EOSINOPHILIC MENINGITIS ASSOCIATED WITH ANGIOSTRONGYLIASIS: CLINICAL FEATURES, LABORATORY INVESTIGATIONS AND SPECIFIC DIAGNOSTIC IgG AND IgG SUBCLASS ANTIBODIES IN CEREBROSPINAL FLUID

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Abstract. Eosinophilic meningitis (EOM) associated angiostrongyliasis mostly induced by the nematode *Angiostrongylus cantonensis*, is a common disease with worldwide prevalence. Heavy infections can lead to chronic disabling disease and even death. This study was conducted to shed light on the overall specific IgG antibody response as well as the specific IgG antibody subclass responses in cerebrospinal fluid (CSF) of patients with EOM. Fifteen patients with EOM associated with angiostrongyliasis were included in the study. Sera were screened by immunoblotting for the presence of IgG antibody to the 29 kDa *A. cantonensis* antigenic polypeptide. CSF was examined by ELISA for the presence of specific IgG and IgG subclass antibodies. Patients presented with headache (100%), neck stiffness (20%), fever (40%), nausea (87%), vomiting (73%), paresthesia (7%), and muscle weakness (7%). Seven of 15 (47%) patients showed peripheral blood eosinophilia and all patients presented with eosinophils in CSF. A sensitivity of 80 % was obtained by combining the diagnostic values of immunoblotting in sera and IgG and IgG subclasses-based ELISA in CSF. The combination of a history of eating raw or semi-cooked infected foods, clinical features, complete blood count, differential cell counts, CSF profiles, and serum and CSF antibodies to *A. cantonensis* can be used to increase the sensitivity for the diagnosis of human angiostrongyliasis.

INTRODUCTION

Eosinophilic meningitis (EOM) associated with angiostrongyliasis is a disease commonly found from Southeast Asia to the Pacific islands, and in Africa, India, the Caribbean, Australia and North America. The major cause is *Angiostrongylus cantonensis*, the rat lungworm. Hundreds of cases infected with this parasite have been recorded from these areas (Punyagupta *et al*, 1970; Yii *et al*, 1975; Chen, 1979; Hwang and Chen, 1991; Witoonpanich *et al*, 1991; Pien and Pien, 1999; Chotmongkol *et al,* 2000; Yeh *et al,* 2001; Tsai *et al,* 2001). Man is an accidental "dead-end" host who becomes infected by ingesting the larvae in infected snails, slugs, paratenic hosts or contaminated, uncooked vegetables. These larvae cause eosinophilia in the cerebrospinal fluid (CSF) and peripheral blood. Humans rarely harbor adult parasites although rats carry the sexually mature worms in their pulmonary arteries and heart. Juvenile worms, however, have been found in the eyes, brain and spinal cord of infected individuals.

Most patients recover fully from the dis-

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ease, although heavy infections can lead to chronic, harmful disease and even death (Pien and Pien, 1999). Much is now known about the antibody responses in *A. cantonensis* infected hosts (Tharavanij, 1979; Yen and Chen, 1991; Nuamtanong, 1996; Chye *et al*, 2000; Eamsobhana *et al*, 2001; Maleewong *et al*, 2001) as well as about the different IgG antibody subclass responses to *A. cantonensis* antigens in infected individuals (Intapan *et al,* 2002, 2003). However, little is known about the specific IgG antibody subclass responses in CSF of EOM associated with angiostrongyliasis.

In this study, we describe the clinical and laboratory features, CSF profiles and computerized tomography of the brain of patients suffering from EOM associated with angiostrongyliasis. Detection of IgG and different IgG subclass responses in CSF and their potential use for supportive investigations were examined. The associations of the antibody responses with clinical parameters and diagnosis are discussed.

MATERIALS AND METHODS

Patients and clinical samples

Fifteen EOM patients (11 males and 4 females) who were admitted at Loei Hospital, northeast Thailand, with severe headache after eating raw snails, shrimps or monitor lizards (*Varanus bengalensis*) were enrolled in the study. A detailed history of eating parasite-contaminated foods, underlying diseases, previous parasitic infections and medications was taken from all patients. The clinical symptoms were recorded. Patients systematically underwent physical, neurological and ophthalmic examinations. The control group (n=10) consisted of four non-meningitis patients whose CSF was sampled during lumbar puncture for anesthesia before surgery and of six symptomatic meningitis-like patients (tension headache) with normal CSF profiles. Their CSF samples were used to determine the cut-off value between positivity and negativity. The serum and CSF samples of all EOM patients were obtained immediately after admission to the hospital during the acute phase. The samples were stored at -20^{o}C before being used for antibody determination.

Ethics approval for the study was received from the Human Ethics Committee of Khon Kaen University. Informed consent was obtained from all adult participants and from parents or legal guardians of minors. During admission, all patients received 8 mg of dexamethasone intravenously, which was followed by 4 mg intravenously every 6 hours for 2-5 consecutive days. Although the severe headache improved dramatically after lumbar tapping in most cases, a combination of appropriate doses of acetaminophen and nonsteroidal anti-inflammatory drugs was given every 6 hours to relieve the fever and headache. Dexamethasone was replaced with prednisolone orally, 30 mg daily in 3 divided doses for 5 consecutive days. Progress of all study cases was good, only one patient needed to have repeated lumbar tapping. The symptoms resolved within a few days to one week and all patients recovered.

Laboratory methods

Laboratory studies were performed on the date of admission. CSF samples were obtained by lumbar puncture on all patients through standard protocol. Blood tests included complete blood and differential cell counts. CSF analysis included leukocyte and differential cell counts, glucose and protein levels, Gram and acid fast stains, Indian ink preparation and larval detection under stereoscope (x40). CSF cultures were accomplished to rule out bacterial infections. Serum VDRL analysis was included to rule out neurological syphilis. Each patient underwent a brain CT scan.

Immunoblotting was performed by methods previously described (Maleewong *et al*, 2001) for the determination of serum IgG anti-

body specific to *A. cantonensis* 29 kDa antigen. In brief, the specific 29 kDa antigen from young adult female *A. cantonensis* worms was revealed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting. The blotted sheet was incubated with serum (dilution of 1:100), followed by an incubation with peroxidase-conjugated goat anti-human IgG (dilution of 1:1,000) (Zymed, South San Francisco, CA). Diaminobenzidine (Sigma Chemical, St Louis, MO) was used as substrate.

IgG and IgG subclass antibodies in CSF were detected by enzyme-linked immunosorbent assay (ELISA) as previously described (Intapan *et al*, 2002). For the determination of parasite-specific IgG and IgG antibody subclasses, microtiter plates were coated with young adult female *A. cantonensis* antigen. Optimum CSF dilution was 1:50 for IgG and 1:5 for IgG_1, IgG_2, IgG_3 and IgG_4, respectively. Horseradish peroxidase (HRP)- conjugated monoclonal anti-human IgG antibodies (DAKO A/S, Glostrup Denmark), diluted 1:50,000 (for IgG), and HRP- conjugated anti-human IgG subclasses (Zymed, South San Francisco, CA, USA), diluted 1:1,000 for IgG_1, IgG_2, and IgG_3 and diluted 1:5,000 for IgG_4 (according to the results of checker-board titration) were added. O-phenylene-diamine dihydrochloride was used as substrate. Optical density (OD) was read at 492 nm with an ELISA reader (Tecan, Salzburg, Austria). Precision of the ELISA was investigated by performing the test on different days using the same pooled positive and negative sera, and the same batch of antigen under the same conditions. Consistent data were obtained from all the tests indicating no day-to-day variation.

Statistical analysis

Mann-Whitney Rank Sum test was used to determine the significance of the difference between two groups of data (Sigma stat, San Rafael, CA). Diagnostic sensitivity and specificity were calculated using Galen's method (Galen, 1980).

RESULTS

The mean age (±SD) of the patients was 27.3±8.4 years (range 16-41). The mean incubation period was 12.8±9.8 days (n=15; range 1-27). Clinical symptoms and signs as well as blood and CSF profiles are shown in Tables 1 and 2. All patients had severe headache combined with a variety of other symptoms and signs (Table 1). The patients had normal or slightly elevated white blood cell counts in peripheral blood and 7 of 15 had blood eosinophilia (≥10%) (Table 2). Twelve of 15 CSF samples were cloudy. The CSF leukocyte counts were elevated in all patients. All CSF samples contained eosinophils ranging from 10% to 81%. Eight patients had elevated CSF protein (>100 mg/dl). CSF glucose was >40 mg/dl in 12 of 15 patients. All stains and bacterial cultures of CSF were negative and no parasite was found in any CSF. All serum VDRL tests were non-reactive. All patients showed normal brain CT scan.

From immunoblotting results, the specific antigenic band of 29 kDa was found to react with 8 out of 15 sera (53%) of EOM patients (Table 3). The response levels of CSF specific

Table 1
Frequency of clinical symptoms and signs among 15 patients with eosinophilic meningitis.

	Number (%)
Symptoms	
Headache	15 (100)
Neck stiffness	3 (20)
Nausea	13 (87)
Vomiting	11 (73)
Paresthesia	1 (7)
Muscle weakness	1 (7)
Signs	
Stiff neck	3 (20)
Fever (body temperature > 38ºC)	6 (40)

Table 2
Blood and cerebrospinal fluid (CSF) finding in 15 eosinophilic meningitis patients.

Patient No.	Blood		CSF profiles[a]					
	White blood cell count ($x10^3$ /μl)	Eosinophil (%)	Appearance	Leukocyte count ($x10^3$ /μl)	Eosinophil (%)	Protein (mg/dl)	Glucose (mg/dl)	Glucose ratio[b]
1	7,800	10	Cloudy	1,980	38	109	36	0.31
2	16,600	0	Cloudy	1,600	49	174	41	0.44
3	16,900	15	Cloudy	1,900	45	134	61	0.58
4	11,800	2	Cloudy	400	22	157	56	0.49
5	14,800	21	Cloudy	3,244	52	106	71	0.23
6	8,400	0	Clear	90	27	62	75	0.66
7	6,100	14	Clear	600	12	133	46	0.44
8	5,700	1	Clear	450	10	228	43	0.44
9	7,600	4	Cloudy	600	14	59	44	0.49
10	9,800	21	Cloudy	1,510	81	124	27	0.29
11	6,500	0	Cloudy	233	42	40	45	0.33
12	6,000	16	Cloudy	310	12	30	51	0.6
13	9,300	3	Cloudy	1,190	36	30	51	0.6
14	9,200	1	Cloudy	670	40	84	50	0.68
15	12,900	12	Cloudy	940	15	45	38	0.47

[a]All CSF samples were colorless, negative for Gram staining, acid-fast bacilli staining and Indian ink staining, bacterial cultures showed no growth.
[b]Cerebrospinal fluid glucose level divided by serum glucose level.

IgG and IgG antibody subclasses to the *A. cantonensis* antigen in EOM as detected by ELISA and other normal CSF profile cases are presented in Tables 3-4. ELISA values in CSF for the specific IgG, IgG_1 and IgG_2 subclasses for EOM were significantly higher than those in the normal CSF profile cases ($p<0.05$), except for the specific IgG_3 and IgG_4 subclasses, which were not significantly elevated ($p>0.05$). Using an absorbance value of the mean plus 2SD of the normal CSF profile cases as the cut-off limit between positivity and negativity of EOM, the sensitivity for detection of specific IgG, IgG_1, IgG_2, IgG_3 and IgG_4 was 40% (6/15), 40% (6/15), 47% (7/15), 13% (2/15) and 40% (6/15), respectively. The specificity calculated from the ELISA values of IgG and IgG subclasses in CSF of the normal CSF profile cases was 90% (9/10), except for IgG_2 where it was 100% (10/10) (data not shown).

DISCUSSION

Although EOM associated with angiostrongyliasis is rarely fatal in adults, patients frequently suffer from severe headache sequelae. An important diagnostic feature of the disease is a history of eating raw or semi-cooked food contaminated with the infective stage of *A. cantonensis* within a period of approximate one month prior to the first symptom (Punyagupta *et al*, 1970). In endemic areas, this disease should be suspected in patients with a history of eating parasite-contaminated foods and who present with any of the following clinical pictures (Punyagupta *et al*, 1970; Tsai *et al,* 2001; Pien and Pien, 1999) : (1) meningitic form with acute severe head-

Table 3
Serological findings of 15 patients with eosinophilic meningitis.

Patient No.	DAH[a]	Immunoblotting[b]	OD ELISA values[c] (results)				
			IgG	IgG_1	IgG_2	IgG_3	IgG_4
1	10	-	0.307 (+)	0.269 (+)	0.092 (-)	0.153 (-)	0.022 (-)
2	4	-	0.123 (-)	0.038 (-)	0.171 (+)	0.018 (-)	0.000 (-)
3	14	+	0.656 (+)	0.205 (+)	0.856 (+)	0.056 (-)	1.851 (+)
4	9	+	0.206 (-)	0.049 (-)	0.092 (-)	0.173 (-)	0.015 (-)
5	5	+	1.120 (+)	1.300 (+)	0.468 (+)	0.153 (-)	0.318 (+)
6	6	-	0.071 (-)	0.010 (-)	0.009 (-)	0.002 (-)	0.024 (+)
7	5	-	0.121 (-)	0.015 (-)	0.030 (-)	0.001 (-)	0.000 (-)
8	3	-	0.135 (-)	0.029 (-)	0.037 (-)	0.074 (-)	0.016 (-)
9	9	+	0.872 (+)	0.761 (+)	0.486 (+)	0.187 (-)	0.005 (-)
10	8	+	2.750 (+)	2.091 (+)	0.776 (+)	0.580 (+)	1.055 (+)
11	6	+	0.162 (-)	0.040 (-)	0.206 (+)	0.006 (-)	0.029 (+)
12	15	-	0.098 (-)	0.025 (-)	0.026 (-)	0.009 (-)	0.024 (+)
13	3	+	0.141 (-)	0.048 (-)	0.077 (-)	0.329 (+)	0.007 (-)
14	2	-	0.073 (-)	0.013 (-)	0.027 (-)	0.007 (-)	0.002(-)
15	5	+	0.405 (+)	0.372 (+)	0.318 (+)	0.004 (-)	0.000 (-)

[a]DAH = day of collecting sera and CSF after first occurrence of clinical symptoms and signs.

[b]Immunoblotting reacting to the 29 kDa *Angiostrongylus cantonensis* antigen performed as previously described (Maleewong *et al*, 2001).

[c]ELISA performed as previously described (Intapan *et al*, 2002); positive (+) = OD values ≥ OD value of the mean plus 2SD of the normal CSF profile cases ; negative (-) = OD values < OD value of the mean plus 2SD of the normal CSF profile cases.

Table 4
Mean and standard deviation of CSF antibody levels by IgG class and IgG subclasses to *Angiostrongylus cantonensis* antigen from patients with eosinophilic meningitis and other neurological disorders

Groups	n	Antibody levels[a]				
		IgG	IgG_1	IgG_2	IgG_3	IgG_4
Eosinophilic meningitis	15	0.483±0.703[b]	0.351±0.601[b]	0.245±0.279[b]	0.117±0.160	0.224±0.527
Normal CSF profiles	10	0.065±0.091	0.029±0.063	0.040±0.036	0.053±0.138	0.008±0.008

[a]Mean optical density values ± standard deviation read at 492 nm.

[b]Value for eosinophilic meningitis which are significantly higher than for normal CSF profiles ($p<0.05$).

ache or subacute severe headache associated with CSF eosinophilia and with or without low grade fever, neck stiffness, cranial nerve involvement and radicular pain; (2) meningoencephalitic form with CSF eosinophilia and acutely impaired consciousness without localizing signs; (3) ocular form, namely failing of vision or diplopia of one or both sides with or without headach; and (4) psychosis with sensorium impairment and severe headaches. However EOM may be confounded with migraine headache, brain tumor, psychoneuro-

sis, purulent meningitis, tuberculosis or viral meningoencephalitis, and occasionally encephalitis caused by sensorium impairment. The gold standard for the diagnosis of EOM caused by angiostrongyliasis is the recovery of *Angiostrongylus* larvae in CSF; however this is difficult to accomplish.

Nowadays, serodiagnostic tests can be used as supportive diagnostic tools (Tharavanij, 1979; Yen and Chen, 1991; Nuamtanong, 1996; Chye *et al*, 2000; Eamsobhana *et al*, 2001; Maleewong *et al*, 2001 Intapan *et al*, 2002, 2003). Intapan *et al* (2003) indicated that immunoblotting to detect IgG_4 subclass antibody to the 29 kDa band of *A. cantonensis* larval extract gives a sensitivity of 75%. Here, we showed that combining the detection of specific IgG antibody to the 29 kDa *A. cantonensis* antigen in the serum by immunoblotting and that of IgG subclass antibodies to *A. cantonensis* in CSF by ELISA could increase the sensitivity up to 80%.

Intrathecal synthesis of IgG and IgA in eosinophilic meningoencephalitis due to *A. cantonensis* could be demonstrated in 85% of the patients seven days after the time of early clinical recovery (Dorta-Contreras and Reiber, 1998). Recently, Dorta-Contreras *et al* (2005) reported IgG_1 and IgG_2 intrathecal synthesis is prominent in *A. cantonensis* meningoencephalitis. The present study revealed an increase of specific IgG, IgG_1 and IgG_2 antibodies to *A. cantonensis* antigen in CSF but no significant elevation of IgG_3 and IgG_4 antibody levels. This immune response in the CSF possibly indicates that the main protective mechanism against *A. cantonensis* infection involves IgG antibody in antibody dependent cell-mediated cytotoxicity (Yoshimura *et al,* 1983). Measurement of IgG subclasses provides some interesting comparisons between EOM and other neurological disorders. The increase in the specific IgG_1 against *A. cantonensis* antigens might be due to a host protective response as has been observed in *Heligmosomoides polygyrus* infection (Monroy and Enriquez, 1992; Ben-Smith *et al*, 1999). IgG_1 subclasses are often developed in early stages of helminthic infections such as fascioliasis (Paz *et al*, 1999). The differential morbidity and mortality of *A. costaricensis* infections in BALB/C and C57BL/10 mice correlated with total IgE and parasite specific IgG_1 production (Geiger *et al*, 1999). IgG_2 that was significantly increased in EOM could possibly be related to carbohydrate antigen as in *Blastocystis hominis* (Hussain *et al*, 1997) and *Schistosomiasis* (Dunne, 1990; Langley *et al*, 1994) infections. However, the low level of IgG_3 subclass antibody is probably due to its labile property and short half-life. Development of IgG_4 subclass antibody is generally due to chronic antigenic stimulation as demonstrated in filariasis (Ottesen *et al*, 1985), schistosomiasis (Van Dam *et al*, 1996) and in allergic conditions (Aalberse *et al*, 1983). This could explain the low levels of IgG_4 subclass antibody of the present result in that they were collected during the early clinical period.

In conclusion, the combination of a history of eating raw or semi-cooked infected food, clinical features, blood examination, CSF profiles, and serological results can be used for the diagnosis of human angiostrongyliasis. Moreover, detection of specific IgG and IgG subclasses in the CSF along with the IgG antibody in patient sera reacting to the *A. cantonensis* 29 kDa antigen can improve sensitivity. However, detection value of the specific IgG antibody in convalescent serum needs to be considered for increasing the diagnostic potential.

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