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Cerebrospinal fluid levels of cytokines in non-herpetic acute limbic encephalitis: Comparison with herpes simplex encephalitis

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ABSTRACT

Background: Recently, non-herpetic acute limbic encephalitis (NHALE) was identified as a new subgroup of limbic encephalitis. The immunological pathophysiology of NHALE is still unclear. *Methods:* We measured the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, and soluble TNF receptor 1 (sTNFR1) in the cerebrospinal fluid (CSF) of 15 patients with NHALE and 13 with herpes simplex encephalitis (HSE) by cytometric bead array or ELISA. *Results:* The CSF concentrations of IL-6 in patients with NHALE and IFN-γ, IL-6, IL-10, and sTNFR1 in HSE patients were significantly higher than those of controls ($p < 0.001$, $p = 0.004$, $p < 0.001$, $p = 0.018$, and $p < 0.001$, respectively). There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients. The CSF concentrations of IFN-γ and sTNFR1 levels of patients with HSE were significantly higher than those with NHALE ($p = 0.001$ and $p = 0.002$, respectively). *Conclusions:* CSF cytokine levels in NHALE were relatively low compared with those in HSE. These results may be related to the favorable prognosis of NHALE.

1. Introduction

In Japan, non-herpetic acute limbic encephalitis (NHALE) was identified as a new subgroup of limbic encephalitis [1–3]. The clinical picture of NHALE is similar to that of herpes simplex encephalitis (HSE). However, the disease is not caused by herpes simplex virus (HSV) infection or a paraneoplastic disease process. Many previously reported patients with NHALE had a rather favorable neurological prognosis compared to those with HSE [2,4]. There have been a few reports on the autopsy cases with NHALE [4,5]. These reports demonstrated that there were neuronal loss and severe gliosis with inflammatory cell infiltrations in the hippocampus and amygdala. The pathogenesis of NHALE is still unclear.

To investigate the immunological pathogenesis of NHALE, we determined the cerebrospinal fluid (CSF) concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, and soluble TNF receptor 1 (sTNFR1) as cytokines related to inflammation in patients with NHALE and HSE.

2. Patients and methods

Informed consent was obtained from the families of the patients and controls enrolled in this study.

2.1. NHALE

CSF samples were obtained from 15 patients with NHALE (five males and 10 females, aged from 12 to 82 years; median, 35 years) admitted to Yamaguchi University Hospital and seven collaborating research hospitals from July 1999 to February 2008 (Tables 1 and 2). The criteria for the diagnosis of NHALE were: (1) acute or subacute onset neurological disorder with limbic-associated symptoms, such as amnesia, delirium, panic, anxiety, excitation, etc., (2) negative HSV DNA in CSF by the nested polymerase chain reaction (PCR) and negative HSV antibodies in CSF determined by the enzyme-linked immunosorbent assay (ELISA), (3) lesions of the temporal lobe, especially hippocampi and amygdalae, on magnetic resonance imaging (MRI) (Fig. 1), (4) absence of malignancy, (5) no bacteria or fungi in CSF culture, and (6) the exclusion of all other neurological, vascular, metabolic, endocrine, toxic, and drug-induced disorders. CSF samples obtained during the acute stage were stored at −70 °C.

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2.2. HSE

CSF samples were obtained from 13 patients with HSE (eight males and five females, aged from 13 to 76 years; median, 61 years) admitted to Yamaguchi University Hospital and two collaborating research hospitals from October 2000 to December 2005 (Table 1). The diagnosis was based on the demonstration of HSV DNA in the CSF by nested PCR. CSF samples during acute stage were stored at −70 °C.

2.3. Control subjects

The control subjects for the CSF levels of the cytokines were 19 afebrile and non-infectious patients with neurological disorders, such as epilepsy, dementia, etc. (11 males and eight females, aged from 13 to 79 years; median, 55 years), as shown in Table 1. CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts.

2.4. Clinical data

The clinical data including age, gender, clinical symptoms on admission, CSF findings at the time of specimen collection, MRI findings during the acute stage, and clinical outcomes in patients with NHALE and HSE were investigated. The outcomes were defined as follows: (1) normal resolution, (2) mild sequelae, (3) severe sequelae necessitating help with daily life activities, and (4) death [6].

2.5. Determination of cytokine concentrations

The concentrations of CSF IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA, USA) according to the manufacturer's manual, as previously described [7–9], with modification of the data analysis using GraphPad Prism software (GraphPad Prism Software, San Diego, CA, USA). Briefly, each series of beads exhibiting discrete fluorescence intensities is coated with a monoclonal antibody against a single cytokine, and a mixture of six series of beads can detect six cytokines in one sample. A secondary phycoerythrin-conjugated monoclonal antibody stains the beads proportionally to the amount of bound cytokine. After fluorescence intensity calibration and electronic color compensation procedures, standard and test samples were analyzed with a FACScan flow cytometer equipped with CellQuest software (BD PharMingen). The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1, 2.8, 2.6, 2.6, 2.5, and 2.8 pg/ml, respectively.

The CSF concentrations of sTNFR1 were determined with a sTNFR1 ELISA kit (Bender Medsystems, Vienna, Austria), as described previously [10]. The lower detection limit for sTNFR1 was 0.05 ng/ml.

2.6. Statistical analysis

All data were log transformed to obtain an approximately normal distribution. The differences in the results between groups were analyzed with a *t*-test and the χ^2 test, and those with a *p*-value of less than 0.05 were considered significant. Correlations were analyzed using Pearson's coefficient correlation. Analyses and calculations were performed using SPSS-12.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Clinical characteristics

Clinical data of patients with NHALE are shown in Tables 1 and 2. There were no significant differences in age or gender among patients with NHALE and HSE and controls (median age, 35, 61, and 55 years, respectively). The CSF cell counts of patients with NHALE were lower than those with HSE ($p = 0.015$, 9/μl vs. 32/μl as a median). The CSF protein levels of patients with NHALE were less than those with HSE ($p = 0.003$, 33 vs. 50 mg/dl as a median). Of the 15 patients with NHALE, 9 (67%) had mild sequelae and 6 (33%) survived without sequelae. Of the 13 patients with HSE, 1 (8%) died and 12 (92%) experienced disability (54% had severe and 38% had mild sequelae).

Table 1
Clinical data of patients with NHALE, HSE, and controls

	NHALE $N = 15$	HSE $N = 13$	Control subjects $N = 19$
Age (median, range)	35 yr, 11–82 yr	61 yr, 13–76 yr	55 yr, 13–79 yr
Sex (male: female)	5:10	8:5	11:8
Comorbid conditions	—	—	Epilepsy, 9; dementia, 5; psychosis, 4; Tic, 1
Prognosis	Normal, 6; mild sequelae, 9	Mild sequelae, 5; severe sequelae, 7; death, 1	—

NHALE, non-herpetic acute limbic encephalitis; HSE, herpes simplex encephalitis.

Table 2
Clinical characteristics of the 15 patients with non-herpetic acute limbic encephalitis

No./age/gender	Main symptoms on admission	Lesions on MRI	CSF findings		Neurological prognosis
			Cell (μl)	Protein (mg/dl)	
1/34 yr/M	Amnesia, delirium	Bilateral temporal lobes	12	39	Normal
2/73 yr/F	Somnolence, convulsion	Bilateral temporal lobes	32	24	Normal
3/35 yr/M	Amnesia, convulsion	Bilateral temporal lobes	9	39	Mild amnesia
4/11 yr/M	Convulsion, delirium	Right temporal lobe	187	33	Intellectual impairment
5/18 yr/F	Convulsion	Bilateral temporal lobes	39	31	Normal
6/49 yr/F	Amnesia, convulsion	Bilateral temporal lobes	42	50	Amnesia, psychopathy
7/31 yr/F	Convulsion	Bilateral temporal lobes	0	27	Epilepsy
8/47 yr/F	Insomnia, convulsion	Bilateral temporal lobes	9	47	Normal
9/82 yr/F	Amnesia, fugue	Bilateral temporal lobes	1	39	Amnesia
10/67 yr/M	Convulsion, delirium	Bilateral temporal lobes	1	35	Amnesia
11/75 yr/F	Convulsion, enuresis	Bilateral temporal lobes	0	32	Amnesia, psychopathy
12/51 yr/M	Amnesia, convulsion	Bilateral temporal lobes	0	28	Amnesia
13/14 yr/F	Panic	Left temporal lobe	121	27	Normal
14/19 yr/F	Excitation, convulsion	Bilateral temporal lobes	8	48	Intellectual impairment, epilepsy
15/12 yr/F	Anxiety, insomnia	Bilateral temporal lobes	14	25	Normal