Original Contribution Kitasato Med J 2011; 41: 160-164

Elevation of interleukin 8 in cerebrospinal fluid in neuropsychiatric systemic lupus erythematosus

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Objective: We investigated the levels of cerebrospinal fluid (CSF) interleukin 8 (IL-8) in neuropsychiatric manifestations in systemic lupus erythematosus (NP-SLE).
Methods: CSF samples were obtained from 48 SLE patients (35 patients with diffuse psychiatric/neuropsychological syndromes [diffuse NP-SLE] and 13 patients with neurologic syndromes or peripheral neuropathy [focal NP-SLE]) as well as from 20 patients with noninflammatory neurological diseases. CSF IL-6 and IL-8 levels were determined using MH60.BSF2 cells or by ELISA (enzyme-linked immunosorbent assay).
Results: CSF IL-8 levels were significantly elevated in focal NP-SLE as well as in diffuse NP-SLE compared with control patients. There were no significant differences in CSF IL-8 levels between diffuse and focal NP-SLE. CSF IL-8 levels were significantly higher in patients diagnosed with acute confusional state than in those with other diffuse NP-SLE. CSF IL-8 levels were significantly correlated with CSF IL-6 levels in NP-SLE.
Conclusions: These results indicate that CSF IL-8 is involved in the development of NP-SLE. These data also suggest that the common mechanism might be involved in the elevation of IL-6 and IL-8 in CSF in NP-SLE.

Key words: central nervous system, cerebrospinal fluid, interleukin, lupus psychosis

Abbreviations: ACR, American College of Rheumatology; CNS, central nervous system; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; NMDA, *N*-methyl-D-aspartate; NP-SLE, neuropsychiatric systemic lupus erythematosus; SEM, standard error of the mean

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with dysfunction in multiple organs and characterized by the presence of a variety of autoantibodies. Neuropsychiatric manifestations occur in approximately one-half of the patients with SLE and may cause substantial impairment of quality of life as well as disability.[1,2] A variety of neuropsychiatric manifestations are seen in patients with SLE. The American College of Rheumatology (ACR) developed a standardized nomenclature system for neuropsychiatric involvement in SLE (NP-SLE) in 1999, in which the central nervous system (CNS) manifestations are grouped into neurologic syndromes (focal NP-SLE) and diffuse psychiatric/neuropsychological syndromes (diffuse NP-SLE).[3] Because there are factors causing psychiatric manifestations other than SLE, the diagnosis of NP-SLE is often difficult and its treatment is challenging.[3-5]

It is noteworthy that abnormalities in cerebrospinal fluid (CSF) have been reported in patients with NP-SLE. Thus, several studies disclosed that CSF interleukin 6 (IL-6) was elevated in patients with NP-SLE.[6-8] Moreover, CSF IL-6 has been shown to be useful for the diagnosis of diffuse NP-SLE.[9] Furthermore, recent studies have revealed that CSF IL-8 was also elevated in patients with NP-SLE.[7,8] However, it remains unclear whether there might be differences in CSF IL-8 levels between patients with focal NP-SLE and those with diffuse NP-SLE. The present study was, therefore, designed to examine CSF IL-8 levels in patients with NP-SLE.

Received 24 January 2011, accepted 26 January 2011
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Patients and Methods

Patients and Samples

There were 48 patients with SLE in the present study. All the patients fulfilled the ACR 1982 revised criteria for the classification of SLE.[10] Of the 48 SLE patients, 35 patients showed diffuse NP-SLE according to the 1999 ACR definition of NP-SLE,[11] whereas 13 patients showed CNS manifestations other than diffuse NP-SLE, including neurologic syndromes and peripheral nervous system involvements (focal NP-SLE) (Table 1). All the patients with NP-SLE were hospitalized in the Teikyo University Hospital and its affiliated hospitals between January 2000 and January 2007. In addition, 20 patients with non-SLE noninflammatory neurologic diseases (6 cervical spondylosis, 6 cerebrovascular diseases, 3 neurodegenerative diseases, 2 headaches, 1 hyperventilation syndrome, 1 diabetic neuropathy, 1 hypothyroidism) were included in the study as the non-SLE control. All 68 patients gave written informed consent, and the study was approved by the institutional ethical committee of the Teikyo University School of Medicine. CSF specimens were obtained from the patients by lumbar puncture on the same day the serum samples were obtained, when the diagnosis of NP-SLE was made by neurologists and rheumatologists. These samples were kept frozen at -30℃ until they were assayed. All assays were performed without knowledge of the diagnosis or clinical presentations. Furthermore, upon entering the present study, the diagnoses of the 48 patients with NP-SLE and its classification were reconfirmed by hospital case records.

CSF IL-6 and IL-8

CSF IL-6 testing was done by ELISA (Human IL-6 US, Invitrogen, Camarillo, CA, USA; detection limit 0.104 pg/ml) or bioassay using IL-6 dependent cell line MH60.BSF2[6] without knowledge of the clinical condition of the patients when they were hospitalized for evaluation for the first time. The IL-6 values determined by MH60.BSF2 cell bioassay have been confirmed to be closely correlated with those determined by enzyme-linked immunosorbent assay (ELISA). CSF IL-8 was also examined by ELISA (Human IL-8 US, Invitrogen; detection limit 0.100 pg/ml).

Results

CSF IL-8 levels were significantly higher in patients with focal NP-SLE and diffuse NP-SLE (20.1 ± 2.6 pg/ml and 108.5 ± 37.2 pg/ml [mean ± SEM (standard error of the mean)], respectively) compared with the control patients (3.25 ± 0.8 pg/ml). There were no significant differences in CSF IL-8 levels between diffuse NP-SLE and focal NP-SLE patients (Figure 1). These results confirm the previous observation that CSF IL-8 is associated with NP-SLE.[7,8]

There are 5 different categories of psychiatric manifestations in diffuse NP-SLE, including acute confusional state, anxiety disorder, cognitive dysfunction, mood disorder, and psychosis.[3] We next examined whether or not CSF IL-8 levels might differ depending on the categories of psychiatric disorders. As shown in Figure 2, CSF IL-8 levels were significantly higher in

Table 1. Profiles of the patients studied

Diagnosis	No. of patients	Gender (male/female)	Age (mean ± SD)
Systemic lupus erythematosus (SLE)	48		
Diffuse NP-SLE	35	4/31	41.3 ± 13.9
Acute confusional state (ACS)	13		
Anxiety disorder	2		
Cognitive dysfunction	7*		
Mood disorder	11		
Psychosis	3		
Focal NP-SLE	13	2/11	39.5 ± 11.6
Cerebrovascular disease	5		
Headache	2		
Movement disorder	1		
Seizure disorder	4		
Polyneuropathy	1		
Non-SLE control	20	16/4	45.6 ± 2.2

*One patient also presented with mood disorder.

patients diagnosed with acute confusional state than in those with other psychiatric disorders, although the difference was slight (P = 0.0436).

Finally, the relationship between CSF IL-8 and CSF IL-6 was examined. As shown in Figure 3, CSF IL-8 levels were significantly correlated with CSF IL-6 (correlation coefficient [r] = 0.7246, P < 0.0001 as determined by using Spearman's rank correlation test) in 48 patients with NP-SLE. These results suggest that the common mechanism might be involved in the elevation of IL-6 and IL-8 in CSF in NP-SLE.

Discussion

Although previous studies disclosed that CSF IL-8 was elevated in patients with NP-SLE,[7,8] there was some report showing conflicting results. Thus, Katsumata et al. did not find any differences in CSF IL-8 levels between NP-SLE and control.[11] In the present study, we confirmed that CSF IL-8 was elevated in NP-SLE. CSF samples were obtained from all the patients with NP-SLE when the patients showed active disease in the present study. However, it is not clear whether all the patients showed

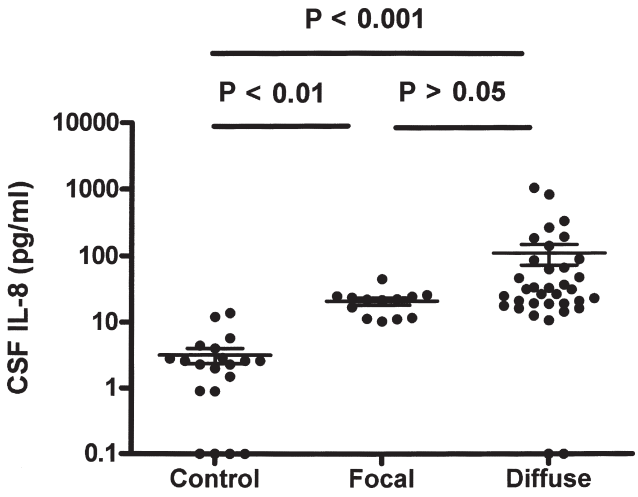

Figure 1. CSF levels of IL-8. IL-8 levels were measured in CSF from 20 patients with non-SLE noninflammatory neurologic diseases (control), 13 patients with focal NP-SLE, and 35 patients with diffuse NP-SLE. Long horizontal bars show the mean; error bars show the standard error of the mean (SEM). Statistical analysis was performed by Kruskal-Wallis test with Dunn's multiple comparison test.

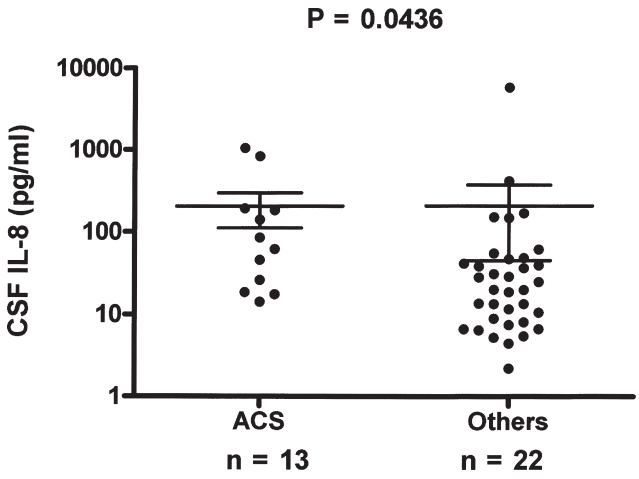

Figure 2. CSF levels of IL-8 in patients with diffuse NP-SLE. CSF IL-8 levels in 13 patients with acute confusional state were compared with those in 22 patients with other psychiatric disorders (others). Long horizontal bars show the mean; error bars show the SEM. Statistical analysis was performed using the Mann-Whitney U test.

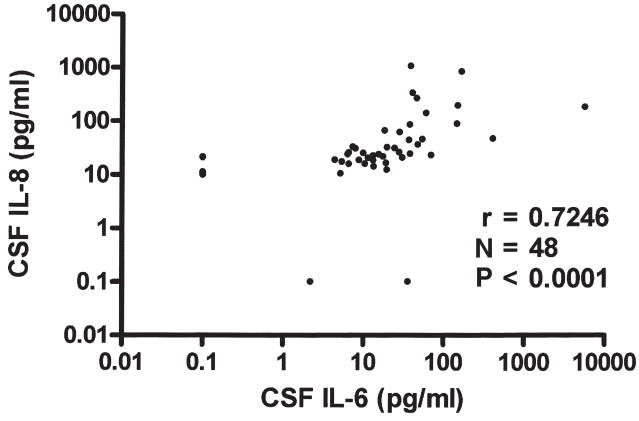

Figure 3. Correlation of CSF IL-8 levels with CSF IL-6 levels in 48 patients with NP-SLE (focal and diffuse). Statistical significance was evaluated by Spearman's rank correlation test.

active CNS disease in the study by Katsumata et al.[11] Moreover, their study did not include non-SLE control patients. Therefore, it is possible that what they call "non-CNS patients" might have actually been, or included among patients with subclinical NP-SLE. In fact, all the patients including non-CNS patients showed CSF IL-8 over 10 pg/ml in the Katsumata et al. study,[11] whereas only 2 of the 20 patients in the control group showed CSF IL-8 over 10 pg/ml in the present study. In addition, approximately 50% of the non-CNS patients in the Shiozawa et al. study showed CSF IFN-α over 10 U/ml, which is considered to be abnormal.[12]

There was no difference in CSF IL-8 between focal NP-SLE and diffuse NP-SLE in the present study. CSF IL-8 levels have been shown to be elevated in a variety of CNS diseases, including stroke.[13] Therefore, it is likely that such damages in the CNS that do not induce psychosis, such as the antiphospholipid syndrome, might result in nonspecific elevation in CSF IL-8 levels. In this regard, CSF IL-8 is comparable to CSF IL-6.[9]

CSF IL-8 levels were significantly higher in patients diagnosed with acute confusional state than in those with other psychiatric disorders, although the difference was slight. This observation reflects the fact that acute confusional state is the most severe manifestation among patients with diffuse NP-SLE. CSF IL-6 was also significantly higher in patients diagnosed with acute confusional state than in those with other psychiatric disorders (data not shown), and the difference was more remarkable compared with CSF IL-8. In this regard, Katsumata et al. showed comparable data.[11] Thus, CSF IL-6, but not CSF IL-8, was elevated in patients diagnosed with acute confusional state compared with non-acute confusional state with diffuse NP-SLE.[11]

Worthy of note, in the present study, we confirmed that CSF IL-8 levels were significantly correlated with CSF IL-6 levels in NP-SLE, in accordance with a previous study by Katsumata et al.[11] It is therefore suggested that the common mechanism might be involved in the elevation of IL-6 and IL-8 in CSF in NP-SLE. It must be pointed out that the mechanism for the elevation of CSF IL-6 and IL-8 in diffuse NP-SLE is different from that in focal NP-SLE.

The expression of autoantibodies is a hallmark of SLE. It should also be pointed out that some autoantibodies in the CSF are specifically elevated in NP-SLE, especially in diffuse NP-SLE. Thus, CSF anti-*N*-methyl-D-aspartate (NMDA) receptor NR2 antibodies have been shown to be higher in patients with diffuse NP-SLE than in patients with focal NP-SLE or non-SLE neurologic disorders.[14] It is noteworthy that previous studies have demonstrated that NMDA and ionomycin (a calcium ionophore) up-regulated IL-6 mRNA, suggesting that neurons may produce IL-6 in response to the calcium influx mediated through NMDA receptors.[15] Moreover, IL-6 dose-dependently protected neurons against NMDA toxicity.[15] We previously demonstrated that IL-6 mRNA was expressed in neurons in the granular layer of the hippocampus in a patient who died of diffuse NP-SLE.[16] It is therefore possible that anti-NMDA antibodies in CSF bind to neurons to produce IL-6. It has been revealed that human neurons also express IL-8 mRNA.[17] Therefore, neurons might express IL-8 mRNA, leading to CSF IL-8 elevation, in diffuse NP-SLE; but to confirm this point, further studies will be necessary.

Acknowledgement

Supported by 2010 research grant-in aid (C) No. 20591175 from the Ministry of Education, Culture, Science and Sports from the Japanese Government.

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