Longitudinal Analysis of Cytokines and Chemokines in the Cerebrospinal Fluid of a Patient with Neuro-Sweet Disease Presenting with Recurrent Encephalomeningitis

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Abstract

Background Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration. However, the pathogenesis of this disease remains unknown. Neutrophil and other inflammatory cell activities are influenced by many cytokines and chemokines, but to date, no studies have examined the levels of these factors in patients with NSD.

Patient and Methods The patient presented with encephalomeningitis twice in one year and was diagnosed with NSD. We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α) and chemokines (i.e., CCL2, CCL3, CCL5, CXCL8, CXCL10 and GM-CSF) in 10 CSF samples from the patient longitudinally for one year including those during two episodes of encephalomeningitis.

Results The elevations of IL-6, IFN-γ, CXCL8 (IL8) and CXCL10 (IP10) were markedly higher than the levels in uninfected control subjects with neurological disorders. The levels of these cytokines and chemokines were statistically correlated with total CSF cell counts (p <0.01).

Conclusion CD4+ helper T (Th) cells can be divided into the Th1 and Th2 subtypes according to their cytokine secretion patterns, and IFN-γ and IP10 are the Th1-type cytokine and chemokine indicating the involvement of Th1 cells in NSD. In addition, the level of IL8, a specific neutrophil chemoattractant, correlated well with the neutrophil cell counts in CSF. Our data suggest the important roles of Th1 cells and IL8 in the pathogenesis of NSD.

Key words: CXCL8 (IL-8), CXCL10 (IP-10), IL-6, IFN-γ, neutrophil cell, Th1 cell

(introduction, background, methods, results, discussion, conclusion)

Introduction

Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration (1, 2). Patients present with painful erythematous plaques on their skin and histological examination of the plaques shows dense dermal infiltration of neutrophils with no signs of vasculitis. This characteristic finding, together with HLA B51 negativity, is important in distinguishing NSD from neuro-Behçet disease (NBD) (2, 3). Japanese patients with NSD also typically show high levels of HLAs B 54 and CW1 (2).

Encephalitis and meningitis are common neurological manifestations of NSD (1). Systemic corticosteroid therapy is highly effective and most patients recover from their neurological deficits without sequelae (1, 2, 4, 5). Despite effective treatment, however, some patients have recurrent episodes indicating that more effective therapies are still needed. A clearly defined pathogenesis for NSD and reliable laboratory markers reflecting disease activity remain elusive. Here, we report the first longitudinal analysis of the levels of cytokines and chemokines in the cerebrospinal fluid...
Figure 1. Magnetic resonance images on admission. FLAIR images show high-intensity lesion in the brainstem (1-1), right thalamus and caudate nucleus (1-2) at first hospitalization, and high-intensity lesion in the cortex and subcortical white matter of the left temporal lobe at second hospitalization (1-3).

(CSF) of a patient with NSD. Our results provide important clues to the pathogenesis of NSD and may contribute to the formulation of more effective preventative NSD therapies.

Patient and Methods

Patient

At first hospitalization

A 59-year-old woman had a sore throat and a fever in late August 2005. Four days later, she visited a local hospital. She was diagnosed with acute tonsilitis, admitted to a hospital and treated with antibiotic therapy in early September 2005. The day after admission, she became drowsy and she was transferred to our hospital. She had a history of acute hepatitis B viral infection. She had a temperature of 36.6°C, a pulse of 78/min, and a blood pressure of 148/65 mm Hg. She had erythematous plaques on both legs. On neurological examination, her consciousness level was semicoma and she presented with right pupillary dilatation and delayed light reflex. The deep tendon reflexes of all four limbs were hypactive except for the bilateral Achilles tendon reflexes. Laboratory evaluation revealed increased numbers of peripheral blood leukocytes and neutrophils: white blood cell (WBC) count, 15.1×10^3/µl (normal range: 3.3×10^3–7.9×10^3/µl) and neutrophil cell count, 13.9×10^3/µl (normal range 1.5×10^3–5.9×10^3/µl). Her serum C reactive protein (CRP) level was 18.8 mg/dl (normal <0.20 mg/dl). CSF examination showed 341 cells/mm³ (mononuclear cells, 298; neutrophilic cells, 43) and a total protein concentration of 172 mg/dl. A culture of a CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus were absent and PCR analysis also showed no herpes simplex virus. A brain MRI scan showed increased signal intensities on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images in the brainstem (Fig. 1-1), right thalamus and caudate nucleus (Fig. 1-2). The electroencephalogram showed slow basic rhythm and diffuse θ activity. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Subsequently, the disturbance of consciousness became progressively worse and mechanical respiratory management was required two days after admission. She suffered a generalized tonic seizure and was treated with phenytoin. The seizures were difficult to control, however, and required treatment with the anesthetic agent propofol. Because a brain MRI scan showed increased signal intensities on T2-weighted and FLAIR images in various subcortical brain structures, a diagnosis of acute disseminated encephalomyelitis (ADEM) was suspected. Thus, four days after admission intravenous dexamethasone (12 mg/day for 5 days) was administered for 4 days and then, ten days later, methylprednisolone (1,000 mg/day for 3 days) was administered for three days. Her condition gradually improved and she did not require respiratory management. However four weeks after admission a brain MRI scan showed an abnormal signal intensity lesion in the periventricular white matter of the left parietal lobe and expansion of the brainstem lesion. Then her symptoms and abnormal brain MRI findings gradually improved and she was discharged from the hospital without any sequelae in early November 2005.

At second hospitalization

The patient had a sore throat and a fever in mid-January 2006. Five days later, she consulted an otolaryngologist and was diagnosed with acute tonsilitis. She was treated with an intravenous infusion of antibiotics. Five days later she suffered a sudden, generalized tonic seizure during infusion and was referred to our department. She had a temperature of 37.8°C, a pulse of 95/min, and a blood pressure of 153/83 mm Hg. Her throat was reddish and the palatal tonsil was swelling with velaque. Erythematous plaques were apparent on her cheek, forearms and legs. On neurological examination, she was disoriented and could not remember her name.
and birthday correctly. The deep tendon reflexes of all four limbs were hyperactive predominantly in left upper and lower limbs. She presented with bilateral Hoffman reflexes and spasticity of the lower limbs. Laboratory tests revealed increased numbers of peripheral blood leukocytes and neutrophils; WBC count, 13.7 × 10^3/μl and neutrophil count, 11.5 × 10^3/μl. Her serum CRP level was elevated at 11.3 mg/dl (normal <0.20 mg/dl). The serum rheumatoid factor and antibodies including antinuclear, anti-SS-A, anti-SS-B, anti-DNA, anti-5m, and anti-RNP antibodies, and the perinuclear anti-neutrophil cytoplasmic antibody (P-ANCA), and the cytoplasmic anti-neutrophil cytoplasmic antibody (C-ANCA) were all absent. Human leukocyte antigen (HLA) typing showed B-54 and CW1. CSF examination showed 108 cells/μl (mononuclear cells, 91; neutrophilic cells, 17), a total protein concentration of 41 mg/dl. A culture of the CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus, varicella zoster virus and toxoplasma were negative. PCR analysis also showed no herpes simplex virus. A brain MRI revealed increased signal intensity on T2-weighted and FLAIR images in the cortex and subcortical white matter of the left temporal lobe (Fig. 1-3). ^99mTc-HMPAO SPECT performed on the third day of hospitalization revealed hyperperfusion in the left temporal lobe. An electroencephalogram showed diffuse slow activity with small spikes and sharp waves in the left temporal region. There were no ocular lesions such as uveitis, episcleritis and conjunctivitis. Neither oral aphthae nor genital ulcers were observed. We performed a malignancy survey including a whole-body CT, an examination by gastrointestinal endoscopy, a bone marrow aspiration study, and a gynecological consultation, all of which showed negative results. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Her consciousness was progressively disturbed and she suffered frequent generalized tonic seizures; therefore, at ten days after admission she required propofol treatment and mechanical respiratory management. A skin biopsy of the erythema on her right forearm was performed. Histological examination showed dense dermal infiltration of neutrophils with no signs of vasculitis, and as a result she was diagnosed with Sweet’s disease. Corticosteroid therapy was initiated with an intravenous administration of methylprednisolone (1,000 mg/day for 3 days) from the tenth day of admission, followed by 50 mg of prednisolone administrated orally. Her symptoms gradually improved by the end of January 2006 she no longer required mechanical ventilation. However, she continued suffering from a slight fever, and elevated levels of CRP and WBCs without signs of infection and presented with aphasia. As a result, she was treated with a second intravenous administration of methylprednisolone (1,000 mg/day for 3 days) in early February 2006. Subsequently, her symptoms and laboratory data improved, and she was discharged from the hospital without any sequela about three weeks later.

### Methods

#### Analysis of levels of cytokines and chemokines

We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α) and chemokines (i.e., CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CXCL8/IL-8, CXCL10/IP-10 and GM-CSF) in 10 CSF samples from the patient throughout the clinical course. We also measured the levels of those cytokines and chemokines in CSF samples from the control subjects. The control subjects for cytokines were 21 noninfected patients with neurological disorders (epilepsy, 8; psychomotor delay, 5; psychogenic response, 5; functional headache, 1; myopathy, 1; agenesia of corpus callosum, 1) and the control subjects for chemokines were 10 noninfected subjects with neurological disorders (functional headache, 3; Parkinson disease, 1; normal pressure hydrocephalus, 2; spinocerebellar degeneration, 2; amyotrophic lateral sclerosis, 2). CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts. All upper values of control subjects are expressed as mean ± 3SD.

#### Determination of cytokine levels

The levels of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 in CSF were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA) as previously described (6-8), with the exception that data analysis was performed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1 pg/mL, 2.8 pg/mL, 2.6 pg/mL, 2.6 pg/mL, 2.5 pg/mL, and 2.8 pg/mL, respectively.

#### Determination of chemokine levels

The levels of CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CXCL8/IL-8, and GM-CSF were measured using ELISA kits (Endogen, Woburn, MA, USA), and the concentration of CXCL10/IP-10 was measured using an ELISA kit (R&D Systems, Minneapolis, MN, USA) on the basis of the quantitative sandwich enzyme immunoassay technique, as previously described (9). The sensitivity of these assays was 10 pg/mL.

#### Statistical analysis

The Spearman rank correlation was calculated to assess the correlation between the levels of cytokines and total CSF cell counts, and the levels of chemokines and total CSF cell counts.

### Results

#### Clinical course (Fig. 2)

Clinical manifestations and brain MRI findings correlated
well with CSF cell counts. The disease activity was divided into active and inactive phases. September 8, 2005, October 3, 2005, January 18, 2006, and January 23, 2006 correspond to the active phases.

**Cytokine levels (Table 1-1, Fig. 3)**

The levels of IL-6 and IFN-γ in CSF were statistically correlated with total CSF cell counts (p <0.01). The elevations of these cytokines were markedly higher than the lev-
Figure 3. Levels of IL-6, IFN-γ (pg/mL) and total cell count ([TCC], cells/mm³) in CSF.

Figure 4. Levels of IL-8, IP 10 (pg/mL) and total cell count ([TCC], cells/mm³) in CSF.

els in 21 uninfected subjects with neurological disorders. The levels of IL-4 and IL-10 in CSF were also statistically correlated with total CSF cell counts. However, the elevations of these cytokines were almost within normal ranges of control subjects. The levels of IL-2 and TNF-α in CSF were equal to or below the detection limits. The levels of CSF cytokines of the control subjects are shown in Table 1-1.

Chemokine levels (Table 1-2, Fig. 4)

The levels of IL-8 and IP-10 in CSF were statistically correlated with total CSF cell counts (p < 0.01). The elevations of these chemokines were markedly higher than the levels in 10 uninfected subjects with neurological disorders. The levels of other chemokines in CSF also showed various changes during the follow-up period; however, there was no significant correlation between these levels and total CSF cell counts. The levels of GM-CSF in all of the CSF samples were below the detection limits. The levels of CSF chemokines of the control subjects are shown in Table 1-2.

Correlations between level of IL-8 in CSF and neutrophilic cell counts in peripheral blood and CSF

The level of IL-8 in CSF correlated with the neutrophilic cell count in CSF (Fig. 5-1). The level of IL-8 in CSF also correlated with the peripheral neutrophilic cell count except for during the active phase (October 3, 2005) at the first time hospitalization (Fig. 5-2).

Discussion

The patient’s symptoms are compatible with probable NSD consistent with the criteria advocated by Hisanaga et al and the Neuro-Sweet Disease Study Group (2). The present patient’s clinical features are summarized according to the following findings: 1. She presented with recurrent encephalomalacia with subsequent acute pharyngitis and tonsilitis. 2. She had erythematous plaques on her cheek, forearms and legs. A histological examination of the skin biopsy revealed predominant neutrophilic infiltration of the dermis, spared epidermis, and the absence of leukocytoclastic vasculitis. 3. On HLA typing, B-54 and CW1 were positive, but B-51
Figure 5. Correlation between level of IL-8 (pg/mL) in CSF and neutrophilic cell counts ([Neutro.], cells/mm³) in CSF (5-1) and peripheral blood (5-2).

was negative. 4. Antibiotics and antiviral therapy were not effective, but systemic glucocorticoids were so effective that the neurologic symptoms and laboratory findings markedly improved. 5. She did not display cutaneous vasculitis and thrombosis, which are seen in Behçet’s disease. 6. Abnormal signal intensities on MRI were demonstrated in various CNS regions without site predilection.

The cytokines and chemokines in CSF that correlated well with the clinical state and total CSF cell counts were IL-6, IFN-γ, IL-8 and IP-10. CD4+ helper T (Th) cells can be divided into the Th1 and Th2 subtypes according to their cytokine secretion patterns (10-12). IFN-γ and IP-10, the levels of which increased in our patient, are Th1-type cytokines. Coincidentally, Th1-type cytokines have previously been implicated as mediators of the pathogenesis of Sweet’s disease (13, 14). Our data suggest an important role of Th1 cells in the pathogenesis of NSD which is Sweet disease with CNS involvement. The levels of IL-4 and IL-10, which are Th2-type cytokines, were also statistically correlated with total CSF cell counts. However, the elevations of these cytokines were almost within normal ranges of control subjects. It is known that these cytokines in turn cause a decrease in the release of the Th1-type cytokines, thereby regulating the inflammatory response. We thought that the elevations of these cytokines were induced by the elevations of the Th1-type cytokines. IFN-γ causes overexpression of adhesion molecules, responsible for neutrophilic adherence and diapedesis (13). There are multiple reports that suggest that neutrophil chemotactic dysfunction may be the basis of Sweet disease (15-17). In this study, the level of IL-8, a specific neutrophil chemoattractant, correlated with the neutrophil cell count in CSF indicating that NSD may also result from neutrophil chemotactic dysfunction. The level of GM-CSF, a neutrophil chemoattractant similar to IL-8, was below the detection limits. We were therefore unable to show any correlation for this chemokine. The increases in the levels of cytokines and chemokines in the CSF of the patient at the second hospitalization were generally higher than those at the first hospitalization. This finding may be attributed to the delay of the systemic glucocorticoid therapy at the second hospitalization.

Recently, the differences between NSD and NBD have been discussed (2, 18, 19). The present patient did not fulfill the criteria of BD (20) and the HLA type (Cw1 and B54) and histology of a skin biopsy from our patient corresponded to NSD, but not to NBD (2). There are several reports of BD that demonstrate an elevation in the levels of Th1-type cytokines in the serum of patients in the active
phases (21, 22) and in turn suggest that IL-8 could be a serological marker of disease activity (23, 24). In addition, elevated levels of IFN-γ and IL-6 in CSF are detectable in patients in the active phase of NBD (25, 26). Our cytokine data suggested that there are common aspect of pathogenesis between NSD and NBD.

Therapy with systemic glucocorticoids is usually effective in improving the neurologic symptoms in patients with NSD; however, like our patient, some patients occasionally experience recurrent episodes of neurological manifestations after glucocorticoid therapy is discontinued (1). Preventive therapies have not been established, but our study demonstrates that the levels of the Th1 cytokines, IL-6 and IL-8 in CSF are important markers of disease activity in patients with NSD. It is known that the treatment with IFN-β reduces the amount of Th1 proinflammatory cytokines and shifts the immune response toward a Th2 profile (27). Therefore, this treatment might have the potential to prevent the recurrence of NSD. We believe that these results provide useful information for clarifying the pathogenesis of NSD, which may contribute to the development of future therapeutic strategies.

This research was partially supported by a Grant-in-Aid for Young Scientists (B), 1690486 from the Japanese Ministry of Education, Culture, Sports, Science and Technology, a Grant-in-Aid for Scientific Research Ino. 1759113 and a Health and Labor Sciences Research Grant for Research on Psychiatry and Neurological Diseases and Mental Health (H18-026).

References


DOI: 10.2169/internalmedicine.47.0370