 53 serum samples obtained from 28 healthy Malaysians and 25 healthy Thais.

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 dot-blot ELISA for the detection of circulating antigens in the sera of patients.

Dot-blot ELISA

Nitrocellulose membrane, 0.45 µm pore size, was obtained from Bio-Rad Laboratories, Richmond, California. The membrane was cut into strips and columns were made about 2 cm apart on the strips with ball-point pen. The strips were soaked for 5 minutes in Tris-buffered saline (TBS), pH 7.4 and were air dried on filter paper. Two-µl of each serum to be tested (diluted 1 in 5 with TBS) were spotted in the center of the column on the strip and allowed to dry for 10 minutes. The membrane strips were rinsed with TBS and subsequently immersed in blocking solution (TBS with 20 mg/ml bovine serum albumin and 5 mM sodium azide) with gentle shaking for 30 minutes. After washing 3 times, by shaking for 5 minutes during each wash in TBS-Tween 20, the membranes were incubated for 1 hour in MAb AW-3c2 (undiluted hybridoma culture supernatant) and again washed as described above. The washed membranes were then immersed in the enzyme conjugate, peroxidase goat anti-mouse immunoglobulins (Capple Laboratories, USA) diluted in 1:1,000 with TBS for 2 hours. The strips were washed as above and transferred to a substrate solution containing 5 mg of 3,3'-diaminobenzidine in 10 ml of TBS plus 3 µl of hydrogen peroxide. After the appearance of the color, the strips were washed with distilled water for several times. The development of well-defined deep brown dots on the nitrocellulose membrane was considered as positive. No color or light brown dots were considered as negative (Fig 1).

With each membrane strip, a positive and a negative control were included. Each serum sample was tested in duplicate.

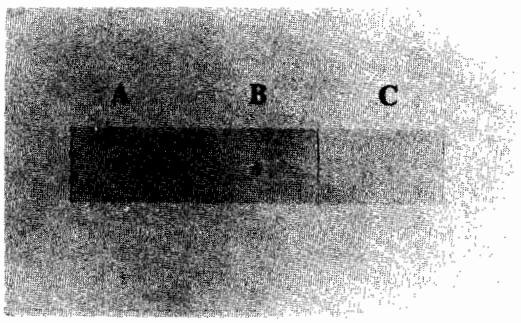

Fig 1–Reactions of dot-blot ELISA on nitrocellulose membrane for the detection of circulating antigens of *P. cantonensis* from sera of patients using AW-3C2 MAb. A, a deep colored dot showing positive reaction. B and C, a light colored dot and no color, respectively, were considered as negative.

Sandwich ELISA

The ELISA system used in the study was a modification of the sandwich ELISA described by Zheng *et al* (1987). This assay was carried out as previously described (Eamsobhana *et al*, 1995).

RESULTS

Sixty percent (6 of 10) patients with parastrongyliasis had detectable circulating antigens with dot-blot ELISA. With the sandwich ELISA, 50% (5 of 10) patients showed positive reactions. One of 5 patients who were negative for *Parastrongylus* antigens in sandwich ELISA responded positively in the dot-blot ELISA (Table 1). When the group of 50 patients with various other parasitic infections (gnathostomiasis, cysticercosis, filariasis, toxocariasis, malaria) were tested by both assays under the same conditions, the two methods produced negative reactions. Likewise, in normal controls all the 53 serum samples were not reactive. Table 2 summarizes the results of the two technics.

Table 1

Sensitivity of dot-blot ELISA and sandwich ELISA for detection of *P. cantonensis* antigens in sera from patients with parastrongyliasis.

Serum sample	ELISA-serum antibodies	Antigen detection assay	
		Sandwich ELISA	Dot-blot ELISA
1	+	+	+
2	+	+	+
3	+	-	+
4	+	-	-
5	+	-	-
6	+	-	-
7	+	+	+
8	+	+	+
9	+	+	+
10	+	-	-

+, positive;-, negative.

Table 2

Detection of circulating antigens of *P. cantonensis* in various human sera using dot-blot ELISA and sandwich ELISA.

Serum group	Sample size	No. positive / No. tested (%)	
		Sandwich ELISA	Dot-blot ELISA
Parastrongyliasis	10	5/10 (50)	6/10 (60)
Cysticercosis	10	0/10 (0)	0/10 (0)
Filariasis	10	0/10 (0)	0/10 (0)
Gnathostomiasis	10	0/10 (0)	0/10 (0)
Malaria	10	0/10 (0)	0/10 (0)
Toxocariasis	10	0/10 (0)	0/10 (0)
Normal control	53	0/53 (0)	0/53 (0)

DISCUSSION

Circulating parasite antigen was first demonstrated in the CSF of *P. cantonensis* infected monkeys in 1973, with the technique of indirect hemagglutination test (Chen *et al*, 1973). However, the conventional antibody preparation as probes for parasite antigen detection has been limited by the variability of the immune response to various antigens and by the heterogeneity of antibody affinity and avidity. Monoclonal antibodies with defined specificities could theoretically be used as immunodiagnostic reagents for the detection of parasite antigens. Recently, such MAbs have been produced and evaluated for their usefulness in the detection of circulating antigens in sera and CSF of parastrongyliasis patients using microtiter plate-based enzyme immunoassays (Shi and Chen, 1991; Eamsobhana *et al*, 1995). Microtiter plate assays do, however, have some limitations. They require long incubation time and special equipment to perform and read the reaction. Nitrocellulose membrane, on the other hand, has been shown to have a high binding capacity as a solid phase antigen carrier (Beyer, 1984). In a dot-blot ELISA, it binds

antigens rapidly and quantitatively with high capacity. It is easy to perform and the results are read visually.

In this study, dot-blot ELISA is comparable to, and appears to be better than, sandwich ELISA for the detection of *Parastrongylus* circulating antigens in human sera. Six of the 10 patients from parastrongyliasis were positive by the dot-blot ELISA. None of the patients with heterologous parasitic infections and normal controls gave positive reactions. Our dot-blot ELISA had a sensitivity of 60% and specificity of 100% for the diagnosis of parastrongyliasis. Corresponding results for the sandwich ELISA on the same patients were 50% and 100% respectively. Dot-blot ELISA thus had slightly better sensitivity in detecting *Parastrongylus* antigens than the sandwich ELISA; however, both the assays are equally specific. Similar results in the sensitivity of dot-blot ELISA over sandwich ELISA in detecting circulating antigens have also been reported in other parasites (Tellez-Giron *et al*, 1987; Zheng *et al*, 1990).

The present study, nevertheless, is limited by the fact that neither the dot-blot ELISA nor the sandwich ELISA will detect all patients with parastrongyliasis. Thus, both assays should be applied to the same CSF, since the somatic and metabolic antigens of *P. cantonensis* are more abundant in human CSF than in serum. It is possible that the detection rate of parasite antigens in the CSF could be greater. Moreover, it should be pointed out that for the purpose of this study, only strong brown coloration on the dot-blot ELISA has been considered as positive. The development of light brown coloration of the dot by this technique has been considered negative; it may however, suggest presence of *Parastrongylus* antigens in very low quantity. An earlier study, using AW-3C2 MAb in dot-blot ELISA has shown that the minimal readable concentration of the *P. cantonensis* antigens in phosphate buffered saline was around 0.10 ng/ml (unpublished data).

In conclusion, it is apparent that our monoclonal antibody-based dot-blot ELISA is a reliable, rapid and practical technique for detecting active *P. cantonensis* infection in man. Additionally, the assay does not require any sophisticated apparatus. It is therefore most adaptable for use in a field test kit. However, due to difficulty in obtaining the confirmed cases with *Parastrongylus* infection, the present study was based on a rather small number of serum samples from patients. A field trial to test the validity of this approach will be required.

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