Granulocyte Colony-Stimulating Factor in Cerebrospinal Fluid From Patients With Meningitis

By Kazuya Shimoda, Seiichi Okamura, Fusayuki Omori, Yumi Mizuno, Toshiro Hara, Tonomobu Aoki, Kohji Ueda, and Yoshiyuki Niho

Granulocyte colony-stimulating factor (G-CSF) in the cerebrospinal fluid from patients with meningitis was measured by our modified enzyme-linked immunosorbent assay for G-CSF. The minimal detection level was 20 pg/mL G-CSF. In patients with bacterial meningitis, the G-CSF levels in the cerebrospinal fluid were extremely elevated, showing a mean value of approximately 1,500 pg/mL. On the other hand, G-CSF levels in the cerebrospinal fluid from 67% patients with aseptic meningitis were moderately increased, showing a mean value of about 80 pg/mL, whereas G-CSF levels in 33% samples remained undetectable. The G-CSF levels and neutrophil counts in the cerebrospinal fluid were proven to be related by Spearman’s rank correlation coefficient analysis (r = .724). These elevations of G-CSF levels at inflammation sites associated with bacterial meningitis may indicate that G-CSF plays an important role in the combat of bacterial infections.

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fied air with 5% CO₂. The cerebrospinal fluids obtained from eight patients were diluted with an equal volume of either IMDM, preimmune IgG (30 µg/mL) in IMDM, or polyclonal antibodies against human rG-CSF (IgG, 30 µg/mL) in IMDM. This polyclonal antibody against rG-CSF completely inhibited the colony formation induced by rG-CSF, whereas preimmune IgG did not have any influence on it. All the colony assays were performed in quadruplicate. The number and type of colonies consisting of more than 20 cells were counted on day 7 with an inverted microscope.

Statistical analysis. A correlation between G-CSF levels and neutrophil counts in the cerebrospinal fluid was estimated by Spearman's rank correlation coefficient analysis. Statistical significance between numbers of colonies was determined by means of the Student's t-test.

RESULTS

G-CSF levels in cerebrospinal fluid from patients with meningitis. Firstly, we evaluated the ELISA for G-CSF. The ELISA, as developed using three kinds of antibodies, could detect rG-CSF in cerebrospinal fluid at more than 20 pg/mL from the standard curve. The recovery of exogenous rG-CSF in cerebrospinal fluid was studied. When two concentrations of exogenous rG-CSF (170 pg/mL and 325 pg/mL) were added to the cerebrospinal fluid from five patients whose G-CSF levels in the cerebrospinal fluid were below 20 pg/mL, the ratios of both the resulting observed and calculated concentration levels were 108% ± 11.1%, and 111% ± 5.4%, which means that most of the added rG-CSF was recovered by the ELISA without any inhibition. The within-run imprecision expressed as a coefficient of variation (CV) was calculated from 76 duplicate estimations as 18% at 20 pg/mL of G-CSF in the cerebrospinal fluid, but below 10% when the G-CSF levels in the cerebrospinal fluid lay between 46 and 2,000 pg/mL.

Figure 1 illustrates the G-CSF levels in the cerebrospinal fluid from patients with bacterial meningitis and aseptic meningitis as estimated by our ELISA. Among the patients with bacterial meningitis, 10 of the 11 patients (91%) had G-CSF levels in the cerebrospinal fluid of greater than 100 pg/mL. The mean value was approximately 1,500 pg/mL with the highest value being 5,707 pg/mL. The changes of G-CSF levels in the cerebrospinal fluid were monitored in five patients with bacterial meningitis during the clinical course of antibiotics therapy. In patients whose G-CSF levels in the cerebrospinal fluid were high before treatment, the G-CSF levels decreased with a concomitant decrease of neutrophil counts in the cerebrospinal fluid after the initiation of antibiotics therapy and they finally came to lie below the detection level (<20 pg/mL) in clinically cured states.

Among 18 patients with aseptic meningitis, G-CSF levels in the cerebrospinal fluid from 12 patients (67%) lay between 22 pg/mL and 366 pg/mL and the mean value was about 80 pg/mL, although the G-CSF levels in the cerebrospinal fluid remained below the detection level in six patients (33%). However, among 10 patients who suffered other diseases besides meningitis, all cases showed G-CSF levels in the cerebrospinal fluid of below 20 pg/mL.

G-CSF values in the cerebrospinal fluid from all the patients with meningitis are plotted in relation to the

neutrophil counts in the cerebrospinal fluid in Fig 2. The relationship between the G-CSF levels and neutrophil counts in cerebrospinal fluid was statistically significant (r = .724, P < .001). On the other hand, no clear correlation was found between G-CSF levels and the monocyte numbers in the cerebrospinal fluid in patients with meningitis.

Biologic assay of the cerebrospinal fluid on in vitro colony formation. The in vitro colony assays were performed by the addition of cerebrospinal fluids from eight patients. The age, clinical diagnosis, neutrophil counts, and the G-CSF

![Fig 1. G-CSF levels in cerebrospinal fluid from patients with meningitis. The mean G-CSF levels in cerebrospinal fluid from patients with bacterial meningitis is significantly higher than in that from patients with aseptic meningitis (P < .01).](image-url)

![Fig 2. A correlation between G-CSF levels and neutrophil counts in cerebrospinal fluid from patients with meningitis.](image-url)
levels in the cerebrospinal fluid as measured by the ELISA, in addition to the final concentrations of G-CSF in the culture well, are shown in Table 1. The numbers of colonies were significantly increased \((P < .01)\) in the presence of the cerebrospinal fluid having final G-CSF concentrations in the well of more than 180 pg/mL (Nos. 1 through 3). Granulocytic colonies were predominant. These increases were completely abrogated by the addition of polyclonal antibodies against human rG-CSF \((P < .01)\). On the other hand, the numbers of colonies in the presence of the cerebrospinal fluid having low concentrations of G-CSF (Nos. 4 through 8) did not increase.

**DISCUSSION**

It was recently reported that the serum G-CSF levels were elevated in bacterial infections accompanied with an increase in absolute neutrophil counts in the peripheral blood.\(^9\) This elevation of G-CSF would stimulate the precursor cells of the granulocytic lineage, resulting in proliferation and maturation in the bone marrow. However, G-CSF levels in sequestered space, such as cerebrospinal fluid space, have not yet been reported.

We have previously reported an ELISA specific for human G-CSF\(^12\) and we applied it for the detection of G-CSF in cerebrospinal fluid with some modifications. The high recovery rate of exogenous rG-CSF to the assay system confirmed that interference was minimum, and the minimum detection level was as low as 20 pg/mL.

Because G-CSF levels in the cerebrospinal fluid from all cases in clinically cured states of meningitis were less than 20 pg/mL, the normal values of G-CSF in the cerebrospinal fluid are considered to be below 20 pg/mL although G-CSF levels in the cerebrospinal fluid in normal healthy people were not examined.

Very high levels of G-CSF in the cerebrospinal fluid from patients with bacterial meningitis were detected by the ELISA as shown in Fig 1. We then measured the biologic activity of G-CSF in the cerebrospinal fluid using murine bone marrow colony formation assays in vitro. A significantly large number of granulocyte colonies were formed with the addition of the samples showing high G-CSF values by means of ELISA. However, the colony formation in the presence of the cerebrospinal fluid showing low G-CSF values as estimated by ELISA from patients with bacterial, aseptic meningitis, and other diseases besides meningitis did not increase. Human G-CSF, but not human GM-CSF nor human IL-3, has colony-stimulating activity of murine granulocytic precursor cells, and this activity was almost completely inhibited by the addition of polyclonal antibodies against human rG-CSF. This experiment also clearly demonstrated that the cerebrospinal fluid from patients with bacterial meningitis showing high G-CSF

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (y)</th>
<th>Diagnosis (infectious microorganism)</th>
<th>N</th>
<th>G-CSF in CSF (G-CSF in the well)</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1.4</td>
<td>Bacterial meningitis (H. influenzae)</td>
<td>3,570</td>
<td>5,707</td>
<td>21.0 ± 2.0</td>
<td>21.0 ± 3.6</td>
<td>3.8 ± 1.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(476)</td>
<td></td>
<td>(15.3 ± 4.0)</td>
<td>(14.5 ± 1.3)</td>
<td>(2.3 ± 0.5)</td>
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<tr>
<td>2</td>
<td>7.7</td>
<td>Bacterial meningitis (Meningococcal)</td>
<td>300</td>
<td>5,107</td>
<td>14.3 ± 3.4</td>
<td>10.8 ± 3.5</td>
<td>0.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(426)</td>
<td></td>
<td>(13.3 ± 3.8)</td>
<td>(9.3 ± 2.1)</td>
<td>(0)</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>Bacterial meningitis (Group B Streptococcus)</td>
<td>27,840</td>
<td>2,184</td>
<td>14.5 ± 1.7</td>
<td>12.8 ± 2.5</td>
<td>6.0 ± 2.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(182)</td>
<td></td>
<td>(11.5 ± 1.3)</td>
<td>(9.5 ± 1.7)</td>
<td>(2.0 ± 2.0)</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>Bacterial meningitis (H. influenzae)</td>
<td>243</td>
<td>—</td>
<td>0.3 ± 0.5</td>
<td>1.0 ± 1.2</td>
<td>0</td>
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<td></td>
<td></td>
<td></td>
<td>(—)</td>
<td></td>
<td>(0.3 ± 0.5)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>5</td>
<td>0.6</td>
<td>Aseptic meningitis (Mumps)</td>
<td>284</td>
<td>—</td>
<td>0.8 ± 1.0</td>
<td>1.0 ± 0.8</td>
<td>0.5 ± 0.6</td>
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<td></td>
<td></td>
<td></td>
<td>(—)</td>
<td></td>
<td>(0)</td>
<td>(0.3 ± 0.5)</td>
<td>(0)</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>Aseptic meningitis (Mumps)</td>
<td>13</td>
<td>—</td>
<td>0.8 ± 1.0</td>
<td>2.7 ± 1.2</td>
<td>2.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(—)</td>
<td></td>
<td>(0)</td>
<td>(1.0 ± 1.0)</td>
<td>(0.8 ± 0.5)</td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>Upper respiratory infection</td>
<td>0</td>
<td>—</td>
<td>0.3 ± 0.5</td>
<td>0.5 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(—)</td>
<td></td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>8</td>
<td>3.5</td>
<td>Feverish convulsion</td>
<td>1</td>
<td>—</td>
<td>0.3 ± 0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(—)</td>
<td></td>
<td>(0)</td>
<td>(0)</td>
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**Abbreviations:** N, neutrophil counts in the cerebrospinal fluid (per μL); G-CSF in CSF, G-CSF in the cerebrospinal fluid estimated by ELISA (pg/mL); G-CSF in the well, G-CSF concentration in the culture well (pg/well).

Each value represents the mean ± SD for quadruplicate assays. The numbers in parentheses show the granulocyte colonies. The in vitro colony assays were performed in the presence of the cerebrospinal fluid samples and the following materials: A, control medium; B, preimmune IgG (30 μg/mL); C, polyclonal antibodies against human rG-CSF (IgG, 30 μg/mL). See Materials and Methods.
values as estimated by ELISA exerted biologically active G-CSF.

G-CSF is known to be a monocyte-derived factor and it is also produced from fibroblasts and endothelial cells. Tweardy et al recently reported that astroglial cell lines also produced from fibroblasts and endothelial cells.

Tweardy et al recently reported that astroglial cell lines also produced from fibroblasts and endothelial cells. As no clear correlation was found between G-CSF levels and monocyte numbers in the cerebrospinal fluid, astroglia within the central nerve system may involve the G-CSF production in cases of bacterial meningitis showing high G-CSF values in the cerebrospinal fluid.

G-CSF exerts an effect not only as a stimulator for the precursor cells in bone marrow but also works on mature granulocytes. It prolongs the survival of granulocytes, works as a chemotactic factor, and stimulates the functional activity of granulocytes such as phagocytosis. All these actions suppressed the spread of bacterial infection and led to a cure. It is possible that this elevation of G-CSF in the cerebrospinal fluid may in fact enhance the migration of neutrophilic granulocytes resulting in a cure of the meningitis.

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