Changes in Biomarkers Focused on Differences in Disease Course or Treatment in Patients with Neuro-Behçet’s Disease

Shunsei Hirohata¹ and Hirotoshi Kikuchi²

Abstract

Objective Neurological manifestations of Behçet’s disease (NB) are serious complications. However, their pathogenesis remains unclear. The current study examined the levels of proinflammatory cytokines, including IL-1β, IL-6, IL-8 and TNF-α, in cerebrospinal fluid (CSF).

Methods CSF cytokines were measured using an enzyme-linked immunosorbent assay. CSF was obtained from 17 patients with acute NB, 19 patients with chronic progressive NB and 20 patients with non-inflammatory neurological diseases, including cerebrovascular disease, cervical spondylosis and degenerative diseases.

Results CSF total cell counts and polymorph nuclear leukocyte counts were significantly lower in the patients with chronic progressive NB than in those with acute NB. The CSF levels of IL-6 and IL-8 were markedly elevated in the NB patients compared with those measured in the control patients. There were no significant differences in the CSF levels of IL-6 and IL-8 between the patients with acute NB and those with chronic progressive NB. In contrast, there were no significant differences in the CSF levels of IL-1β and TNF-α among the control, acute NB and chronic progressive NB patients. Consistently, the CSF levels of IL-6 and IL-8 were significantly decreased following successful treatment in both acute NB and chronic progressive NB patients, whereas the CSF levels of IL-1β and TNF-α were not changed significantly. Of note, the CSF levels of IL-6 were significantly correlated with the CSF levels of IL-8 in the patients with acute NB (r =0.7647, p =0.0003) but not in the patients with chronic progressive NB (r =0.1343, p =0.5835).

Conclusion These results indicate that CSF IL-6 and IL-8 play important roles in the pathogenesis of NB. However, the data also suggest that the mechanisms underlying the elevation of CSF IL-6 and IL-8 might be different in patients with acute NB and those with chronic progressive NB.

Key words: neuro-Behçet’s disease, cerebrospinal fluid, IL-1β, IL-6, IL-8, TNF-α

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Introduction

Behçet’s disease is characterized by recurrent episodes of remission and exacerbation of various symptoms, whereas chronic sustained inflammation in certain tissues is rare (1). Central nervous system (CNS) involvement in patients with Behçet’s disease is either caused by primary neural parenchymal lesions [neuro-Behçet’s disease (NB)] or is secondary to major vascular involvement (2, 3). The most common manifestations of NB consist of different combinations of cranial nerve palsy, dysarthria, unilateral or bilateral pyramidal tract signs and ataxia with or without consciousness disturbance (4). Less common CNS manifestations include hemiparesis, cognitive-behavioral changes, emotional changes, extrapyramidal signs and seizures (3-5).

We recently disclosed that NB can be classified into acute and chronic progressive types (6). Acute NB responds to...
corticosteroid therapy and is usually self-limiting, although recurrence of attacks sometimes takes place. In contrast, the chronic progressive type of NB is characterized by intractable, slowly progressive neurobehavioral changes, ataxia and dysarthria (1, 4), with persistent marked elevations in the cerebrospinal fluid (CSF) levels of IL-6 (>20 pg/mL) (7). Of importance, chronic progressive NB is resistant to conventional treatment with corticosteroids, cyclophosphamide or azathioprine; however, it responds to low-dose weekly methotrexate (7-9).

Accumulating reports of patients with Behçet’s disease show that infliximab, an anti-TNF-α chimeric monoclonal antibody, is effective for the treatment of intractable oral-genital ulcerations (10), skin lesions (11), gastrointestinal lesions (12) and sight-threatening panuveitis in patients with Behçet’s disease (13). In addition, recent studies have also suggested the efficacy of infliximab for treating acute NB as well as recalcitrant chronic progressive NB (14, 15). However, the drug’s mechanism of action remains unclear. Of note, increasing attention has been paid to the role of proinflammatory cytokines in the pathogenesis of NB (16). The current study was performed to explore the levels of various proinflammatory cytokines in CSF obtained from patients with NB in order to delineate the roles of these cytokines in the pathogenesis of NB.

**Materials and Methods**

**Patients**

Thirty-six NB patients who satisfied the diagnostic criteria of the International Study Group for Behçet’s disease (17) and gave their informed consent were enrolled in the study (27 men and 9 women, aged 45.3±13.4 years [mean ± SD]). NB was further classified into acute NB and chronic progressive NB based on the patients’ clinical courses (3, 5-8). Acute NB was defined as acute meningoencephalitis with or without focal lesions that improved spontaneously or with treatment with corticosteroids, although there might be recurrence of the attacks, residual permanent damage or disability without progression (18). Chronic progressive NB was defined as intractable, slowly progressive neurological manifestations leading to severe disability and deterioration in spite of the administration of empirical immunotherapy (18). Of the 36 NB patients, 17 had acute NB (11 men and 6 women, aged 45.1±14.4 years) and 19 had chronic progressive NB (16 men and 3 women, aged 45.4±12.4 years). All NB patients were extensively evaluated using neurological examinations as well as laboratory tests and were found to lack features of systemic diseases other than Behçet’s disease that cause neurological manifestations, including infections, hypothyroidism, uremia, liver cirrhosis, atherosclerosis and HIV infection.

In addition, 20 patients with non-inflammatory neurologic diseases (6 with cerebrovascular diseases, 6 with cervical spondylosis, 4 with neurodegenerative diseases, 2 with headaches, 1 with hyperventilation syndrome and one with diabetic neuropathy) were studied as non-NB controls (16 men and 4 women, aged 40.1±12.3 years). All 56 patients provided their informed consent, and the study was approved by the institutional ethics committee of Teikyo University School of Medicine and was therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CSF specimens were obtained from the patients using lumbar puncture performed at the time of diagnosis of NB of either type by neurologists or rheumatologists and after the administration of successful treatment. The samples were kept frozen at -30°C until assay. All assays were performed without knowledge on the part of the investigator of the patient’s diagnosis or clinical features.

**Assay of IL-1β, IL-6, IL-8 and TNF-α**

The IL-6 activity in CSF was determined using IL-6-dependent murine hybridoma MH60.BSF2 cells, as previously described (7). The concentrations of IL-1β, IL-8 and TNF-α were determined using the Human IL-1β US, Human IL-8 US and Human TNF-α US Immunoassay kits (Invitrogen, Carlsbad, CA).

**Statistics**

Statistical significance was evaluated using the Mann-Whitney U test, the Kruskal-Wallis test with Dunn’s multiple comparison test or the Wilcoxon signed-rank test where appropriate.

### Table. Profiles of the Patients with Acute or Chronic Progressive Neuro-Behçet’s Disease

<table>
<thead>
<tr>
<th></th>
<th>Acute NB</th>
<th>Chronic progressive NB</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Age (mean±SD)</td>
<td>45.1±14.4</td>
<td>45.4±12.4</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>11:6</td>
<td>16:3</td>
</tr>
<tr>
<td>Years from the onset of Behçet’s disease (mean±SD)</td>
<td>10.4±10.1</td>
<td>8.5±5.1</td>
</tr>
<tr>
<td>Neurological manifestations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache and/or fever</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Hemiparesis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ataxia</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Dementia and/or psychiatric problems</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Incontinence</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dizziness and/or vertigo</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CSF cell count (×10³/mm³)</td>
<td>160.8±248.9</td>
<td>8.2±7.2</td>
</tr>
<tr>
<td>PMN count (×10³/mm³)</td>
<td>24.4±33.3</td>
<td>2.9±2.5</td>
</tr>
<tr>
<td>MRI findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 high intensity lesions</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Cerebrum (bgg, IC, etc.)</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Brainstem atrophy</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>No abnormalities</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

NB: neuro-Behçet’s disease, PMN: polymorphonuclear leukocyte, bgg: basal ganglia, IC: internal capsule
Results

Table summarizes the features of patients with acute or chronic progressive NB. There was a tendency toward male dominance, especially among the patients with chronic progressive NB, as is consistent with previous reports (18). Moreover, headaches and fevers were more common in the patients with acute NB, whereas ataxia and disorders of higher cerebral functions were more prevalent in the patients with chronic progressive NB. The frequencies of high intensity lesions on T2-weighted MRI images were not significantly different between the acute and chronic progressive NB patients, while brainstem atrophy was detected in more than 70% of patients with chronic progressive NB. Finally, CSF cell counts as well as CSF polymorphonuclear leukocyte (PMN) counts were significantly higher in the patients with acute NB than in the patients with chronic progressive NB (Fig. 1).

The levels of various proinflammatory cytokines in CSF were determined in the control, acute NB and chronic progressive NB patients. The levels of IL-1β, IL-6, IL-8 and TNF-α in the CSF obtained from the 20 control patients were 0.568±0.755 pg/mL [mean ± SD] 1.317±0.997 pg/mL, 3.109±3.660 pg/mL and 3.940±2.563 pg/mL, respectively. The CSF IL-6 and IL-8 levels were markedly elevated in the NB patients compared with those measured in the control patients. There were no significant differences in the CSF IL-6 and IL-8 levels between the acute and chronic progressive NB patients. In contrast, there were no significant dif-
Figure 3. Changes in the cerebrospinal fluid (CSF) levels of IL-6 and IL-8 before and after successful treatment (Tx) in 17 patients with acute neuro-Behçet’s disease (Acute NB). Statistical significance was analyzed using the Wilcoxon signed-rank test.

Figure 4. Changes in the cerebrospinal fluid (CSF) levels of IL-6 and IL-8 before and after successful treatment (Tx) in 19 patients with chronic progressive neuro-Behçet’s disease (CP NB). Statistical significance was analyzed using the Wilcoxon signed-rank test.

Figure 5. Correlation of the CSF IL-6 levels and the CSF IL-8 levels in patients with acute neuro-Behçet’s disease (Acute NB) and in patients with chronic progressive NB (CP NB). Statistical significance was analyzed using the Spearman’s rank correlation test.

ferences in the CSF TNF-α and IL-1β levels among the control, acute NB and chronic progressive NB patients (Fig. 2). These results indicate that the CSF IL-6 and IL-8, but not the IL-1β or TNF-α, levels are associated with both acute and chronic progressive NB.

Most patients with acute NB were successfully treated with corticosteroids, whereas the patients with chronic progressive NB were treated with low-dose methotrexate alone (13 patients) or with methotrexate and infliximab (6 patients) to prevent the progression of neurological manifestations. The CSF IL-6 and IL-8 levels were significantly decreased after successful treatment in both the acute (Fig. 3) and chronic progressive NB groups (Fig. 4). In contrast, neither the CSF IL-1β levels nor the CSF TNF-α levels were changed significantly after successful treatment in the patients with acute or chronic progressive NB (data not shown). These results confirm that the CSF IL-6 and IL-8, but not the CSF IL-1β or TNF-α, levels play a pivotal role in the pathogenesis of acute and chronic progressive NB.

We next compared the CSF IL-6 and CSF IL-8 levels in each patient with acute or chronic progressive NB. As can be seen in Fig. 5, the CSF IL-6 levels were significantly correlated with the CSF IL-8 levels in the patients with acute NB ($r=0.7647$, $p=0.0003$). In contrast, there were no significant correlations between the CSF IL-6 levels and the CSF IL-8 levels in the patients with chronic progressive NB.
Figure 6. Correlation of the CSF IL-6 levels or the CSF IL-8 levels with the CSF total cell counts or polymorphonuclear leukocyte (PMN) counts in patients with acute neuro-Behçet’s disease (Acute NB) and in patients with chronic progressive NB (CP NB). Statistical significance was analyzed using a linear regression test.

- A. Correlation of IL-6 with total cell count
  - Acute NB: r = 0.5569, p = 0.0250
  - CP NB: r = 0.6106, p = 0.0059

- B. Correlation of IL-8 with polymorphonuclear leukocyte (PMN) count
  - Acute NB: r = 0.5437, p = 0.0445
  - CP NB: r = 0.4831, p = 0.0362

Discussion

The current study clearly demonstrated that the levels of IL-6 and IL-8, but not IL-1β or TNF-α, are elevated in CSF obtained from patients with NB, irrespective of the clinical types of acute and chronic progressive NB. Moreover, in this study, the CSF IL-6 and IL-8 levels were significantly decreased following successful treatment, whereas the CSF IL-1β and TNF-α levels were not changed. Although CSF IL-8 has been found to be elevated in patients with NB (16), the relationship between CSF IL-6 and NB has not been delineated. Furthermore, it remained unclear whether there might be any differences in CSF IL-8 between acute and chronic progressive NB patients. Although all patients with NB presented with abnormal elevations of CSF IL-6, approximately 20% of the patients with acute or chronic progressive NB did not show elevations of CSF IL-8. Moreover, in the patients who did not show elevations of CSF IL-8, the CSF IL-8 levels did not decrease following successful treatment. These results indicate that, among the proinflammatory cytokines, IL-6 plays the most important role in the pathogenesis of NB, as is consistent with the results of previous studies (7, 8, 19, 20).

Elevation of CSF PMN is a characteristic feature of NB (1-5). Consistently, the CSF PMN counts were elevated in the patients with acute NB as well as in the patients with chronic progressive NB. Moreover, the data also suggest that CSF IL-8 might play a role in the elevation of CSF PMN in acute and chronic progressive NB. (r = 0.1343, p = 0.5835). These results indicate that the mechanisms underlying the elevation of CSF IL-8 are different from those of CSF IL-6 in patients with chronic progressive NB, whereas CSF IL-6 and IL-8 are upregulated through a common mechanism in patients with acute NB.

The CSF IL-6 levels were significantly correlated with the CSF total cell counts in the patients with acute NB as well as in the patients with chronic progressive NB (Fig. 6A), whereas the CSF IL-6 levels were significantly correlated with the CSF PMN counts in the patients with acute NB (r = 0.8505, p = 0.0001) but not in the patients with chronic progressive NB (r = 0.3210, p = 0.1802) (data not shown). In contrast, the CSF IL-8 levels were significantly correlated with the CSF PMN counts in the patients with acute NB as well as in the patients with chronic progressive NB (Fig. 6B), whereas the CSF IL-8 levels were significantly correlated with the CSF total cell counts in the patients with chronic progressive NB (r = 0.4774, p = 0.0387) but not in the patients with acute NB (r = 0.2775, p = 0.2980) (data not shown). These results indicate that the CSF total cell counts reflect elevations in the CSF levels of IL-6, presumably through intrathecal IL-6 production, in patients with acute NB as well as in patients with chronic progressive NB. Moreover, the data also suggest that CSF IL-8 might play a role in the elevation of CSF PMN in acute and chronic progressive NB.
ing chemotaxis of PMN into the CNS. As to the differential patterns of the correlation between CSF IL-6 and CSF PMN counts and that between CSF IL-8 and CSF total cell counts in patients with acute or chronic progressive NB, further studies are needed.

Previous studies have suggested an analogy between acute NB and bacterial meningitis. CSF pleocytosis with increased numbers of neutrophils, the breakdown of the blood-brain barrier and low CSF glucose levels have been noted in patients with acute NB (4, 18). It should be pointed out, however, that the CSF IL-β and TNF-α levels as well as the CSF IL-6 and IL-8 levels are found to be markedly elevated in patients with bacterial meningitis (21, 22). The lack of elevation of either CSF IL-1β or TNF-α in patients with NB, as shown in the present study, clearly indicate that the pathogenesis of acute NB is completely different from that of bacterial meningitis.

Recent studies have revealed that the CSF levels of IL-6 and IL-8 are significantly elevated in patients with neuro-myelitis optica (NMO) compared with that observed in patients with multiple sclerosis or non-inflammatory neurological diseases (23). None of our patients with NB exhibited optic neuritis or spinal cord lesions. Therefore, the difference between NB and NMO remains unclear, although both diseases involve elevations of CSF IL-6 and IL-8. Presumably, the mechanisms underlying the elevations of CSF IL-6 and IL-8 are different between these diseases. In this regard, anti-aquaporin 4 antibodies have been shown to play a pivotal role in the pathogenesis of NMO (23). However, there is no evidence for the involvement of B cells in NB (24). Although it is possible that Th17 might be involved in the pathogenesis of NB, we could not detect CSF IL-17 in the patients with acute or chronic progressive NB in the present study (data not shown). Further studies are required to clarify the mechanisms underlying the elevations of CSF IL-6 and IL-8.

The CSF IL-6 levels were significantly correlated with the CSF IL-8 levels in the patients with acute NB but not in the patients with chronic progressive NB. The data indicate that the mechanisms underlying the elevation of CSF IL-8 are different from those of CSF IL-6 in patients with chronic progressive NB. It should be noted that the CSF cell counts were significantly lower in the patients with chronic progressive NB than those observed in the patients with acute NB, although there were no significant differences in the CSF IL-6 levels, as is consistent with the results of previous studies (18). Taken together, these findings strongly suggest that the pathogenesis of chronic progressive NB might be different from that of acute NB. It should be noted that the frequencies of HLA-B51 and cigarette smoking are extremely high in patients with chronic progressive NB (18, 25). On the other hand, recent studies have revealed that cigarette smoking induces the production of cytokines, including IL-6, from CD8+ T cells in patients with chronic obstructive lung disease (26). Since MHC class I antigens are involved in antigen presentation to CD8+ T cells, it is possible that the continuous activation of CD8+ T cells by cigarette-related antigens in the context of HLA-B51 leads to sustained production of IL-6 in patients with chronic progressive NB. Further studies are required to delineate this issue.

Previous studies have shown that low-dose methotrexate therapy has beneficial effects in the treatment of patients with chronic progressive NB who present with persistent marked elevations of CSF IL-6 (8, 9). Accordingly, in this study, the CSF IL-6 levels significantly decreased following successful treatment with methotrexate. However, it should be pointed out that there were a fraction of patients who did not adequately respond to methotrexate and who deteriorated with persistent elevations of CSF IL-6. Interestingly, it has been reported that patients with chronic progressive NB refractory to methotrexate respond to treatment with infliximab (27). More importantly, administration of infliximab decreases the CSF IL-6 levels without affecting the TNF-α levels in these patients (27). This suggests that the mechanisms underlying decreases in the levels of CSF IL-6 might not be accounted for by neutralization of TNF-α. The lack of elevation of CSF TNF-α in patients with chronic progressive NB shown in the present study further supports this conclusion.

It has been suggested that one of the mechanisms of action of infliximab involves cytotoxic effects on monocytes/macrophages in corporation with complements (28). In fact, anti-tumor necrosis factor therapy induces apoptosis of macrophages (29). Moreover, infliximab significantly increases apoptosis in activated monocytes through transmembrane TNF reverse signaling and inhibits the constitutive activation of NFκB, resulting in suppression of the production of IL-1β (30). It is highly likely that infliximab suppresses IL-6 production via a similar mechanism. On the other hand, one of the characteristic histopathological features of NB is scattered perivascular cuffing of CD45RO+ T cells and CD68+ activated monocytes (24), which may contribute to persistent elevation of CSF IL-6. It is therefore likely that infliximab acts on activated monocytes and suppresses their production of IL-6, although further studies are needed to clarify this point.

Accumulating evidence suggests that, among a variety of cytokines, IL-6 is the most important in the pathogenesis of NB. Recently, a number of studies indicated beneficial effects of tocilizumab on IL-6 receptor inhibition in the treatment of various rheumatic diseases, including rheumatoid arthritis (31). In addition, tocilizumab was recently shown to be effective in a patient with Behçet’s disease who had recalcitrant uveitis resistant to infliximab (32). It is therefore possible that tocilizumab may be a potential addition to the weapons against NB (33), although further clinical trials are required to confirm the drug’s efficacy in patients with NB.

The authors state that they have no Conflict of Interest (COI).
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References