Chapter 6
Cytokines and Chemokines

Taku Yoshio and Hiroshi Okamoto

Abstract Neuropsychiatric syndromes of systemic lupus erythematosus (NPSLE) is a life-threatening disorder and early diagnosis and proper treatment are critical for the management of patients with this disease. Brain magnetic resonance imaging, electroencephalogram, neuropsychological tests and routine cerebrospinal fluid (CSF) examination are used clinically for the diagnosis of NPSLE. In addition to these tests, cytokine and chemokine levels in the CSF have been reported as useful diagnostic markers of NPSLE. This chapter provides an overview of the roles of cytokines and chemokines in NPSLE.

Keywords NPSLE · Cytokines · Chemokines · BBB · CSF · IL-6 · IL-8 · MCP-1 · IP-10 · G-CSF · TNF-α · IL-10 · IFN-α · RANTES · Fractalkine · The IP-10/MCP-1 ratio

6.1 Introduction

Systemic lupus erythematous (SLE) is an autoimmune disease characterized by widespread immunologic abnormalities and multi-organ involvement, including the skin, joints, and kidney, as well as the peripheral and central nervous systems (CNS). Neuropsychiatric syndromes in systemic lupus erythematosus (NPSLE) may occur at any time during the course of the disease, and symptoms are extremely diverse, ranging from depression, psychosis, and seizures to stroke [1]. The origin of minor clinical symptoms, such as headaches and mood swings are not specific for NPSLE. In fact, SLE patients may be under the influence of other conditions...
capable of causing neuropsychiatric symptoms, such as infections, severe hyperten-
sions, metabolic complications, steroid psychosis, and other drug toxicities [2].
Without proper treatment, neuropsychiatric involvement in SLE is known to
increase morbidity and mortality, and therefore the availability of beneficial treat-
ments increases the need for the early recognition of neuropsychiatric manifesta-
tions in SLE. Along with more specific diagnostic tools and an effective method of
monitoring disease activity, therapeutic responses are crucial in the management of
NPSLE. Currently, tests for diagnosing NPSLE include brain magnetic resonance
imaging, electroencephalogram, neuropsychological tests and routine cerebrospinal
fluid (CSF) examination. The results of these tests are reported to be abnormal in
some but not all patients, and therefore none of the findings are specific for
NPSLE. The large discrepancy in the reported frequency of neuropsychiatric
involvement in SLE patients (14–75%) further proves there is no single confirma-
tory diagnostic tool [3, 4].

Increased levels of proinflammatory cytokines and chemokines have been
reported in the CSF of patients with NPSLE. Thus, several reports have shown cyto-
kines and chemokines, such as interleukin (IL)-6, IL-1, IL-8/CXCL8, IL-10, tumor
necrosis factor (TNF)-α, interferon (IFN)-α, IFN-γ, monocyte chemotactic protein
1 (MCP-1)/CCL2, interferon-gamma inducible protein-10 (IP-10)/CXCL10,
Fractalkine/CX3CL1 and granuocyte-colony stimulating factor (G-CSF), to be
elevated intrathecally, thereby allowing these cytokines and chemokines to be used
as diagnostic tools [5–10].

Cytokines and chemokines are considered to be therapeutic targets in several
chronic inflammatory disorders such as SLE. Based on the number of recently pub-
lished studies, this chapter focuses on the use of cytokines and chemokines as bio-
markers as well as pathogenic factors in NPSLE.

6.2 The Blood-Brain Barrier

The blood-brain barrier (BBB) is a highly specialized, multi-cellular structure that
functions as a selective diffusion barrier between the peripheral circulation and the
CNS. The BBB is composed of specialized endothelial cells (ECs) that are linked
by complex tight junctions and adherens junctions. These cells are also surrounded
by astrocytes and pericytes. Under normal conditions, the specialized structure of
the BBB hinders paracellular transport of most hydrophilic compounds across the
cerebral endothelium and restricts migration of blood-borne cells into the CNS. As
a result, microglia, the resident immune cells of the CNS, are the initial responders
to pathogens or tissue damage. However, prolonged tissue insult triggers inflamma-
tory conditions that cause the BBB to lose its restrictive features, resulting in the
subsequent infiltration of peripheral immune cells.

Reactive microglia, astrocytes, and pericytes, as well as ECs, release numerous
molecules that promote invasion of peripheral immune cells into the CNS. Secreted
inflammatory mediators, including IL-8/CXCL8, MCP-1/CCL2, TNF-α, IL-1β,
recruit immune cells and stimulate the expression of adhesion molecules on ECs that participate in integrin-mediated leukocyte tethering, rolling and activation. These pro-inflammatory molecules also trigger the dynamic reorganization of junction complexes between ECs, thereby promoting the formation of paracellular gaps. Matrix metalloproteases, which are also released, degrade proteins present in the extracellular matrix and may contribute to the loss of pericytes. These events lead to an increase in the permeability of the BBB and invasion of peripheral immune cells.

6.3 Cytokines

Cytokines are small substances secreted by specific cells of the immune system which mediate local communication between cells and play important roles in the development and functioning of both the innate and adaptive immune response.

Several cytokines such as IL-1, soluble IL-2R, IL-6, IL-10, TNF-α, IFN-γ, IFN-α, and G-CSF have been reported to be elevated in the CSF from patients with NPSLE [5, 10–22]. A summary of the reported results (IL-1, soluble IL-2R, IL-6, IL-10, TNF-α, IFN-γ, IFN-α, and G-CSF) is shown in Table 6.1.

6.4 Cytokines as Biomarkers

In this section, the role and diagnostic tools of respective cytokine as a biomarker in NPSLE are described.

6.4.1 Tumor Necrosis Factor

The role of TNF-α in lupus is still controversial. TNF-α may be protective in patients with lupus, since low TNF-α activity is associated with increased disease activity. Some patients with rheumatoid arthritis who were treated with anti-TNF-α antibodies, expressed anti-double-stranded DNA antibodies, and even lupus developed in a few of these patients. By contrast, TNF-α may promote the pathogenesis of lupus, since the level of TNF-α messenger RNA was high in kidney-biopsy specimens from patients with lupus nephritis and there is a report showing that giving the anti-TNF-α antibody agent, infliximab, to six patients with lupus led to resolution of joint swelling in three patients with arthritis and a 60% reduction of urinary protein loss in four patients with renal lupus [22, 23].

There is a report studying the expression of IL-4, IL-10, TNF-α and IFN-γ in both peripheral blood lymphocytes (PBLs) and CSF from NPSLE patients whereby the authors found that mRNA for IL-10, TNF-α and IFN-γ were increased in PBLs while only IL-10 and IFN-γ were elevated in CSF [6]. Our group showed that the
mean CSF levels of TNF-α were significantly higher in the 30 patients with central NPSLE as compared to the 22 non-NPSLE patients. However, a comparison of cytokine and chemokine levels in the CSF and serum samples of 30 patients with central NPSLE from whom the CSF and serum samples were obtained at the same time showed that CSF TNF-α levels were much lower than serum TNF-α levels [10]. One report showed that CSF TNF-α levels in patients with NPSLE were higher than those in healthy controls [18], however two other reports did not show an

Table 6.1 Cytokines in CSF of NPSLE

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>NPSLE</th>
<th>Control group</th>
<th>Authors</th>
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<tbody>
<tr>
<td>IL-1</td>
<td>Increased</td>
<td>None</td>
<td>Alcocer-Varela et al. [11]</td>
<td>1992</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Same</td>
<td>Neurlogical symptoms without neurological diseases</td>
<td>Gilad et al. [12]</td>
<td>1997</td>
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<tr>
<td>Soluble IL-2R</td>
<td>Increased</td>
<td>Neurological symptoms without neurological diseases</td>
<td>Gilad et al. [12]</td>
<td>1997</td>
</tr>
<tr>
<td>IL-6</td>
<td>Increased</td>
<td>Non-NPSLE, HI</td>
<td>Jonsen et al. [13]</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>HI, neurocysticercosis</td>
<td>Jara et al. [14]</td>
<td>1998</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>Non-NPSLE</td>
<td>Hirohata and Miyamoto [15]</td>
<td>1990</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>Cerebral infarction</td>
<td>Hirohata and Hayakawa [16]</td>
<td>1999</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>None</td>
<td>Alcocer-Varela et al. [11]</td>
<td>1992</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>CNS inflammation, non-inflammatory CNS diseases</td>
<td>Tsai et al. [17]</td>
<td>1994</td>
</tr>
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<td>Trysberg et al. [5]</td>
<td>2009</td>
</tr>
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<td></td>
<td>Increased</td>
<td>HI</td>
<td>Dellalibera-Joviliano et al. [18]</td>
<td>2003</td>
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<tr>
<td></td>
<td>Increased</td>
<td>Non-NPSLE, SM, non-AID</td>
<td>Fragoso-Loyo et al. [19]</td>
<td>2007</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>Non-NPSLE</td>
<td>Yoshio et al. [10]</td>
<td>2016</td>
</tr>
<tr>
<td>IL-10</td>
<td>Same</td>
<td>Non-NPSLE, HI</td>
<td>Jonsen et al. [13]</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>HI</td>
<td>Dellalibera-Joviliano et al. [18]</td>
<td>2003</td>
</tr>
<tr>
<td>TNF-α</td>
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<td>HI</td>
<td>Dellalibera-Joviliano et al. [18]</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td>Same</td>
<td>Neurological symptoms without neurological diseases</td>
<td>Gilad et al. [12]</td>
<td>1997</td>
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<tr>
<td>IFN-α</td>
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<td>Non-NPSLE</td>
<td>Jonsen et al. [13]</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>Non-NPSLE</td>
<td>Winfield et al. [20]</td>
<td>1983</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>Non-NPSLE</td>
<td>Shiozawa et al. [21]</td>
<td>1992</td>
</tr>
</tbody>
</table>

*AID* autoimmune diseases, *HI* healthy individuals, *MS* multiple sclerosis, *SM* septic meningitis
increase of CSF TNF-α levels in patients with NPSLE [12, 19]. It is therefore uncertain whether CSF TNF-α is associated with the pathogenesis of NPSLE.

### 6.4.2 Interleukin-10

Serum levels of IL-10 are consistently high in patients with lupus, and they correlate with the activity of the disease. IL-10 has a number of biologic effects, including stimulation of polyclonal populations of B lymphocytes. In fact, blocking this cytokine could reduce the production of pathogenic autoantibodies [23].

There is a report studying the expression of IL-4, IL-10, TNF-α and IFN-γ in both PBLs and CSF from NPSLE patients whereby the authors found that mRNA levels for IL-10, TNF-α and IFN-γ were increased in PBLs while only IL-10 and IFN-γ were elevated in CSF [6]. Our group showed that the mean CSF levels of IL-10 were significantly higher in the 30 patients with central NPSLE as compared to those in the 22 non-NPSLE patients. Interestingly, these results are in contrast to a previous study which demonstrated that CSF IL-10 levels were significantly lower than serum IL-10 levels [10]. One report showed that CSF IL-10 levels in patients with NPSLE were higher than those in healthy controls [18], however two other reports did not show increased CSF IL-10 levels in patients with NPSLE [13, 19]. Again, it is thus controversial whether CSF IL-10 is associated with the pathogenesis of NPSLE.

### 6.4.3 Interferon-α

Serum levels of interferon-α (IFN-α) are elevated in patients with active lupus and microarray studies showed that 13 genes regulated by IFN were up-regulated in peripheral-blood mononuclear cells from patients with lupus, as compared with healthy controls [23].

IFN-α was also detected in the CSF of patients with NPSLE, and is of particular interest in the pathophysiology of NPSLE, given its ability to promote an autoimmune response and its recognized role in the etiopathogenesis of SLE [13, 20, 21].

As SLE is an autoimmune disorder characterized by numerous autoantibodies, a pathogenetic role for autoantibodies is theoretically suspected. Immune complexes in SLE can stimulate IFN-α production and there is strong evidence in humans and in mice that IFN-α can cause neuropsychiatric manifestations. Santer DM et al. used a bioassay containing plasmacytoid dendritic cells to demonstrate that NPSLE CSF induced significantly higher IFN-α compared with CSF from patients with multiple sclerosis or other autoimmune disease controls [24]. When normalized for IgG concentration, NPSLE CSF was 800-fold more potent at inducing IFN-α compared with paired serum, due to inhibitors present in serum. In addition to IFN-α, immune complexes formed by CSF autoantibodies produced significantly increased levels of IP-10/CXCL, IL-8/CXCL8 and MCP-1/CCL2. From these results they proposed a
two-step model of NPSLE whereby CSF autoantibodies bind to antigens released by neurocytotoxic antibodies or other brain cell injury, and the resulting immune complexes stimulate IFN-α, proinflammatory cytokines and chemokines [24].

Indirect support for the role of IFN-α in NPSLE comes from the untoward side effects of this cytokine when used as a therapeutic modality for treatment of hepatitis or malignancy, with approximately one third of patients receiving IFN-α exhibiting CNS symptoms [25]. Although depression is the most common feature, other symptoms, such as psychosis, confusion, mania, and seizures have also been reported. Of note, IL-6 may potentiate the depressive propensity of IFN-α, as high serum levels of IL-6 prior to administration of IFN-α has been reported to predict the development of depression [26].

6.4.4 Interleukin-6

Among reported cytokines, IL-6 has been shown to have the strongest positive association with NPSLE. An exhaustive study of cytokines and chemokines recently reported that IL-6 and IL-8/CXCL8 were elevated in NPSLE compared with non-NPSLE and non-autoimmune disease patients [19]. This study also found that IL-2, IL-4, IL-10, TNF-α, and IFN-γ were low in all groups examined [19]. In other reports, no association was found between IL-2, IL-6, IL-10, TNF-α and IFN-γ with NPSLE [12, 13]. A recent study showed that the sensitivity and specificity of CSF IL-6 for diagnosis of lupus psychosis was 87.5% and 92.3%, respectively, indicating that CSF IL-6 might be an effective marker for the diagnosis of lupus psychosis [27].

More recently, a comparison of cytokine and chemokine levels in the CSF and serum samples of 30 patients with central NPSLE from whom the CSF and serum samples were obtained at the same time, suggested that the intrathecal concentrations of IL-6, IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCR2 and G-CSF were not influenced by the serum concentrations in patients with central NPSLE [10]. These data indicated that production of these cytokines and chemokines might take place in the CNS. To confirm the role of these small molecules in the pathogenesis of NPSLE, the levels of IL-6, IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCL2 and G-CSF in the CSF from 30 patients with central NPSLE were compared with those in 22 patients with non-NPSLE. The mean levels of CSF IL-6, IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCL2 and G-CSF were significantly higher in 30 patients with central NPSLE as compared to those in 22 patients with non-NPSLE [10]. Importantly, the largest differences occurred in the level of IL-6 in the CSF [10]. Thus IL-6 in the CSF might be the most useful diagnostic marker of central NPSLE among the cytokines and chemokines investigated.

IL-6 levels in the CSF of NPSLE were reported to be elevated without damage of the BBB. In addition, the expression of IL-6 mRNA was elevated in the hippocampus and cerebral cortex, suggesting that IL-6 expression was increased within the entire CNS of NPSLE [15, 16].
On the other hand, our group demonstrated the *in vitro* activation of human ECs by anti-NR2 glutamate receptor antibodies (anti-NR2) (the enhanced production of cytokines such as IL-6 and IL-8/CXCL8 and the up-regulated expression of adhesion molecules such as ELAM-1, ICAM-1 and VCAM-1) through the activation of the NF-kB pathway [28]. Consistently, the production of IL-6, IL-8, IP-10, MCP-1 and G-CSF by ECs has been reported [28–31]. Therefore, the BBB damage might be caused by autoantibodies such as anti-NR2 or antiribosomal P protein antibodies that react with ECs in NPSLE patients. Thus, this damage in ECs of the BBB leads to the increased concentrations of IL-6, IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCR2 and G-CSF in the CSF, allowing access to the CNS by autoantibodies, immune complexes and immune cells such as leukocytes in the circulation, resulting in inflammation in the brain.

In addition, intrathecal production of these cytokines and chemokines by neuronal or glial cells might also take place. Furthermore, these cytokines and chemokines might increase the permeability of the BBB, thus providing access to the CNS for autoantibodies, immune complexes and immune cells such as leukocytes in the circulation. It is conceivable that both the degree of the BBB dysfunction and the type and titer of autoantibodies might be the determining factors in the development of certain diffuse NPSLE, such as psychosis and acute confusional state.

The TNF family ligands BAFF (B-cell activating factor of TNF family) and APRIL (a proliferation-inducing ligand) are essential for B-cell proliferation, differentiation and function. Intrathecal IL-6 in NPSLE is associated with the CSF immunoglobulin (Ig) G Index, a measurement of intrathecal IgG production, suggesting that IL-6 in concert with BAFF and APRIL, which are also elevated in CSF from patients with diffuse NPSLE, may increase B-cell activation within the CNS [32].

Elevated serum levels of BAFF and APRIL have been reported in patients with SLE. Recently BAFF and APRIL were studied in the CSF of NPSLE patients. They found that levels of APRIL in CSF were more than 20-fold higher and levels of BAFF in CSF were more than 200-fold higher than those of healthy controls [33]. Comparing the levels of APRIL in CSF between NPSLE and non-NPSLE patients, enhanced levels of APRIL were noted in NPSLE. Moreover, they found that CSF levels of APRIL correlated with BAFF but not with IL-6 [33].

There is a report regarding the association between cytokine levels and acute confusional state (ACS) of NPSLE [32]. The authors performed a prospective study using a cohort of 59 patients with SLE and compared patients with and without ACS as well as associations between ACS and each CSF test (IL-6, IL-8/CXCL8, IFN-α, IgG index, and Q-albumin). In this study, ACS was diagnosed in 10 patients (ACS group), NPSLE except ACS in 13 patients, and non-NPSLE in 36 patients (non-NPSLE group). CSF IL-6 levels in the ACS group were significantly higher than those in the non-NPSLE group (p < 0.05) and a positive IgG index (p = 0.028) was significantly associated with ACS. No other test showed a significant association with ACS. The positive and negative predictive values for the diagnosis of ACS in SLE were 80% and 85% for elevated CSF IL-6 levels (greater than 31.8 pg/ml), and 75% and 83% for the IgG index, respectively. From these results, the authors concluded that no single CSF test had sufficient predictive value to diagnose ACS in SLE, although CSF IL-6 levels and the IgG index showed statistical associations with ACS [32].
Increased levels of intrathecal IL-6 have been reported in numerous inflammatory conditions, such as other autoimmune diseases (Neuro-Behçet’s syndrome, mixed connective tissue disease) and neurologic conditions such as CNS infections, cerebrovascular events and myelitis [34, 35]. Therefore, the possibility of these conditions must be excluded to confirm that a CSF IL-6 elevation is indeed attributed to NPSLE.

6.4.5 **Granulocyte-Colony Stimulating Factors**

Recently the results in comparison of granulocyte-colony stimulating factors (G-CSF) levels in the CSF and serum samples of 30 patients with NPSLE, in whom the CSF and serum samples were obtained at the same time, suggested that in the patients with NPSLE the intrathecal concentrations of G-CSF were not influenced by the serum concentrations, indicating that production of G-CSF might take place in the CNS [10]. Furthermore, the mean level of CSF G-CSF was significantly higher in 30 patients with NPSLE than that in 22 patients with non-NPSLE [10]. Recently CSF G-CSF levels have been reported to be significantly higher in patients with neuromyelitis optica than in patients with other non-inflammatory neurological diseases [36]. G-CSF has been shown to be released from ECs [29] and to pass across the intact BBB [37]. Besides its role in hematopoiesis, G-CSF could also act as a neurotrophic factor, inducing neurogenesis, as well as a protein to counteract apoptosis. These properties play a major role in the development of treatments for neurological diseases such as cerebral ischemia [37, 38]. Taken together, it is suggested that G-CSF might act to treat the damaged CNS intrathecally in patients with NPSLE.

6.5 **Chemokines**

Chemokines are chemoattractant cytokines which play key roles in the accumulation of inflammatory cells at the site of inflammation. Chemokines in humans comprise more than 50 small (8-to-10-kDa) heparin-binding proteins with 20–70 percent homology in amino acid sequences. Chemokines were originally identified by their chemotactic activity on bone marrow–derived cells [39, 40]. They are classified into at least four families according to the location of their cysteine residues. The four chemokine groups are CC, C, CXC, and CX3C, where C is a cysteine and X is any amino-acid residue, and their receptors are consequently classified as CCR, CR, CXCR, and CX3CR. The chemokine receptors are bound to the cell membrane through seven transmembrane helical segments coupled with a G-protein which transduces the intracellular signal. The two major subclasses include the CC chemokines where the cysteines are neighboring and the CXC chemokines where the cysteines are separated by one amino acid. The CXC chemokines mainly act on neutrophils and lymphocytes, whereas the CC chemokines mainly act on monocytes and lymphocytes without affecting neutrophils [41]. Fractalkine, in the CX3C family, is a cell-surface-bound protein, in which the first two cysteine residues are
separated by three amino acids. Fractalkine has potent chemoattractant activity for T cells and monocytes [42]. One characteristic feature of chemokines is the redundancy of the system. Several chemokines bind to more than one receptor and the majority of chemokine receptors have multiple ligands leading to the generation of multiple pathways directing similar cellular responses.

Several chemokines such as IL-8/CXCL8, the IP-10/CXCL10, fractalkine/CXCL1, regulated upon activation, normal T-cell expressed and secreted (RANTES)/CCL5 and monocyte chemoattractant protein (MCP)-1/CCL2 as well as IP-10/MCP-1 ratios have been reported to be elevated in the CSF from patients with NPSLE (Table 6.2) [5, 7–10, 19, 24, 43, 44].

Table 6.2 Chemokines in CSF of NPSLE

<table>
<thead>
<tr>
<th>Chemokines</th>
<th>NPSLE</th>
<th>Control group</th>
<th>Authors</th>
<th>Year</th>
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</tr>
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<td></td>
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<td>Yoshio et al. [10]</td>
<td>2016</td>
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<td>Trysberg et al. [5]</td>
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<td>Okamoto et al. [8]</td>
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</table>

AID autoimmune diseases, HI healthy individuals, MS multiple sclerosis, SM septic meningitis

6.6 Chemokines as Biomarkers

In this section, the role and diagnostic tools of each chemokine as a biomarker in NPSLE are described.

6.6.1 Monocyte Chemoattractant Protein-1/CCL2 (A Ligand of CCR2)

MCP-1/CCL2 (a ligand of CCR2) can attract monocytes, T cells, NK cells, and basophils [7, 29]. It is a high-affinity ligand for the CCR2 chemokine receptor that is constitutively expressed in monocytes but is expressed on lymphocytes only after stimulation by IL-2. Expression of CCR2 on monocytes can be down-regulated by lipopolysaccharides.
Our group and others have reported that CSF MCP-1/CCL2 levels are higher in NPSLE patients than in non-NPSLE patients [7, 19]. In addition, we reported that levels of MCP-1/CCL2 decreased after immunosuppressive treatment. Furthermore, we compared the levels of MCP-1/CCL2 among various neuropsychiatric symptoms. However, due to the paucity of sample size in some patient groups, we were unable to conclude which type of symptom was associated with the increase of CSF MCP-1/CCL2 levels in our study [7].

Recently, a comparison of MCP-1/CCL2 levels in the CSF and serum samples of 30 patients with NPSLE, in whom the CSF and serum samples were obtained at the same time, suggested that in the patients with NPSLE the intrathecal concentration of MCP-1/CCL2 were not influenced by the serum concentrations, indicating that production of MCP-1/CCL2 might take place in the CNS [10]. Furthermore, the mean level of CSF MCP-1/CCL2 was significantly higher in 30 patients with NPSLE than that in 22 patients with non-NPSLE [10].

### 6.6.2 Regulated Upon Activation, Normal T-Cell Expressed and Secreted (RANTES)/CCL5 (A Ligand of CCR1, CCR3, and CCR5)

RANTES/CCL5 is another CC chemokine which attracts monocytes, memory T cells and NK cells and is implicated in the pathophysiology of SLE, rheumatoid arthritis (RA) and multiple sclerosis [45]. Chemokine receptor CCR5 is preferentially expressed on T helper 1 (Th1) lymphocytes and has been reported to have an important role in the pathogenesis of RA. It has been reported that systemic administration of a small molecular weight antagonist of CCR5, SCH-X, suppressed the development of collagen-induced arthritis in a monkey model of RA [46]. Our group also provided evidence showing that systemic administration of TAK-779, a small molecular weight nonpeptide compound, inhibits the development of adjuvant-induced arthritis in rats [47].

Two reports showed that CSF level of RANTES/CCL5 in patients with NPSLE were higher than those in patients with non-NPSLE [5, 19]. Although the mean CSF level of RANTES/CCL5 was significantly higher in 30 patients with NPSLE than those in 22 patients with non-NPSLE, CSF RANTES/CCL5 levels were 1/100 of serum RANTES/CCL5 levels in 30 patients with NPSLE [10]. It is uncertain whether RANTES/CCL5 in the CSF contributes to the pathogenesis and appearance of NPSLE in the patients with SLE and whether the increased levels of RANTES/CCL5 in the CSF are caused by NPSLE.

### 6.6.3 Interleukin-8/CXCL8 (A Ligand of CXCR1 and CXCR2)

IL-8/CXCL8 was the first chemokine identified to be involved in leukocyte chemotaxis such as polymorphonuclear neutrophils and specific T cells [48, 49]. There are several reports showing that IL-8/CXCL8 levels in the CSF are elevated in NPSLE [19, 24].
Recently our group reported that in NPSLE patients intrathecal concentrations of IL-8/CXCL8 were not influenced by their serum concentrations, indicating that production of IL-8 might take place in the CNS [10]. Furthermore, the mean level of CSF IL-8/CXCL8 was significantly higher in 30 patients with NPSLE as compared to that in the 22 patients with non-NPSLE [10].

Our group also demonstrated that anti-NR2 induced the *in vivo* activation of human ECs, resulting in the enhanced production of cytokines such as IL-6 and IL-8/CXCL8 as well as the up-regulated expression of adhesion molecules such as ELAM-1, ICAM-1 and VCAM-1 through the activation of NF-kB pathway [28] as described elsewhere in the Sect. 6.4.4.

### 6.6.4 Interferon-Gamma Inducible Protein-10/CXCL10 (A Ligand of CXCR3)

IP-10/CXCL10 is expressed and secreted by monocytes and fibroblasts following stimulation with IFN-γ [50]. IP-10/CXCL10 is a high-affinity ligand for the CXCR3 chemokine receptor which is mainly expressed on natural killer cells and activated T cells, especially on Th1 cells. The predominance of Th1 versus Th2 cells in NPSLE patients remains unresolved. Okamoto et al. and other investigators have reported that IP-10/CXCL10 was up-regulated in the CSF of NPSLE [9, 19].

Recently a comparison of IP-10/CXCL10 levels in the CSF and serum samples of 30 patients with NPSLE, in whom the CSF and serum samples were obtained at the same time, disclosed that in the patients with NPSLE the intrathecal concentration of IP-10/CXCL10 were not influenced by their serum concentrations, indicating that production of IP-10/CXCL10 might take place in the CNS [10]. Furthermore, the mean level of CSF IP-10/CXCL10 was significantly higher in 30 patients with NPSLE as compared to that in the 22 patients with non-NPSLE [10].

Furthermore, as mentioned in the previous Sect. 6.6.3, the increased IP-10/CXCL10 in the CSF of NPSLE might be derived from the activation of ECs of the BBB, neuronal or glial cells.

### 6.6.5 Fractalkine/CX3CL1 (a Ligand of CX3CR1d)

The C chemokine family is represented by two chemokines, lymphotactin/XCL1 and SCM-1β/XCL2, whereas the CX3C chemokine family contains only one member, called fractalkine/CX3CL1 [51].

Fractalkine/CX3CL1 is synthesized by EC as a type 1 transmembrane protein which is then cleaved by proteolysis, possibly mediated by TNF-α-converting enzyme and ADAM 10, thereby yielding the soluble form of Fractalkine/CX3CL1 (sFKN). Fractalkine/CX3CL1 binds to a receptor known as CXCR1 and signals via the G protein pathway in NK cells, macrophages and a certain proportion of T cells.
Fractalkine/CX3CL1 plays important roles in the pathogenesis of RA by attracting pro-inflammatory cells, such as activated macrophages and T cells [52].

There is a report showing that levels of sFKN/sCX3CL1 were elevated in the CSF of NPSLE. In this report, both serum and CSF sFKN/sCX3CL1 levels declined along with successful treatment [43]. However, our group did not find a significant increase of sFKN/sCX3CL1 in CSF from NPSLE patients when compared with that of non-NPSLE patients [44].

6.6.6 Ratio of Two Different Chemokine Levels (The IP-10/MCP-1 Ratio)

The IP-10/MCP-1 ratio was reported to be a useful marker to detect NPSLE [8]. In this study, the IP-10/MCP-1 ratio in the NPSLE group was significantly higher than that in the non-NPSLE group (P = 0.0000014). The discriminative ability (area under the curve) of various ratios between NPSLE and non-NPSLE on Receiver Operating Characteristic (ROC) curve analysis was 0.63111 (IP-10/CXCL10), 0.67626 (MCP-1/CCL2) and 0.82672 (IP-10/MCP-1 ratio). These results supported the conclusion that CSF IP-10/MCP-1 ratios are higher in NPSLE patients than in non-NPSLE patients and that this index is a useful diagnostic marker of NPSLE [8].

6.7 Cytokines and Chemokines as Pathogenic Factors

6.7.1 Cytokines as Pathogenic Factors

Although some cytokines and chemokines are important biomarkers of NPSLE, the mechanism for the elevated levels of cytokines and chemokines is thus far unknown. As SLE is an autoimmune disorder characterized by numerous autoantibodies, a pathogenetic role for autoantibodies is theoretically suspected. Immune complexes in SLE can stimulate IFN-α and there is strong evidence in humans and in mice that IFN-α can cause neuropsychiatric manifestations as described in the section of 6.4.3 Interferon-α. Santer DM et al. used a bioassay containing plasmacytoid dendritic cells to demonstrate that CSF from patients with NPSLE induced significantly higher IFN-α production compared with CSF from patients with multiple sclerosis or other autoimmune disease controls [24]. In addition to IFN-α, immune complexes formed by CSF autoantibodies significantly increased levels of IP-10/CXCL, IL-8/CXCL8 and MCP-1/CCL2. From these results they proposed a two-step model of NPSLE whereby CSF autoantibodies bind to antigens released by neurocytotoxic antibodies or other brain cell injury, and the resulting immune complexes stimulate IFN-α and proinflammatory cytokines and chemokines [24]. Recently, our group showed that IgG anti-NR2 from SLE patients directly activated ECs through the activation of NF-κB signaling, resulting in the
up-regulation of adhesion molecules and cytokine production [28]. Thus, it is evident that autoantibodies alone can induce the production of cytokines without forming immune complexes. Further immunological studies are expected to show how autoantibodies in SLE patients work to promote the cytokine storm associated with the pathophysiology of NPSLE.

### 6.7.2 Chemokines as Pathogenic Factors

Our group reported that CSF MCP-1/CCL2 and IP-10/CXCL10 levels are higher in NPSLE patients than in non-NPSLE patients, indicating possible involvement of these chemokines in the pathogenesis of NPSLE [8, 9]. The receptor of IP-10/CXCL10, CXCR3, is predominantly expressed on natural killer cells and activated T cells, especially Th1 cells. On the other hand, the receptor of MCP-1/CCL2, CCR2, is expressed not only on activated T cells and natural killer cells but also on monocytes, basophils, and dendritic cells. CD4+ T cells populations that upregulate expression of the transcription factor RORγt can be differentiated into IL-17 producing CD4+ T cells (Th17 cells) that differ in phenotype and function from Th1 or Th2 cells. Th17 cells are thought to protect against bacteria and fungi and these cells are also involved in the pathogenesis of autoimmune diseases [50]. Interestingly, CCR2 is expressed on a subpopulation of Th17 cells which produce a large amount of IL-17 but little IFN-γ [53]. These results thus implicate the differential contribution of both CXCR3 and CCR2 signaling in the pathogenesis of NPSLE, especially on effector T cells such as Th1, Th2, and Th17 cells.

### 6.8 Summary

Although a large number of studies have been performed, the precise pathophysiology of NPSLE is not completely understood. As we described here, various cytokines and chemokines are highly expressed in the brain of NPSLE patients, and it is believed that these small molecules have important roles in the pathogenesis of NPSLE. However, the molecular mechanisms by which these molecules work in the course of the development of NPSLE have not yet been completely revealed. Cytokines and chemokines are expressed by the stimulation of NF-κB signaling as well as by signal transduction pathways involving other transcription factors [54]. As mentioned above, our group showed that IgG anti-NR2 from SLE patients direct NF-κB signaling in ECs, resulting in the up-regulation of adhesion molecules and cytokine production [28]. Therefore, autoantibodies which are characteristic feature of SLE bind to corresponding autoantigens on the EC surface and these interactions may stimulate signaling cascades, resulting in the activation of certain transcription factors (Fig. 6.1). Activation of signal transduction pathways involving these transcription factors might activate transcription and expression of cytokines and
chemokines, resulting in a cytokine/chemokine storm and the development of NPSLE pathophysiology. Further molecular studies are required to prove this proposed mode of action for cytokines and chemokines. In addition, cytokines and chemokines are considered to be therapeutic targets of NPSLE. As most of the cytokines and chemokines involved in NPSLE have pleiotrophic roles in other biological processes, inhibition of these cytokines and chemokines might invite unexpected side effects in vivo. Therefore, cooperative contribution of both clinical studies and molecular biological studies is required for the development of ideal therapeutic strategies against NPSLE.

References


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