



## 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- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), 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- $\gamma$ , 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- $\gamma$  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.

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## 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- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), 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  $-70^{\circ}\text{C}$ .

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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  $-70^{\circ}\text{C}$ .

## 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- $\gamma$ , TNF- $\alpha$ , 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- $\gamma$ , TNF- $\alpha$ , 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  $\chi^2$  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 ( $p = 0.015$ ,  $9/\mu\text{l}$  vs.  $32/\mu\text{l}$  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

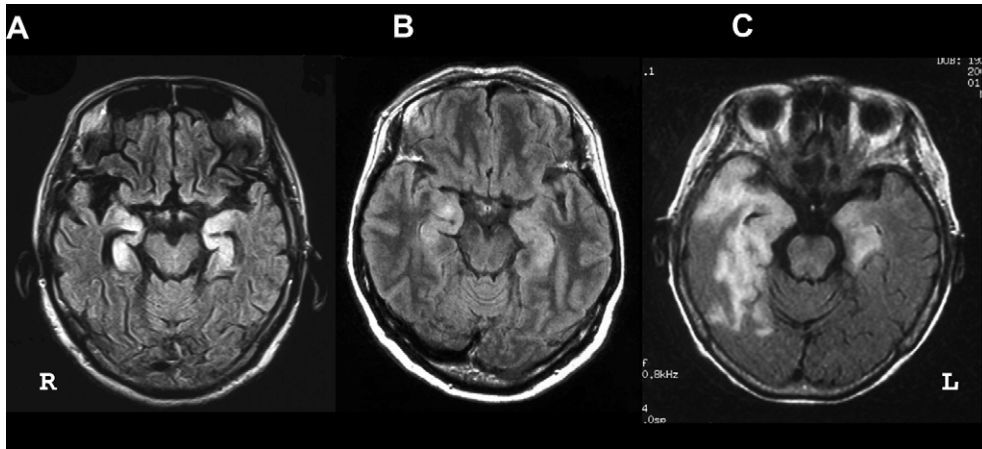
	NHALE N = 15	HSE N = 13	Control subjects N = 19
Age (median, range)	35 yr, 11–82 yr	61 yr, 13–76 yr	55 yr, 13–79 yr
Sex (male: female)	5:10	8:5	11:8
Comorbid conditions	—	—	Epilepsy, 9; dementia, 5; psychosis, 4; Tic, 1
Prognosis	Normal, 6; mild sequelae, 9	Mild sequelae, 5; severe sequelae, 7; death,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



**Fig. 1.** FLAIR MRI of Patient 2 (A), Patient 6 (B), and Patient 9 (C) demonstrated high signal intensity lesions in the bilateral temporal lobes.

**3.2. CSF concentrations of cytokines**

In patients with NHALE, the CSF concentrations of IL-6 were significantly higher than those of controls ( $p < 0.001$ ), but those of IFN- $\gamma$ , TNF- $\alpha$ , IL-2, IL-4, IL-10, or sTNFR1 were not (Fig. 2).

In patients with HSE, the CSF concentrations of IFN- $\gamma$ , IL-6, IL-10, and sTNFR1 were significantly higher than those of controls ( $p = 0.004$ ,  $p < 0.001$ ,  $p = 0.018$ , and  $p < 0.001$ , respectively), but those of TNF- $\alpha$ , IL-2, or IL-4 were not (Fig. 2). There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients (IL-6 and IL-10,  $p = 0.008$ ; IL-6 and sTNFR1,  $p < 0.001$ ; IL-10 and sTNFR1,  $p = 0.030$ ) (Fig. 3).

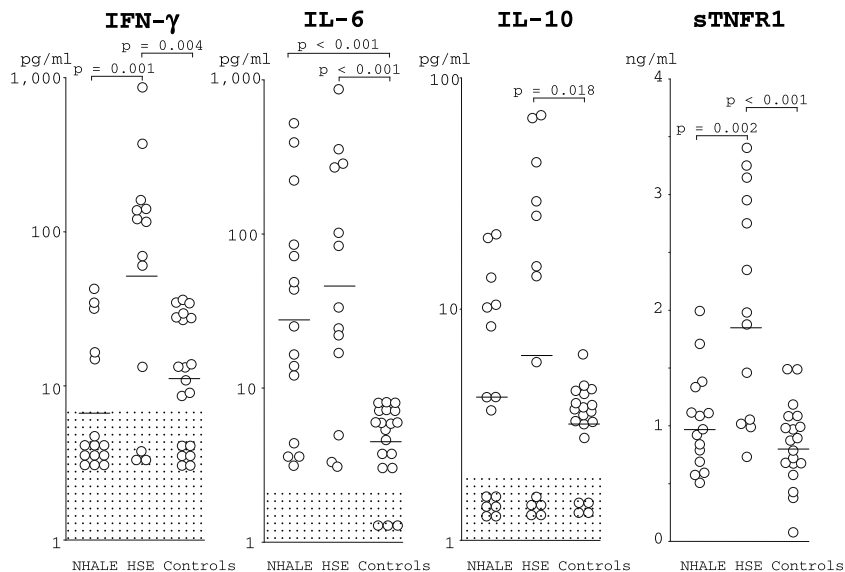
The CSF concentrations of IFN- $\gamma$  and sTNFR1 levels of patients with HSE were significantly higher than those with NHALE ( $p = 0.001$ , and  $p = 0.002$ , respectively) (Fig. 2).

**4. Discussion**

Main lesions in NHALE were in the bilateral temporal lobes, especially the hippocampus and amygdala, similar to those in HSE. However, HSV DNA or anti-HSV antibodies were not detected

in the CSF of patients with NHALE. Previous reports on autopsy cases of NHALE revealed that HSV-1 or -2 were not detected in the brain [4,5]. Therefore, NHALE has been identified as a new type of encephalitis, especially in Japan [1–4]. Several autoantibodies, including those against the *N*-methyl-D-aspartate glutamate receptor and voltage-gated potassium channel, were detected in patients with NHALE [4,11–14]. Moreover, patients with limbic encephalitis associated with autoimmune disease, including Hashimoto’s disease, Sjögren’s syndrome, and systemic lupus erythematosus, have been reported [15–17]. These previous studies suggest that NHALE is immune-mediated encephalitis.

The clinical outcomes of patients with NHALE were relatively favorable compared with those with HSE. Moreover, CSF cell counts and protein concentrations of patients with NHALE were significantly less and lower than those with HSE, suggesting that inflammation in the CNS in NHALE is milder than that in HSE. In this study, we demonstrated CSF cytokine profiles of NHALE compared with HSE. In patients with NHALE, the CSF concentrations of IL-6 were significantly higher than those of controls, but those of IFN- $\gamma$ , TNF- $\alpha$ , IL-2, IL-4, IL-10, or sTNFR1 were not. IL-6 is well-known as a cytokine that plays important roles in inflammatory re-



**Fig. 2.** The CSF concentrations of IFN- $\gamma$ , IL-6, IL-10, and sTNFR1 in patients with NHALE, HSE, and controls. Horizontal lines indicate geometric means. Shaded areas indicate values below the detection limits.

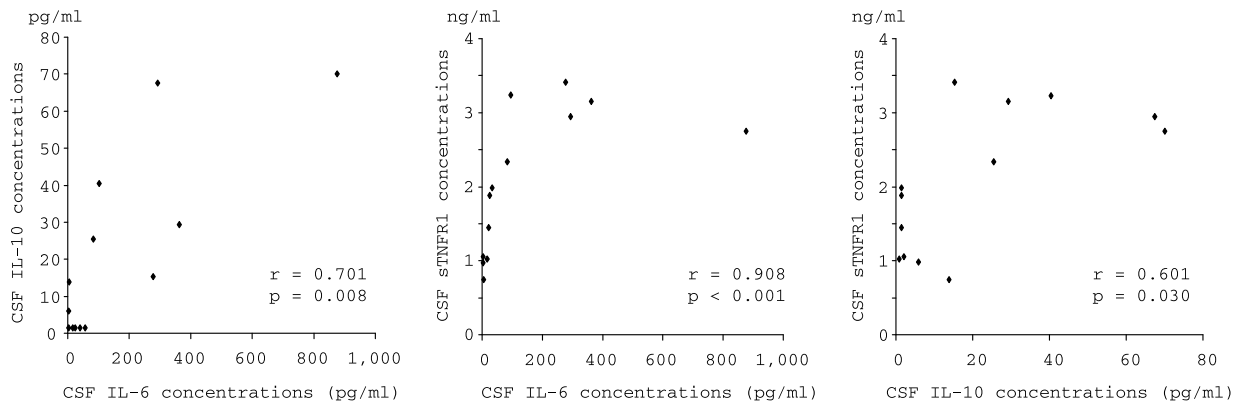


Fig. 3. The relationship among CSF IL-6, IL-10, and sTNFR1 concentrations in patients with HSE. *r*, Pearson's coefficient.

sponses [18,19]. Our results suggested that NHALE involves mild inflammation modified by IL-6 in the central nervous system (CNS). IFN- $\gamma$ , which is produced by NK cells and CD8<sup>+</sup> and Th1 type CD4<sup>+</sup> T lymphocytes, plays an important role in host defense against viral infection, and inhibits viral replication [20]. We previously demonstrated that CSF IFN- $\gamma$  levels were elevated in CNS disorders due to direct viral invasion, such as viral meningitis and HSE [2,21,22], but not in immune-mediated CNS disorders, such as acute disseminated encephalomyelitis, influenza-associated encephalopathy, acute encephalopathy following prolonged febrile seizures, and hemolytic uremic syndrome with encephalopathy [23–26]. Taking our findings into consideration, NHALE without elevated IFN- $\gamma$  levels in the CSF in this study is not caused by direct viral infection.

In patients with HSE, the CSF concentrations of IFN- $\gamma$ , IL-6, IL-10, and sTNFR1 were significantly higher than those with controls. Our present data that CSF IFN- $\gamma$  levels were elevated in HSE were consistent with a previous study [2]. There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients. In addition, CSF sTNFR1 levels in HSE were significantly higher than those in NHALE. TNF- $\alpha$  increases blood–brain vascular permeability, injures vascular endothelial cells, and induces the necrosis of myelin and oligodendrocytes [29–31]. Previous studies have suggested that TNF- $\alpha$  mediates the pathogenesis of acute encephalitis/encephalopathy [23,32–35]. It is believed that sTNFR reflects the true biological activity of TNF- $\alpha$  [36–38]. CSF sTNFR1 levels are related to the neurological prognosis in bacterial meningitis and acute encephalopathy/encephalitis [10,33]. CSF sTNFR1 levels may reflect the neurological outcome in HSE. IL-10 as an anti-inflammatory cytokine decreases the production of IL-1, IL-6, and TNF- $\alpha$  induced by an endotoxin or bacteria [27,28]. Therefore we suggest that IL-10 is induced in the CNS to modulate pro-inflammatory cytokine-mediated inflammation in the CNS of patients with HSE. Patients with HSE showed elevated pro-inflammatory and anti-inflammatory cytokines in the CSF, suggesting that there was severe inflammation in the CNS of these patients.

In conclusion, the CSF concentrations of IL-6 in patients with NHALE and IFN- $\gamma$ , IL-6, IL-10, and sTNFR1 in HSE patients were significantly higher than those in controls. Patients with HSE had many elevated cytokines in the CSF, but those with NHALE showed only an elevated CSF level of IL-6. These findings may be related to the fact that the clinical outcome of NHALE is relatively favorable compared with that of HSE.

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#### References

- [1] Kusuhara T, Shoji H, Kaji M, Ayabe M, Hino H. Non-herpetic acute limbic encephalitis. *Rinsho Shinkeigaku* 1994;34:1083–8 [In Japanese].
- [2] Asaoka K, Shoji H, Nishizaka S, Ayabe M, Abe T, Ohori N, et al. Non-herpetic acute limbic encephalitis: cerebrospinal fluid cytokines and magnetic resonance imaging findings. *Intern Med* 2004;43:42–8.
- [3] Shoji H, Asaoka K, Ayabe M, Ichiyama T, Sakai K. Non-herpetic acute limbic encephalitis: a new subgroup of limbic encephalitis? *Intern Med* 2004;43:348.
- [4] Mochizuki Y, Mizutani T, Isozaki E, Ohtake T, Takahashi Y. Acute limbic encephalitis: a new entity? *Neurosci Lett* 2006;394:5–8.
- [5] Maki T, Kokubo Y, Nishida S, Suzuki H, Kuzuhara S. An autopsy case with non-herpetic acute limbic encephalitis (NHALE). *Neuropathology*, in press.
- [6] Morishima T, Togashi T, Yokota S, Okuno Y, Miyazaki C, Tashiro M, et al. Collaborative study group on influenza-associated encephalopathy in Japan. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. *Clin Infect Dis* 2002;35:512–7.
- [7] Chen R, Lowe L, Wilson JD, Crowther E, Tzeghai K, Bishop JE, et al. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clin Chem* 1999;45:1693–4.
- [8] Cook EB, Stahl JL, Lowe L, Chen R, Morgan E, Wilson J, et al. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. *J Immunol Methods* 2001;254:109–18.
- [9] Metelitsa LS, Naidenko OV, Kant A, Wu HW, Loza MJ, Perussia B, et al. Human NKT cells mediate antitumor cytotoxicity directly by recognizing target cell CD1d with bound ligand or indirectly by producing IL-2 to activate NK cells. *J Immunol* 2001;167:3114–22.
- [10] Ichiyama T, Hayashi T, Furukawa S. Cerebrospinal fluid concentrations of soluble tumor necrosis factor receptor in bacterial and aseptic meningitis. *Neurology* 1996;46:837–8.
- [11] Kimura A, Sakurai T, Suzuki Y, Hayashi Y, Hozumi I, Watanabe O, et al. Autoantibodies against glutamate receptor  $\epsilon_2$ -subunit detected in a subgroup of patients with reversible autoimmune limbic encephalitis. *Eur Neurol* 2007;58:152–8.
- [12] Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 2004;127:701–12.
- [13] Fauser S, Talazko J, Wagner K, Ziyeh S, Jarius S, Vincent A, et al. FDG-PET and MRI in potassium channel antibody-associated non-paraneoplastic limbic encephalitis: correlation with clinical course and neuropsychology. *Acta Neurol Scand* 2005;111:338–43.
- [14] Iranzo A, Graus F, Clover L, Morera J, Bruna J, Vilar C, et al. Rapid eye movement sleep behavior disorder and potassium channel antibody-associated limbic encephalitis. *Ann Neurol* 2006;59:178–81.
- [15] Ide T, Iizuka T, Suzuki N. Limbic encephalitis associated with autoimmune diseases. *Shinkei Naika* 2003;59:31–7 [In Japanese].
- [16] Mocellin R, Walterfang M, Velakoulis D. Hashimoto's encephalopathy: epidemiology, pathogenesis and management. *CNS Drugs* 2007;21:799–811.
- [17] Stübgen JP. Nervous system lupus mimics limbic encephalitis. *Lupus* 1998;7:557–60.
- [18] Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990;265:621–36.
- [19] Tracey KJ, Vlassara H, Cerami A. Cachectin/tumor necrosis factor. *Lancet* 1989;1:1122–6.

- [20] Samuel CE. Antiviral actions of interferon. Interferon-regulated cellular proteins and their surprisingly selective antiviral activities. *Virology* 1991;183:1–11.
- [21] Matsubara T, Matsuoka T, Katayama K, Yoshitomi T, Nishikawa M, Ichiyama T, et al. Mononuclear cells and cytokines in the cerebrospinal fluid of echovirus 30 meningitis patients. *Scand J Infect Dis* 2000;32:471–4.
- [22] Ichiyama T, Maeba S, Suenaga N, Saito K, Matsubara T, Furukawa S. Analysis of cytokine levels in cerebrospinal fluid in mumps meningitis: comparison with echovirus type 30 meningitis. *Cytokine* 2005;30:243–7.
- [23] Ichiyama T, Shoji H, Kato M, Sawaishi Y, Ozawa H, Matsubara T, et al. Cerebrospinal fluid levels of cytokines and soluble tumor necrosis factor receptor in acute disseminated encephalomyelitis. *Eur J Pediatr* 2002;161:133–7.
- [24] Ichiyama T, Morishima T, Isumi H, Matsufuji H, Matubara T, Furukawa S. Analysis of cytokine levels and NF- $\kappa$ B activation in peripheral blood mononuclear cells in influenza virus-associated encephalopathy. *Cytokine* 2004;27:31–7.
- [25] Ichiyama T, Suenaga N, Kajimoto M, Tohyama J, Isumi H, Kubota M, et al. Serum and CSF levels of cytokines in acute encephalopathy following prolonged febrile seizures. *Brain Dev* 2008;30:47–52.
- [26] Shiraishi M, Ichiyama T, Matsushige T, Iwaki T, Iyoda K, Fukuda K, et al. Soluble tumor necrosis factor receptor 1 and tissue inhibitors of metalloproteinases-1 in hemolytic uremic syndrome with encephalopathy. *J Neuroimmunol* 2008;196:147–52.
- [27] Howard M, Muchamuel T, Andrade S, Menon S. Interleukin 10 protects mice from lethal endotoxemia. *J Exp Med* 1993;177:1205–8.
- [28] Paris MM, Hickey SM, Trujillo M, Ahmed A, Olsen K, McCracken Jr GH. The effect of interleukin-10 on meningeal inflammation in experimental bacterial meningitis. *J Infect Dis* 1997;176:1239–46.
- [29] Mustafa MM, Lebel MH, Ramilo O, Olsen KD, Reisch JS, Beutler B, et al. Correlation of interleukin-1 $\beta$  and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. *J Pediatr* 1989;115:208–13.
- [30] Salmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. *Ann Neurol* 1988;23:339–46.
- [31] Sato N, Goto T, Haranaka K, Satomi N, Nariuchi H, Mano-Hirano Y, et al. Actions of tumor necrosis factor on cultured vascular endothelial cells: morphologic modulation, growth inhibition, and cytotoxicity. *J Natl Cancer Inst* 1986;76:1113–21.
- [32] Aurelius E, Andersson B, Forsgren M, Sköldenberg B, Strannegård O. Cytokines and other markers of intrathecal immune response in patients with herpes simplex encephalitis. *J Infect Dis* 1994;170:678–81.
- [33] Ichiyama T, Hayashi T, Nishikawa M, Furukawa S. Cerebrospinal fluid levels of soluble tumor necrosis factor receptor in acute encephalitis. *J Neurol* 1996;243:457–60.
- [34] Ichiyama T, Nishikawa M, Yoshitomi T, Hayashi T, Furukawa S. Tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$  and interleukin-6 in cerebrospinal fluid from children with prolonged febrile seizures. Comparison with acute encephalitis/encephalopathy. *Neurology* 1998;50:407–11.
- [35] Ichiyama T, Isumi H, Ozawa H, Matsubara T, Morishima T, Furukawa S. Cerebrospinal fluid and serum levels of cytokines and soluble tumor necrosis factor receptor in influenza virus-associated encephalopathy. *Scand J Infect Dis* 2003;35:59–61.
- [36] Duncombe AS, Brenner MK. Is circulating tumor necrosis factor bioactive? *N Engl J Med* 1988;319:1227.
- [37] Seckinger P, Issaz S, Dayer JM. A human inhibitor of tumor necrosis factor  $\alpha$ . *J Exp Med* 1988;167:1511–6.
- [38] Engelmann H, Novick D, Wallach D. Two tumor necrosis factor-binding proteins purified from human urine; evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptor. *J Biol Chem* 1990;265:1531–6.