IL-17 is elevated in cerebrospinal fluids in bacterial meningitis in children

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1. Introduction

Bacterial meningitis is potentially one of the most serious infections occurring in infants and older children. This infection is associated with a high rate of acute complications and a risk of long-term morbidity [1]. Host reactions initiated to eliminate bacterial pathogens appear to be responsible for damage to neurons surrounding the inflammatory focus. Accumulation of leukocytes is an important part of the inflammatory response to foreign pathogens, and degenerative enzymes, as well as nitric oxide and reactive oxygen intermediates released from activated neutrophils and mononuclear cells, may also contribute to the initiation of tissue injury, although the precise manner in which the recruitment of leukocytes to the site of inflammation is regulated remains unclear.

The recent development of multiplex fluorescent immunoassay now allows the simultaneous measurement of multiple cytokines and chemokines using only small volumes of material, and is therefore particularly suited to assaying cytokines in cerebrospinal fluid (CSF). Here, we measured 17 cytokines/chemokines, namely interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, interferon-γ, tumor necrosis factor-α, granulocyte colony-stimulating factor, granulocyte monocyte colony-stimulating factor, monocyte chemoattractant protein-1 and macrophage inflammatory protein-1β, were measured simultaneously in CSF supernatants. We found that, IL-17 was significantly elevated in CSF with bacterial meningitis. We believe that IL-17 plays a key role in neutrophil infiltration into CSF and neuronal protection in bacterial meningitis.

Abstract

Bacterial meningitis has a poor prognosis and neurologic complications. The present study aimed to investigate the cytokine/chemokine network in cerebrospinal fluid (CSF) from children with bacterial meningitis and aseptic meningitis. Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, interferon-γ, tumor necrosis factor-α, granulocyte colony-stimulating factor, granulocyte monocyte colony-stimulating factor, monocyte chemoattractant protein-1 and macrophage inflammatory protein-1β, were measured simultaneously in CSF supernatants. We found that, IL-17 was significantly elevated in CSF with bacterial meningitis. We believe that IL-17 plays a key role in neutrophil infiltration into CSF and neuronal protection in bacterial meningitis.

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nosed as follows: number of cells >6 leukocytes/μl, initially with predominance of neutrophils, followed by predominance of lymphomononuclear cells; normal or increased protein; normal glucose concentrations; and absence of bacteria on culture [2].

Diagnosis was made by at least two pediatric neurologists based on clinical findings and routine examinations. All specimens were collected for diagnostic tests and the remaining portions were used for our cytokine investigations. Following collection, samples were centrifuged at 1500 rpm for 5 min in order to remove cells, were divided into aliquots, and were immediately frozen on dry ice for storage at –80 °C. The Institutional Review Board of Nippon Medical School, Chiba Hokusoh Hospital approved the collection and investigation of samples and written informed consent was obtained from all subjects.

2.2. Multiplexed immunoassay

Cytokine measurement of CSF was analyzed simultaneously for 17 different cytokines and chemokines, namely IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, IFN-γ, TNF-α, G-CSF, GM-CSF, MCP-1 and MIP-1β, using the Bio-Plex Cytokine Assay System (Bio-Rad Laboratories, Inc., San Diego, CA, USA), according to the manufacturer’s instructions. Briefly, 50 μL of each CSF supernatant and various concentrations of each cytokine standard (Bio-Rad) were added to a 96-well filter plate. After 60-min incubation, the plate was washed and 25 μL of biotinylated antibody solution (Bio-Rad) was added to each well, followed by another 30-min incubation. The plate was then washed and 50 μL of streptavidin-conjugated phycoerythrin (PE; Bio-Rad) was added to each well, followed by incubation for 10 min. After a final wash, the contents of each well were resuspended in 125 μL of assay buffer (Bio-Rad) and were analyzed using Bio-Plex Array Reader (Bio-Rad). Cytokine concentrations were calculated by reference to standard curves for each cytokine derived using various concentrations of cytokine standards assayed in the same manner as CSF samples. All samples were analyzed undiluted in duplicate. All CSF samples were undiluted and all serum samples were analyzed diluted fourfold and analyzed in duplicate and all of these samples were measured at once. The lower detection limit for each cytokine in CSF was as follows: 0.30 pg/ml for IL-1β, 0.14 pg/ml for IL-2, 0.03 pg/ml for IL-4, 0.22 pg/ml for IL-5, 0.21 pg/ml for IL-6, 0.28 pg/ml for IL-7, 0.68 pg/ml for IL-8, 0.79 pg/ml for IL-10, 0.27 pg/ml for IL-12 (p70), 0.29 pg/ml for IL-13, 0.19 pg/ml for IL-17, 0.15 pg/ml for IFN-γ, 0.50 pg/ml for TNFα, 0.10 pg/ml for GM-CSF, 0.79 pg/ml for G-CSF, 0.66 pg/ml for MCP-1 and 0.59 pg/ml for MIP-1β. The lower detection limit for each cytokine in serum was one forth of CSF. Cytokine concentrations in CSF and serum were within the linear part of the standard curve. The data of undetectable concentrations of each cytokine defined as zero. We used Bio-plex Manager 4.0 software (Bio-Rad) for analysis, and defined as working range within 20% for intra-CV assay. In terms of inter-assay variability, we performed the assay at once. So there was no inter-assay variability. Percent of recovery was defined 70–130% as working range using Bio-plex Manager 4.0.

2.3. Statistical analysis

Statistical analysis was performed using the Kruskall–Wallis H test. When differences were significant, the Mann–Whitney U test was used to determine the significance of differences between each group. Uncorrected p values were corrected by multiplying them by the number of comparisons (Bonferoni–Dunn correction) to calculate corrected p values. Comparisons between serum and CSF were performed using paired Wilcoxon analysis. Fisher’s exact probability test was employed for comparison of the detection rates of cytokines and chemokines in each group.

3. Results

3.1. Patient demographic data

We analyzed CSF samples both patients with bacterial meningitis (n = 10), aseptic meningitis (n = 19) or fever without neurological complications (n = 8). Mean age of bacterial and aseptic meningitis patients showed a significant difference, as did cell number, total protein and total sugar content of CSF (Table 1). The identified bacterial species in bacterial meningitis was Hemophilus influenzae in three cases, Staphylococcus pneumonia in two cases and unknown agents with distinct suppurative manifestations in five cases. In aseptic meningitis, eight cases were mumps meningitis, and the remaining 11 cases were caused by an unknown virus. Children with fever lacking neurological complications had pneumonia (three cases), bronchitis (two cases), pericarditis (one case), urinary tract infection (one case) and gastroenterocolitis (one case).

3.2. Cytokine/chemokine profiles of CSF from bacterial meningitis, aseptic meningitis, and fever without neurological complications

Significant differences were seen in IL-4, IL-6, IL-8, IL-17, GM-CSF and MIP-1β levels in CSF from bacterial meningitis, aseptic meningitis and fever without neurological complications (Fig. 1 and Table 2). These cytokines/chemokines were highest in bacterial meningitis, followed by aseptic meningitis and fever without neurological complications. Furthermore, IL-7 and TNF-α showed higher levels in bacterial meningitis (bacterial meningitis > aseptic meningitis = fever without neural complications), while IL-2, IL-10, IL-12, IL-13 showed significantly higher levels in bacterial and aseptic meningitis when compared to fever without neurological complications (bacterial meningitis = aseptic meningitis > fever without neural complications; Fig. 1 and Table 2).

3.3. Comparison of cytokine/chemokine expression between serum and CSF

IL-1β, IL-6, IL-8 and IL-13 expression was higher in CSF than in serum of patients with bacterial meningitis (n = 4), however, not statistically significant (data not shown).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Dermographic details of subjects in the CSF.</th>
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<tr>
<td></td>
<td>Age (months)</td>
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<tr>
<td>Bacterial meningitis (n = 10)</td>
<td>25.1 ± 4.5</td>
</tr>
<tr>
<td>Aseptic meningitis (n = 19)</td>
<td>86.4 ± 15.1</td>
</tr>
<tr>
<td>Fever (n = 8)</td>
<td>17 ± 9.5</td>
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</table>

* Bacterial meningitis vs. aseptic meningitis: p < 0.001.
** Bacterial meningitis vs. aseptic meningitis: p < 0.01.
*** Bacterial meningitis vs. aseptic meningitis: p < 0.05.
4. Discussion

The mortality rate in bacterial meningitis remains high and many survivors exhibit severe neurologic sequelae as a result of irreparable destruction of nervous tissue. Several reports have demonstrated significant elevation of cytokines and chemokines, including IL-1β, IL-6, IL-8, IL-10, IL-12, TNF-α and MCP-1, in bacterial meningitis, although the entire cytokine network remains uncertain [2–6].

IL-6 is produced by monocytes, macrophages, endothelial cells, T lymphocytes and fibroblasts, which are stimulated by IL-1 and TNF and are associated with fatal outcome in meningitis [7]. IL-12 is thought to contribute to immunity against microorganisms in bacterial meningitis [8]. High levels of TNF-α in CSF are associated with seizures, whereas high levels in serum are associated with a high mortality in bacterial, but not viral, meningitis [7].

IL-4, IL-10 and IL-13 have an inhibitory effect on the production of pro-inflammatory cytokines. IL-10 also inhibits the
production of chemokines by leukocytes and down-regulates the expression of intercellular adhesion molecule 1 and chemoattrac-
tant proteins by endothelial cells, thus inhibiting leukocyte migration to the site of infection [7]. It is generally believed that pro-inflammatory responses prevail at the site of the infection, whereas the systemic response is anti-inflammatory. This idea partially conflicts with our findings of highly elevated levels of anti-inflammatory mediators (IL-4, IL-10 and IL-13) in CSF from bacterial meningitis [9]. Our results showed the simultaneous activation of intrathecal pro- and anti-inflammatory immune re-
sponses in CSF, which may reflect the specific mechanisms of host responses in the brain. There are some possibilities that these dif-
fferences of cytokines/chemokines in CSF between with bacterial and aseptic meningitis are due to age-related because the average age of both meningitis was significantly difference (25.1 months vs. 86.4 months). We could not exclude the possibility because there is no report on age depended levels of CSF cytokines/che-
mokines in healthy children.

IL-8 is produced by monocytes, macrophages, endothelial cells and fibroblasts, and is a chemotactic stimulus for neutrophils during infection. Intracerebral administration of IL-8 exerts dramatic polymorphonuclear leukocyte recruitment to the central nervous system [10], although two studies in patients with bacterial meningitis showed no relationship between IL-8 levels and granulocyte count in CSF [7], which was also seen in our study (data not shown). MIP-1β contributes to recruiting monocytes from blood
flow to CSF [11]. Injection of MIP-1 into the central nervous system compartment leads to neutrophil accumulation [12].

IL-17 is a potent pro-inflammatory cytokine produced by a subset of memory CD4+T cells. IL-17 is mainly produced by Th17 cells, although some Th1 cells produce both IL-17 and IFN [13]. Astrocytes and oligodendrocytes also express IL-17 mRNA and protein in active multiple sclerosis lesions [14]. Our study showed that IL-17 in CSF with bacterial and aseptic meningitis increased significantly, despite conflicting results in previous studies [15]. IL-17 triggers the local production of downstream cytokines and chemokines, such as IL-6, IL-8 and GM-CSF, in a variety of cells [16]. In our study, IL-6, IL-8 and GM-CSF showed a similar profile to IL-17 although there was no significant difference between serum and CSF IL-17 levels with increases of a variety of pro- and anti-inflammatory cytokines.

**Table 2** Summary of cytokines and chemokines expression in CSF with bacterial and aseptic meningitis.

<table>
<thead>
<tr>
<th>Expression pattern</th>
<th>Cytokines and chemokines</th>
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</thead>
<tbody>
<tr>
<td>Bacterial &gt; aseptic &gt; fever control</td>
<td>IL-4, IL-6, IL-8, IL-17, GM-CSF, MIP-1β</td>
</tr>
<tr>
<td>Bacterial &gt; aseptic &gt; fever control</td>
<td>IL-7, TNF-α</td>
</tr>
<tr>
<td>Bacterial &gt; aseptic &gt; fever control</td>
<td>IL-2, IL-10, IL-12, IL-13</td>
</tr>
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Bacterial, bacterial meningitis; aseptic, aseptic meningitis; fever control, fever without neurological complications.
Inflammatory cytokines/chemokines in CSF from patients with bacterial meningitis. These findings suggest that the increases in IL-6, IL-8 and GM-CSF seen in the present study may be triggered by IL-17. In addition, IL-17 stimulates the production of matrix metalloproteinases, inducible Nitric Oxide Synthetase, Nitric Oxide and Prostaglandin E2, which enhance the local inflammatory environment and lead to inflammatory destruction [13]. IL-17 also causes neutrophil recruitment mainly through the release of IL-8 and induces neutrophil activations, i.e., increases myeloperoxidase and elastase activity [17].

The increases in IL-17 and IL-8 seen in this study could lead to severe destruction of tissue by neutrophic inflammation and may be related to severity of inflammation in spinal roots and the destruction of the blood–brain barrier, as well as the formation of inflammatory of intrathecal lesions. Thus, neutrophilia in CSF may be related to the increases in IL-17 and IL-8 in CSF. On the other hand, IL-17 expression is induced by IL-23, a product of activated dendritic cells and macrophage/microglial cells [17]. The IL-23/IL-17 axis is reported to be important in host defense against Gram-positive and Gram-negative bacterial infections, as well as Mycoplasma pneumoniae infections, by recruiting neutrophils to inflammatory sites [18]. Previous reports and the present study also suggest that increased IL-17 has important protective effects in bacterial meningitis.

In conclusion, we investigated the cytokine network in bacterial meningitis and found that IL-17 may play a key role in neural destruction by neutrophil infiltration into CSF, as well as in CNS protection, in bacterial meningitis. Further investigation on the protective and/or destructive roles of IL-17 in CNS bacterial infections is thus required.

Acknowledgments

This work was supported in part by grants from the ministry of Education, Science, Sports and Culture of Japan (C-18591171).

References