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

By Kazuya Shimoda, Seiichi Okamura, Fusayuki Omori, Yumi Mizuno, Toshiro Hara, Tomonobu 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.

© 1991 by The American Society of Hematology.

GRANULOCYTE colony-stimulating factor (G-CSF) is one of the cytokines that regulate hematopoiesis.1 It stimulates the proliferation of the precursor cells specific for granulocytic lineage and augments the functional activities of granulocytes. Granulocytosis is observed in the peripheral blood from patients with bacterial infections and in the cerebrospinal fluid from patients with bacterial meningitis. Although the serum levels of G-CSF in some diseases have been reported,2,3 the levels in the cerebrospinal fluid have not yet been documented. We present here the first report of the G-CSF levels in the cerebrospinal fluid from patients with meningitis as measured by our sensitive enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Patients. From 11 patients with bacterial meningitis, 18 patients with aseptic meningitis, and 10 patients punctured for the exclusion of meningitis (upper respiratory infections, four cases; feverish convulsions, three cases; otitis media, two cases; systemic lupus erythematous, one case), 45 cerebrospinal fluid samples were obtained during routine examinations. All the patients examined were children and the mean age of patients with bacterial meningitis was 2.5 ± 2.3 years, whereas that of those with aseptic meningitis was 4.9 ± 2.9 years, and in the case of other diseases besides meningitis, 2.4 ± 3.3 years. Cerebrospinal fluid was obtained by atraumatic lumbar puncture, and collected in sterile tubes. One tube of cerebrospinal fluid was centrifuged immediately at 3,000 rpm for 15 minutes, and the supernatant was frozen at −20°C until needed for determinations of G-CSF. The second and the third tube were processed in the clinical laboratory for the determination of the numbers of leukocytes, leukocyte differential count, and bacterial culture.

Measurement of G-CSF in cerebrospinal fluid by ELISA. We have already reported elsewhere a sandwich immunosassay for G-CSF using murine monoclonal antibodies (MoAbs) on the solid phase and rabbit polyclonal antibodies for the second layer. To increase the sensitivity, we modified the assay used in this study as follows. All the antibodies used here were the F(ab)2 fragment of IgG prepared by pepsin digestion. Wells of a flat-bottomed, 96-well microtiter plate (Linbro/Titretek; Flow Lab, Inc, McLean, VA) were incubated with 100 µL of MoAbs (1.25 µg/mL) in phosphate-buffered saline (PBS) at room temperature overnight followed by three washings with PBS containing 0.05% Tween 20 (PBS-T). The MoAbs used here did not crossreact with either recombinant granulocyte-macrophage CSF (rGM-CSF) or recombinant interleukin-6 (rIL-6). The plate was then incubated with the blocking solution (5% skim milk in PBS) for 2 hours at room temperature followed by a further washing. To the wells were added 100 µL of cerebrospinal fluid samples diluted with the same volume of dilution buffer (0.2% Tween 20 in PBS), and a serial concentration of rG-CSF in standard buffer (0.5% bovine serum albumin [BSA], 0.1% Tween 20 in PBS), followed by overnight incubation at 4°C. After three washings with PBS-T, 100 µL of rabbit polyclonal antibodies (IgG F(ab)2; fraction; 5 µg/mL) in PBS containing 0.1% Tween 20 and 1% skim milk (PBS-TS) were added to the wells, and the plate was incubated for 2 hours at room temperature. After three further washings, the wells were incubated with 100 µL of peroxidase-conjugated goat antirabbit IgG (Bio-Rad, Richmond, CA) diluted 1/3,000 in PBS-T for 2 hours at room temperature. Again after three washings, 75 µL of 42 mmol/L 3,3′,5,5′-tetramethylbenzidine (Katayama Chem Co, Ltd, Osaka, Japan) in 0.1 mol/L sodium acetate buffer, pH 5.5, and 25 µL of 5.6 mmol/L H2O2 were then added to the wells. Twenty minutes later, the coloring reaction was stopped by the addition of 25 µL of 2 mol/L H2SO4. The amount of G-CSF bound was then quantified by measuring the absorbance at a wavelength of 450 nm with a microplate colorimeter (Multiskan MCC ELISA reader; Titertek; Flow Lab), and the level of G-CSF was calculated from the rG-CSF standard curve. Measurements were performed in duplicate.

Biologic assay for G-CSF in cerebrospinal fluid in vitro. The in vitro colony assay used here was presented in an earlier report. Briefly, bone marrow nuclear cells (3 × 109/mL), extracted from the femurs of female Balb/c mice (8 weeks old; Charles River Japan Inc, Kanagawa, Japan), were cultured in 100 µL of a mixture containing Iscove’s modified Dulbecco medium (IMDM; Gibco, Grand Island, NY) supplemented with 20% fetal calf serum, 0.8% methycellulose (Shinetsu Chemicals, Tokyo, Japan) and 16.7% cerebrospinal fluid samples in plastic 96-microwell plates (Falcon 3072; Becton Dickinson Labware, Oxnard, CA) at 37°C in humid-
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

**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
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 \( (P < 0.01) \) 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 \( (P < 0.01) \). 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.\(^{10}\) This elevation of G-CSF would stimulate the precursor cells of the granulocyte 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 1. In Vitro Colony Assay of Mouse Bone Marrow Cells**

<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>
</thead>
<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 ± 4.2</td>
<td>21.0 ± 3.6</td>
<td>3.8 ± 1.0</td>
</tr>
<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>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<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>5</td>
<td>0.6</td>
<td>Aseptic meningitis (Mumpus)</td>
<td>284</td>
<td>—</td>
<td>0.8 ± 1.0</td>
<td>1.0 ± 0.8</td>
<td>0.5 ± 0.6</td>
</tr>
<tr>
<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 (Mumpus)</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>0.6</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>
</tbody>
</table>

**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/mL).

*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 produce from fibroblasts and endothelial cells. 

The authors thank Kirin Brewery Co Ltd (Tokyo, Japan) for providing rG-CSF. We are also grateful to K. Akazawa for the statistical analysis, to Dr Y. Asano for the colony formation assays, to Drs S. Kondo and C. Kawasaki for valuable advice, to K. Haga and H. Baba for their excellent technical assistance, and to K. Miller for proofreading the English in this manuscript.

REFERENCES

Granulocyte colony-stimulating factor in cerebrospinal fluid from patients with meningitis

K Shimoda, S Okamura, F Omori, Y Mizuno, T Hara, T Aoki, K Ueda and Y Niho