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Journal of the Neurological Sciences 267 (2008) 17–21

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Cerebrospinal fluid cytokine responses in human eosinophilic meningitis associated with angiostrongyliasis

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Received 30 May 2007; received in revised form 14 September 2007; accepted 17 September 2007
Available online 24 October 2007

Abstract

The levels of interleukin 5 (IL5), IL10, and IL13 in the cerebrospinal fluid (CSF) were markedly higher in 30 patients with eosinophilic meningitis associated with angiostrongyliasis (EOMA) than in the controls ($P<0.001$). IL2, IL4, interferon γ (IFNγ), and tumor necrosis factor α (TNFα) levels were not significantly different ($P>0.05$). IL5, IL10, and TNFα levels correlated with eosinophil levels ($P=0.023$, $P=0.018$, and $P=0.005$, respectively) while IL2, IL4, IL13, and IFNγ did not ($P>0.05$). Our data suggest that local T-helper-2 (TH2) cytokine responses are predominant in the CSF of patients with EOMA. Data on T lymphocyte-parasite interactions are important for the design of effective vaccines and immunotherapies. The measurement of T-helper-1 (TH1)/TH2 cytokines in the CSF may also have some potential for the diagnosis of parasite associated meningitis.

Keywords: Angiostrongyliasis; Cerebrospinal fluid; Cytokine responses; Eosinophilic meningitis; Human; T-helper-2

1. Introduction

Human eosinophilic meningitis associated with angiostrongyliasis (EOMA) is a disease found worldwide [1]. The disease is widespread in Southeast Asia and the Pacific islands, as well as in Africa, India, the Caribbean, Australia, and North America. The major cause is the rat lungworm *Angiostrongylus cantonensis*. Many cases infected with this parasite have been reported from these areas [1–9]. Humans are accidental hosts who become infected by consuming infected larvae in snails, slugs, paratenic hosts or contaminated uncooked vegetables. These larvae migrate to the brain, spinal cord, and nerve roots causing eosinophilia in the cerebrospinal fluid (CSF) and peripheral blood. Humans rarely find host adult parasites, unlike rats that harbor 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. Infected patients experience severe headaches, vomiting, paresthesia, weakness, and occasionally visual disturbances and extraocular muscle paralysis. Most patients recover fully, although heavy infection can lead to chronic, harmful disease and even death [9].

Several reports already documented the antibody responses in *A. cantonensis*-infected hosts [10–15] as well as the different IgG antibody subclass responses in infected individuals [16,17]. Systemic and local T-helper-2 (TH2) cytokine responses, particularly involving interleukin 5 (IL5), are predominant in *A. cantonensis*-infected mice and IL5 is an important cytokine underlying the innate resistance of mice against the worm [18]. IL5 can stimulate the function

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doi:10.1016/j.jns.2007.09.023

of mature eosinophils and act as a potent and selective chemoattractant for eosinophils [19]. However, little is known about cytokine responses in the CSF of human EOMA. Thus, the objective of this study was to evaluate the expression of cytokines responsible for the immune responses in the CSF of EOMA. The associations of the cytokine responses with CSF eosinophilia are also discussed. The results increase our understanding of T-helper-1 (TH1) and TH2 roles in human EOMA.

2. Patients and methods

2.1. Patients and clinical samples

The 30 EOMA patients (24 males and 6 females) who were admitted at Loei, Nampong, and Srinagarind Hospitals, Northeastern Thailand, with severe headaches after eating raw shrimps, snails or monitor lizards (*Varanus bengalensis*) were included in the study. A detailed medical history, including food habits (eating possible parasite-contaminated food), underlying diseases, previous parasitic infections, as well as current and past medication, was obtained from all patients. The clinical symptoms were recorded and the patients underwent detailed physical, neurological, and ophthalmic examinations. The mean age ± SD of the patients was 33.27 ± 11.09 years (range 11–55). The mean incubation period ± SD was 18.89 ± 19.82 days (median ± 14, range 1–90 days). The patients presented with headache (30/30; 100%), neck stiffness (8/30; 26.7%), fever (16/30; 53.3%), nausea (19/30; 63.3%), vomiting (13/30; 43.3%), paresthesia (1/30; 3.3%), and muscle weakness (1/30; 3.3%). 21 of 30 (70%) patients showed peripheral blood eosinophilia and all patients presented with eosinophils in the CSF. The diagnostic criterion of EOMA of the present study was based on finding ≥ 10% eosinophils in the CSF [7]; all CSF samples contained eosinophils ranging from 10% to 84%. The CSF leukocyte counts were elevated in all patients (mean ± SD = 817 ± 729 wbc/μL; range 48–3244 wbc/μL). 17 of 30 (56.67%) patients had elevated CSF protein (>100 mg/dL). The CSF glucose was >40 mg/dL in 22 of 30 patients (73.33%). All stains and bacterial cultures of the CSF samples turned out negative. No parasite was found in any CSF sample either. Serum VDRL tests were all non-reactive but all EOMA patients were positive for specific IgG antibody to *A. cantonensis* antigen in the CSF by enzyme-linked immunosorbent assay (ELISA) [16]. The ELISA values in the CSF for the specific IgG antibody ranged from 0.247 to 2.750 (mean ± SD = 0.486 ± 0.735). A specific *A. cantonensis* antigenic band at a molecular mass of 29 kDa was found to react with all of 30 EOMA sera by immunoblotting [15]. The CSF samples of all EOMA patients were obtained immediately after admission to the hospital during the acute phase before treatments. The samples were stored at −70°C before being used for cytokine determination.

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. All control patients were negative for specific IgG antibody to *A. cantonensis* antigen in the CSF by ELISA [16]. The specific IgG ELISA values ranged from 0.007 to 0.192 (mean ± SD = 0.04 ± 0.05). An ELISA absorbances value greater than the mean plus 2SD of the control group (0.14) was used as the cut-off limit between positivity and negativity for specific IgG antibody to *A. cantonensis* in CSF. Controls were also non-reactive for the specific *A. cantonensis* antigenic band at a molecular mass of 29 kDa by immunoblotting in the sera [15]. Their CSF samples were used to determine the cut-off value between positivity and negativity for parasite-specific antibody and cytokine determinations.

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 the parents or legal guardians of minors. During admission, all EOMA patients received a standard corticosteroid treatment as previously described [7].

2.2. ELISA and immunoblotting

The IgG antibody in the CSF was detected by ELISA as previously described [16]. For the determination of parasite-specific IgG antibody, microtiter plates were coated with young adult female *A. cantonensis* antigen. CSF at its optimal dilution of 1:50 and horseradish peroxidase conjugated monoclonal anti-human IgG antibodies (DAKO A/S, Glostrup Denmark), diluted 1:50,000 (according to checker-board titration) were added. *O*-phenylene-diamine dihydrochloride was used as substrate. The optical density (OD) was read at 492 nm with an ELISA reader (Tecan, Salzburg, Austria). The precision of the ELISA was investigated by performing the test on different days using the same pooled positive and negative reference sera, the same batch of antigen under the same conditions. Consistent data were obtained from all the tests indicating no day-to-day variation.

Immunoblotting was performed as previously described [15] for the determination of serum specific IgG antibody to the *A. cantonensis* 29 kDa antigen. Briefly, the specific 29 kDa antigen from young adult female *A. cantonensis* worms was revealed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting. The blot pattern was mixed with serum (dilution of 1:100), followed by an incubation with peroxidase conjugated goat anti-human IgG (dilution of 1:1000) (Zymed, South San Francisco, CA). Diaminobenzidine (Sigma Chemical Co., St. Louis, MO) was used as substrate.

2.3. Determination of cytokine concentrations

The IL2, IL4, IL5, IL10, IL13, interferon γ (IFNγ), and tumor necrosis factor α (TNFα) concentrations were detected using enzyme immunoassay kits with intra- and inter-