SPECIFICITY OF ANTIBODIES IN CEREBROSPINAL FLUID OF HUMAN CEREBRAL GNATHOSTOMIASIS CASES

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Abstract. Specificity of antibodies in cerebrospinal fluid (CSF) of human cerebral gnathostomiasis cases were examined by indirect fluorescent antibody technique against paraffin sections of *Gnathostoma spinigerum* larva. Specific greenish fluorescence was observed at cuticle, esophagus, muscle cells, intestinal cell cytoplasm and microvilli. CSF of confirmed cerebral cysticercosis cases gave fluorescence mostly at the cuticle. It is suggested that parasite-specific antigen may be present on intestinal cell microvilli and CSF would be a good source of antibodies in studying specificity of antibodies to gnathostome infections.

INTRODUCTION

A target of serum antibodies from human gnathostomiasis cases has been demonstrated by indirect fluorescent antibody technique (IFAT) (Morakote *et al*, 1989). Similar fluorescent staining pattern was also observed with human angiostrongyliasis serum, but not cysticercosis serum. Using the same approach, the present study examined the specificity of antibodies in CSF of cerebral gnathostomiasis, angiostrongyliasis, and cysticercosis cases.

MATERIALS AND METHODS

Cases

Patients were diagnosed as having cerebral gnathostomiasis (7 cases) or angiostrogyliasis (6 cases) from signs and symptoms (Boongird *et al*, 1977; Punyagupta, 1979), and cysticercosis (5 cases) from surgery or computed tomography. Cerebrospinal fluids (CSF) were obtained by spinal tapping and stored at -70°C until used. Negative control CSF was pooled from specimens obtained from children with febrile convulsion.

Serological test

Enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to *Gnathostoma* larval antigens was similar to that described previously (Maleewong *et al*, 1988). The assays for anti-*Angiostrongylus* and anti-cysticercus antibodies were similar, with substitution of *Gnathostoma* antigen with adult *Angiostrongylus cantonensis* (2.5 μg/ml) or *Taenia solium* cysticercus extract (2.5 μg/ml) as coating antigen. Each assay system was performed under pre-determined optimal conditions. CSF specimens were tested at 1:4 dilution.

Indirect fluorescent antibody technique (IFAT)

The procedures were the same as described in detail previously (Morakote *et al*, 1989). Briefly, encapsulated third-stage larva of *Gnathostoma spinigerum* were fixed in cold alcoholic formalin and processed through paraffin tissue-processing technique. The paraffin-embedded worm was cut into 6 μm-thick sections, mounted on glass slides, dried at 60°C for 30 minutes. Sections were deparaffinized in xylene, passed through absolute ethanol and air dried. They were next covered with 1% BSA for 10 minutes, washed with PBS, CSF diluted 1:4 in 1% BSA for 30 minutes, washed twice with PBS, FITC-conjugated anti-human IgG optimally diluted in PBS containing 0.2% Evans blue for 30 minutes, washed twice with PBS, and finally mounted in buffered glycerol. The sections were examined under fluorescence microscope (Olympus, Vanox-S, Japan).

RESULTS

ELISA values of CSF from cases are summarized in Table 1. Examination of sections treated with CSF revealed patterns of apple-green fluorescence as shown in Table 2. All specimens from cerebral gnathostomiasis cases showed consistent pattern of staining. Relatively bright greenish fluorescence was observed at cuticle, esophagus, and intestinal cell microvilli (brush border). Weaker fluorescence was detected at muscle cells and intestinal cell cytoplasm (Fig 1A). CSF from angiostrongyliasis cases produced similar fluorescent staining patterns (case 8) (Fig 1B) or fluorescence at cuticle, esophagus, intestinal cell cytoplasm but not at intestinal cell microvilli (cases 9, 10, 12, 13), or no fluorescence (case 11). CSF from cysticercosis cases produced fluorescence only at cuticle of *Gnathostoma* section (case 14, 17, 18) (Fig 1C) or weak staining of the esophagus (case 16). Control sections treated with PBS or control CSF were completely reddish under the fluorescence microscope (Fig 1D).

DISCUSSION

In a previous study, serum antibodies of parasitologically confirmed human gnathostomiasis were found to react with cuticle, esophagus, and intestinal cell cytoplasm of *Gnathostoma* larva (Morakote *et al*, 1989). Using CSF of human cerebral gnathostomiasis cases as antibody source, the above findings were confirmed. In addition, intestinal cell microvilli and muscle cells were found to be a target of antibodies in CSF. The pattern of staining was similar in most of the cases, suggesting a homogeneity of antibody responses.

Table 1

ELISA values of cerebrospinal fluid employed in the study.

Case	ELISA Values		
	Gn[1] Ag	Angio Ag	Cystic Ag
Pooled negative control	0.027	0.018	0.024
Gnathostomiasis			
1	0.914	0.005	ND[2]
2	0.792	0.038	ND
3	1.031	0.073	ND
4	0.839	0.030	ND
5	1.400	0.046	ND
6	1.228	0.120	ND
7	0.550	0.016	ND
Angiostrongyliasis			
8	0.367	0.898	ND
9	0.430	0.720	ND
10	0.260	0.917	ND
11	0.088	0.258	ND
12	0.301	0.764	ND
13	0.336	0.877	ND
Cysticercosis			
14	0.232	ND	0.970
15	0.036	ND	1.088
16	0.469	ND	1.646
17	0.173	ND	1.323
18	0.168	ND	1.488

[1]Gn = *Gnathostoma*; Angio = *Angiostrongylus*; Cystic = cysticercus
[2]ND = not done

Positive reactions were also observed with CSF from angiostrongyliasis cases. In this case, esophagus, cuticle, and intestinal cell cytoplasm were common targets. Intestinal cell microvilli and muscle cells, however, reacted to antibodies in only one out of 6 cases. CSF of cysticercosis cases produced fluorescence mostly at the cuticle, demonstrating nonspecificity of cuticular antigen. Immune electronmicroscopy is required to identify the ultrastructure of target organs which bind the antibody.

Specific antibodies have been detected in CSF in angiostrongyliasis and neurocysticercosis (Perez *et al*, 1989; Rosas *et al*, 1986; Corona *et al*, 1986), and cerebral gnathostomiasis (Tuntipopipat *et al*, 1989). CSF appears to be a good source of antibodies for studying antigenic complexity of gnathostome larval antigens. Normal CSF has a low level of immunoglobulins which results in completely reddish fluorescence of the treated sections in the present study even at undiluted concentration. The situation permits clear-cut study results and extends the ability to examine many CSF specimens without interpretation difficulty. Future investigation should be made to confirm that antibodies in CSF are synthesized intrathecally.

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Table 2

Specific fluorescence pattern of worm sections reacted with cerebrospinal fluids.

Case	Cuticle			Esophagus	Intestinal cell		Muscle
	surface	inner	basal		cyto[1]	microvilli	
Gnathostomiasis							
1	+ + +	−	−	+ + +	+	+ + + +	+ + +
2	+ + +	−	−	+ + +	+	+ + + +	+
3	+	−	−	+	+	+ + + +	+/−
4	+ + +	−	−	+ + +	+	+ + + +	+ + +
5	+ + +	−	−	+ + +	+	+ + + +	+ +
6	+ + +	−	−	+ + +	+	+ + + +	+ + +
7	+ + +	−	−	+ + +	+	+ + + +	−
Angiostrongyliasis							
8	+ + +	−	−	+ + +	+ +	+ + +	+
9	+ +	−	+	+	−	−	−
10	+ +	−	−	+ + +	−	−	−
11	−	−	−	−	−	−	−
12	+ + +	−	+ +	+ + +	+ +	−	−
13	+ + +	−	+ +	+ + +	+ +	−	−
Cysticercosis							
14	+ +	−	+ +	−	−	−	−
15	−	−	−	−	−	−	−
16	+/−	+ +	+/−	+	−	−	−
17	−	+ +	−	−	−	−	−
18	+/−	+ +	+/−	−	−	−	−

[1]cytoplasm

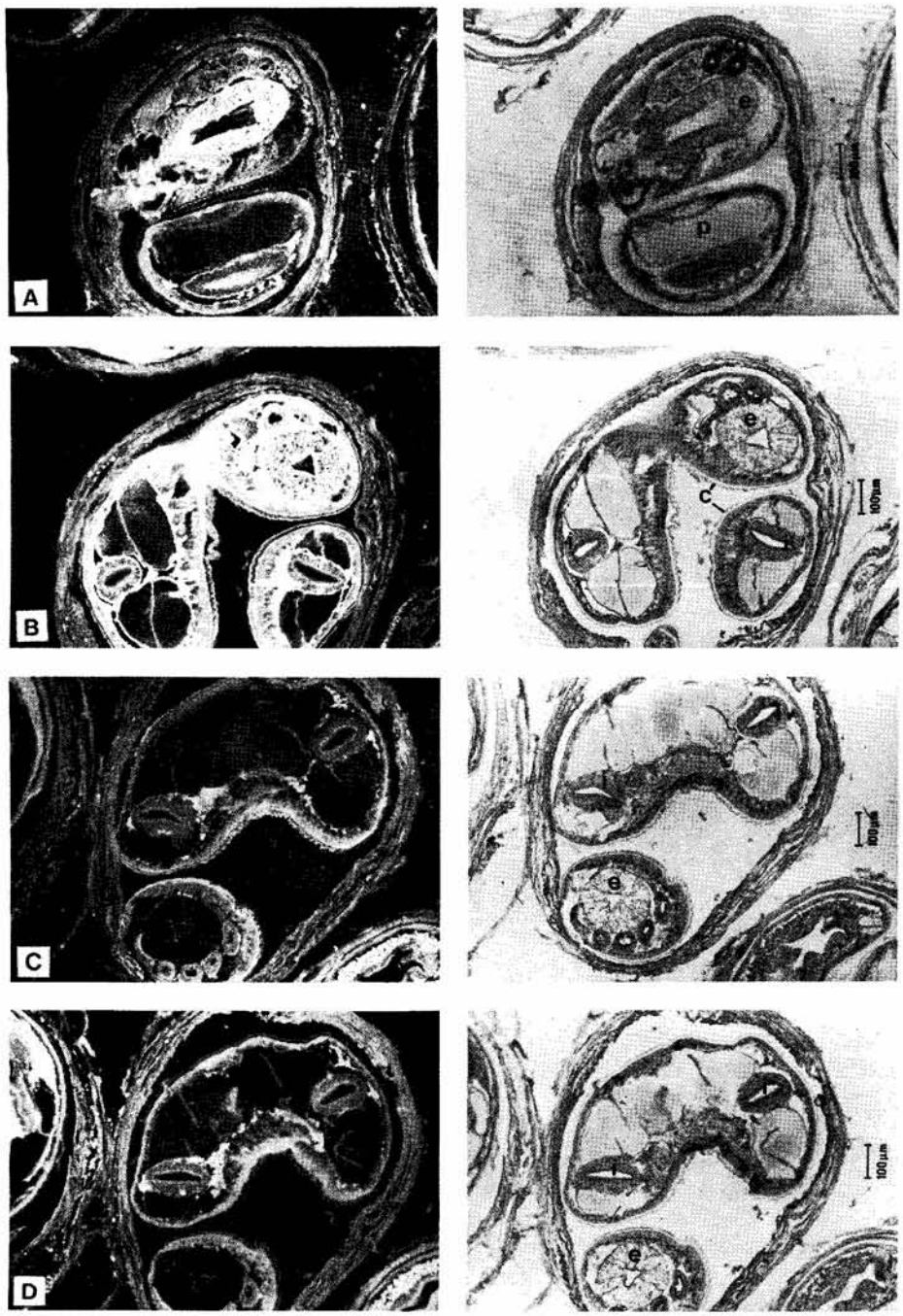

Fig 1—Fluorescence (left column) and light microscopy (right column) of gnathostome larval sections reacted with CSF of (A) gnathostomiasis, (B) angiostrongyliasis, (C) cysticercosis, and (D) negative control cases. Abbreviations : c = cuticle; cg = cervical glands; cw = cyst wall; e = esophagus; i = intestine; p = pseudocoelom.

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